Abstracts of Oral Presentations

B E S
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Award Lecture

Protein electrochemistry: From mercury to MIPs

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Protein electrochemistry started with the polarographic investigations of proteins at mercury electrodes. This approach demonstrated the „unfolding“of globular proteins at the electrode surface but also the bioelectrocatalysis of oxygen reduction with heme proteins. Both processes are still of high relevance in protein electrochemistry. Later on, hybrid structures composed of a functionalized conductive support and biocatalysts like enzymes, organelles, cells or photosystems, have been developed as building blocks for biosensors, biofuel cells and micro-reactors. The effective electron transfer between the inorganic and the biological component is the key process in these devices and requires the adaption of both parts.
In this talk the evolution of protein electrochemistry from protein-covered mercury electrodes to electro-synthesized protein-MIPs will be presented including the following aspects:
(i) Elucidation of the interfacial behaviour of globular proteins at metal electrodes,
(ii) Establishment of the direct electron transfer of heme proteins,
(iii) Signal amplification by coupled enzyme reactions in bio(mimetic)sensors
(iv) New generation of Molecularly imprinted polymers.

Acknowledgement: FWS thanks all present and former co-workers.

Hybrid biological electric power sources: from blue bioelectrochemistry to green bioelectronics

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The lecture will overview the critical advances in electric power biodevice technology. It will start from the bioelectrochemistry of blue copper containing redox enzymes [1-5], widely used as cathodic biocatalysts of biological electric power sources [6-15]. Then, generally accepted and novel classifications of biological electric power devices, such as conventional and charge-storing biofuel cells and biobatteries, as well as conventional and self-charging biosupercapacitors, will be presented and exemplified based on a comprehensive literature analysis [6-14]. The lecture will be finished with a brief presentation of recently disclosed novel bioelectronic devices – charge-storage bio-solar cells (solar biosupercapacitors) [15, 16]. Because of their properties, i.e. real green colour and positive environmental impact, these biodevices exemplify intrinsically green bioelectronics.

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Observing, Quantifying and Understanding Vesicular Exocytosis with Micro- and Nanoelectrodes

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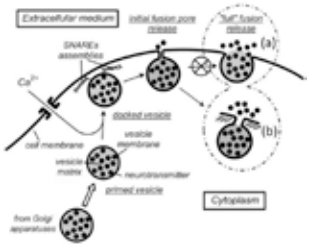
Vesicular exocytosis is a natural nanoscale process. This involves a connection between nanometric vesicles contained inside a cell with the cell membrane occurring by creation of a fusion nanopore across the two membranes through which biologically active molecules contained inside the vesicle are released into the extracellular environment (synaptic cleft, circulating fluids). Despite the minute released amounts (attomoles), single exocytotic events can be studied by means of the 'artificial synapse' amperometric method [1], in which a cell is interrogated by a carbon fiber microelectrode collecting and oxidizing released molecules so that the finely-structured electrochemical current tracks quantitatively the exocytotic flux. Our purpose is to derive dynamic information about these phenomena.

Such information is obtained by theoretical deconvolution of the experimental current to obtain the time-dependent radius of the fusion nanopore [2]. However, reconstruction requires that one of the characteristic dimensions is known as an independent entry. To this end, we resorted to initial fusion nanopore radius values (1.2±0.35 nm), which are well established by patch-clamp measurements. This allowed determining the average neurotransmitter diffusion rate within the vesicle, which in turn permitted reconstructing the fusion nanopore dynamics from any given spike [3-5].



Owing to the large number of spikes available in amperometric traces this afforded statistically significant distributions of initial and final fusion pore sizes [5,6]. This established unambiguously for the first time that the "full fusion" stage does not end into full fusion but stops after less than ca. 1% of the vesicle membrane surface area is integrated in that of the cell. The expansion of the fusion pore appears to be blocked by some mechanical constraint, presumably imposed by extra-membrane proteins [4-8]. Such quantitative results are in line with Ewing's previous qualitative observations pointing out that the fusion mechanism is more complex than thought and is most certainly regulated by an active participation of the actin cytoskeleton and possibly also by proteins complexes. The same approach was used to investigate neurotransmitter release from single functional neuronal synapses, probed with nano-conical carbon fiber electrodes. This evidenced that inside synapses release occurs mostly through Kiss-and-Run sequences [9-11].

Acknowledgements

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High-Frequency Irreversible Electroporation for Brain Cancer Treatment

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High-Frequency Irreversible Electroporation (H-FIRE) is a new, minimally invasive ablation technique that involves delivering bursts of low-energy bipolar electric pulses to targeted tissue for about 5 minutes. These intense (~1000V) yet short 200-us bursts of ~1-us pulses destabilize the cell membranes of the targeted tissue, inducing cell death. The non-thermal nature of cell death preserves important tissue components such as scaffolds, myelin sheaths, blood vessels, and connective tissue. Through treatment planning algorithms, the H-FIRE zone can accurately be predicted to optimize treatment outcome. Treatment planning is complicated by the fact that field distribution, the greatest single factor controlling the extent of H-FIRE, depends on electrode geometry, pulse parameters and tissue properties. We are currently developing H-FIRE for the treatment of glioblastoma multiforme (GBM), which has a patient median survival of only 15 months. One of the reasons for poor survival is that glioma cells typically infiltrate up to 2cm beyond the volume of the visible tumor. For tumor cells outside the direct zone of ablation, there is a transient increase in blood-brain barrier (BBB) permeability, making these cells more susceptible to administered agents and thus, making the combination of H-FIRE and adjuvant agents synergistic. We have also shown in vitro that malignant cells are more susceptible to H-FIRE than healthy cells. Our preclinical work to date has focused on helping canine patients with naturally occurring malignant glioma (MG), which are excellent translational models of human MG. Our results show that H-FIRE can be planned and monitored with techniques used routinely in neurosurgical practice, and administered in a minimally invasive fashion. Results of our ongoing trials have been extremely positive, further supporting that H-FIRE is effective for the treatment of MG, including tumors refractory to surgery, radio- and chemotherapies.

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Unconventional Use of Biofuel Cells - From Implantable Devices to Drug Release Systems and Memristors

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Biofuel cells are usually considered as miniaturized and potentially implantable power sources for biomedical applications. However, some other unusual applications are also possible for similar systems.

A new approach to bioelectronic *Sense-and-Act* systems was developed with the use of modified electrodes assembled in a system similar to a biofuel cell and performing sensing and substance-releasing functions. The sensing electrode was activated by biomolecular/biological signals ranging from small biomolecules to proteins and bacterial cells. The activated sensing electrode generated reductive potential and current, which stimulated dissolution of an Fe^{3+}-cross-linked alginate matrix on the second connected electrode resulting in the release of loaded biochemical species with different functionalities. Drug-mimicking species, antibacterial drugs and enzymes activating a biofuel cell were released and tested for various biomedical and biotechnological applications. The studied systems offer great versatility for future applications in controlled drug release and personalized medicine. Their future applications in implantable devices with autonomous operation are proposed.

An electrochemical memristor based on a pH-switchable polymer-modified electrode integrated with a biofuel cell was designed and proposed for interfacing between biomolecular information processing and electronic systems. The present approach demonstrates a new application of biofuel cells in information processing systems, rather than for electrical power generation.

Wearable Bioelectronic Devices based on Advanced Materials

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Epidermal electrochemical sensors and biofuel cells have received considerable attention in the fields of wearable devices and mobile health. A challenge unique for realizing such wearable electrochemical devices is mechanical resiliency. Mechanical damage-induced device failure is a common occurrence that can limit the operational lifespan of wearable bioelectronic devices. Recognizing these issues and challenges, this presentation will describe the use of advanced materials for imparting remarkable stretchability and self-healing abilities that enable epidermal bioelectronic devices to endure extreme deformations experienced by the human skin without compromising their bioelectronic characteristics. Particular attention will be given specially-engineered inks for creating printable electrochemical biosensors and biofuel cells that can endure strains as high as 500% and offer autonomous healing ability without impacting their sensing or energy-harvesting ability.

Creating and Using Electronically-Controlled Microorganisms

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Since both microorganisms and devices use electrons as information and energy carriers, interfacing living cells with electrodes offers the opportunity to control key biological processes electronically. In the first part of my talk, I will describe how we have transformed the industrial microbe *Escherichia coli* into an electroactive bacterium, whose behavior we can control and readout electronically. In the second part of my talk, I will describe new materials and devices that are optimized for electronically interfacing with these microbes. Together this work paves the way for employing rationally constructed electronically-controlled microorganisms in sensing and bioenergy applications.

Figure 1. Schematic showing engineered microorganisms that interface with inorganic materials.

Systems Integration in Bioanalysis: Oriented Immobilization of Biomolecules for Affinity Sensors

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Biosensors have been a first step towards integration by merging biotechnology with electronics to facilitate easy to use and fast chemical analysis. Additionally, microfluidics was invented to empower Lab-on-Chip applications and raised a new field of technologies. The merger of biomolecular analysis with electronics and telecommunication will be the next step of integration for daily use. The ultimate integration will be done on the molecular level: Sensor-actor molecules are capable of recognising and signalling within a single molecular unit.

Here, we will present recent results on immobilization of biomolecules by virtue of second order electrical forces. Immobilisation of active biomolecules is a key task for biosensor manufacturing. Usual techniques based on chemical coupling relay on statistical distribution of molecules attached to the surface. Only a reduced portion of molecules remain active after this kind of immobilisation, reports give estimates of only 10% of all antibodies fixed at a surface to remain active. This feature of chemical coupling rises concerns about miniaturisation, because the number of active molecules may be too low to gain a significant response. Therefore oriented immobilization is required. Biochemical immobilisation by use of affinity tags is widely used and molecular biologists are inventive to develop new tags.

An alternative to chemical coupling has recently introduced by our group employing second order electrical forces: Dielectrophoresis, i.e. the action of an electric field gradient on polarisable objects, is well known to act on microscopic particles like cells (Pohl 1978). It has been demonstrated to be applicable even on the molecular scale at room temperature (Hölzel et al. 2005). To achieve this effect nanoscale electrodes are required. The curvature of the electrode tip, which is responsible for the strength of the field gradient, has to scale in the same order of magnitude as the objects that shall be attracted. Moreover, the molecules are not only attracted, but also show orientation according to the induced electrical dipole (Laux et al. 2016). This effect can be used for a method applicable to biomolecules with the result of orientation.

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Keynotes

The role of DNA-specific pattern recognition receptors in electrotransfer of plasmid DNA into tumor cell lines

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Electrotransfer is an effective method for introduction of plasmid DNA into different cell types. Although the use of electrotransfer already reached clinical trials, the effects of electrotransfer at cellular level are not completely understood. Electrical parameters of electrotransfer vary greatly between different applications, but in general, the electrotransfer of plasmid DNA can cause cell death. To explore the possible underlying mechanisms involved in the observed phenomenon, potential activation of DNA-specific pattern recognition receptors (PRRs) were studied in different tumour cell lines *in vitro* and B16.F10 melanoma *in vivo* using two different protocols of gene electrotransfer. In addition, production of proinflammatory cytokines, type I IFN cytokines and the mode of cell death were studied.

In vitro, in B16.F10 melanoma, WEHI 164 fibrosarcoma and TS/A mammary adenocarcinoma cells, both protocols of electrotransfer upregulated mRNA of cytosolic DNA sensors, predominantly, DAI/ZPB1, DDX60 and p204. Furthermore, IFNβ and TNFα mRNA and proteins were detected in all cell lines, but to different levels depending on the electrotransfer protocol used. These observations were confirmed by determination of the mode of cell death. Gene electrotransfer protocol using 8 electric pulses of 600 V/cm, 5 ms duration at frequency 1 Hz resulted in higher percentage of necrosis compared to electrotransfer protocol with 6 pulses, 1300 V/cm, 100 μs duration at frequency 4 Hz. However, the latter protocol lead to higher percentage of apoptosis. *In vivo*, in B16.F10 tumours, the upregulation of DNA sensors was not detected; however production of IFN 1β was increased, indicating on the paracrine/autocrine signalling leading to cell death.

In conclusion, the results of our study demonstrate that gene electrotransfer of plasmid DNA devoid of therapeutic gene lead to increased IFN1β, TNFα and DNA sensor expression, thus indicating that this mechanism might be the putative for the observed cytotoxicity and tumour regressions obtained in gene electrotransfer studies.

Electrochemical and Computational Study of the Reactivity of a Diiron Azadithiolate Complex towards Protons

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One of the catalytic key features of the organometallic active site of diiron hydrogenases is the presence of an amino group in the dithiolate-bridge (Figure 1). Its role is qualitatively explained by considering that, relative to proton transfer directly to the metal center, protonation of the second coordination sphere involves lower reorganization energy and thus proceeds at a faster rate. However, the factors that influence the thermodynamics and the kinetics of the intramolecular proton transfer from the ligand to the metal center remain often elusive.

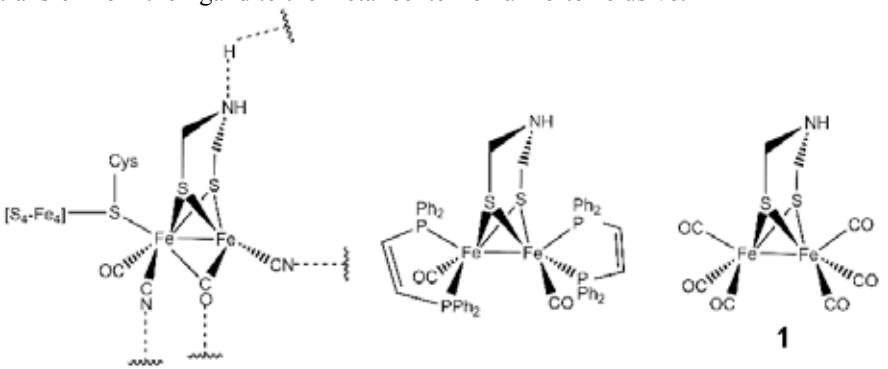

Figure 1 From left to right: Structure of the active site of [FeFe]-hydrogenase, $[Fe_2(\mu\text{-}adt^H)(CO)_2(\kappa^2\text{-}dppv)_2]$, and $[Fe_2(\mu\text{-}adt^H)(CO)_6]$ (**1**).

Here we will present recent studies suggesting that the electrochemical reduction of the N-protonated form of $[Fe_2(\mu\text{-}adt^H)(CO)_6]$ (**1**) triggers a tautomerization reaction leading to a putative hydride intermediate [1,2]. The reactivity of complex **1** towards protons in the presence of coordinating anions will also be discussed [3].

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Bioenergy from Microbial Fuel Cells for Practical Implementation

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Microbial fuel cells (MFCs) are a kind of renewable energy technology, which can convert chemical energy locked in organic matter, directly into electricity [1], and at the same time treat (or clean) the feedstock supplied as fuel. This is the result of metabolism from electro-active microorganisms, which utilise organic matter, found in the fuel/feedstock, as an electron source, and the anode electrode (rather than e.g. oxygen) as the electron acceptor [2]. This microbial anaerobic respiration results in electrons 'excreted' as part of electroactive metabolites, or conducted via biological conduits, which may also be used for attachment on the electrode surface. When an external circuit is connected between the negative anode and the positive (abiotic) cathode, electrons flow through the circuit, to produce current.

In the field of MFCs, it well known that individual units produce relatively low levels of electrical output - due to thermodynamic limits. Over the years, workers have attempted to address this issue (and achieve scale up) either by enlarging individual units, or by assembling stacks of a plurality of units, to produce useful levels of electricity [3], and it is the latter approach that has been followed in the current long-term study. When stacked, MFCs can produce sufficient power to run various applications, either directly or intermittently through recharging batteries or super capacitors [4]. The main objectives of this work are therefore: (i) to improve the energy density of individual units, with the incorporation of additional electrodes – also called "pins" - and (ii) optimise the configuration and performance of MFC stacks for generating useful power and running practical applications. This presentation will introduce the MFC technology, and demonstrate its development from a lab-based technology to a system undergoing field trials.

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Multilayered Lipid Membrane Stacks for Bioelectrocatalysis Using Membrane Enzymes

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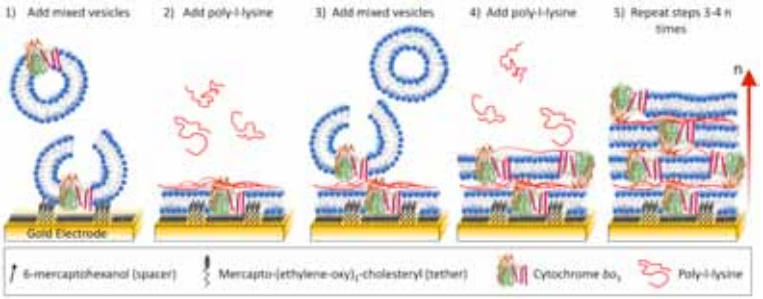
Multilayered or stacked lipid membranes are a common principle in biology and have various functional advantages compared to single lipid membranes, such as their ability to spatially organize processes, compartmentalize molecules and greatly increase surface area and hence membrane protein concentration. Here we present a supramolecular assembly of a multilayered lipid membrane system in which poly-L-lysine electrostatically links negatively charged lipid membranes. When suitable membrane enzymes are incorporated, either an ubiquinol oxidase (cytochrome bo_3 from *Escherichia coli*) or an oxygen tolerant hydrogenase (the membrane-bound hydrogenase from *Ralstonia eutropha*), cyclic voltammetry (CV) reveals a linear increase in biocatalytic activity with each additional membrane layer. Electron transfer between the enzymes and the electrode is mediated by the quinone pool that is present in the lipid phase. We deduce by atomic force microscopy, CV and fluorescence microscopy that quinones are able to diffuse between the stacked lipid membrane layers via defect sites where the lipid membranes are interconnected. This assembly is akin to that of interconnected thylakoid membranes or the folded lamella of mitochondria and have significant potential for mimicry in biotechnology applications such as energy production or biosensing.



Enhancing nanopore sensing with DNA nanotechnology

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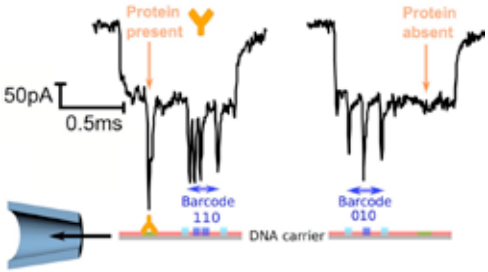

Solid-state nanopores are single molecule sensors for detection and analysis of biomolecules like DNA, RNA and proteins. The idea is as simple as intriguing since the sensing relies on measuring the changes in ionic current as a macromolecule passes.

We introduced DNA carriers that have specific protein binding sites to control protein translocation. Protein detection down to the single protein level is achieved, allowing for identification a single protein species within complex mixture [1]. We used our DNA carriers to detect nanomolar protein concentrations and compare performance of antibody samples acquired from a range of manufacturers [2].

Using ideas from DNA origami self-assembly, we design a library of DNA carrier with digitally encoded markers that allow for unambiguous identification. The DNA carrier method is extended to fully multiplexed protein sensing and identification [3].

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From metalloenzymes to bioinspired catalysts for Noble Metal-Free hydrogen fuel cells

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Conventional hydrogen fuel cells require noble metal such as platinum to harvest energy from the electrocatalytic H_2 oxidation and O_2 reduction. Nature rely on iron, nickel and copper-based enzymes in order to oxidize H_2 or reduce O_2 with minimal overpotential requirement and exceptional catalytic activity. The electrical wiring of enzymes on electrodes implies the development of efficient strategies to maximize enzyme concentration and electron transfers between the surface of the electrode and the enzyme active site. This presentation will show our recent advances to integrate such enzymes at fuel cell electrodes. We have especially investigated the wiring of hydrogenases and multicopper oxidases at nanostructured electrodes for their integration in conventional fuel cell systems.[1–3] Another strategy lies in the design of novel bioinspired molecular catalysts and their immobilization on electrodes. These catalysts have to be designed by taking into account the important features of metalloenzymes which are responsible for their exceptional catalytic efficiency. Our efforts in the design of novel bioinspired catalysts for both H_2 oxidation and O_2 reduction will be discussed as well as their immobilization on carbon nanotubes and their integration in noble-metal-free hydrogen fuel cells.[4–6]

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Elucidating Neurochemical and Circuit Based Mechanism of Action of Neuromodulation

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Despite its widespread clinical success, there is a limited understanding of the therapeutic mechanism behind Deep Brain Stimulation, Spinal Cord Stimulation, and Cortical Stimulation. At Mayo Clinic, we have deployed these technologies to the treatment of Parkinson's Disease, Essential Tremor, Dystonia, Obsessive Compulsive disorder, Chronic Pain, Tourette's Syndrome, and Paralysis from spinal cord injury. Further, to fully understand the neurochemical and neural circuit effects of DBS, we developed the Wireless Instantaneous Neurochemical Concentration Sensing (WINCS) system, Mayo Investigational Neuromodulation Control System (MINCS), WINCS Harmoni, and fMRI monitoring during neuromodulation. WINCS and Harmoni employs fast scan cyclic voltammetry (FSCV) to characterize the neurochemical interactions events during DBS. Harmoni incorporates a wirelessly controlled synchronizable neurostimulator, and four-channel integrated circuit for simultaneous neurochemical and electrophysiological measurements. PC based software provides real-time control of stimulation, neurotransmitter detection, data acquisition, and data visualization. Harmoni successfully evoked and detected striatal dopamine release by DBS, as well as provided closed loop stimulation based on FSCV recordings. Notably, the synchronization of stimulation with interleaved FSCV scans eliminated the stimulus artifact that would have otherwise obscured the neurochemical measurements. In addition, the stereotactic targeting of the recording electrode was identified by fMRI BOLD activation during DBS in the pig, monkey, and human. Taken together, our results suggest the activation during DBS corresponds to neurotransmitter release in distant sites of the neural circuit. Our results suggest the exciting possibility of fMRI based stereotactic sensor implantation combined with neurochemical (FSCV) based closed loop DBS for the treatment of variety of neuropsychiatric disorders in the future.

Electroporation without Electropermeabilization and Electropermeabilization without Electroporation ? A Reflexion on the Consequences of Cell Exposure to Electro(magnetic)fields and its Major Consequences

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Electropulsation, which consists in exposing to short and intense electric pulses either cells or spheroids or tissues or organisms or, on the contrary, very simple objects as Giant Unilamellar lipidic Vesicles, is a technique known since decades and largely applied in medicine and in food and environment industries. It allows the transport of a large variety of molecules across the membrane. The electropermeabilized state, which lasts for durations that are orders of magnitude longer than the pulse duration, usually follows the electroporated state that is achieved during the delivery of the electric pulses. Nevertheless, in experimental models, hydrophilic pores (electroporation) can be generated during electric pulses delivery, without the generation of an electropermeabilized state. Thus electroporation and electropermeabilization can be dissociated.

The molecular changes occurring at the cell membrane during the electric pulses and persisting during the post-pulse period (the electropermeabilized state) start to be deciphered. However, there are still many questions open: for example, are the same molecular species transported in the electropermeabilized and in the electroporated states? is it possible to achieve electropermeabilization in the absence of electroporation?

Keynotes

Photoelectrochemistry of the water oxidation enzyme Photosystem II: From basic understanding to semi-artificial photosynthesis

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In natural photosynthesis, light is used for the production of chemical energy carriers to fuel biological activity and the first protein in the photosynthetic chain is the water oxidation enzyme Photosystem II. This presentation will summarise our progress in the development of protein film photoelectrochemistry as a technique for the light-dependent activity of this enzyme adsorbed onto an electrode surface to be studied.[1] Materials design enabled us to develop 'tailor-made' 3D electrode scaffolds for optimised integration of the 'wired' enzyme and these investigations yielded valuable insights into the performance of Photosystem II and interfacial charge transfer pathways. Examples are the identification of unnatural electron escape routes to the electrode and a recently elucidated O_2 reduction pathway that short-circuits the known water-oxidation process.[2]

The integration of Photosystem II in a photoelectrochemical circuit has also enabled the in vitro re-engineering of natural photosynthetic pathways. We succeeded in assembling an efficient enzyme-based full water splitting cell driven by light through the rational wiring of Photosystem II to a [NiFeSe]-hydrogenase.[3] This hydrogenase displays unique properties for water splitting applications as it displays good H_2 evolution activity, little product (H_2) inhibition and some tolerance towards O_2.[4] The semi-artificial water splitting cell shows how we can harvest and utilise electrons generated during water oxidation at Photosystem II electrodes for the generation of renewable H_2 with a wired hydrogenase through a direct pathway unavailable to biology. This work is currently supported by an ERC Consolidator Grant 'MatEnSAP' (682833).

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Porous carbon materials for enzymatic biofuel cells

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A combination of electron transfer technology and porous carbon material would be helpful in achieving a much higher and stable current output, thus contributing to a practical advance in the sustainable energy field. In this presentation, recent developments in enzymatic biofuel cells (EBFCs) technology using porous carbon materials, especially Magnesium oxide (MgO)-templated porous carbons (MgOC), are highlighted. Our recent papers demonstrated that MgOCs are promising candidates as electrode materials for the elaboration of efficient bioelectrochemical devices. MgO is a suitable template because it is both thermally and structurally stable during carbonization, and is easy to remove from the resulting carbon by washing with a dilute acid. Another advantage of MgOC over other mesoporous carbons is its tunable pore size distribution (2–150 nm), which can be modified by changing the crystalline structure of the template MgO [1]. Furthermore, the interconnected mesopores of MgOC provide a high effective surface area per volume for enzyme immobilization.

The effect of pore size of MgOC were elucidated by using MgOCs with mean pore diameter 5, 10, 20, 40, 100, 150 nm. To create a glucose bioanode, an MgOC electrode was further coated with a biocatalytic hydrogel composed of a redox polymer, flavin adenine dinucleotide-dependent glucose dehydrogenase (FAD-GDH), and a cross-linker. For fructose bioanode, fructose dehydrogenase was modified on the electrode without a redox mediator. MgOC-modified electrode can be applied for the biocathode using bilirubin oxidase (BOD) as an oxygen reduction reaction catalysts. The large surface area of mesoporous materials can increase the enzyme loading and electron transfer efficiency, and the macropores enable the efficient fuel transport.

MgOC with a three-dimensional (3D) hierarchical pore structure were designed to further improve the current production efficiency and stability of bioelectrodes. The 3D hierarchical meso/macro-porous carbon was fabricated from dual MgO templates: small MgO template (like 10, 20 or 40 nm) and large MgOC (150 nm). The morphology of pores are also important, and the optimal pore composition for a BOD-catalyzed oxygen reduction cathode was a mixture of 33% macropores and 67% mesopores [2].

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Keynotes

Mechanistic insight into enzymatic electrocatalysis via surface enhanced vibrational spectro-electrochemistry

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For utilisation of enzymes in electrocatalytic applications immobilisation of the enzyme on electrodes hast to be done in a way that the structure of the active site is preserved. Furthermore substrate and proton accessibility from solution and electron supply from the electrode has to remain efficient. The latter point becomes very important as in many cases electrocatalytic activity of immobilized enzymes is lowered not because the active site is damaged but due to a change in the electron transfer properties in the adsorbed state. To understand these processes on electrodes techniques are needed that show the structure of the enzyme when attached to the electrode surface in presence of an electrochemical potential.
Surface enhanced Raman spectroscopy (SERS) and surface enhanced infrared absorption (SEIRA) are able to analyse the structure of surface bound molecules under light illumination if nanostructured plasmon active noble metal electrodes are used as support material. We have combined these surface sensitive spectroscopic methods with electrochemistry to understand the correlation between electrocatalytic activity and electron transfer properties of several enzyme/electrode systems [1]. The redox enzymes cellobiose dehydrogenase (CDH) [2], cytochrome c oxidase (CcO) [3] and the hexameric tyrosine coordinated heme protein (HTHP) have been investigated by comparing SERS and SEIRA spectral changes with the electrochemical signal of the enzyme. It turned out that in all cases electron transfer properties were crucial for the activity of the system. The results are compared to bioinspired metal complexes that show similar electrocatalytic properties [4].

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Coupling enzymes and electrodes for bioelectrocatalysis: Coupling strategies and electrochemical reactions of heme- and molybdopterin containing enzymes

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Bioelectrocatalysis involves the enhancement of an electrode reaction by a (bio) catalytic process and is thus the basis for sensitive and selective electroanalytical devices. For effective bioelectrocatalysis it is essential to achieve a fast communication between the redox protein and the electrode, while the biocatalytic activity is preserved. The signal transduction is performed by direct electronic communication between the protein and redox electrodes and by mobile or polymer bound redox mediators. For direct electron transfer the surface interaction for immobilization plays the most important role. We will discuss recent examples of enhanced electron transfer in such electrochemical biosensors, i.e., cytochromes and heme peptides at metal oxides, sulfite oxidase bioelectrocatalysis on nanoparticles and formate dehydrogenase modified electrodes. These approaches are used to contribute to the understanding of the mechanism of enzymes and their biotechnological exploitation.

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Enzymatic Bioelectrosynthesis of Ammonia and Hydrocarbon

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Bioelectrochemical systems working with isolated enzymes have been extensively studied for the development of biofuel cells and biosensors. Such devices can operate at room temperature, ambient pressure and near-neutral pH, all of which are highly desirable for alternative production of chemicals and fuels. Therefore, enzyme-electrodes can offer the possibility to produce electricity, fuels and chemicals, simultaneously with low cost. Alternative production of chemicals and fuels of great interest, such as ammonia and hydrocarbons, is of high interest to scientists globally. The Haber-Bosch process is an industrial process with high energetic cost that produces around 66% of total ammonia (NH_3) produced in the world from molecular hydrogen (H_2) and molecular nitrogen (N_2).[1] The synthesis of hydrocarbon that lack any chemical functional groups is also still challenging.

Nitrogenase and aldehyde decarbonylase (AD) are two promising redox enzymes for the field of bioelectrosynthesis. The first one is the only know single enzyme capable to catalyze the reduction of nitrogen (N_2) to ammonia (NH_3). The AD, recently renamed aldehyde-deformylating oxygenase, can catalyze the decarbonylation of fatty aldehyde to alkane by converting the aldehyde carbon to formate with a broad range of substrates (C18 to C3). It was shown that this enzyme undergoes a redox oxygenation requiring an auxiliary reducing system such as NADH/PMS.[2]

On one hand, we demonstrate the coupling of hydrogenase (anodic) with nitrogenase (cathodic) with methyl-viologen as a unique electron mediator. This coupling resulting in NH_3 production from H_2 and N_2 while simultaneously producing an electrical current.[3] On the other hand, we show the possibility to produce alkanes by bioelectrocatalysis by using an electrochemical reducing system with AD.

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Assembly and stacking of enzymatic bioelectrodes for high power glucose fuel cells

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Fuel cell technology relies on the conversion of chemical energy into electrical energy. One of the biggest challenges in the field of enzymatic biofuel cells is the choice of the catalyst and also the stability of the bioelectrodes. At the anode, the use of glucose oxidase from *Aspergillus Niger* (GOx) is promoted with a well-known redox mediator, 1,4-naphthoquinone (NQ) in order to transfer the generated electrons during glucose oxidation to the electrode. The stability of this anode has already been demonstrated[1]. At the cathode, the effective immobilization of bilirubin oxidase from *Myrothecium Verrucaria* (MvBOD) has been performed on carbon nanotube electrodes. Efficient orientation was obtained thanks to the use of protoporphyrin IX as a direct electron transfer promoter for the reduction of oxygen in water[2]. This work is dedicated to the enzymatic biofuel cells design for glucose and O_2 supply in order to obtain a sufficient power to run nomad devices such as a timer or a watch without using energy storage devices or DC/DC converters. Different configurations have been tested to fully characterize our devices. The optimized configuration of our biofuel cell design allows the connection of several biofuel cells in series and in parallel. The configuration of two cells connected in parallel shows an open-circuit voltage (OCV) of 0.669 V and supplies a maximal power (P_{max}) of 1.75 mW at 0.381 V. In series, an OCV of 1.35 V is measured providing a P_{max} of 1.82 mW at 0.675 V. Moreover, the long-term stabilities and the produced energy increase markedly in charge / discharge mode. While applying 3 kΩ of resistance induces the energy production of 1 mW.h in 48 hours under discontinued discharges, 0.72 mW.h was generated within 24 hours at 6 kΩ under continuous discharge.

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Electrochemical monitoring of the reversible folding of unlabelled DNA i-motif structures at gold electrodes

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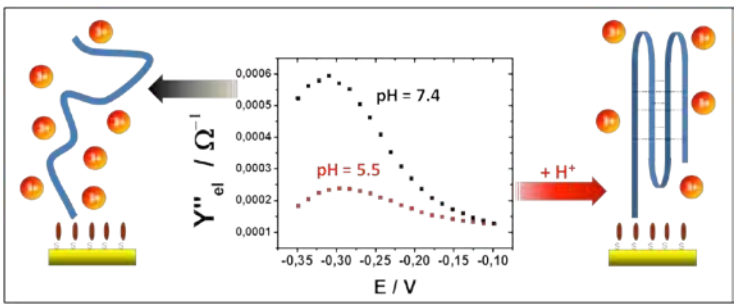
Non-canonical DNA structures are nucleic acid arrangements that differ from the classical Watson and Crick double helical structure and can be observed in some specific DNA sequences depending on the molecular environment (temperature, ionic composition, pH…). Because of their good stability and reversible responsiveness, these secondary structures are attracting a lot of interest in the field of nanotechnology and for the design of biosensors. In this work, we present an electrochemical method that allows the *in situ* monitoring of the formation of i-motif structures with cytosine rich sequences (*e.g.* the human telomeric sequence) immobilized on gold electrodes as self-assembled monolayers. Folding of i-motifs is highly sensitive to the pH and is directly related to the protonation of cytosines. We will demonstrate that electrostatic interactions between the electroactive $[Ru(NH_3)_6]^{3+}$ and the negatively charged DNA backbone are sensitive enough to probe the variation of the effective charge of the i-motifs between the folded and unfolded states. This allows us to quantify the charge on the DNA and to estimate the surface pK_a of the immobilized sequences. This work is an important contribution for the study of i-motif DNA structures at the surface of electrodes.



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Star-shaped gold nanoparticles provide improved interfaces for electrochemical biosensors

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Gold nanoparticles (AuNPs) are among the most used nanostructured materials in the field of (bio)electroanalytical detection. Unique physical and chemical properties, such as high surface-to-volume ratio, high electrical conductivity and excellent biocompatibility, contributed for their successful use in electrochemical sensors and biosensors with enhanced performance [1]. Traditionally, AuNPs are spherically shaped. Herein we have explored AuNPs of different shapes as promoters of the direct electron transfer of redox proteins and enzymes. We focused on star-shaped particles (AuNS), which have different curvatures and enhanced surfaces areas and consequently have been associated with improved adsorption of proteins in comparison with spherical AuNPs. The NPs were modified with bifunctional alkanethiol capping agents (e.g. mercapto-undecanoic acid, MUA), which enable strong binding to the AuNPs and at the same time provide a biocompatible surface that can be further used to bind redox proteins [2]. In this way, cytochrome *c* based bionanoconjugates were prepared using MUA coated AuNS and 15 nm spherical AuNPs. The biocomposite materials were deposited on pyrolytic graphite (PG) electrodes and probed by cyclic voltammetry. The cyt *c*/MUA/AuNS modified PG electrodes displayed improved electrocatalytic activity towards H_2O_2 reduction in comparison with spherical AuNPs based bionanoconjugates. The analytical performance of different electrode designs is currently being optimized. In parallel work, these nanostructured materials were used as platforms for the immobilization of glucose oxidase. The response of the enzyme to glucose was evaluated via the consumption of oxygen. The activity of this glucose biosensor was at least 5x higher on AuNS modified glassy carbon electrodes than in control electrodes without NPs.

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Biosupercapacitors Based on Enzymes Entrapped in Os-Complex Modified Redox Polymers

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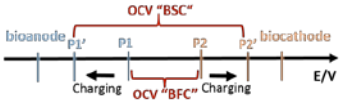
We present a self-charging biocapacitor based on Os-complex modified polymers that are used to entrap FAD-dependent enzymes at the anode and O_2 reducing bilirubin oxidase at the cathode. A first device was fabricated by using a high potential redox polymer for the bioanode and a low potential redox polymer for the biocathode; $E_{anode} > E_{cathode}$ (in the absence of the substrate at open circuit). Upon charging the a_{ox}/a_{red} ratio is changed by conversion of the Os-species within the polymer matrix driven by the catalytic reaction. Hence, the potential of the anode and the cathode are shifted to lower and higher values, respectively, and a potential inversion occurs ($E_{anode} < E_{cathode}$). This results in the establishment of an effective OCV of > 0.4 V for the presented devices [2].

The high thermodynamic driving force originating from the large potential difference between the designed redox polymers and the enzyme ensures fast and efficient charging of the redox polymer at the anode and the cathode side.

Moreover, to achieve a higher OCV a high-potential biocathode and low-potential bioanode was developed in analogy to a conventional biofuel cell design. For this the potential of the redox polymers were tuned by modulation of the ligand sphere of the polymer-tethered Os-complexes.



In order to enhance the stability of the Os-complexes, which is of particular importance for device fabrication, we synthesized inert and stable N6-coordinated Os-complexes bearing stable alkyl chain based tethers for the covalent attachment to the polymer backbones.

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High throughput electrochemical assays for oxidase activity measurements

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The identification of enzyme inhibitors from large chemical libraries is usually achieved by performing efficient high throughput screening (HTS) assays. Electrochemical methods for the detection of biological activity require the optimization of experimental conditions (electrode interface, immobilization matrix, reaction media…). This approach reveals time-consuming because only few samples can be measured at the same time and large reaction volumes are required. Therefore, electrochemical HTS assays are necessary.
Previously, such method has been proposed by our lab to perform 96 different independent electrochemical measurements within 1 minute using 96 screen-printed carbon electrodes and small reaction volume (30-50 µl) (1, 2).
In the present work we focus on transketolase (TK), a target enzyme in the treatment of cancer, neurodegenerative diseases and diabetes (3), whose activity cannot be directly detected by electrochemical methods. Therefore, an indirect assay has been developed using an auxiliary enzyme, namely galactose oxidase (GAOx) (4). Hydrogen peroxide, produced after the oxidation of TK products by GAOx, is detected using Prussian-blue modified electrodes.
Here, we report recent results using our electrochemical HTS assay to optimize the detection of free or immobilized GAOx activity, in only 50 min by the mean of 5 different sets of 96 screen-printed electrodes, and by using two electrochemical methods: intermittent pulse amperometry using Prussian blue as mediator and electrochemiluminescence using luminol (5). This assay may be extrapolated to other oxygen dependent oxidases.

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Tunable Self-Assembled Scaffolds on Gold-Interfaced Transducers as Supports for Biosensing Applications

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Affinity sensors built-up on gold surfaces are probably the most versatile platforms for real time monitoring of biomolecular events and detection of structurally complex analytes through molecular recognition. Due to their inherent properties, thin gold films uphold tunable 1D, 2D and 3D scaffolds of alkylthiols, peptides, molecularly imprinted polymers [1] etc., prevalently trough self-assembling in a variety of immobilization formats. At the same times, gold films are amenable for interfacing electrochemical, optical or piezoelectric transducers. Herein, surface plasmon resonance (SPR) and surface acoustic wave (SAW) sensors are the suitable choice for the detection of small toxins [2], antibiotic residues or even proteins from complex matrices, due to their enhanced sensitivity to mass changes near the surface (SPR sensors) [3] or mass, density and viscosity variations (SAW sensors) [4]. Voltammetric sensors based on redox-labeled self-assembled monolayers (SAMs) can also be used for developing affinity bioassays [5]. Our research group has focused on developing multifunctional supports for a wide range of targets or receptors, which can be used with for electrochemical, SPR and SAW transducers. In this context, we optimized alkylthiolated supports for SPR and SAW transducers for the direct detection Aflatoxin B1-protein bioconjugates in serum samples and redox peptide SAMs for direct antibody detection through voltammetric measurements. Our aim is to extend these achievements for developing affinity formats for the indirect detection of small analytes such as Ochratoxin A and chloramphenicol.

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Electrochemical Monitoring of the Interactions between DNA and Bioinorganic Compounds

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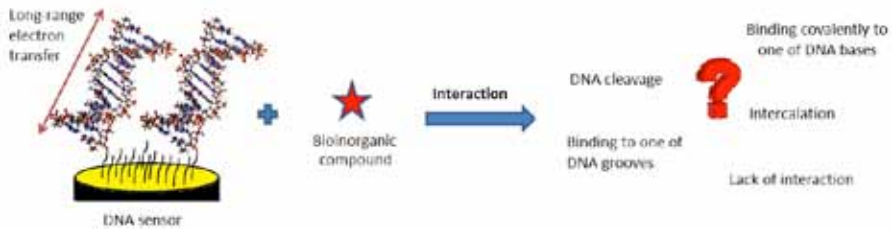
Some bioinorganic compounds (BC), during interaction with DNA, can change the conformation of DNA and block binding sites. Additionally, some of the BC after binding to DNA can promote a Fenton-like reaction that leads to a creation reactive oxygen and nitrogen species (ROS/RNS). Radicals can cause serious DNA damage, such as strand breakage. Thus, DNA-BC interaction can inhibit DNA replication and, consequently, result in cell death. Hence, some BC have the potential to be used as anticancer drugs as they can efficiently kill cancer cells.

The aim of this research project is to use electrochemical DNA sensors to investigate the interactions between DNA and BC, proposed as candidates for cancer therapeutics.

An electrochemical DNA sensor, fabricated through the immobilization of DNA onto the electrode surface, can deliver information about changes in DNA structure caused by drug interaction. DNA layers immobilized covalently to electrode surfaces are quite robust and DNA is fully accessible for interaction with BC.

The DNA sensors are immersed in a solution containing the BC of choice. During the redox cycling of bioinorganic molecules at DNA sensors, electrons must be transferred to and from the electrode surface to the compound. Oxidation or reduction of the metal centre is thought to occur *via* a long-range electron transfer mechanism – stacked DNA base pairs behave as the electron conduit. Changes in the measured current response of the sensor, in the presence of the BC, can provide information about the type of binding interaction.

The nuclease activity of the BC is investigated through the measurement of changes in the DNA electrode surface coverage before and after interaction with the BC.



Development of an Electrochemical Biosensor Based on Nanomodified IDA Biochip for High Sensitivity Non-Labeled Biomarker Detection

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Sensitive detection of protein biomarker molecules is very potential strategy for the next generation diagnostic and evaluation of the treatment methods [1]. Nevertheless, preparation of electrodes with high sensitivity and specificity is a challenging task because of non-specific binding of analyte [2]. Different techniques were combined: microcontact printing (μCp) of self-assembler monolayers (SAM), wet etching, electroplating, etc., to assure faster and cheaper preparation of sensitive platinum and gold Interdigitated-array (IDAs) electrodes [3, 4].

In this work, we use label-free techniques for the detection of ovarian cancer biomarker (CA-125) avoiding modification of antibodies with additional labels such as fluorescent dyes, redox enzymes or radioactive labels.

Characterization of the electrodes was performed using scanning electron microscopy, energy-dispersive X-ray spectroscopy and atomic force microscopy before immobilization of antibody. Alternative methods for antibody immobilization such as μCp and electrochemical deposition of SAM's as well as application of immunoassay using cyclic voltammetry, electrochemical impedance spectroscopy and electrochemiluminescence analysis will be presented.

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Optimization of A biocompatible bioanode based glucose dehydrogenase for implantable glucose biofuel cell

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In the past few decades, we have witnessed tremendous development in electronics, micro- and nano-fabrication, and wireless technology which have greatly enhanced the quality and efficacy of healthcare as well as life-science research. Medical Devices (IMDs) are used to improve healthcare, aiding or delivering the functions of certain malfunctioning organs have been sustained for years. However, to ensure proper operation, most IMDs need to rely on a permanent and sufficient power supply, thus numerous power sources for IMD have been widely investigated in the last decades. The existing technology is to utilize a rechargeable lithium battery, but a battery with an acceptable lifetime and size is only indicated for implanted devices consuming a few tens of microwatts. In this context biochemical energy harvesters, also called **biofuel cells, that convert the energy stored in chemical bonds into electrical energy are very promising**. In medical implants, a biofuel cell generates the power by complementary chemical reactions at a pair of electrodes. Oxidation occurs at the anode electrode and reduction takes place at the cathode [1]. Chemical reactions are accelerated by the participation of biocatalysts (enzymes). Due to its omnipresence in body fluids, glucose is the most commonly used fuel in biofuel cells. The most used enzymes for the oxidation of glucose is the enzyme oxidase (GOx), which is a hard enzyme with a good stability. However, in the presence of oxygen and glucose, GOx produce hydrogen peroxide which is toxic inside body [2]. To overcome to this problem, we propose in this present work, the use of bioanode based the glucose dehydrogenase which is insensitive to the presence to the oxygen. Our bioanode is fabricated by a mechanical compression of GDH and its cofactor NADP, carbon nanotube particles (CNTs) coated with poly (methylene green) (PMG) for the regeneration of NADP. CNTs coated PMG where fabricated by chemical polymerization of the methylene green on CNTs nanoparticles (Figure 1). Moreover, we have optimized the synthesis of Nafion polymer with a controlled porosity to prevent GDH and NADP lunching from the bioanode to the electrolyte solution. To ensure the biocompatibility the bioanode surface was covered with a biocompatible membrane of Poly-Vinyl Alcohol (PVA) (Figure 2.A). Electrochemical performances show that this anode is capable to produce a current density of 500µA/cm^2 in physiological conditions (glucose 5mM) (Figure 3). Moreover, the bioanode was capable to produces continuously a current density of 50µA/cm^2 during three weeks. Finally, first in vivo test inside rat body shows a good biocompatibility of the bioanode and no inflammation or rejection reaction were observed. Besides ex-vitro measurement shows that after one month of implantation the bioanode still active.

Neural Engineering Next Generation of DBS Technology

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Since Deep Brain Stimulation (DBS) systems have become available (FDA approval 2002), there have been approximately 110,000 implantations, primarily for debilitating neurologic disorders such as essential tremor and Parkinson's disease. While DBS is believed to regulate neurochemical release, the stimulation effect is not known. A variety of brain characteristics have been proposed for providing a feedback mechanism to allow the extension of DBS to a variety of disease states including psychological issues such as depression, obsessive compulsive disorder and Tourette's among others. Feed back mechanisms include electrophysiology, amperometery and fast scan cyclic voltammetry (FSCV). We, at the Mayo Clinic have developed WINCS (Wireless Instantaneous Neurochemical Concentration Sensing), MINCS(Mayo Investigational Neuromodulation Control System) and Harmoni (the combination of WINCS and MINCS) as a staged approach in the design and building of closed loop DBS based upon FSCV electrochemical detection. The monitoring system utilizes a real time, wireless, in vivo neurochemical detection process which is capable of monitoring and reporting in real time the release in neurochemicals during DBS surgery. By imposing a voltage waveform that ramps through potentials allowing oxidation and reduction of chemical species of interest, detection and measurement of chemical changes can be determined by monitoring the nanoampere amount of electrical current flowing at specific voltages. Further, the carbon fiber microelectrode, long the mainstay of FSCV, erodes with the imposed voltage waveform. It appears that carbon is oxidized to carbon dioxide which dissolves in the interstitial fluid and is dispersed to the body. This issue prevents the use of CFM for any permanently implanted device. As we compared results of CFMs to boron-doped diamond, both electrodes demonstrate sensitivity detecting neurochemicals in vitro and in vivo, the diamond based electrodes have increased longevity and mechanical strength, much needed for long term implantation. We anticipate that coupling the developed, multichannel neurotransmitter detection device with the interleaved stimulation pulse subsystem and diamond sensing electrode, will provide the means for the long term detection of neurotransmitters. This combination is expected to provide the required information needed for a DBS control algorithm allowing intervention in a greater number of debilitating neurologic disorders.

Knowledge-Based Design of the Electrode-Separator Assembly Boosted Microbial Fuel Cell Power Density

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Power density provided by microbial fuel cells (MFCs) equipped with air-breathing cathodes does generally not exceed one or two W/m^2 with respect to the cathode surface area [1]. This record has recently been raised to 4.7 W/m^2 [2]. Several causes of power limitations have been identified [3]: microbial anodes require pH values close to neutrality, at which oxygen-reduction catalysts lose their catalytic efficiency; microbial anodes do not accept high salinity and impose the use of an electrolyte of low ionic conductivity; microbial anodes are sensitive to local acidification that occurs during operation and to oxygen crossover from the cathode; the composition of the culture media required by microbial anodes favors fouling and biofouling of the cathode, etc. All these limiting causes will be presented and analyzed on the basis of a literature review coupled with theory.

This analysis led to the development of a new separator-electrode assembly design, including a 3-dimensional anode, a separator and an air-cathode that can be removed and replaced during operation [4]. Several kinds of separators were checked and, surprisingly, the highest power density of 6.4 W/m^2 was obtained by using a simple, large-mesh, plastic grid. Electrode kinetics were monitored by voltammetry during a 1-month operation period with 6 MFCs. It was thus proved that cathode biofouling was a major rate-limiting phenomenon, so that replacing the air-cathodes allowed 5 W/m^2 to be still produced after 1-month operation. The microbial communities identified by 16S metagenomics showed that oxygen crossover did not affect the bioanodes thanks to their 3-dimensional structure. The theoretical analysis explained that the large-mesh grid allowed free mass transfer of the buffering species between the anode and the cathode, which resulted in an efficient pH balance of both electrodes, and was the main key of success.

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Electrochemical studies of membrane proteins reconstituted in lipidic liquid crystalline cubic phase

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Retaining the activity of proteins incorporated in the artificial membranes essential for their use in membrane - based sensors or biofuel cell devices remains a true challenge. Direct adsorption of enzymes at electrode surfaces often results in partial loss of their activity. Membrane proteins, generally are not stable outside the cell membranes, however, we reveal their stabilization by entrapment in a matrix that resembles natural lipidic environment. Selected proteins: fructose dehydrogenase [1], chloride-conducting ion channel from *Escherichia coli* (EcClC) and Na^+/K^+-ATPase transmembrane protein were incorporated into the monoolein (MO) based liquid crystalline phase (LCP) and studied using cyclic voltammetry, chronoamperometry and chronocoulometry. The structures of the LCPs without and with the proteins were determined by small-angle X-ray scattering (SAXS).
Since ecCLC is a potential dependent protein, chronocoulometry was used to study the charge passing through the LCP film as a function of potential applied to the electrode. From the charge vs. potential plots the difference between charge passing through the LCP and LCP with ecClC channel protein was evaluated. Results indicate that more negative charge is transferred through the electrode covered with LCP containing ecClC which confirms that ecClC acts as Cl^- ions transporter. Diode-like behavior of cubic phase containing the protein is observed. More negative charge was found to flow through the LCP covered electrode loaded with ecClC at pH 4.5 than at pH 7.4. The results of our studies demonstrate that the membrane proteins studied can be reconstituted in the lipidic cubic mesophase, and retain their functionalities in this biomimetic environment.

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Flexible Transparent Nanostructured Photoelectrodes for Solar Biofuel Cells and Biosupercapacitors

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Electrode nanostructuring mainly has the advantage of a significant increase of the surface area, as well as an enlarged interfacial area between electrolyte and electrode surface. This results in a higher number of binding sites for e.g. biocatalysts and an enhancement in the double layer capacitance. Thin nanostructured layers of nanoparticles (NP), such as indium tin oxide (ITO) deposited on ITO glass electrodes, are sufficiently conductive and transparent for utilizing them as a support in biophotoelectrochemical charge storing devices as we have suggested recently[1]. Combining this concept with the concomitant integration of photoactive biomolecules, such as e.g. photosystem I and II in redox-polymer hydrogels, provides the basis for a significantly higher current output and stability as compared to non-modified systems[2,3].

Here, we propose an optimized type of spray-coated layered NP-modified electrode based on flexible materials and covered with a top layer of redox-polymer hydrogels embedding photoactive bio-molecules. A self-developed automatized spray-coater allows us to precisely cover the flat electrode surfaces with controlled and homogeneous NP-layers. The resulting transparent and flexible photobio-electrodes are utilized in a model system[1] demonstrating the applicability of this electrode type. After implementation of suitable cathodic and anodic biocatalysts, the device can be operated as a solar biofuel cell in a continuous mode or as a solar biosupercapacitor in a pulsed charge/discharge mode during light irradiation. Due to their high flexibility, the developed electrodes can be formed in numerous different shapes, e.g. for attachment to functional clothes or a cylindrical structure which allows a 360° irradiation (see sketch).

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A Novel Self-Powered Glucose Biosensor based on *Ct*CDH and AuNPs modified Screen-Printed Carbon Electrode

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The electrical wiring of redox enzymes with electrodes is one of the main issues to develop biosensors, bioelectrocatalyzed transformations, and biofuel cells [1]. For this purpose, nanomaterials (NPs) and in particular, metal NPs modified electrodes provide a lot of advantages compared to macroelectrodes, such as enhancement of mass transport and catalysis, high effective surface area and control of the electrode conductive microenvironment. However, the derived electrical power from enzymatic biofuel cells (EFCs) is usually in the order of μW, making the system questionable as a source of energy, especially compared to fuel cells. However, an EFC configuration consisting of two electrodes may act as a self-powered biosensor device, since the open-circuit voltage of the system (OCV) depends on the fuel concentration. In this work AuNPs were directly electrodeposited onto graphite screen-printed-electrodes, further modified with *Corynascus thermophilus* cellobiose dehydrogenase C291Y (*Ct*CDH 291Y) [2] to realize a biofuel cell bioanode, using glucose as fuel, and with *Myrothecium verrucaria* bilirubine oxidase (*Mv*BOD) to realize the biocathode, using O_2 as oxidant [3]. A complete characterization of the EFCs by was carried out evaluating several parameters such as OCV, power output and life-time stability. Finally, the EFC was miniaturized in order to obtain a self-powered glucose biosensor, which was tested in blood.

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Inoculation process and assessment of different carbon–based anode materials for Microbial Desalination Cells

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Microbial Desalination Cell (MDC) technology is a novel energy saving, cost-effective an eco-friendly desalination process, that comes out from merging Microbial Fuel Cells (MFCs) with desalination technology. MDC consists in a three chambers reactor: anode and cathode chambers are equal to MFC and in-between them a chamber containing salt water is placed. The desalination process is carried out due the potential gradient between cathode and anode. Anions (Cl^-) migrate to the anodic chamber and cations (Na^+) migrate to the cathode chamber through ion exchange membranes. The main advantage of MDC is the lower energy required to drive the desalination process (potentially 0.5 kWh m^{-3}) compared to the conventional processes (e.g. reverse osmosis, thermal-based distillation), which require up to 2-15 kWh m^{-3} to produce drinking-water-quality. The performance of the anode is a key parameter that must be optimized to obtain a high desalination rate in MDCs. In the present work, the inoculation process of the anode has been optimized, to achieve a stable anodic biofilm in a short time frame. Also, different carbon-based anode materials have been assessed. During the inoculation phase, the anode has been polarized at sequential and decreasing potentials (ranging from -100 mV to -350 mV vs. Ag/AgCl). Biofilm growth has been continuously evaluated through chronoamperometry, cyclic voltammetry (CV) and open circuit potential (OCP) techniques. Results demonstrated that the anode could be efficiently inoculated in less than 20 days. The inoculation was considered as complete when these three parameters were achieved: 1) the current output was higher than 0.15 mA cm^{-2}; 2) the OCP was around -550 mV Vs. Ag/AgCl; and 3) the CV indicated the presence of biofilm grown onto the anode. Different carbon-based anode materials have been tested as well as different physical and chemical activation methods in order to modify their surface properties (surface area, roughness and hydrophobicity) in views to improve the inoculation process by facilitating microbial attachment. The results of this study are likely to be transferrable to others BES systems.

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3-Dimensional Bipolar Bioelectrochemistry

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Electrogenerated chemiluminescence (ECL) is a type of luminescence which involves the *in-situ* formation of an excited state of a luminophore at the electrode surface initiated by an electron transfer step. ECL has now become an exceptionally useful analytical technique employed daily in ultrasensitive immunoassays for healthcare clinical diagnostic [1]. Bipolar electrochemistry (BPE) is based on a polarization potential gradient established along a conducting object exposed to an electric field [2]. The promotion of ECL reactions at bipolar electrodes addressed electrochemically in a wireless fashion will be presented. In particular, BPE allows to control the motion of conducting particles capable to emit ECL light when travelling in a capillary filled with an aqueous solution [3]. This strategy is applied to dynamic bioanalytical sensing with the possibility to probe locally an inhomogeneous solution exhibiting a concentration gradient of biochemical target [3]. Then it will be demonstrated that the intrinsic limitation of ECL as a 2 dimensional surface-confined process can be overcome by taking advantage of BPE [4]. Indeed, this very unique technique offers the possibility of wireless electrochemical addressing of large ensembles of conducting particles dispersed in solution. Such a simultaneous BPE-addressing allows the generation of 3-dimensional bulk ECL in the whole volume of an analytical solution. This will be exemplified with several luminophores in order to perform successfully multiple-analyte detection with enzymatic systems [4].

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The Role of Cytoskeleton in Electroporation of Cellular Membranes

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Electroporation is a popular physical method to introduce foreign biomolecules into a living cell, by applying strong electric pulses that temporarily increase the membrane permeability . Although this technique has been routinely employed in different laboratories for biological and biomedical applications, many of the mechanisms underlying membrane electroporation and DNA transport processes remain to be addressed. Understanding of the cellular phenomena in the presence of external forces (such as electric field) using fundamental and physical laws based on soft matter physics is still far away. To overcome this limitation, giant unilamellar vesicles (GUVs) are used as a simplified system to study the fundamental mechanisms behind electroporation processes. Experiments on empty GUVs have displayed peculiar phenomena such as lipid loss, size reduction, and the creation of macropores (micrometer-sized pores), which have not been observed in real living cells [1-3]. These observations have suggested that the electroporation mechanism is strongly altered by the intracellular components, such as the cytoskeleton. To address this key issue, we successfully created a model GUV system that is closer to natural cells than the classical (empty) GUV model. Recently, we have encapsulated actin inside GUVs and created the actin-netwerk/-cortex structure to investigate the effect of cytoskeleton on the response of GUVs during/after the electroporation process. In this talk, I will discuss the effect of actin-cortex, cytoskeleton microstructures, on the dynamic response of GUVs, during/after the exposure to electric pulses.

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Fast-EIS Applications on Dynamic In-Vivo Tissues

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Electrical impedance spectroscopy (EIS) has been widely used to characterize biological materials, from cell cultures to whole human body. Stepped sine based EIS shows limitations when measuring in organs which have movement. Fast-EIS measurements allow characterizing the in-cycle impedance spectrum of dynamic phenomena.

This talk will review the basics of this technique and describe the materials, methods and results obtained by our group, together with Hospital de Sant Pau, in two main applications: the in-vivo and in-cycle characterization of myocardium in acute and healed ischemia and the in-vivo and in-cycle characterization of lung tissue obtained through broncoscopy. [1], [2]. By using a multisine-based fast-EIS technique, in-situ characterization of myocardium tissue in acute and healed infarct was performed in pig models. The measurements allowed confirming the already known average changes in the impedance spectrum along the ischemia time course but also showed changes in the in-cycle impedance shape at different frequencies. The measurements allow defining an impedance-pressure diagram which provides information about the passive or active behavior of local regions of the tissue respect to the phase of the cardiac cycle. Indicators of evolving acute myocardial ischemia or healed scar can be deduced from multifrequency impedance values, their shapes and their delays respect to physiological variables. The second application that will be described consists on the in-vivo and in-situ characterization of lung tissue in humans by a catheter introduced through a bronchoscope in routine bronchoscopy procedures.

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Investigation of the Chemical Mechanisms Involved in the Electropulsation of Membranes at the Molecular Level

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The description of membrane electropulsation at the molecular level as a purely physical phenomenon is not satisfactory yet since it only considers nanoseconds kinetics whereas electric pulses display long-term effects. Our study considers the chemical mechanisms of cell electropulsation. The chemical consequences of electropulsation on giant unilamellar vesicles (GUVs), in particular the possible oxidation of unsaturated phospholipids have been investigated by mass spectrometry, flow cytometry and absorbance methods. Pulse application induced the oxidation of the GUV phospholipids and the oxidation level depended on the duration of the pulse. UV-light and O_2 increased the level of pulse-induced lipid peroxidation whereas the presence of antioxidants either in the membrane or in the solution completely suppressed peroxidation. The presence of metal ions did not impact the peroxidation. Importantly, pulse application did not create additional reactive oxygen species (ROS) in GUV-free solution. Lipid peroxidation seems to result from a facilitation of the lipid peroxidation by the ROS already present in the solution before pulsing, not from a direct pulse-induced peroxidation. The pulse would facilitate the entrance of ROS in the core of the membrane, allowing the contact between ROS and lipid chains and provoking the oxidation. Our finding demonstrate that the application of electric pulses on cells could induce the oxidation of the membrane phospholipids since cell membranes contain unsaturated lipids. The chemical consequences of electropulsation will therefore have to be taken into account during the development of future biomedical applications of electropulsation since oxidized phospholipids play a key role in many signaling pathways and diseases.

Electrochemical Determination of Electroactive Film Thickness Distribution in the Solvated State

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Redox active films are important in energy conversion related catalysis applications because of their ability to mediate the electron transfer of redox catalysts. In practice, the surface of a prepared film could be highly inhomogeneous, so that there is the possibility that parts of the film could have highly variable catalytic performance. Although Atomic Force Microscopy is a popular method for the characterization of film surfaces, the redox active portion of the film thickness cannot be differentiated from the total film thickness when electrochemical breaks within in the film are present. Here, we introduce the use of an electroanalytical approach based on linear sweep voltammetry (LSV) to extract the electroactive film thickness distribution. The peak currents from the LSV depend on the experimental time scale and the electron transfer distance from the electrode to the film boundary. Hence, the peak current depends also on the distribution of the distances from the electrode surface to the film boundary, and is therefore useful for extraction of the film thickness distribution. The method is in principle applicable to any redox-active material for which the current responses depend on diffusion-like processes such as electron hopping or counter ion transport. More generally, the LSV method is expected to greatly impact the way film thickness distributions are measured since the method intrinsically takes into account the entire sample regardless of its size instead of the limited portion of the surface that is probed in classical methods for surface roughness determination.

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Extracellular Osmotic Stress Reduces the Vesicle Size While Keeping a Constant Neurotransmitter Concentration

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Secretory cells respond to hypertonic stress by shrinking and reduces exocytosis activity and the amount signaling molecules released from each single exocytosis event. It has been assumed this is due to alteration in biophysical properties of cell membrane and cytoplasm and that the secretory vesicles are not affected by osmotic stress. We used intracellular electrochemical cytometry[1] to quantify the amounts of catecholamine and together with vesicle size measurements using transmission electron microscopy the catecholamine concentration in dense core vesicles before and after cell exposure to osmotic stress was determined. In addition, single cell exocytosis amperometry recording at chromaffin cells was used to monitor the affect on exocytosis activity and to quantify the change in amount catecholamine released during exocytosis when cells were exposed to osmotic stress. Here we show that hypertonic stress hampers exocytosis secretion after the first pool of ready releasable vesicles has fused and causes catecholamine filled vesicles to shrink, mainly by reducing the halo solution surrounding the dense core protein matrix.[2] In addition, the vesicles demonstrate the ability to control and perform adjustment in neurotransmitter content during shrinking to maintain a constant catecholamine concentration within the vesicle compartment.

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Redox Polymer Bearing Plastoquinone Mimics for Efficient Wiring of Photosystem II

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Photosystem II (PSII) is a photosynthetic enzyme able to achieve light driven water oxidation. Due to its extraordinary photocatalytic activity, application in biosensors and biophotovoltaic systems were proposed [1],[2]. The fundamental understanding of the PSII function, as well as its use in the mentioned applications, have motivated the integration of the PSII into electrode platforms. In previous works, redox polymers bearing osmium complexes [1],[2] or phenothiazines [2],[3] as electron mediators were used to support and electrically connect the PSII with an electrode surface. With the aim of improving the electron transfer process between the enzyme and the electrode, we report the synthesis of a redox hydrogel using trimethylbenzoquinone as a bioinspired mediator mimicking the structure of the plastoquinone. An isothiocyanate benzoquinone was synthesized and attached to a polyethyleneimine (PEI) polymeric backbone. The electrochemistry of the resulting quinone-modified polymer displays a redox potential of 250 mV vs SHE at pH 6.5 and quasireversible behavior demonstrating its suitability as electron mediator. A photocurrent density of 35 μA cm^{-2} on planar electrodes and an onset potential of about 250 mV vs SHE were obtained when evaluating an immobilized PSII sample within the quinone-based polymer at pH 6.5. This polymer favorably compares with previously reported systems possibly owing to the adjusted redox potential of the covalently tethered plastoquinone mimic, which is retained in high local concentration in the vicinity of the PSII protein complex.

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From Nanometer-Smooth to 500-µm Pillars: New Surface Topography to Improve *Geobacter sulfurreducens* Bioanodes

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The ability of microorganisms to build efficient and durable electroactive biofilms is directly related to the topography of the electrode surface. Strong effect of the surface roughness has often been observed on the efficiency of electroactive biofilms but no correlation has been established yet on a large range of roughness sizes. The purpose of the study was to assess the influence of the electrode surface topography using well-controlled surface patterns from nanoscale roughness to micro-structured surfaces. From a practical standpoint, the objective was to increase the biofilm stability and the current produced per square centimeter of projected surface area.

Gold surfaces were created by electrochemical growth leading to different surface roughness with nanometer sizes ranging from Ra = 0.8 to 3nm. Similar gold electrodes were designed with micro-pillars of 500 µm height and 100 µm side with different spacing from 100 to 200 µm. The bioanodes were formed under chronoamperometry at 0.1 V/SCE in reactors inoculated with *Geobacter sulfurreducens* with acetate 10 mM as the substrate. The bioanodes were characterized by cyclic voltammetry (catalytic and non-turnover) and the biofilm coverage ratios were determined by epifluorescent microscopy.

The nanometer-sized rough electrodes produced current densities around 2 A/m² and showed proportionality between the current density and the biofilm coverage ratio. The micro-pillar structured electrodes led to substantially higher current densities of the order of 6-8 A/m². The difference in current production was directly linked to the increased surface area. Moreover, the micro-pillar patterns always led to the integral coverage of the electrode surface by the biofilm, whereas the nanometer-sized rough electrodes showed erratic microbial colonization and current production. By improving the surface attractiveness for biofilm formation, surface micro-structuring revealed to be a powerful way to boost microbial anode performance.

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Brain monitoring with Minimally Invasive Microelectrode Biosensors based on Platinized Carbon Fibers

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Monitoring the chemical composition of the brain interstitial fluid is an important challenge for both pre-clinical and clinical research on brain injury. Microelectrode biosensors are a promising technique with a temporal resolution in the order of seconds. Here, ultra-microelectrodes based on platinized carbon fibers were fabricated to obtain biosensors with less than 15 µm external diameter. Platinization was achieved by sputtering a 10 nm Cr adhesion layer followed by 100 nm of platinum. Platinized carbon fibers were then encased in a glass micropipette and covered with electropolymerized poly-phenylenediamine for selectivity, and covalently immobilized oxidase enzymes (glucose oxidase, lactate oxidase, D-amino acid oxidase or glutamate oxidase). After implantation in the rat parietal cortex, such biosensors detected a smaller basal lactate concentration and a slower diffusion of glucose and D-serine through the blood brain barrier compared to more conventional biosensors with 90-100 µm external diameter. Interestingly, spreading depolarizations (SD) induced by gently pricking the surface of the brain, produced a smaller increase in lactate, a larger decrease in glucose with platinized carbon fibers microelectrode biosensors compared to larger sensors. Therefore, estimated basal concentrations of neurochemicals, their diffusion rate across the blood-brain barrier as well as the neurochemical signature of SDs differed when estimated with these new minimally invasive biosensors. Such small devices avoid major mechanical injury to blood vessels, preserve the blood brain barrier at the site of implantation, and consequently, provide more accurate measurements from the brain interstitial fluid. To conclude, developing smaller, less invasive probes for brain monitoring is key to obtaining meaningful information about the cellular mechanisms at work during brain injury.

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Scaffolding of enzymes on virus nanoarrays: searching for nanoscale confinement effects on enzymatic activity

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In this presentation, we will describe a bioinspired platform devoted to study the nanoscale confinement effects on the catalytic activity of enzymes. Our strategy is to use filamentous fd virus as enzymatic nanocarrier material by decorating the protein shells (capsids) with the glucose oxidase (GOx) model enzyme.[1] fd virus are first adsorbed onto a gold electrode surface, to form either randomly oriented or directionally ordered nanoarrays. Then, GOx enzymes are nano-scaffolded on the fd virus capsids using a robust immunological strategy,[2] by making use of virus-specific antibodies, further recognized by GOx-conjugated antibodies. The step-by-step enzymatic fd decoration is monitored by atomic force microscopy (AFM) imaging in liquid, while cyclic voltammetry is carried out to probe the glucose oxidation catalysis of nano-scaffolded GOx patterns. Finally, the total amount of surface-confined enzymes is independently determined with an optimized fluorescence protocol, where FAD cofactors (released from denaturated GOx), are assayed. Thus, a full characterization of the kinetic parameters can be carried out, in order to reveal a clear modulation of the activity of GOx enzymatic scaffolds according to the orientation of the fd-nanocarriers at the surface (random or oriented virus nanoarrays).

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Monitoring of oxidation during electroporation via endogenous chemiluminescence

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Pulsed electric fields have already wide use and further great potential for novel applications in biomedicine and food industry[1]. Although not fully explored, one mechanism of the electric pulses action in biological samples is through the electric field-induced lipids oxidation. Here we report monitoring of oxidation of DC-3F cell suspensions via endogenous chemiluminescence. Endogenous chemiluminescence arises from oxidative processes in the biosamples without any external stimuli or additionally applied external luminophores[2] and enables label-free and real-time monitoring of oxidative processes. In our experiments, we have found that a train of several short pulses tends to induce stronger response of endogenous chemiluminescence from cells than the single long pulse while total duration and other conditions are the same. Specifically, 20, 200 kV/m, 250 μs, pulses fired at 1 Hz tend to induce higher chemiluminescence signal than a single 200 kV/m, 5 ms pulse. See typical chemiluminescence signals from DC-3F cells in electroporation buffer (SHM: sucrose-HEPES-Mg+) in Fig. 1. Our results support hypothesis[3] that the time gaps between the pulses will cause larger surface of the cells to be electroporated (hence more intracellular biomolecules can be released and oxidized) because the lipids in the membrane will have enough time to diffuse along the membrane between the pulses.

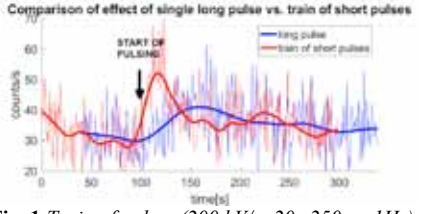

Fig. 1 *Train of pulses (200 kV/m 20x 250 μs 1Hz) induces stronger chemiluminescence (oxidation) response than the single 200 kV/m 5 ms pulse.*

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Evaluation of Stability of PS1-based Photocathodes. Suppressing the Effect of Reactive Oxygen Species

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Photosystem 1 (PS1) is of great interest since it is a highly abundant and robust protein complex, capable of generating light-induced charge separation with high quantum yield.[1] To date, successful integration of the isolated redox protein into photo-electrochemical devices has been demonstrated in the design of advanced architectures and engineered electron-transfer chains for an efficient coupling of the photo-synthetic protein with different electrode surfaces.[2] However, light-induced damage of the photosystem induces degradation of the protein with a consequent drop in activity over time, therefore substantially limiting the applicability of biohybrid devices. Particularly, when methyl viologen is used as free-diffusing electron acceptor, the fast mediated electron transfer to oxygen causes the formation of highly reactive superoxide anion radicals and hydrogen peroxide,[3] that may lead to inactivation of PS1.

Based on a recently presented new analytical methodology for the simultaneous monitoring of the photocurrent generated by the PS1-based photocathode and concomitant collection of H_2O_2 and O_2 under localized irradiation conditions, we present further insights in light-induced stress processes at redox polymer films comprising isolated PS1 and the removal of potentially harmful species by co-immobilization of superoxide dismutase and catalase in the hydrogel film.

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New Customized Platform Based on Electrochemically Generated Polypyrrole Nanoparticles Decorated with Gold Nanoparticles for the Highly Selective and Sensitive Electrochemical Detection of Serotonin

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Electrochemical sensing strategies for the detection of neurotransmitters are envisaged due to their rapid, sensitive, selective and low cost achievements. Several nanomaterials as well as electrochemical techniques are currently employed in the selective quantification of neurotransmitters. Serotonin is a neurotransmitter with crucial implications in the clinical field. Changes in its concentration in cerebrospinal fluid have been directly linked to various neurological and psychiatric disorders. Moreover, serotonin levels have been related to many gastrointestinal illnesses, higher concentrations of serotonin in the plasma indicating serotoninergic malfunctions [1]. This presentation will report a novel platform based on PPy nanoparticles (PPyNPs) decorated with AuNPs on graphite-based screen-printed electrodes (GSPE) and the development of an electrochemical sensor for selective and sensitive determination of serotonin. The AuNPs@PPyNPs nanocomposite produces a synergistic effect towards the detection of serotonin, in the presence of its common interferents. The detection limit and the sensitivity of 33.22 nM (S/N=3) and 0.3316 $\mu A\ \mu M^{-1}$, respectively, were calculated (R^2=0.9914; RSD=4.49%). Serotonin determination in serum was performed in triplicate, without any pretreatment, and excellent recoveries between 100.27 % and 103.06 % were obtained [2]. The analytical characteristics obtained suggest that this sensor could be usefully incorporated in point-of-care devices for use in clinical analysis.

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Anaerobic and aerobic processes synergistically promoted in biocathodes of microbial fuel cells

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In a microbial fuel cell (MFC), electroactive anodophilic bacteria anaerobically oxidize biodegradable organics dissolved in the media. The products of the anodic semi-reaction are delivered to a terminal acceptor, usually the oxygen.

Depending on the architectural features of the MFC (single- or double-chamber) and of the cathode itself (air-breathing or water-submerged), the oxygen reduction reaction (ORR) and other related red-ox reactions concur to the overall electrons flow, through both biotic and abiotic mechanisms.

In this study, biocathodes operating in MFCs under three different conditions: i) air –breathing (A-MFC); ii) water-submerged (W-MFC) and iii) assisted by a photosynthetic microorganisms *Spirulina arthrospira* (P-MFC) were investigated. Photosynthetic microorganisms cultures in the cathodic chamber act as oxygen supplier to enhance cathodic reactions in MFCs.

MFCs were operated for more than two months, until reaching a maximum and stable electrochemical performance. The anodes and cathodes of each MFC were observed by confocal microscopy and cryosectioning of cathodic biofilms were used in combination with epifluorescence microscopy to investigate the structure of the biofilms through their thickness. The anodic and cathodic biofilms were then collected and processed by Illumina sequencing tools.

The microbial composition of different electroactive biocathodes were analyzed and the aerobic and anaerobic processes potentially involved in ORR sustained from the principal groups of detected bacteria are discussed.

High oxygen concentration (up to 20 mg_{O2} L^{-1}) produced by *Spirulina arthrospira* culture allowed biocathodes of P-MFC to work at more aerobic condition than air-breathing or water –submerged cathode ones.

Based on the microbiological data achieved and the electrochemical behavior of each cathode, a model of aerobic and anaerobic mechanisms, both promoting the ORR as final electron acceptor, are proposed.

Label-Free Electrochemical Sensors for Total Antioxidant Capacity Monitoring in Plant Extracts

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Hydrogen peroxide is formed in human and animal organisms as a short-lived product in biochemical processes. Peroxide molecules are highly reactive and may lead to oxidative stress and chain reactions that damage cells. Various plants and fruits contain high concentrations of several redox-active antioxidants, which can inhibit the oxidation of biomolecules by the peroxide ions and terminate these chain reactions.

In this work six different hydrosoluble extracts with antioxidant capacity from *Hippophae rhamnoides L* and *Lavandula angustifolia Mill. si L.* were obtained via two extraction methods. Nanoparticle modified screen-printed carbon electrodes have been successfully used to develop sensitive, label-free electrochemical sensors for total antioxidant capacity monitoring of studied extracts. Voltammetry and fixed potential amperometry measurements were performed using carbonic and gold nanoparticles; studies of pH and working potentials together with nanoparticles type and extract concentrations influence were carried out for sensors optimization.

The relative antioxidant capacity was determined using optimised electrochemical sensors for all extracts and compared with the results obtained by standard methods. Best extracting method which conducts to the highest antioxidant activity of plant extracts and optimized sensors configuration have been outlined.

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ATP synthesis coupled to the electroenzymatic activity of a hydrogenase immobilized at an electrode/biomimetic membrane interface

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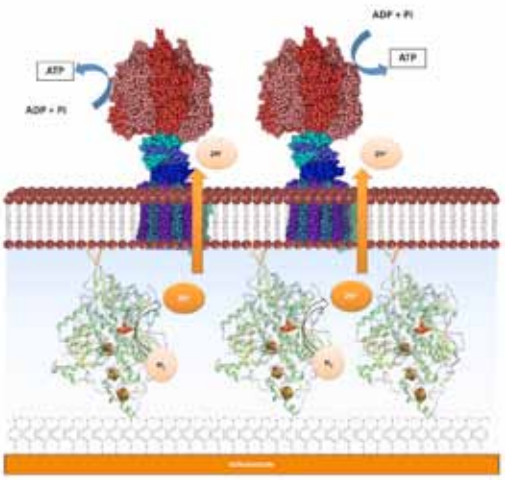
Cells generate energy by coupling a proton gradient across a phospholipid bilayer membrane with the activity of a cross-membrane ATP synthase enzyme. In an effort to mimic this process in an artificial environment, we show that ATP can be efficiently produced starting from molecular hydrogen as a fuel.

The proton concentration in an electrode/phospholipid bilayer interface can be controlled and monitorised electrochemically by immobilizing the membrane-bound [NiFeSe]-hydrogenase from *Desulfovibrio vulgaris* Hildenborough.[1] The electro-enzymatic oxidation of H_2 generated a proton gradient across the supported biomimetic membrane that can be coupled to the *in vitro* synthesis of ATP by reconstituting ATP-synthase from *E. coli* on the biomimetic system.[2]



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Differential Electrochemical Mass Spectrometry (DEMS) Applied in Enzyme Electrochemistry

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NAD-dependent alcohol dehydrogenase (ADH) enzymes that catalyze the oxidation of a variety of primary, secondary, branched, and cyclic alcohols to the respective aldehydes with the concomitant reduction of NAD^+ to NADH is a very important biocatalyst for metabolic engineering, food science and biofuel cell technology. There is evidence that the rate-limiting step in ethanol ADH-biocatalyzed reaction is the dissociations of the acetaldehyde-coenzyme complexes; however, to test this hypothesis, NADH and acetaldehyde should be concomitantly measured under bioelectrocatalysis control. Here we describe an original setup that combines the precise control of volatile metabolic formation on enzyme electrode and differential electrochemical mass spectrometry (DEMS) measurements. Alcohol dehydrogenase (ADH) from Saccharomyces cerevisiae was adsorbed on flexible carbon fiber[1], and we were able to providing precise monitoring acetaldehyde formation from bioelectrocatalytic oxidation of ethanol by ADH. Mass spectrometry data was recorded concurrently with chronoamperometry for different mass/charge ratios. Acetaldehyde formation was recorded with ionic currents at m/z = 29, that corresponds to COH^+ fragment of ionized acetaldehyde molecule. When the enzyme electrode was polarized at 0.6 V *vs.* $Ag/AgCl/Cl^-_{sat}$ it was observed the increase of ionic current for m/z =29, evincing the acetaldehyde formation. Thus, this is a proof-of-concept that DEMS applied to enzyme electrochemistry can giving insights about enzyme kinetics and mechanisms.

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Miniature Enzymatic Fuel Cells for Healthcare Applications

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Self-powered wearable biodevices are highly attractive for a number of applications, including non-invasive detection of biomarkers and fitness monitoring. Enzymatic fuel cells (EFCs) hold great potential for the development of such devices. This particular type of fuel cells uses redox enzymes as catalysts to convert organic substrates, such as carbohydrates, into useful electricity at body temperature. In the past few years, electricity generation from human physiological fluids *via* EFCs has been reported, which proved the possibility to harvest energy from tears, serum and saliva. Through a careful choice of the enzymes used, EFCs can work as effective amperometric biosensors for target biomarkes.

We have developed innovative miniature flow-through glucose/oxygen enzymatic fuel cells for continuous power generation. The EFCs use highly-porous gold (hPG) as electrodes. hPG is characterised by a very high specific surface area, with a pore size distribution ranging from the micro to the nano scale. This property, in combination with high conductivity and biocompatibility, makes hPG electrodes the ideal support for enzyme immobilisation.

To scale-up the power density, we developed a cascade of EFCs embedded in a compact and handy single channel device. To prove its use with a physiological fluid, the device was tested for the first time with transdermal extracts obtained by iontophoresis. The miniature EFCs generate power outputs in the orders of 1-5 μW cm^{-2}. Glucose detection in the sweat range (5.6 and 2,200 μM) was demonstrated, thus paving the way for self-powered painless glucose monitoring applications.

We also proved for the first time that EFCs can be used to detect the neurotransmitter acetylcholine, identified as a biomarker for the Alzheimer's disease, when acetylcholinesterase is used as the anode catalyst.

An Impedimetric Biosensor for Early Rapid Diagnosis of Invasive Fungal Infections in Critically-ill Patients

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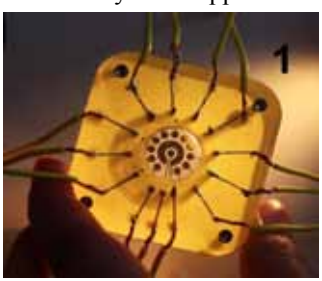
Candida albicans is responsible for the most frequently encountered invasive fungal infections (IFIs)[1], which is the major cause of morbidity and mortality among immunocompromised patients[2]. Diagnostic methods such as blood culture[3], PCR[3], ELISA[4] and mass spectrometry[5] are either complex/tedious or lack sensitivity. Thus, timely and specific diagnosis of IFIs remains a challenge. Here we present a membrane-based impedimetric biosensor as a sensitive tool for early rapid diagnosis of *C. albicans*. Gold electrodes are patterned on the membrane by metal deposition and functionalized with polyclonal anti-Candida antibodies as recognition elements (fig.1). Increasing concentrations of *C. albicans* are incubated on the sensor and detected by electrochemical impedance spectroscopy (EIS) in 10mM solution of potassium ferri/ferrocyanide in PBS. When tested with *Saccharomyces cerevisae,* the biosensor shows high selectivity. The lowest 'limit of detection' (LOD) of 150 CFU/ml (15 cells binding to the sensor) is achieved (fig.2). As the aim is to enable early diagnosis of IFI, we are currently working towards lowering the LOD to 10 *Candida* cells in 10 ml of blood. A simple sample preprocessing is applied, which involves lysis of blood cells and preconcentration of the present yeast cells. The proposed biosensor could be used for the diagnosis of IFIs caused by other opportunistic pathogens such as *Aspergillus*.



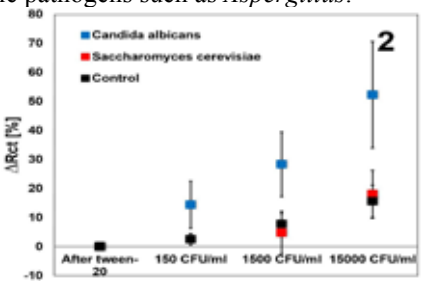


Figure 1. Biosensor prototype interfaced with PCB board. **Figure 2.** EIS data presented as relative charge transfer resistance. The biosensor shows high selectivity to *C. albicans*.

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Advanced Electrochemical Cell for the Study of the Highly Active Enzymes: CO- Dehydrogenase

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CO dehydrogenase, CODH, is a promising enzyme, that catalyzes the reversible reaction of reduction of carbon dioxide into carbon monoxide: $CO + H_2O \leftrightarrow CO_2 + 2e^- + 2H^+$, which is the first step to the production of fuel[1,2]. Our group has studied the mechanism of this enzyme using protein film voltammetry (PFV)[3] in which the enzyme is absorbed at the electrode surface where the electron transfer is direct (**Figure**). With the classical setup using the rotating disk electrode, the transport of chemical species toward the enzyme is insufficient to match its very high activity; this blurs the enzymatic response and prevents mechanistic studies. To enhance the transport toward the active sites of the enzymes at the electrode, we are designing a new type of a flow cell. We use fluid mechanics simulation to study a variety of geometrical configurations, to choose a suitable prototype of electrochemical cell. Based on our numerical simulations, we will build this cell, then we will calibrate the flow in the new system, and later use it for the study of the mechanism of CODH and other enzymatic catalytic reactions.

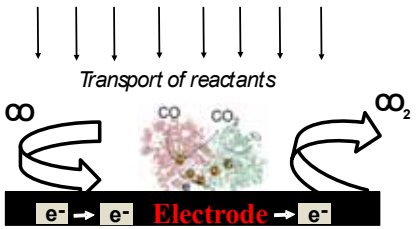

Figure: Schematic representation of a protein film voltammetry technique, CODH is the colorful part deposited at the electrode surface.

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Bio-modified nanoband array electrodes for sensing in complex fluids

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Many biosensors employ different nanomaterials in the design, which can provide considerable improvements in sensitivity, selectivity, and accuracy. However, the use of nanoparticles or mediators can sometimes be problematic, *e.g.* for *in vivo* applications where their use could raise health concerns. Instead of employing nanomaterials-modified macro-scale electrodes in the biosensor design, an alternative approach is to employ nanoelectrodes or nanoelectrode arrays.[1, 2] Nanoelectrodes provide several benefits, such as enhanced mass transport, increased signal-to-noise ratio and high sensitivity. Such approaches have been shown to provide high sensitivity for biorecognition reactions.[2, 3] Thus, an important potential application of nanoelectrodes is in bioanalytical measurement systems, where improved sensitivity can expand the range of potential target analyte molecules, as well as improve the overall performance of the biodevice. As one example, here we present data from lactate oxidase modified nanoband array electrodes employed as a first generation type glucose biosensor, using platinum and Prussian blue modified gold (Fig. 1). Electrodes were investigated in complex buffers and physiological fluids, showing a high analyte sensitivity (sub μM LOD).

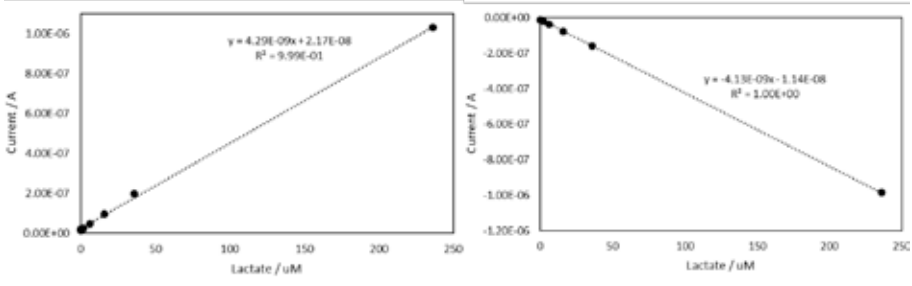

Fig. 1. Calibration curve of enzyme modified Pt (left) and Prussian blue modified gold (right) nanoband array electrode towards lactate, operated at 0.55 V and 0.05 V vs Ag/AgCl (respectively).

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Electrochemical Hairpin Biosensor Based on Peroxidase-mimicking Covalent Hemin-G4 quadruplex complexes for Ultrasensitive Detection of *E.coli* DNA and rRNA

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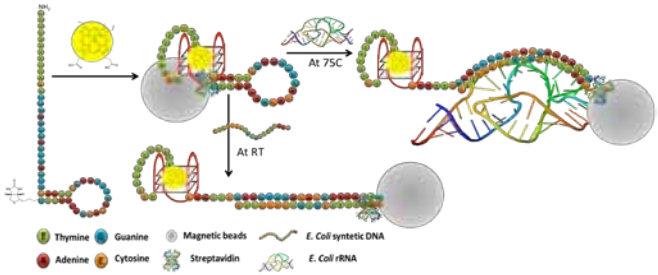
Recently, nucleic acid DNAzymes gained popularity as catalytic labels for DNA analysis (1). Here, we explored the horseradish peroxidase-mimicking DNAzyme as a catalytic label for analysis of bacterial DNA and RNA sequences specific to *E.coli* DNA and rRNA. The catalytic label consisted of hemin covalently attached and stacked into a G-quadruplex embedded in the hairpin nucleic acid structure and attached to magnetic beads through the biotin-streptavidin binding (2). The *E.coli*–specific short DNA sequences were obtained from the conserved flanking region of the 16s rRNA, the internal transcribed spacer region (ITS) and the 23s rRNA genes. The DNA hairpin was exclusively designed to complement the *E.coli* conserved flanking genes, and also allowed the formation of a G-quadruplex. Briefly, hemin was covalently linked to the flexible region of the hairpin at 5′ end, allowing hemin to freely interact with G-quadruplex sequences (3). This biosensor architecture allowed the robust and fast detection of down to 50 attomole *E.coli* DNA and 2 femtomole *E.coli* rRNA in the presence of O_2 or H_2O_2 substrates and without any additional mediator. This result is comparable with the best result for bacterial DNA/rRNA detection using sandwich assay on magnetic beads (4).



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Electrochemical Detection of Nitrite Reductase Activity of Hemoglobin

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Multiple physiological studies have revealed that nitrite represents a biologic reservoir of nitric oxide that can regulate hypoxic vasodilation, cellular respiration, and signaling.[1,2] These studies suggest a vital role for deoxyhemoglobin-dependent nitrite reduction. Hemoglobin has been shown that hemoglobin can reduce nitrite to nitric oxide. The heme iron of the protein combines rapidly and irreversibly with the endogenous vasodilator, nitric oxide, causing constriction of blood vessels. Thus, studying the mechanism of nitrite reductase activity of hemoglobin has been a topic of utmost interest.[3,4]

In this study we applied different electrochemical techniques to study nitrite reduction by hemoglobin. Different types of hemoglobins were deposited on the glassy carbon electrode surface using surfactant molecules. The behavior of electrodes was studied under various conditions to understand the mechanisms underlying the biological framework of nitric oxide signal transduction.

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Oxygen-Reducing Biocathodes from Wastewater Inoculum: Insight from 16S rRNA Gene Sequencing

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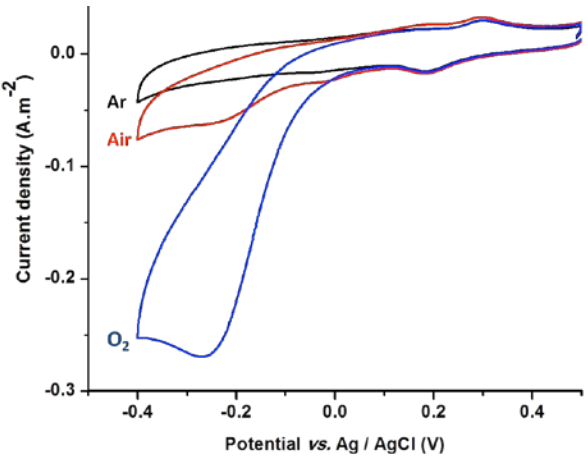
Microbial fuel cells have attracted attention due to their ability to generate electricity while treating wastewater.[1] Dioxygen is an interesting strong oxidant for the reduction reactions at the cathode of fuel cells since it is free, widely available, and can be reduced to innocuous water. A few reports of oxygen reducing microbial biocathodes appeared in the literature since the pioneering work of Bergel *et al*.[2] These reports highlight a large potential scale at which oxygen can be reduced (ca. from +0.2 to -0.4V vs. Ag/AgCl) which implies a diversity of communities and mechanisms. In this paper we report the development of a reproducible and stable (> 6 months) microbial oxygen biocathode using an aerobic inoculum from a wastewater treatment plant (Beaurade, Rennes, France). Chemical analyses of the evolution of nitrogenous species in the catholyte are discussed as well as insights from biofilm 16S rRNA gene sequencing.



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Alternative DNA Structures Studied By Voltammetric Methods: Guanine Quadruplex Models

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Nucleic acids (NAs) are electrochemically active. At mercury and amalgam electrodes, adenine (A) and cytosine (C) in natural NAs produce a cathodic peak CA while guanine (G) gives an anodic signal (peak G^{HMDE}) due to oxidation of 7,8-dihydroguanine generated at the electrode at highly negative potentials. At carbon electrodes, all DNA bases can be oxidized and particularly G and A produce analytically useful signals, peaks G^{ox} and A^{ox}. At the mercury-based electrodes, NAs give also a series of capacitive signals due to adsorption/desorption of specific parts of its chains. Importantly, intrinsic electrochemical responses of NAs are structure-sensitive, reflecting structural transitions-dependent changes in the accessibility of nucleobase residues for contact with the electrode.

Besides the Watson-Crick double helix, DNA and RNA can adopt alternative structures such as non-canonical duplexes, hairpins, triplexes or guanine or cytosine quadruplexes. These structures have recently attracted attention of electrochemists as new molecular models suitable for general studies of NA interactions with electrodes. Several papers have been devoted to electrochemical studies of oligonucleotides (ONs) differing in nucleobase composition and/or in secondary structure. We studied influence of the length of central $(dG)_n$ stretch in 15-mer ONs using voltammetry at the mercury and pyrolytic graphite electrode (PGE). We observed anomalous dependence of the peak G^{HMDE} on the number of G, changes of the peak G^{ox} at PGE and a transition of adsorption/desorption curves at the mercury electrode that correlated with observation of G quadruplex structures by CD spectroscopy and electrophoretic methods. More recent data have shown marked effects of the length and nucleobase composition of single-stranded tails flanking the $(dG)_n$ segment. Results of these studies represent the ground for the development of simple electrochemical techniques suitable for rapid label-free monitoring of DNA (or RNA) structural transitions.

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Reactivity with O_2 of H_2-producing and CO_2-reducing enzymes

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Metalloenzymes are enzymes that contain metallic cofactors. A number of them catalyze reactions that have potential impact in biotechnologies, such as the production or oxidation of H_2 or the reduction of CO_2. However, metalloenzymes are often very sensitive to O_2, which prevents their use in biotechnological devices. We have used protein film voltammetry[1] (figure, center), a technique in which a redox enzyme is immobilized on an electrode and its activity is monitored over time, to study the reactivity with O_2 of FeFe hydrogenases (figure, left) and CO dehydrogenases (figure, right). We showed that FeFe hydrogenases are able to reduce O_2 directly to water[2,3], and that some CO dehydrogenases are much more resistant to O_2 than what was thought before[4].

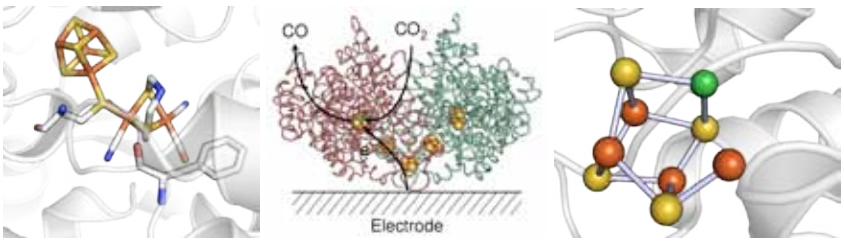

Figure: Left: active site of the FeFe hydrogenase. Center: schematic representation of protein film voltammetry. Right: active site of CO dehydrogenase.

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Biological photovoltaic building blocks for solar energy harvesting

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The primary processes of photosynthesis consist of light absorption, excitation energy transfer and electron transfers that take place within a network of various types of pigment-protein complexes (1). Decades of research has elucidated this process to highest detail from the molecular level to systems biology. In recent years a large number of researchers are investigating hybrid photosynthetic designs where photosynthesis is interconnected to electrodes to explore the application of the photosynthetic machinery for bioelectronic devices and solar cells (2). Here we report on our recent efforts to study the process outside the biological cell, to maximize functionality and to pinpoint bottlenecks in electron transfer reactions between electrodes and proteins (3). We find that many characteristics of the biological process within individual proteins, between proteins and within networks of proteins are maintained within the artificial environment. Crafting the electrode material into a nanoporous structure reveals an enhanced stability and turnover rate. Photo bio-electrochemical experiments discussed include Langmuir Blodgett films, plasmonic structures, membranes and genetically modified proteins (4-7).

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Electron Transfer from *G. sulfurreducens* to ω-Functionalized Alkanethiol-Modified Gold Electrodes

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The dissimilatory metal reducing microorganism *Geobacter sulfurreducens* (*Gs*) is a well-established model for the study of exoelectrogenic bacteria. These bacteria developed an extracellular electron transfer mechanism, which consists of a redox chain formed by c type cytochromes. These proteins catch the electrons at the inner cell membrane and transport them through the periplasm to the outer cell membrane. There, the electrons are used to reduce extracellular electron acceptors. In microbial fuel cells (MFCs) this mechanism is redirected to electrodes for electricity production. For optimal electricity production, the understanding of the interfacial electron transfer (ET) between the microorganism and the metal electrode is crucial. Here, we studied a *Gs* submonolayer (subML) on a bare Au electrodes and ω-functionalized alkanethiol-modified Au electrodes to understand and to optimize the ET processes at the interface between the outer membrane cytochromes and the Au electrode.

When *Gs* were directly attached to a Au electrode by electrostatic interactions, a potential dependent conformational change of the heme units of the cytochromes was observed for a *Gs* subML. This change was not observed for a *Gs* subML attached to a carboxylate-terminated alkanethiol-modified Au electrode. Additionally, the electrochemical activity of the subML was comparable to a subML attached to a bare Au electrode.

Changing the linker length of carboxylate-terminated alkanethiols from $n = 1$ to $n = 15$ methylene units had no effect on the initial attachment of *Gs* to these surfaces but a strong dependence of the growth of a *Gs* biofilm on the linker length has been found. While *Gs* on linkers with $n = 1$ performed similar to bare Au electrodes, the current production and coverage decreased exponentially with increasing chain length.

These results indicate that the interfacial ET depends strongly on the length of the linkers and give indications that the ET through the biofilm is the current-limiting step in *Gs* biofilms grown on bare Au electrodes.

Polymer-supported electron transfer of PQQ-dependent glucose dehydrogenase at carbon nanotubes modified by electropolymerized polythiophene

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Conductive polymers are very attractive in the field of electrochemical biosensors. The intrinsic conductivity and the possibility to equip the chains with different functional groups just by choosing the proper monomers make them very useful to improve the connection between the electrode and the redox center of an enzyme. In this context, polythiophene derivatives have only recently been employed in biosensing field in order to immobilize glucose oxidase, catalase or laccase. Anyway the use as conductive wiring matrix has not been addressed yet.
Pyrroloquinoline quinone dependent glucose dehydrogenase (PQQ-GDH) has been extensively studied for biosensors and biofuel cells development thanks to its high catalytic activity and oxygen insensitivity. The redox active site is however embedded in the protein structure, so it is not easy to achieve an efficient direct electron transfer (DET).
In this work, polythiophene copolymers have been evaluated and used as modifier for electrode surfaces in order to allow the immobilization of active PQQ-GDH and to simultaneously improve the direct electrical connection of the enzyme with the electrode. Polymer films were electrosynthesized onto carbon nanotubes modified gold electrodes from mixtures of 3-thiopheneacetic acid ($ThCH_2CO_2H$), 3-methoxythiophene ($ThOCH_3$) and 2-thiophenesulfonic acid ($ThSO_3^-$) using a pulsed deposition method. Polythiophene deposition significantly improves the bioelectrocatalysis of PQQ-GDH. The process starts at -0.2V vs. Ag/AgCl and allows glucose detection at 0V vs. Ag/AgCl. Several parameters of the electro-polymerizing method were evaluated to maximize the anodic current output after enzyme coupling. Polymer deposition has been performed first in acetonitrile which required then a transfer into the aqueous system. However, electrosynthesis performed in an aqueous solution verified that also in this case successful electrode modification is feasible. Catalytic currents for the coupled PQQ-GDH have been found to be much higher. The electrode modifications were characterized by SEM, FTIR and UV-Vis. The obtained results suggest a further investigation of this kind of polymers and, in particular, the study of the interaction with other enzymes in order to employ them in building up biosensors and biofuel cells.

Understanding the anti-oxidant activity of Epigallocatechin gallate and its derivatives using square-wave voltammetry

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Oxidative stress has been implicated in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease (AD)[1]. Epigallocatechin gallate (EGCG), a polyphenol found in green-tea has recently garnered widespread attention for its anti-oxidant activity and its ability to interact with AD proteins such as amyloid-beta resulting in off-pathway non-toxic oligomers[2-4]. We examine the redox activity of EGCG and its derivatives using square-wave voltammetry. Electrochemical measurements were performed using screen-printed gold electrodes (SPEs). Square-wave voltammograms of epigallocatechin gallate (EGCG), epigallocatechin (ECG), epicatechin (EC), catechin hydrate (CH), catechin gallate (CG) and gallocatechin (GC) were obtained at pH 2, 5 and 7.4, respectively. Ferric reducing anti-oxidant power assay (FRAP) was also performed to assess anti-oxidant properties of the molecules and absorbance was measured at 595 nm using a BioTek Powerwave XS plate reader. EGCG and its derivatives exhibited strong redox activity when tested using SWV, with maximum peak current response at pH 2 and decreasing peak current with increasing pH. EGCG and its derivatives showed powerful anti-oxidant activity on the FRAP assay, with EC and CG demonstrating the most powerful anti-oxidant activity. **References: 1**. Chriten, Y., J Clin. Nut., 2000, 71(2), 621-629 **2**. Ladiwala, A.R.; Dordick, J.S.; Tessier, P.M., J Biol Chem, 2011, 286(5) 3209-18 **3**. Ehrnhoefer, D.E.; Bieschke, J.; Boeddrich, A.; Herbst, M.; Masino, L.; Lurz, R.; Engemann, S.; Pastore, A.; Wanker, E.E., Nat Struct Mol Biol, 2008, 15(6) 558-66 **4**. Bieschke, J.; Russ, J.; Friedrich, R.P.; Ehrnhoefer, D.E.; Wobst, H.; Neugebauer, K.; Wanker, E.E., Proc. Nat. Acad. Sci, 2010, 107(17), 7710-5

Electroporation with sine signals for an accurate control of cell permeabilization

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The interaction of the cell membrane with high intensity pulsed electric fields is routinely used to provoke the transient permeabilization of the cell membrane in a phenomenon termed electroporation. This technique has traditionally used short square direct current (DC) pulses. On the contrary, although theoretically described, few experimental reports have deeply studied the ability of alternating current (AC) signals to cause cell permeabilization [1]. According to Schwann equation, the induced transmembrane potential (ITP) by an oscillating electric field varies with frequency and it is negligible for frequencies above 1 MHz [2]. If the ITP reaches a given threshold, the cell membrane is permeabilized.

In order to correctly assess the frequency dependence of membrane permeabilization, it is necessary to concentrate the energy delivered to cells in a narrow spectral band. The complex spectral content of square pulses or oscillating biphasic square waves implies the inability to separate the coupled effects at the different frequencies excited and are not suitable for a precise study of this dependence. On the contrary, narrow band signals as sine waves are the most appropriate option for this goal.

In the present work we studied the permeabilization of cells using single bursts of AC sine waves in the frequency band from 8-130 kHz. The efficacy of electropermeabilization of cell membrane was studied using Ca^{2+} as indicator.

Results show the expected frequency dependence and how by only modifying the frequency of the applied signal it is possible to control the extent of permeabilization.

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Pt-free Hydrogen/Air fuel cell integrating carbon-nanotube-supported Copper Enzyme at the Cathode and a Bio-Inspired Nickel Complex at the Anode

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Hydrogen is a new emerging energy vector alternative to fossil energies. Proton exchange membrane fuel cells (PEMFC) use platinum, as a catalyst, to oxidize dihydrogen at the anode and reduce dioxygen at the cathode. As world platinum reserves are not unlimited (approx. 40 000 tones)[1], one key issue would be to replace it by non-noble metals. Hydrogenases, composed of nickel and iron in their active site, reversibly oxidize H_2 into H^+ at low over potential. Multicopper oxydases reduce O_2 into H_2O by a $4H^+/4e^-$ mechanism. Enzymes are limited by their instability and specific working conditions, such as pH and temperature. Mimiking the active site of enzymes would overcome these limitations.

Recently, a new type of hydrogen-oxidizing arginine-based nickel bis-diphosphine complex $Ni[P_2^{Cy}N_2^{Arg}]^{7+}$ has been developed.[2] This bio-inspired model of Hydrogenases was immobilized onto carbon nanotube electrodes. This anode oxidizes H_2 into H^+ over a wide range of pH, at high current densities from 5 to 16 $mA.cm^{-2}$,and with near-zero overpotential. Associated with a Pt/C doped- cathode (1mg cm^{-2}) in a PEMFC, a power density of 16 mW cm^{-2} and an OCV of 1V, near the expected thermodynamic value of 1.2V, were reached. Finally, the bio-inspired anode was integrated in hybrid Pt-free H_2/air fuel cell with the enzyme bilirubin oxydase from *Myrothecium verrucaria* at the cathode. Hence, we measured a power density of 1.85 mW cm^{-2} at pH = 5 and at room temperature.[3]

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Nanostructured Choline Oxidase Biosensor for Sensitive Choline Detection

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Choline is an essential physiological component of the cerebral spinal fluid, which is distributed in the peripheral and central nervous systems of mammals. It is often considered as a biomarker of cholinergic activity in the brain tissue, especially in the clinical detection of neurodegenerative disorder diseases, such as Parkinson's and Alzheimer's diseases [1].

Amperometric biosensors based on choline oxidase (ChOx) offer some advantages such as simplicity, reliability, rapid response, high sensitivity and low cost. In their design, the enzyme may be immobilised together with conducting polymers, nanoparticles, carbon nanotubes, graphene, or their combinations in order to enhance the biosensor functionality and sensitivity [2].

A novel, nanostructured choline biosensor has been developed based on the immobilization of choline oxidase onto gold nanoparticle/multiwalled carbon nanotube modified glassy carbon electrodes (ChOx/GNP/MWCNT/GCE).

The influence of incorporating each nanostructured component in the biosensor architecture was probed by fixed potential amperometry and electrochemical impedance spectroscopy. Experimental parameters were optimized and the performance compared with other choline biosensors. The biosensor was used for the quantitative amperometric detection of choline and exhibited a wide linear range, good intra and inter-electrode precision, and high stability and selectivity. This study also presents the first impedimetric electrochemical semi-quantitative detection of choline with nanostructured enzyme modified electrodes, demonstrating good response at 0.0 V vs Ag/AgCl reference electrode.

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A novel inoculation and adaptation procedure to improve the performance of microbial anodes operated with complex industrial wastewater

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Despite high content in organic carbon, complex industrial effluents can be difficult to treat by means of a microbial anode producing electricity. In this contribution we report on a specific inoculation and adaption procedure that improves current density and treatment efficiency of a microbial anode operated with an industrial effluent rich in organic acids. Thereto, two different reactor types (bottle and flat plate) were equipped with a graphite felt anode operated at –200 mV vs. the SCE reference electrode. The cells were inoculated with *Alkaliphilus, Paenibacillus, Escherichia,* and *Clostridium* strains specifically isolated from the industrial wastewater, as well as *Geobacter* s*ulfurreducens*. Following initial operation for one week with synthetic medium containing acetate and propionate, the cells were operated with a slowly increasing share of industrial waste water. After two weeks the transition from synthetic medium to 100% wastewater was achieved.

Without the adaption period, the addition of *Geobacter* to the isolates in the bottle-type reactors already resulted in a doubled current density. With the adaption procedure, this current density could be increased approx. 80-fold to a final value of 0.12 mA cm^{-2}. With the flat-plate reactors (36 cm^2 projected electrode area), this inoculation and adaptation procedure resulted in a current density of up to 0.5 mA cm^{-2}. This value is three times higher than the current density reached in a cell that was inoculated with a consortium originating from an anaerobic digester and operated without the adaptation period. Similarly, with inoculation and adaptation a higher COD removal of 80% was achieved, as compared to only 25% in the control experiment.

In summary, our results show that the combination of specific inoculation and slow adaptation can be a promising strategy to achieve superior performance of microbial anodes operated with a complex and difficult to degrade wastewater.

Electrochemical Study of the Extracellular Electron Transfer of Wild Type and Mutants of *Enterococcus faecalis* to Electrodes

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Only few bacteria are able to transfer electrons from the cell metabolism to an electrode surface directly. For most bacteria mediators are necessary and only some of the extracellular electron transfer (ET) mechanisms have been studied in depth. We have previously shown that flexible $Os^{2+/+}$-redox polymers can communicate with viable bacterial cells and facilitate ET to electrodes [1,2]. The aim of this study was to investigate the role of each component of the respiratory chain of the Gram-positive *Enterococcus faecalis* and accordingly find out the mechanism of the extracellular ET from the cells to the electrode via both $Os^{2+/+}$ and quinone redox polymers. *E. faecalis* is a facultative anaerobe and aerobic respiration depends on the presence of heme serving as a cofactor for cytoplasmic catalase and membrane bound cytochrome *bd* oxidase. *E. faecalis* does not require heme to grow and lacks the genes for its synthesis but is able to take up heme from the environment. When supplied with heme, a minimal respiratory chain is built up, including several NADH dehydrogenases, a demethylmenaquinol pool in the membrane and membrane bound cytochrome *bd* oxidase. Wild type and three mutant strains of *E. faecalis* with mutations within the ET chain were investigated electrochemically and under different experimental and culture conditions to identify possible ways of the cell-redox polymer-electrode communication.

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Myoglobin immobilized graphene oxide nanoribbons/chitosan nanobiocomposite for biosensing H_2O_2 release in living cells and NO_2 in meat sample

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We have described graphene oxide nanoribbons (GONR)/chitosan (Mb) nanobiocomposite for the efficient immobilization of myoglobin (Mb). Direct electron transfer of myoglobin was attained at this nanobiocomposite and its biosensing applicability towards determination of hydrogen peroxide (H_2O_2) and nitrite was established. The practical application of the biosensor is successfully explored in the real-time tracking H_2O_2 secretion by living cells. GONR possess large surface area, high electrical conductivity and electrochemical stability which make it suitable for electrochemical applications. (1) The presence of abundant oxygen functionalities make GONR as versatile matrix for Mb immobilization, (2) the higher area-normalized edge-plane structures and chemically active sites make GONR as superior electrocatalysts among other carbonaceous materials, and (3) excellent biocompatibility of chitosan provide the necessary microenvironment for the enzyme. The Mb direct electron transfer characteristics such as, redox properties, efficiency of immobilization, electrical communication; electron transfer efficiency, biocompatibility and biosensing ability have studied in detail at GONRs/chitosan/Mb. The amount of electroactive enzyme and heterogeneous electron transfer rate constant were calculated to be 2.5×10^{-10} mol cm^{-2} and 4.47 s^{-1} indicating high enzyme loading and fast electron shuttling, respectively. Being as good immobilization matrix, GONR/chitosan is also an excellent signal amplifier which helped in achieving low detection limits to the determinations of H_2O_2 and nitrite. The developed sensor displayed ultra-sensitivity for H_2O_2 detection with a detection limit of 0.5 nM and linear range of 0.5 nM–1625 μM. Besides, the sensor showed wide linear range of 0.01–2080 μM and low detection of 10 nM for nitrite detection. The practical utility of the nitrite biosensor was successfully demonstrated in hot dog meat sample. The biosensor acquires good selectivity, reproducibility and stability as well.

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Halotolerant Bacteria for Treatment and Monitoring of Hypersaline Solutions in Microbial Fuel Cells

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Hypersaline wastewater (salinity higher than 3.5%) accounts for approximately 5% of effluents worldwide and their contribution is expected to increase as new saline wastewater industries are emerging.[1] This type of wastewater cannot be treated in normal biodegradation plants, since the biological process can be deactivated due to the presence of high salinity.[2] The possibility to use microorganisms able to degrade organic wastes in a hypersaline environment is then of critical interest.
A microbial fuel cell (MFC) is a bio-electrochemical system that uses the metabolic activities of bacteria to convert the chemical energy of organic pollutants into electrical energy.[3] MFCs can be applied to different purposes, such as wastewater treatment, power generation and biosensing.[4] However, MFCs are normally studied and operated in conditions of low salinity, preventing their application in saline and hypersaline solutions.
In order to develop MFCs capable to operate in hypersaline conditions, the Great Salt Lake (GSL, Utah) was selected as a source for bacteria samples due to its elevated salinity (ranging between 50 and 280 g L^{-1} [5]). The collection points revealed different bacteria composition that were able to generate power in Single Chamber-Microbial Fuel Cell with different performances.[6] Furthermore, with the aim to apply the technology on-field, new electrode materials were developed. Surface-modification approaches were investigated to optimize biofilm colonization and current output.

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Lipidic cubic phase for hosting enzymes and improving their catalytic activity

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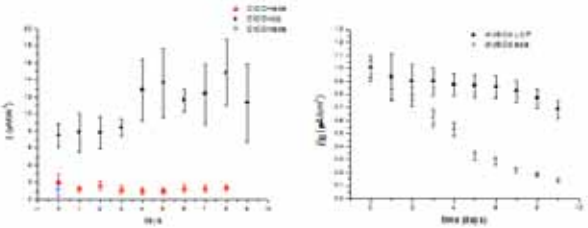
Lipid cubic phase systems are excellent immobilizing carriers for enzymes due to their biocompatibility and well-defined pore nanostructure[1]. They have been proposed as a convenient matrix for incorporating enzymes and holding them on the electrode surfaces in fully active forms. Biofuel cells based on cubic phase do not need additional separating membranes and can be easily miniaturized. The lipidic membrane is stable in the presence of water[2].

Advantages are multiple when hosting anodic and cathodic enzymes. If bilirubin oxidase from *Myrothecium verrucaria* (*Mv*BOd) is included in the mesophase the stability of the biocathode is improved[3]. When *Corynascus thermophius* cellobiose dehydrogenase (*Ct*CDH) is trapped in the monoolein cubic phase, not only stability but also catalytic performance are enhanced both for mediated and direct electron transfer conditions. Ruthenium ammonium chloride, $Ru(NH_3)Cl_2$, with formal potential at pH 7.4 equal to -136 mV, was successfully used the mediator.



The long term stability of *Ct*CDH and *Mv*BOd in cubic phase vs. drop casted on the electrode surface conveniently modified.

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Self-Assembly of Redox-Active Glyconanoparticles for Bioelectrocatalytic Energy Conversion

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The controlled self-assembly of electrochemically and photochemically active carbohydrate-coated nanoparticles offers the exciting prospect of biocompatible nanoassemblies for catalytic energy conversion, bio-sensing and bio-labeling applications. Here an aqueous nanoparticle system has been developed with a versatile outer layer for host-guest encapsulation via β-cyclodextrin inclusion complexes[1]. A biosourced β-cyclodextrin-modified polystyrene was synthesised and self-assembled into encapsulated nanoparticles via nanoprecipitation with tetrazine and pyrene derivatives. One advantage of the nanoparticle architecture is the ability to enhance the solubilisation of hydrophobic molecules in aqueous solution. Characterisation of the nanoparticles has been performed using techniques including dynamic light scattering (DLS), UV-vis, fluorescence methods, nanoparticle tracking analysis and cyclic voltammetry. These experiments reveal evidence for molecular encapsulation and differences as a function of preparation method such as size, stability, polydispersity, radius of gyration and shape. Application of redox-active nanoparticles for electrical wiring of freely-diffusing enzymes for bioelectrocatalysis in solution has been explored and their use of the nanoparticles for the development of enzymatic biofuel cells will be discussed.

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Cu-TPA-Based Redox-Polymers as Catalysts for the Oxygen Reduction Reaction

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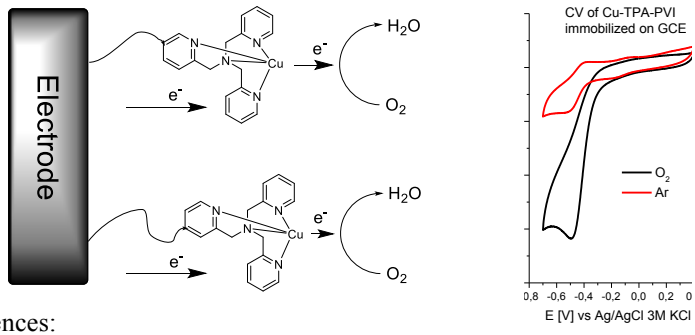
Efficient electro-catalysts based on earth-abundant elements are necessary for the O_2-reduction reaction (ORR). Molecular system based on Cu-ions complexed by the tetradentate ligand tris[(2-pyridyl)-methyl]amine (TPA) were reported as promising alternatives for Pt-based electro-catalyst for the ORR [1]. Here, we show that Cu-TPA complexes can be immobilized to various polymer backbones by different linking methods, e.g. by "click-chemistry" and amide bond formation. The resulting redox polymers serve as immobilization matrix for the electro-catalysts on electrode materials. The redox-polymer based on Cu-complex that were attached by "click-chemistry" exhibits a quasi-reversible redox signal at -240 mV vs SHE under Ar-atmosphere. In the presence of O_2 the potential onset for the ORR is at -179 mV vs SHE, which is significantly more positive compared to the ORR at a blank GCE-surface (-250 mV vs SHE). The redox polymer synthesized via amide bond formation between the Cu-complex and the carboxy-modified polymer backbone showed an even more positive redox-potential of -30 mV vs SHE under an Ar-atmosphere. In presence of O_2 the polymer exhibits a potential for ORR of -91 mV, illustrating the effect of matrix design for the optimization of ORR catalyzed by Cu-complexes.



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Development of 2.5th Generation Biosensors

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Introduction

Enzyme based electrochemical biosensors are divided into three generations according to their electron transfer processes. The first-generation sensing systems use oxygen as the electron acceptor of the enzyme reaction. The second-generation systems use free artificial electron acceptors (mediators) to transfer electrons to the electrode and are currently the most commonly used. The third-generation systems employ enzymes with the ability to transfer electrons directly to the electrode, without the need for toxic free mediators. In this study, we propose a "2.5th" generation type sensing system, based on mediator-modified enzymes without free mediators. Here, we report the preparation and evaluation of mediator modification of flavin adenine dinucleotide (FAD)-dependent glucose dehydrogenase (FADGDH) and electrochemical measurements with mediator-modified GDH immobilized electrodes.

Method

A mediator was bound to amine residues of the enzyme via amine coupling. Success of the modification was evaluated by activity assays using phenazine methosulfate (PMS) and 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), or only MTT as electron acceptors. Next, mediator-modified GDH was immobilized on electrodes and the dependency of the current on the glucose concentration was observed after applying a potential.

Results and Discussion

FADGDH cannot utilize MTT as the primary external electron acceptor. However, mediator-modified GDH showed significant activity with MTT as sole electron acceptor. Electrochemical measurements showed catalytic currents upon addition of glucose when mediator-modified GDH immobilized electrodes were used, without the addition of any free mediators. This shows that this system does not require an addition of free mediators, as is the case in third generation systems. Furthermore, this system is not limited to specific enzymes and thus can be applied for the measurement of a variety of target molecules.

The intrinsic reactivity of cell culture medium acts on the biocompatibility of metallic alloys

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The biocompatibility of metallic alloys is closely related to the surface reactivity, itself governed by biological and electrochemical reactions. Most of biocompatibility and corrosion assessments are done in highly simplified physiological solutions without organic compounds [1,2]. While those solutions make interpretation of experiments easier, they induce experimental bias by not taking into account various reactions of organic/metallic system. Moreover the growing of cells is only possible solutions providing a sufficient quantity of nutrients [3].

We investigated the intrinsic behavior of several cell culture media by electrochemical and biological tests. The electrochemical reactivity and kinetic of organic compounds was studied using platinum electrodes. Viability and mineralization assessments have been performed on osteoblasts-like cells in different culture media to better understand how complex biological systems may interact with the environment.

We notice that the cell culture media has a specific electrochemical reactivity under potential field. Organic species modify the adsorbed layer on platinum electrodes and are sensitive to the level of dissolved CO_2. The osteoblastic cells growth depends on the culture medium formulation: for example the Saos-2 osteoblasts grow quicker in RPMI-1640 than DMEM/F12 medium. The changes of cellular activity can be accompanied by oxidizing agents secretion that may induce variations of the corrosive nature of the medium. Thus the metallic alloys biocompatibility can be more understood thanks to the upstream advanced investigation of the intrinsic cell culture media behavior.

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Coupling Rolling Circle Amplification and SECM Read-out for Label-free DNA Detection

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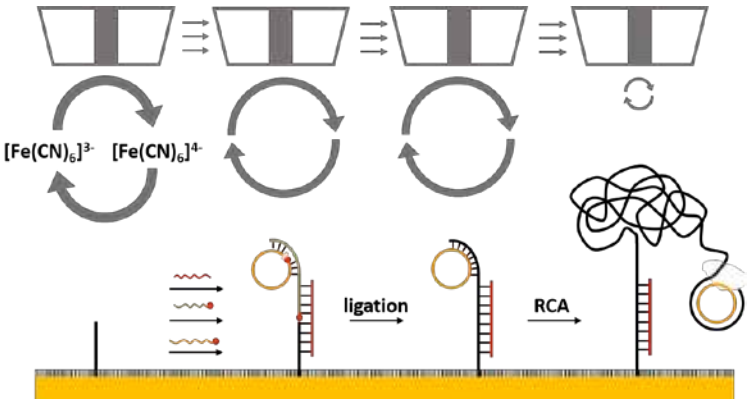
Isothermal DNA amplification approaches are rapidly developing over the course of the last two decades. Among them, rolling circle amplification (RCA) is the simplest and the most widely used as a diagnostic tool. It uses a circular DNA sequence as a template for DNA polymerase that produces a long repeating product, which presents an amplified copy of that circular DNA.
Scanning electrochemical microscopy (SECM) can locally visualize electrochemical properties of a surface with at least micrometer resolution. Therefore, it is considered a promising technique for high throughput localized read-out of DNA arrays. It has been previously employed for DNA detection, however, DNA analysis by means of SECM still did not reach the desired sensitivity.



This study couples for the first time RCA with SECM read-out for label-free DNA hybridization detection. Significant increase in the blocking of the electron-transfer rate of a free-diffusing and negatively charged redox species at the DNA-modified surface by the formed RCA product makes this approach very suitable for SECM read-out. Negligible unspecific adsorption as required for high signal contrast, is achieved with carefully designed surfaces obtained by a previously developed potential-assisted surface modification method.

Surfactant-Free Gold Nanoparticles on Graphene: Optical and Electro-catalytic Properties

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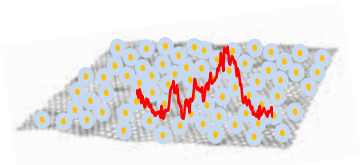
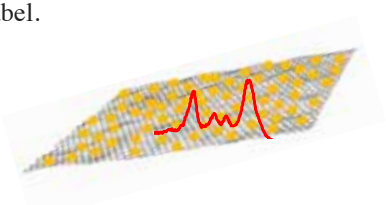
Composites of gold nanoparticles and graphene are of intense interest due to their enhanced catalytic, magnetic, and optoelectronic properties. For example, bare metal nanoparticles have been immobilised onto graphene films using wet chemistry (*in-situ* synthesis of metal nanoparticles using reduction from metal salt solution) and exhibit useful optical properties.[1] However, this method results in a heterogeneous distribution of nanoparticles on the graphene sheet.

In this work, homogeneous, ligand free gold nanoparticles were synthesised using a laser ablation technique. This method provided great control over particle size and morphology and these features of the nanoparticles are crucial which is controlling the optical and catalytic properties of metal nanoparticles. These undecorated gold nanoparticles have been physisorbed onto graphene sheets and their electro-catalytic activity investigated. These ligand free gold nanoparticles show two fold increases in electro-catalytic activity for the decomposition of H_2O_2 compared to conventional citrate stabilised particles. In addition, in presence of graphene 40 % enhancement in the electro-catalytic properties of gold nanoparticles is observed. The surface of these particles produced by laser ablation can be easily modified, e.g., with a monolayer of the surface active metal complex $[Ru(bpy)_2Qbpy]^{2+}$. These functionalised particles exhibit more intense surface enhanced Raman spectroscopy responses and their performance significantly exceeds that achievable using nanoparticles produced using wet chemical methods. Significantly, deposition of the nanoparticles onto graphene further enhances the Raman signal intensity. The luminescence signal intensity enhancement is also observed in presence of graphene film. In summary, a novel composite material is reported that exhibits synergistic interactions leading to more intense Raman and luminescence responses for immobilised dyes. These enhanced optical and electrocatalytic properties are potentially useful for highly sensitive detection of target analytes using the composite as a label.





An Approach to Treat Dairy Waste in Microbial Fuel Cell

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This work studied the feasibility of bioelectricity generation and dairy waste treatment using a two chamber electron mediated Microbial Fuel Cell (MFC) fabricated with graphite felt as an electrode and paneer whey as anodic substrate. 0.1M phosphate buffer was used as cathodic solution. Result showed that the treatment of waste in MFC found to reduce total sugar by 95.06%, phosphorous by 65.67%, ammonical nitrogen by 67.13% and COD by 58.27% indicating the efficiency of MFC in removal of pollutants and waste water treatment. Mixed culture of bacteria and *lactobacillus* spp showed the maximum open circuit voltage of 528.4mV and power of 13.510W/m^3 on the fifth day using external resistor of 1000Ω; in comparision to single strain of *Klebsiella* sps and *Lactobacillus* sps. This indicates that the electrogenic mixed culture of bacteria are more electrochemically active than the single culture bacteria in MFC. When 0.1M of potassium ferricyanide (2ml/min) was added to the phosphate buffer the MFC showed the maximum OCV of 609.5mV and maximum power of 22.261W/m^3 on the fourth day using the 1000Ω resistor.
The graphite electrodes coated with CNT composite treated with absolute ethanol showed the maximum power of 25.869W/m^3 with 1000Ω resistor on the fifth day using the phosphate buffer enriched with potassium ferricyanide.

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Point-of-Care Diagnostics using Inkjet-printed Microtiter Plates

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Developing reliable and efficient Point-of-Care (POC) devices for unindustrialized countries is one of the current goals of a big part of the scientific community. POC platforms applying electroanalytical methods, often in combination with immunoassay-based detection, could make a great contribution for improving qualitative and quantitative diagnostics in health care as well as environmental sensing.

Inkjet printing has become an attractive approach for microfabrication of state-of-the-art electrodes, as well as, new functional materials in POC platforms. This digital and contact-less printing technique offers great possibilities for mass production and flexibility in design with a high precision of material deposition. Different post-treatment techniques, such as thermal curing, photonic curing and UV polymerization, can be implemented for transformation of the inks into solid functional electrode materials.

Herein, we present a fast and efficient way of the large-scale fabrication of microtiter plates that empower the implementation of a broad range of immunoassay techniques with amperometric detection in POC devices. Microtiter plates were fabricated through a fully automated multilayer inkjet printing process and they consist of eight independent electrochemical cells composed of carbon nanotube working and counter electrodes, silver/silver chloride quasi-reference electrode and insulating dielectric materials used to precisely define the electrode areas. We demonstrate the successful application of such plates in a portable POC platform containing a multichannel potentiostat for a broad range of samples including the detection of analytes in sandwich and competitive immunoassay strategies. The results demonstrate that the printed system is comparable and can even be superior to conventional platforms.

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Signal Switch and Signal Amplification for Electrochemical Biosensing

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Recently, the investigation on electrochemical biosensing has made great progress. The developed biosensors have extensively been used in different fields, particularly, the sensitive and accurate detection of DNA, protein and metal ions. The designs of specific signal switch and signal amplification strategies are becoming the main topic of electrochemical biosensing research. This presentation will introduce new signal switches and signal amplification strategies developed in last two years. For example, we prepared two electrochemical catalytic probes to design two molecular switches for electrochemical DNA sensing by coupling with porphyrin-encapsulated metal-organic frameworks. Several new signal amplification strategies based on nanotechnology and molecular biology have been presented for DNA detection and immunoassay. By the recognition of nucleic acids to metal ions, some signal switches and label-free methods have been developed for specific and sensitive detection of heavy metal ions. Some new electrochemiluminescent emitters have been synthesized and used for design of new ECL biosensing mechanisms. A wavelength-resolved ratiometric photoelectrochemical technique has also been developed for sensing applications. These works led to a series of amplified methods for sensitive detection of DNA, microRNA and protein.

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Identification of single nucleotide polymorphisms by means of electrochemistry combined with SERS

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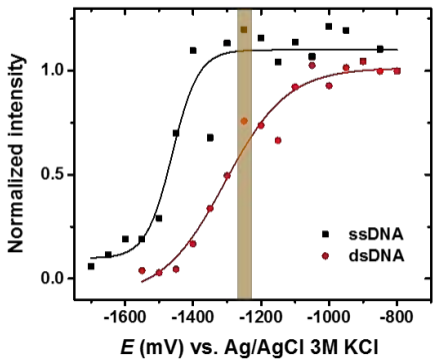
Modulations of various parameters such as temperature, ionic strength or electrical field may induce dehybridization of double-stranded DNA immobilized at the electrified interface of a suitable electrode material. Thermodynamic stability of dsDNA, which crucially depends on the DNA sequence, defines the threshold of DNA dehybridization. Therefore, DNA melting analyses provide the required information to differentiate full-matched dsDNA and mismatched dsDNA molecules. Apart from the temperature-dependent melting analysis, it was demonstrated that potential-assisted DNA melting analysis provides the required information [1].
In this study, a specific dsDNA denaturing potential is derived by comparing potential-assisted dsDNA melting curves with ssDNA desorption curves for a chosen 20 bp oligonucleotide. Using this potential, SNP analysis is achieved by observing dsDNA dehybridization kinetics of fully matched and single mismatched dsDNA sequences. The analyses were performed on a Au nanovoid modified SERS-active electrode surface using in-situ SERS measurements. Cy3-labeled target DNA was employed as a Raman reporter to observe dehybridization and the corresponding desorption curves. Rapid and uniform surface modifications were achieved via the recently suggested potential-pulse assisted DNA immobilization method [2].



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Electrochemical Methods to Understand Protein-Protein Interactions in Alzheimer's Disease

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Apolipoprotein E (ApoE) has been suggested to be involved in plaque formation, a major pathological hallmark of Alzheimer's disease (AD).[1] One of the proposed mechanisms of ApoE involvement in plaque formation is through a protein-protein interaction with amyloid-beta (Aβ).[2] The hypothesis currently gaining widespread support is that ApoE is involved in the deposition or clearance of Aβ by directly interacting with the peptide. The various ApoE isoforms such as ApoE4, ApoE3, ApoE2 have been shown to interact differently with Aβ.[3] ApoE4 has been shown to promote plaque formation by stronger association with Aβ compared to ApoE3 and ApoE2, which are, instead, thought to be involved in the clearance of amyloid deposition.[1-3] In this study, electrochemical impedance spectroscopy and voltammetry were utilized to detect how ApoE isoforms interact with Aβ in the presence of small-molecule modulators. Surface plasmon resonance (SPR)-based methods supported our electrochemical findings. Electrochemical methods are versatile platforms that are promising in the development of multiplexed drug screening assays for AD therapy.

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Fructose dehydrogenase electron transfer pathways in direct and mediated electron transfer mechanisms

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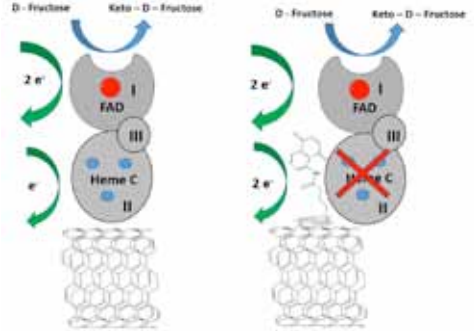
One of the most intriguing phenomena in bioelectrocatalysis, is the direct electron transfer (DET) and mediated electron transfer (MET) between the electrode material and redox active protein.

We present the results of our studies on the kinetics and mechanism of fructose oxidation by fructose dehydrogenase (FDH). It comprises 3 subunits: I contains Flavin adenine dinucleotide (FAD), where catalytic process strictly occurs, subunit II with three heme c moieties as redox prosthetic groups. Subunit III has no cofactors, however most probably it plays significant role in maintaining stability of molecule. FDH was either physically adsorbed on non-modified multiwalled carbon nanotubes (MWCNT) to study the direct electron transfer parameters or at surfaces modified with pyrene derivative of naphtoquinone to investigate the mediated electron transfer properties. Kinetic parameters: number of electron transferred, rate constants of the enzymatic reaction were calculated based on cyclic voltammetry and rotating disc experiments. Comparison of the voltammetry results under noncatalytic and catalytic conditions reveals the role of heme c as the active site in the overall electron transfer pathway. Shift of the catalytic onset potential and linear dependence on pH in the presence of naphtoquinone suggests that the mediator with sufficiently low formal potential can substitute heme c in its function in the intramolecular electron transfer chain. Comparison of the kinetic parameters of the DET and MET processes shows clearly that addition of mediator significantly increases the rate of catalytic oxidation of fructose making MET always a favorable approach for the applications in biosensing and biofuel cell.



Surface Chemistry-Dependent Effect of Superparamagnetic Iron Oxide-Based Nanoparticles on Biomimetic Membranes. Electrochemical and Spectroscopic Studies.

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Hydrophilic and hydrophobic superparamagnetic, iron oxide-based nanoparticles (SPIONs) effect on biomimetic membranes were followed with a palette of electrochemical and spectroscopic techniques from the point of view their potential use for medical applications, such as targeted drug delivery, magnetic resonance imaging. **Hydrophilic**, citrate-coated SPIONs were dispersed in an aqueous environment and interacted with lipidic biomimetic mono-and bilayers, whereas **hydrophobic**, oleic acid-coated SPIONs were confined solely within the bilayer lipid membrane of liposomes, forming magnetoliposomes (MLUVs). MLUVs were then loaded with doxorubicin – a potent anticancer drug and tested as potential carriers for magnetic field-dependent, targeted drug delivery. Our results indicate that hydrophilic SPIONs affect the phase behavior of lipid layers, altering their organization, and inducing changes in the domain formation, whereas hydrophobic SPIONs in the lipid membrane lead to its stiffening, rendering MLUVs prone to mechanical rupture and drug release in a low-frequency alternating magnetic field. The kinetics of drug release was evaluated with electrochemical and fluorescence techniques. Both techniques point toward the Korsmeyer-Peppas mechanism of drug release with mechanical rupture of MLUVs, visualized with TEM. We think that the drug release proceeds through the MLUV rupture induced by mechanical vibration of SPIONs rather than through the localized heating of SPIONs vicinity.

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Calcium ions and photosensitizer co-loaded in biocompatible nanocarriers dedicated for electroporation-supported photodynamic treatment of melanoma cells

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Melanoma is considered as the most serious type of skin cancer. In spite of various therapeutic approaches such as chemotherapy, radiotherapy or immunotherapy, the final treatment outcome is still poor because of the resistance of melanoma cells to these strategies (*Oncologist. 2011,16:5*). Thus, alternative methods are still in demand and a promising one is based on the generation of reactive oxygen species (ROS) after electroporation-supported photodynamic therapy (ES-PDT) of cancer cells (*Bioelectrochemistry 2016,110:19*). New developments in ES-PDT aiming at enhanced tumor selectivity and biocompatibility encompass an application of a third generation photosensitizer, i.e. chlorine-based which is used as a structural matrix for co-precipitation with a polymer in the micellar pseudophase (*Br J Pharmacol. 2008,154:1*).

In our studies, we applied ES-PDT to improve photodynamic effect of various photosensitizing agents. For this purpose we employed biocompatible components, i.e. biodegradable polymer: poly-D,L(lactide), PDLLA and anionic dicephalic type surfactant: (disodium N-dodecyliminodiacetate), $C_{12}(COONa)_2$ for encapsulation of Chlorin e6 using nanoemulsion embedding technique. Moreover, calcium ions ($CaCl_2$) were also incorporated in nanoparticles to enhance the cell death effect. After the process of engineering, controlled by dynamic light scattering technique, ζ-potential measurements, transmission electron and atomic force microscopy, loaded spherical polyester nanocarriers with average size < 200 nm were subjected to *in vitro* studies. Biological activity evaluation was performed using melanoma cells (Me45) and the control CHO-K1 cell line. Therapeutic effect enhanced by EP was assessed by oxidoreductive potential measurements, fluorescent microscopy and photosensitizer uptake studies. The optimization of nanoparticle uptake in function of temperature was also performed. Our work indicated an efficient photosensitizer delivery into the cancer cells and a good photodynamic efficiency enhanced by the electroporation mode.

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Molecular Imprinting for Some Toxin Selective Determination in Processed Food of Animal Origin

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Meat food processing may generate toxic heteroaromatic amines and nitrosamines (NAs), thus causing several diseases. For determination of these toxins, we devised and fabricated chemical sensors. Conducting polymers molecularly imprinted (MIPs) with the *N*-nitroso-L-proline (Pro-NO)[1] and *N*-nitroso-thiazolidine-4-carboxylic acid (NT4A)[2] toxins, used as templates, served as recognition units of these chemosensors. We used computational molecular modeling for selection of most appropriate functional monomers. A phenolic derivative of bis(bithiophene) and a thiosalenCo(III) or thiosalenNi(II) complexes formed stable pre-polymerization, 1 : 2 template : monomer, complexes with Pro-NO and NT4A, respectively. The Gibbs free energy gain due to formation of these complexes with a series of NAs was proportional to stability constants of these complexes, determined by UV-vis spectroscopy. We electropolymerized these complexes under potentiodynamic conditions and the resulting thin MIP films deposited on different electrode substrates. Then, after extracting the Pro-NO and NT4A templates, we used the MIPs for determination of Pro-NO and NT4A analytes in grilled pork neck samples. For analytical signal transduction, we used piezoelectric microgravimetry at Au-QCR/MIP electrodes of EQCM as well as DPV and EIS at the Pt/MIP electrodes. Limits of detection were 36.9 nM Pro-NO (with EIS) and 46 nM NT4A (with DPV). The chemosensors selectively responded to the target toxins in the presence of several interferences of similar composition and structure. Successful selective determination of Pro-NO and NT4A in food extracts substantiate usefulness of the chemosensors for monitoring these toxins in processed meat.

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Electrospun Nanofibers Embedding Carbon Nanotubes and Enzyme as Efficient Conductive Matrix for Biosensor Application

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Electrospinning is a very simple, versatile and high-throughput technique to prepare continuous and ultrafine polymeric nanofibers (NFs) with diameters ranging from some tens nanometers to several microns. The high specific surface area and porosity of electrospun NFs make them particularly suited for surface functionalization and creation of porous structures at the nanometer scale. A number of physical and chemical sensors based on electrospun NFs have been already reported [1] and electrospun NFs have been used for enzyme immobilization and stabilization [2]. However, very few enzyme-based biosensors based on electrospun NFs have been reported [3].

In the present work, we propose an easy and rapid surface nanobiostructuring method using enzyme-based electrospun NFs. NF mats were produced at the surface of gold electrodes from blends of the photochemically cross-linkable polyvinyl alcohol styrylpyridinium polymer (PVA-SbQ), carboxylated multiwall carbon nanotubes (MWCNT-COOHs) and glucose oxidase (GOx) as model enzyme for electrochemical biosensor application. Different parameters were tailored to produce PVA-SbQ/MWCNT-COOH NFs with minimal beading and enhanced electrical properties. We demonstrated that the combination of MWCNT-COOHs (5 wt%) and PVA-SbQ polymer (6.5 wt%) significantly improved electron transfer through the generated nanofibrous mats and preserved enzyme activity and accessibility to the substrate (glucose). The resulting enzyme biosensor, based on cyclic voltammetry detection, was linear in a wide range of glucose concentration (up to at least 4 mM) and a very low LOD (2 µM) was achieved.

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Cytochrome *c* Electroactivity Revealed by Pure Cardiolipin on the Electrode or in the Electrolyte

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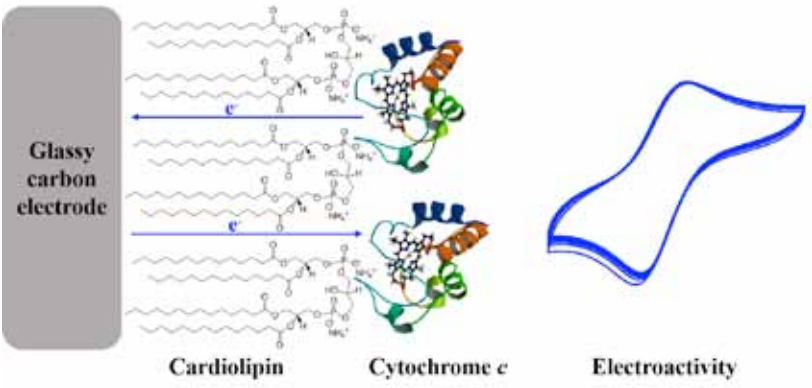
Cardiolipin is present as a fraction (10-20%) of the lipids constituting the inner membrane of mitochondria and is closely associated to the bioenergetic processes.[1] Its specific function arises from its unique structure containing four alkyl chains and a net negative charge. The electrochemical detection of the protein redox signal is greatly improved by the specific interaction between cardiolipin and cytochrome *c*.[2] However, pure cardiolipin films (without any other lipid) have never been used as supported lipid membrane on electrodes for the immobilization and electrochemical study of cytochrome *c*.

We found that the pure cardiolipin film structure and organization onto glassy carbon surface is strongly dependent on the amount of deposited lipid. This parameter is crucial for the detection and stability of the electroactive response of cytochrome *c* immobilized on the modified electrode surface. Optimization of the cardiolipin amount yields a compact structure with low permeability as demonstrated by electrochemical impedance spectroscopy results.



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Direct Electron Transfer Type Glucose Sensor with Self-Assembled Monolayer (SAM) Immobilized FAD Glucose Dehydrogenase Complex

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Introduction

Continuous glucose monitoring (CGM) is the term for a glucose sensing system measuring the glucose concentration in the interstitial fluid continuously. Currently, glucose oxidase (GOx) is used as CGM sensor element. However, GOx is not able to transfer electrons directly to the electrode without oxygen or a mediator.

We previously reported of a direct electron transfer type FAD dependent glucose dehydrogenase complex (FADGDH)[1] and a first continuous glucose sensing system employing FADGDH[2]. In this study, we have developed a highly sensitive CGM sensor employing direct electron transfer type FADGDH, which is not affected by interfering substances. To construct this highly sensitive CGM sensor, we controlled the immobilization state of the enzymes on the electrode surface by immobilizing FADGDH on a self-assembled monolayer (SAM).

Method

We prepared 3 types of SAMs of different lengths using three different carboxy disulfide NHS esters. Cleaned Au electrodes were incubated over night at 25 °C in SAM reagent solutions, and subsequently in FADGDH solutions.

Results and Discussion

With the employment of direct electron transfer type FADGDH and SAM, high current densities were achieved without being affected by interfering substances such as acetaminophen. The current of the enzyme electrodes decreased depending on the SAM length, suggesting that the distance between the enzyme and the electrode can be controlled with SAMs. These results indicate the feasibility of designing the immobilization state of the enzymes on the electrode surface. This novel glucose sensor can be applied as CGM sensor.

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Development of alkaline phosphatase fused zinc finger protein for target DNA detection

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Introduction

Detection of specific DNA is important for the food industry, clinical diagnosis, and environmental control. We previously reported a DNA sequence-specific detection system using a zinc finger protein fused with luciferase or glucose dehydrogenase as a reporter enzyme. In this study, as a new versatile labeling enzyme, we focused on alkaline phosphatase (ALP), which is applicable to wide range of detection system including electrochemical detection.

Method

We used mouse derived zinc finger protein, Zif268, which recognizes 5′-GCGTGGGCG-3′, and fused *Escherichia coli* derived ALP to its C-terminus. We produced the recombinant protein in *E. coli*, and purified it through affinity chromatography. After purification, ALP activity was checked towards *p*-nitro phenyl phosphate hydrolysis activity. Finally, interaction of the new label with double strand DNA immobilized on micro titer plate was evaluated.

Results and discussion

After culturing the *E. coli* bearing an expression vector for the designed protein at 20 ºC for 36 h, we found the expression of Zif268-ALP in a soluble fraction. Also, we succeeded in a purification of the protein via strep-tag. The ALP maintained approximately 10% of specific activity after fusion with Zif268.

As a result of double strand DNA detection using Zif268-ALP label, chemiluminescence signal was found to be related to the amount of immobilized target DNA. These results indicate that Zif268 maintained its binding ability and specificity. The detection limit was 10^8 copies of target DNA in the reaction medium, corresponding to 10 copies of DNA per PCR reaction. This limit of detection is usually considered to be sensitive enough for practical use.

Engineering of Glucose Dehydrogenase for Direct Electron Transfer via Site-Specific Gold Binding Peptide

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Fast development of biomedical technology has led us to the invention and use of implantable medical devices to be utilized for direct monitoring of human health. Battery is generally used as the main micropower supply of these implanted devices, however it has some drawbacks as it has to be replenished in due time. One of the promising continuous energy supplies to replace the use of batteries is enzyme biofuel cell (EBFC) which harvests electrical energy from the glucose oxidation and oxygen reduction; in which two of its main substrate are abundant and essential components in living things physiological fluid. Bioelectrocatalytic applications including enyzme biofuel cell, biosensors are expected to be minimal in size but optimizing the electrical communication between enzymes and electrodes is one of the primary concerns for real applications. One approach to address this limitation is the attachment of redox mediators or relays to the enzyme. Here we report a simple genetic modification of a glucose dehydrogenase to display gold binding peptide near its active site. Gold binding peptide binds to Au electrode, the basis of microelectronic and magnetic thin films, which facilitates the stable immobilization. In addition, site specific peptide on enzyme lead to close between enzyme cofactor and electrode, causing to enhance direct electrical communication between the conjugated enzyme and an electrode. Glucose dehydrogenase is of particular interest in biofuel cell and biosensor applications, and the approach of "prewiring" enzyme conjugates in a site-specific manner will be valuable in the continued development of these systems

Electroanalysis at a Single Biomimetic Microreactor for Studying NO-Synthase Activities

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As the only source of nitric oxide (NO·) in mammals, NO-Synthases are enzymes of major biomedical interest. Although NO-Synthases are firstly dedicated to produce NO·, their precise enzymatic mechanisms are not well understood. This is related to the second role of NO-Synthases in the production of diverse Reactive Oxygen and Nitrogen Species (ROS and RNS) [1]. The usual analytical methods dedicated to study NO-Synthase mechanisms are conducted in bulk and steady-state conditions. In order to decipher more efficiently their reactivities and mechanisms, it would be very interesting to keep the enzyme in its active state and at the biologically-related scales (volume, time).

In this context, the aim of our project is to develop biomimetic microreactors allowing the characterization of NO-Synthase reactional pathways via *in situ* detection of produced species by electrochemistry and fluorescence microscopy. Indeed, if reactions take place within a microreactor, the time-scales of diffusion and reactivity of the species should not be limiting anymore, allowing the direct detection of ROS-RNS generated by the enzyme. Furthermore, a Nanotube Vesicle Network (NVN) can be used to trigger the enzymatic reaction in a controlled manner [2-4]. The *in situ* detection of produced species is achieved by combining electrochemistry and fluorescence microscopy. Owing to pseudo-selective fluorescent probes for ROS-RNS, the localization and diffusion of species is imaged at the microreactor scale. Then, platinized ultramicroelectrodes (UME) are used to monitor low concentration variations (nM-µM) of ROS and RNS [5]. The UME is placed at the vicinity of the microreactor membrane and species diffusing through lipidic bilayer, including H_2O_2 and NO· are measured by chronoamperometry. True fluxes of species generated by NO-Synthase under various activation/inhibition conditions can be quantified with these micrometric methodological approaches.

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MFC bioanodes constituted of nickel sulfide nanoparticles and electroactive biofilm.

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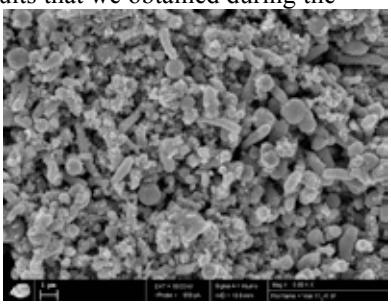
Microbial fuel cells (MFCs) are eco-friendly and sustainable energy bioelectrochemical systems (BES), which convert chemical energy directly to electricity, through the bio-catalytic oxidation of organic compounds by electro-active bacteria[1,2]. The improvement of microbial fuel cell (MFC) performances requires the increase in cell voltage as well as discharge current densities. The choice of an efficient cathode is a problem[3] and often limits the performance of the MFC. Nevertheless, it is possible to increase the whole performance by improving bioanode operating. The modification of bioanode surface by nanoparticles[4] is a suitable way to increase the efficiency of electron transfer from the bacteria and anodic current collector. For this purpose, metal sulfides are interesting compounds because they involve various redox processes occurring in natural anaerobic environments. Very few studies reported these compounds as bioanodes materials[5]. In this communication, we will present the results that we obtained during the study of nickel sulfide / biofilm bioanodes formed by polarization at -0.2V/AgCl/Ag in KCl-garden compost solution filled with sodium acetate as an organic substrate : long-term current time transients of these bioanodes and comparison to classical carbon or stainless steel bioanodes, microscopy observations of the surface of bioanodes, discussion on the electrochemical coupling between nickel sulfide nanoparticles and electroactive bacteria.



SEM image of nano NiS – electroactive bacteria bioanode.

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Transferring Monodispersity from the Nanoscale to the Microscale – Dendrimers as Building Blocks for Redox Hydrogel Films

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Redox hydrogel films built from polymeric materials are one of the most commonly used matrices for the immobilization and electrical wiring of redox enzymes in electrochemical systems [1]. Since the film thickness defines the contributions from mass transport, electron transfer and catalysis to the total catalytic current, reproducible formation of films with controlled and homogeneous thicknesses is essential toward their technological application. We have previously proposed a novel in situ gelation procedure for the formation of viologen modified hydrogel films for the integration of hydrogenase in biofuel cells [2]. The method is highly reproducible for film thicknesses in the range of 30 to 300 µm. However, thinner films display an increased surface roughness related to the size of polymer aggregates. Here, we introduce redox viologen-based dendrimers as alternative scaffolds for film formation. The monodispersity of these molecular building blocks results in better control of the in-situ gelation and opens the possibility for homogenous film formation in the range of 0.1 to 10 µm. An apparent electron transfer coefficient of $(1.15 \pm 0.1)*10^{-8}$ cm^2 s^{-1} was reached within the dendrimer hydrogel film, which is more than two times faster than that of the analogue polymer-based hydrogel [2]. We used FNR for NADPH oxidation as a model system to test the applicability of the dendrimer hydrogel for bioelectrocatalytic processes. The resulting electrodes display unprecedented catalytic current densities (icat) for NADPH oxidation with FNR (600 µA/cm^2). The precise control of the film thickness was demonstrated by a clear transition from a catalysis limitation for thin film (< 2 µm) to electron transfer limitation for thicker films in agreement with the predicted icat for the reaction/diffusion model in redox films.

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Photoactive biohybrid electrodes based on photosystem I and a small redox protein

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Artificial systems, which can effectively convert light energy into electrical or chemical energy and exploit the unique features of natural photosynthesis, are increasingly in the focus of current research. Particularly the two supramolecular protein complexes photosystem I and II (PSI and PSII) of the oxygenic photosynthesis have attracted the attention of researchers to build up new biohybrid systems. In this context an efficient coupling of PS with the electrode is essential. Besides the light-to-current conversion, the PS may also be useful for light-driven enzymatic reactions resulting in photobioelectrocatalysis.

Different basic principles can be used by establishing communication between PS and electrode surfaces. Here we present approaches which are based on the interaction of the trimeric PSI from *Thermosynechococcus elongates* and the small redox protein cytochrome c [1]. Although not natural partners, cyt c can effectively reduce the light-activated PSI. Furthermore cyt c shows fast electron transfer with electrodes - even in an immobilised state - and allows self exchange to occur. PSI can be immobilised on a cyt c monolayer on gold in an oriented way resulting in a cathodic photocurrent. The amount of active PSI can be enhanced by the construction of multilayers, the co-assembly of PSI and cyt c and the combination of co-assembly and multilayer formation by means of the natural, negatively-charged polyelectrolyte DNA [2]. Alternatively, a 3D electrode configuration can be exploited. Here mesoporous ITO has been used allowing electrode thicknesses of several tens of µm [3]. In all these electrodes purely a cathodic photocurrent has been found the magnitude of which can be enhanced from few $\mu A/cm^2$ up to values of $150 \mu A/cm^2$. The analysis of rate-limiting steps is advantageous in order to overcome photocurrent limitations, e.g. the heterogenous electron transfer of cyt c at ITO. Cyt c based systems further exhibit a rather high quantum efficiency in the range from 4 to 39% (IQE) thus belonging to the most efficient systems to date.

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Biotemplated Synthesis of Nanoarchitectures and Their Electrochemical Glucose Biosensing Applications

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The nature provides an enormous library of natural biomaterials with an astonishing variety of nano-structures, which are hard to be obtained even by the advanced synthesis strategies. As the requirements for nano-materials with controllable morphologies and improved performances are becoming increasingly significant, researchers have been exploring different biomaterials as scaffolds to fabricate novel types of nano-structures for biosensing applications. In this talk, we focus on our recent advancements in developing transition metal oxide based hierarchal structure using leaves and genetically engineered phage as templates, separately, under mild and green conditions. Further, their applications in developing electrochemical glucose biosensor at neutral condition with a wide linear range, a low limit of detection, good reproducibility and satisfactory storage stability are highlighted.

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Efficient Biofuel Cells Based on Microbial Surface Displaying Enzyme and Novel Nanostructures

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The power output and stability of biofuel cells (BFCs) are greatly dependent on the properties of both the biocathode and bioanode. Microbial surface display is a biotechnology that protein, peptide or antibody could be expressed on the surface of microorganisms, which could find potential applications in biocatalysis, environmental governance and bioanalysis. In this talk, we present our recent progress in developing efficient enzyme biofuel cells. using glucose dehydrogenase, xylose dehydrogenase surface displayed bacteria as well as sequential enzymes displayed bacteria as anode catalysts, which significantly improved the stability of the monosugars oxidation at the bioanode. To improve the cathode, highly uniform three-dimensional (3D) macroporous gold (MP-Au) film was developed. The laccase/MP-Au biocathode exihibited an onset potential of 0.62 V versus SCE (or 0.86V vs. NHE) toward O_2 reduction, which was very close to the redox potential of laccase from *Trametes versicolor*, indicating that the direct electron transfer of laccase was successfully achieved. The strategy overcomes the conventional limitations such as the undesirable long-term durability and electron transfer issue in the development of BFCs. Thus, our strategy holds great potential to directly convert degradation products of biomass into electrical energy.

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Interdigitated Array Microelectrodes as Novel Platform Technology for Disposable Enzyme Sensor Strips

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Introduction

The market for medical devices such as for the self-monitoring of blood glucose is growing. Therefore, novel platform technologies realizing more accurate and faster measurements are on demand, as alternatives to conventional screen printed electrodes or planar gold electrodes. Recent progress in fabrication techniques has put a mass-production of interdigitated array microelectrodes (IDAMs) within reach and cheap, disposable IDAM-strips are expected to become available soon.

In here, we propose an alternative platform technology based on IDAMs for disposable enzyme sensor strips suitable for POCT and self-monitoring devices. We demonstrate our new technology with sensors for the measurement of glucose and of glycated albumin (GA).

Methods

Sensors were fabricated by drying fungal FAD-dependent glucose dehydrogenase [1,2] or fructosyl amino acid oxidase (FAOx) together with a mediator onto IDAM-strips (Tanaka Kikinzoku Kogyo K.K.). A spacer and cover were attached to the thus prepared electrodes to form a sub-micro-liter capillary space. For the measurements, samples with different analyte concentrations were injected into the capillary space. GA samples were pre-treated with protease. Chronoamperometric measurements were carried out with one of the interdigitated electrodes set as working electrode (WE) and the other as counter electrode (CE).

Results and Discussion

With this new type of sensor, fast, accurate and sensitive measurements were achieved for both glucose and GA. With common plate-type electrodes, the time course of chronoamperometric measurements strongly depends on the time passed since application of a potential, and thus small disturbances in the measurement might lead to significant errors in the measured values. In contrast to this, with interdigitated WE and CE, the time course reaches a relatively high plateau current within seconds after applying the potential and the current does not depend on the time passed. This should lead to more accurate sensing devices, which can be fabricated more easily.

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An O_2 insensitive self-powered biosensor based on a polymer multilayer bioanode for instrument-free detection of glucose

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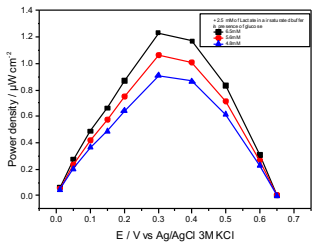
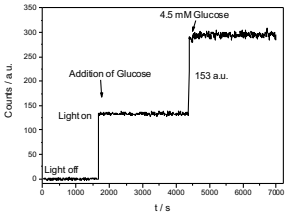
The use of biofuel cells as a basis to design self-powered miniaturized devices for *in-vivo* sensing and point-of-care diagnostics is considered a promising approach. Moreover, a simple readout strategy of such sensing devices highly facilitates their potential application in self diagnostics by the patients.

Here, we report on a self-powered biosensor based on an O_2 insensitive polymer multi-layer based bioanode comprising glucose oxidase in the sensing layer and a lactate oxidase/catalase system in the top layer. In order to ensure a high open circuit voltage (OCV) of the biofuel cell, glucose oxidase was electrically wired via a low-potential phenothiazine-modified redox polymer (TB-polymer) bearing crosslinker capabilities that allow for an electrochemical induced deposition process via the in-situ activation of an initially protected crosslinker. To prevent parasitic current losses due to O_2 reduction at the low-potential polymer or via the biorecognition element a lactate oxidase/catalase based O_2 removal system was introduced as a protecting layer on top of the sensing layer. The protection layer is powered by lactate which is present in blood in concentrations of up to 2.5 mM. This low-potential bioanode was combined with an O_2 reducing bilirubin based biocathode.

The self-powered device showed an OCV of around 650 mV which was high enough to induce a color change in an electrochromic window in the presence of the analyte glucose without applying an external potential and under ambient conditions. The novel self-powered sensor was able to detect glucose in the presence of O_2 and without significant loss in efficiency compared to measurement under argon atmosphere. This novel sensor concept may become the basis of a simple monitoring device for the determination of the glucose level in biological fluids.





left: Dependence of the power density of the device based on a GOx/TB-polymer//LOx/CAT/P(SS-GMA-BA) bioanode and BOD-GE modified biocathode in air saturated PBS buffer at pH 7 containing (2.5 mM) on lactate and glucose. **center**: Change of optical transmission at 650 nm of the electrochromic window (optical readout) recorded for glucose concentrations from 0.5 mM to 4.5 mM in the presence of 2.5 mM of lactate and air. **right**: Photograph of the electrode design for the suggested self-power biosensor. The bioanode and the biocathode were immobilized on the surface of the black carbon electrodes, respectively. Methylene green was deposited on the trasparent PEDOT window.

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Direct electron transfer of cellobiose dehydrogenase anisotropically orientated on gold electrodes

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Cellobiose dehydrogenase (CDH) is an extracellular flavocytochrome involved in fungal biomass degradation, which has a distinct flavodehydrogenase domain (DH) and a cytochrome domain (CYT). It is one of the few redox proteins that are capable of direct electron transfer (DET). To study DET in regard to CDH's orientation on the gold electrode, cysteines were introduced to different surface areas of the DH by site-directed mutagenesis, resulting in seven variants with an surface exposed thiol group (from the introduced cysteine residue). The mutations were located at front, back, top, bottom or both sides of the DH domain. A reaction between the free thiol group and maleimide groups pre-attached to the electrode surface was used to covalently bind the enzyme to the electrode surface. This led to anisotropically orientated CDH on the electrodes with a limited rotational freedom. The immobilization of CDH variants on the electrode surface was monitored by surface plasmon resonance (SPR) and cyclic voltammetry (CV) in absence or presence of the CDH substrate lactose was performed. Interestingly, the variants with cysteine located at the sides of the DH give 2-5 fold higher DET currents than the variants with cysteine at top, bottom or back sides. This probably attributes to the shorter distance the CYT domain has to move between the DH domain and the electrode surface to mediate electrons. Further optimization of the enzyme immobilization will be based on the crystal structure of CDH, for which an efficient electron transfer is important for both biosensor and biofuel cell development.

Polymer-Immobilized Hybrid Enzyme-TEMPO Electrocatalytic Systems for Enhanced Oxidation of Glycerol

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Enzyme-based catalytic systems represent a class of bioelectrocatalysts that have found remarkable use in the field of biofuel cells, as a consequence of the inherent high catalytic activity of such systems towards oxidation of biofuels. Integration of these bioelectrocatalysts with organic oxidation catalysts extends their utility by enabling significant amplification of the electrocatalytic activity, which increases the rate of electrocatalytic oxidation of biologically available biofuels. Herein, we describe the development of a hybrid enzyme-poly(TEMPO) catalytic system to illustrate a synergistic enhancement in the electrocatalytic production of CO_2 from the oxidation of glycerol. Specifically, we demonstrate that surface-immobilized TEMPO-LPEI/oxalate decarboxylase (TEMPO/OxDc) and TEMPO-LPEI/oxalate oxidase (TEMPO/OxOx) hybrid systems permit enhanced electrocatalytic oxidation of glycerol by allowing the organic redox polymer (TEMPO-LPEI) to catalyze the oxidation of glycerol to mesoxalate, followed by cleavage of a carbon-carbon (C-C) bond of mesoxalate - a crucial step in the complete oxidation of glycerol – and conversion of oxalate to CO_2 by the enzymes, thereby collecting up to 14 electrons per molecule of glycerol. We report on the catalytic activity of the hybrid systems with mesoxalate, as TEMPO-LPEI is not capable of cleaving a C-C bond. Furthermore, the hybrid catalytic systems impart greater stability and generate comparable catalytic currents of approximately 3 mA/cm^2 in the presence of 10 mM mesoxalate and 5 mA/cm^2 in the presence of 200 mM glycerol. This study illustrates the promising potential of surface-immobilized, hybrid enzyme-poly(TEMPO) catalytic systems, and thus, offers a simple methodology for fabricating bioanodes for enzymatic fuel cells.

OECT-based (Bio)logic circuits for sensing

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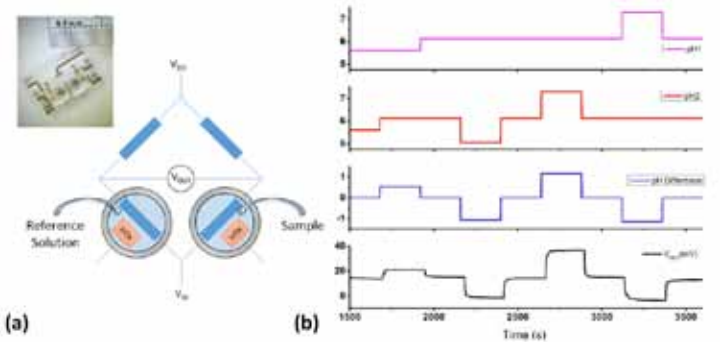
In a context of patient survey at home, the demand in wearable biosensors for continuous body monitoring strongly rises. Thereby, these biosensors have to be minimally invasive and should provide accurate sensing whatever are the patient activities. Consequently the need of flexible solid state sensors grows up. Organic electronic is an excellent candidate for such sensing application thanks to the use of low cost flexible materials that could be easily processed using large scale facilities such as screen-printing. More precisely, organic electrochemical transistors (OECT) offer original properties for bio-sensing applications. Thus, we first focused on the development of fully printed biosensors for lactate and glucose[1].

Away from classic biosensing, OECT offer the unique possibility to be combined for the design of more complex electronic circuitry. Thereby, some basic electronic architectures, Wheastone bridge, as well as NAND and NOR gates, were designed. The former circuit was implemented for the referenceless measurement of pH[2] in sweat. The later NOR gate was assayed for the design of an enzymatic logic gate using lactate and glucose as input signals..



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An O_2 insensitive glucose oxidase bioelectrode

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Glucose oxidase (GOx) has been one of the most frequently used enzymes in enzymatic biosensors and biofuel cells but tend to be replaced by O_2-insentitive enzymes such as cellobiose dehydrogenase or PQQ-dehydrogenase. The performances of GOx based biosensors are limited by the competition between redox mediators and molecular oxygen, which is the natural substrate for the oxidative half-reaction. Different strategies have been proposed to overcome the competitive oxidation by O_2 ; *i.e* by cofator redesign or by using bienzymatic systems.

GOx is still an enzyme of interest to elaborate anode in biofuel cells because of its high activity towards glucose and its low redox potential. To decrease the effect of O_2, we identified keys amino acids near the FAD redox centers. Site directed mutations allows us to generate mutants almost insensitive to O_2. By combining UV vis, stopped flow and electrochemical experiments we proved that the new mutants were less sensitive to O_2 than the Wild type GOx.

At pH 7 in presence of 5mM glucose under O_2 and forced convection, to maximize the effect of O_2, the current densities of electrodes modified with the new mutants and osmium based redox polymers only decrease by 2% compare to experiments made under Ar. For the WT, the current densities decrease by 95% upon switching Ar to O_2.

Enabling Bioelectrochemistry for In Vivo Analysis

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The development of new strategies and methodologies to directly, selectively, and sensitively record chemical signals of neurons involved in brain functions has drawn more and more attention because recording of the dynamics of chemical signals affords a platform to understanding the chemical essence involved in various brain functions, for example, neurotransmission and the diagnosis and therapy of diseases. Electrochemical methods based on bioelectrochemical mechanisms are particularly attractive for probing brain chemistry with a high temporal and spatial resolution. This topic will focus on our attempts on enabling bioelectrochemistry for in vivo analysis, covering the fundamental aspects of bioelectrochemistry with direct electron transfer of laccase as example, recent updates in biofuel cells, and new electrochemical methods for in vivo analysis based on new biosensors and biogenerators. The biogenerators are envisaged to be one kind of novel neurochemical sensing technology, providing a new tool to understanding chemical essences underlying physiological and pathological processes. With the development of the interfacial bioelectrochemistry and energy conversion technologies, some kinds of new biogenerators will be developed to work efficiently in living systems.

Keywords: Bioelectrochemistry, Biosensors, In Vivo Electrochemistry

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Self–powered wireless carbohydrate sensitive radio based biodevice operating in human blood stream

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Here, we present three-dimensional (3-D)–hierarchically structured carbon microfibers (CMFs) decorated with dense carbon nanotubes (CNTs), grown by means of chemical vapor deposition (CVD) on carbon cloth (CC), as a support for bioelectrodes fabrication [1]. Carbon nanotube/carbon microfiber-modified carbon cloth (CNT/CMF/CC) electrodes provide a 150-fold larger surface than unmodified CC, allowing an enhanced enzyme loading and hence to improve the electrochemical connection between the enzyme and the electrode. This approach was combined with a PTFE coating on one side of the material, allowing gas breathing through the electrode and, at the same time, confining the solution inside the electrochemical cell. This electrode, modified with bilirubin oxidase, was used as an air-breathing enzymatic cathode. When operating the biodevice in a blood mimicking buffer, the following parameters were registered: open-circuit voltage equal to 0.8 V and 0.55 mA cm^{-2} at operating voltages of 0.5 V vs. NHE. The air-breathing biocathode, combined with a Zn based anode, generated sustained electricity enough to power a wireless carbohydrate sensitive biodevice based on radio signal transmission, when operating in an authentic human blood stream.

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Development and Characterisation of Amperometric Enzymatic Nanobiosensors

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Carbon nanoelectrodes (CNEs) fabricated by pyrolytic deposition of carbon in laser-pulled nanopipettes are a versatile tool for various applications such as intracellular detection of reactive oxygen species[1] or immobilisation of single nanoparticles[2].

Here, we present the development of nanometer-sized amperometric biosensors based on CNEs. Glucose converting redox enzymes were embedded in a hydrogel film into etched nanocavities at the electrode tip, which were crucial to enhance the sensor stability. Different sensor designs using a variety of different functional polymers, enzymes and bifunctional crosslinkers were evaluated and optimised sensor architectures for the determination of glucose in nanoconfined volumes were obtained.

Due to their small size, these nanosensors exhibit several advantages in comparison to conventional microelectrodes including lower glucose consumption, thus minimal invasiveness, and improved spatial resolution. Using the obtained nanobiosensors, we envisage their application for monitoring glucose consumption of single cells.

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H_2/O_2 Enzymatic Fuel Cell Based on the Thermostable Enzymes in Direct Connection with 3D-Electrodes

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Emergence of renewable energy sources such as solar and wind ones induces the need of sustainable energy career that would allow to deliver and to store this energy notably during intermittent production. Hydrogen appears to meet this requirement representing at the same time high energy density and harmless water generation upon reaction with abundant oxygen. This concept is incarnated in H_2/O_2 fuel cells that produce electricity directly from chemical energy. However, the sluggish reactions of both hydrogen oxidation and oxygen reduction on conventional electrodes require the use of a catalyst. So far, platinum catalysts remain unbeaten in terms of performance but its limited availability and other economic and political issues push researchers to look for alternative inorganic, biological and bioinspired catalysts.

In the previous works we explored the biodiversity and ability of some organisms to survive in extreme environmental conditions and demonstrated the feasibility of the use of thermostable enzymes hydrogenase from *Aquifex aeolicus* and bilirubin oxidase from *Bacillus pumilus* in the design of high temperature H_2/O_2 enzymatic fuel cell [1]. At the same time, we developed the approach allowing to control orientation and therefore the ratio of electrically connected enzymes in the carbon nanotubes network [2,3]. In this work, by combining this approach with macroporous carbon felt and rational electrode nanostructuration, we report H_2/O_2 enzymatic fuel cell delivering more than 2 mW of power at 50 °C. Due to the thermostable nature of enzymes, such EFC demonstrates excellent operational stability. The amounts of incorporated and electrochemically active immobilized enzymes were determined allowing to deduce current densities in the order of hundreds of mA per mg of catalysts and being comparable with platinum at similar conditions. We perform the modelling and discuss some issues appearing when highly active enzymes are incorporated into porous electrodes such as electrode geometry, ohmic drop and mass-transfer limitations. Finally, we evaluate the prospects of such biocatalysts to replace platinum.

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Kinetics and Electrocatalytic Properties of Cellobiose Dehydrogenase Covalently Immobilised at Electrode Surfaces

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The present work aims to study the kinetics and electrocatalytic features of cellobiose dehydrogenase from *Myriococcum thermophilum* (*Mt*CDH) covalently immobilised at electrode surfaces. CDH is a promising redox enzyme in the field of biosensors and biofuel cells, suitable for the detection of some essential carbohydrates, such as glucose and lactose, and the construction of efficient bioanodes. This is due to its great ability of exchanging electrons with electrodes, in a mechanism known as direct electron transfer (DET), without using any redox mediators. In this study, CDH mutants genetically engineered to bear a free cysteine at their surface were covalently immobilised on carbon nanotubes electrodes chemically modified with maleimide groups. This should ensure site-specific immobilization since maleimide readily reacts with the only free cysteine at the enzyme surface, ideally producing a uniform monolayer of CDH molecules lying with the same orientation on the electrode surface. The kinetics and electrocatalytic properties of the immobilised CDH were studied using mainly cyclic voltammetry, considering also the effects of different substrates, solution pH and presence of divalent cations. The influence of the position of the free cysteine on the enzyme surface was also evaluated using different CDH variants bearing the mutated amino acid in different sites.

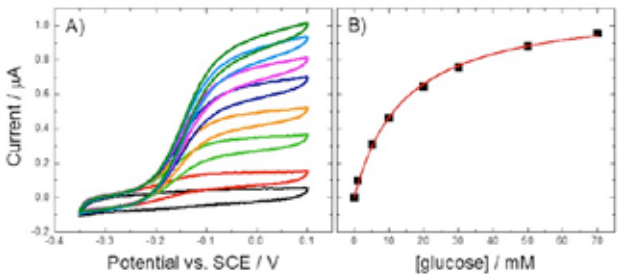

Figure 1. A) Cyclic voltammograms for a CDH-modified carbon nanotubes electrode at different glucose concentrations (0-70 mM). B) Plot of the current *vs*. sugar concentration.

A wide-field CARS Setup for spectroscopy under electropulsation of biological objects

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To deep insight into basic phenomena occurring during and after electropulsation of biological object, a new experimental setup is presented in this abstract. It combines a wide field CARS microscope [1] with a wide band coplanar waveguide (CCPW) [2]. This setup allows to acquire CARS hyper-spectra of specific Raman bands of cells and giant unilateral vesicles (GUV), simultaneously exposed to high amplitude ultra-short (microsecond or nanosecond duration) electric pulses. The electrodes gap of the CCPW was fixed to 0.5 mm to allow the CARS (pump and Stokes) beams (diameters of 100 µm) focusing at the biological object. The illumination scheme followed a non-phase-matched geometry as suggested in [1]. The pump laser axis was kept parallel to the microscope objective axis, while the Stokes beam was tilted by 7° to efficiently attenuate the non-resonant signal. CARS spectra of a phosphate buffer saline (PBS) solution (used to suspend cells and GUVs) was analyzed at first. Spectra of the PBS solution were acquired under unexposed and exposed conditions (8 electric pulses of 100µs and a field amplitude of 1200 V/cm). These spectra were retrieved from a set of 146 images recorded by scanning the Stokes frequency between 2915 and 3453 cm^{-1}. The CARS spectrum of unexposed PBS shows a main peak at 3215 cm^{-1} shifted towards the O-H asymmetric stretch vibration. While the exposed buffer presents a main peak at 3135 cm^{-1}. This last shorter spectral component seems attributable to the so called ice water Raman band. This spectral location is a marker of "ordered" organization of the water at the interface more evident after electric pulses application and the subsequent water molecules polarization. CARS images and spectra acquisitions of unexposed and exposed DC3F cells (a human fibroblast line) and GUV are ongoing.

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Cell Membrane Permeabilization by Pulsed Electromagnetic Fields in vitro

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Cell membrane permeabilization by pulsed electromagnetic fields (PEMF) has been suggested as a novel contactless method which results in effects similar to conventional electroporation (1-3). The non-invasiveness of this technique represents high potential for its use in biomedicine, food processing, and biotechnology. Currently reported PEMF permeabilization effectiveness when compared to standard electroporation and the lack of clear description of the induced transmembrane transport are of major concern. We investigated the feasibility to increase membrane permeability of Chinese Hamster Ovary (CHO) cells in vitro using 5.5 T short microsecond (15 μs) pulse bursts (100 or 200 pulses) at low frequency (1 Hz) and high dB/dt (>10^6 T/s). The effectiveness of the treatment was evaluated by fluorescence microscopy and flow cytometry using two different fluorescent dyes - propidium iodide (PI) and YO-PRO®-1 (YP). The results were compared to conventional electroporation (single pulse, 1.2 kV/cm, 100 μs), i.e., positive control. The PEMF protocols used (both for 100 and 200 pulses) resulted in increased number of permeable cells (70±11% for PI and 67±9% for YP). Both cell permeabilization assays also showed a significant (8±2% for PI and 35±14% for YP) increase in fluorescence intensity indicating membrane permeabilization. The survival was not affected. The results demonstrate the potential of PEMF as a contactless treatment for achieving reversible permeabilization of biological cells. Similar to electroporation, the PEMF permeabilization efficacy is influenced by pulse parameters in a dose-dependent manner.

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Designing highly organized porous electrodes for miniaturized Biofuel Cells

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The increasing demand on small-dimension portable and implantable devices has led to the development of new technologies to elaborate new electrode materials and methods of immobilization of bio components. In order to enhance the performance of biofuel cells (BFC) we propose highly organized porous material (providing high surface area) which allow the efficient hosting of specifically tailored enzymes without impeding the diffusion of substrate molecules thus leading to design of miniaturized implantable devices [1]. To improve lifetime and stability of BFC we are using different methods of immobilization of enzymes based on direct electron transfer (DET), mediated electron transfer (MET) as well as biocompatible hydrogels (GNF) [2] as an outer protective layer of the bioelectrode. In preliminary experiments, bilirubin oxidase was covalently immobilized on macroporous and bare gold electrodes. The bioelectrocatalytic reduction of O_2 was significantly higher for the macroporous gold electrodes. Also coverage of the modified macroporous gold electrode with the GNF hydrogel improved the stability of the bioelectrode in comparison to uncoated one [3].

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UV/VIS-Spectroelectrochemical Investigation of Cellobiose Dehydrogenase from *Corynascus thermophilus*

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In our lab we are using a homemade spectroelectrochemistry setup in order to determine the redox potential of different oxidoreductases which are of interest for applications in electrical biosensors and biofuel cells. In the past we have determined the redox potentials of glucose oxidase from *Aspergillus niger* at different pH.[1]
Currently we are investigating the redox potentials of other important enzymes such as cellobiose dehydrogenases (CDH) from ascoymcetes. These CDHs catalyze the oxidation of glucose and are able to undergo direct electron transfer at different electrode materials. We will present details of our spectroelectrochemical measurements performed with CDH from *Corynascus thermophilus* at several pH values. Due to a LabView routine controlling the setup, long periods for the establishment of electrochemical equilibrium can be chosen and thus, measurements with low concentrations of mediator mixtures may be performed. Further we have programmed a singular value decomposition (SVD) routine to evaluate our spectroelectrochemical measurements more in detail. In order ensure electrochemical equilibrium, the composition of the mediator had to be carefully optimized and will thus be discussed in detail. In addition it turned out that reversible spectroelectrochemical measurements can only be carried out, if residual oxygen is avoided because otherwise the potential of the flavin domain is shifted to more negative values (probably because of degradation). Some of the mediators that are able to electrochemically communicate with the enzyme can also be co-adsorbed together with the enzyme leading to modified electrodes that comprise a high bioelectrocatalytic current in the presence of substrate.
Depending on the progress of our research, we may also present the results of spectroelectrochemical measurements performed with other enzymes.

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Nanostructured platforms for sensitive electrochemical immunosensors

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The development of stable and sensitive immunosensing interfaces for monitoring small target analytes, present in trace amounts in clinical or environmental samples, remains a challenge. Using the natural antibody-antigen affinity, most often in a sandwich based configuration, in conjugation with nanomaterials (e.g. metal nanoparticles, graphene oxide), it is possible to achieve immunosensors with enhanced optical [1] and electrochemical [2] detection signals. In this work, we developed innovative methodologies to biofunctionalize Au and Fe_3O_4 nanoparticles with antibodies, that can be employed in both capture and detection layers, through the facile *in situ* dithiocarbamate chemistry [3,4]. In the presence of a gold surface, the reaction between CS_2, proteins and nanoparticles also enables the direct attachment of the nanostructured bioassemblies. Whenever required, protein A or G have been used to properly orientate antibody molecules. In addition, to amplify the detection of the biorecognition event, nanoparticles have been modified with optical and electrochemical signal inducers (e.g. porphyrin) [5]. Thus, the new platforms allow the application of electrochemical methods, but also optical techniques, namely surface plasmon resonance and imaging ellipsometry. Overall, the developed immunosensing interfaces, with notorious capability to inhibit protein non-specific adsorption, can be tailored to any target molecule (e.g. toxins) by using specific antibodies.

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Considering the Excitability of Cancer Cells in Electropulsation and Electroporation Effects

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Tissue excitability is expected and well understood in neuroscience, muscle and cardiac physiology, however, less so in malignant tissues. Many types of cancers aberrantly express voltage-gated ion channels, which confers some aspects of cellular excitability and raises a number of questions. What is the purpose of these ion channels in malignant cells? Can we exploit the excitability of cancer cells with electrical therapeutics? The sensitivity of cancer cells to pulsed electric fields will be discussed in the context of electropulsation and electroporation effects with particular focus on our recent research applying imaging and optophysiology tools developed in the systems neuroscience community.

Outer Membrane Cytochromes for Extracellular Electron Uptake in Sulfate Reducing Bacteria

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Sulfate-reducing bacteria (SRB) commonly play a major role in promoting microbially induced corrosion (MIC) under anaerobic conditions. The MIC damages important energy infrastructure such as underground oil and gas pipelines, resulting in enormous economic losses. One of major mechanisms for SRB to enhance the rate of anaerobic corrosion is the depletion of molecular hydrogen formed on iron or FeS surfaces, in addition to producing corrosive H_2S during dissimilatory sulfate reduction. However, the relatively slow kinetics of hydrogen evolution has raised questions regarding the role of hydrogen as a major electron carrier for MIC processes. Recently, using Fe(0) as a sole electron donor, Dinh *et al.* isolated several novel SRB strains that induce corrosion significantly faster than hydrogen-consuming microbes.[1] Here, we report that an intensely iron-corroding microbe, *Desulfovibrio ferrophilus* strain IS5, is capable of extracting electrons from an indium tin-doped oxide electrode via outer-membrane *c*-type cytochromes without consuming electrochemically generated hydrogen as an electron carrier. Electrochemical measurements using intact microbes were conducted in a single-chamber anaerobic 3-electrode system with *D. ferrophilus* strain IS5 precultivated in butyl-rubber-stoppered glass vials in DSMZ medium 195c at 303 K with an anoxic headspace of CO_2/N_2 (20:80, v/v) for 5 days. Upon the addition of IS5 cells into the reactor in the presence of sulfate as a sole electron acceptor, significant cathodic current was produced at an onset potential of –200 mV vs. SHE, which was approximately 750 mV more positive than the onset for hydrogen evolution in our experimental condition. [2] This finding indicates that hydrogen is not required for the cathodic reaction of IS5, suggesting that IS5 accelerates anaerobic iron corrosion likely through direct electron uptake. We will show differential voltammetric data, in addition to our analysis of the outer-membrane fractions by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and the whole genome of IS5, and discuss about the electrochemical property of *c*-type cytochromes located at cell surface in IS5.

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Calcium Induced Calmodulin Conformational Change. Electrochemical Evaluation

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Calmodulin (CaM) is an essential protein present in all eukaryote cells, ranging from vertebrates to unicellular organisms. CaM is the most important Ca^{2+} signaling protein, composed of two domains, N- and C-terminal domains, linked by a flexible central α-helix. CaM can aggregate up to four calcium ions (two Ca^{2+} per domain) and exists in two forms: without calcium (ApoCaM) and in the calcium saturated form (HoloCaM), changing its conformation and determining how it recognizes and regulates its cellular targets.

The development of effective new methods, for the determination and quantification of CaM, is essential for drug development, clinic diagnosis, and disease etiology investigation, due to the importance of CaM in the control of numerous physiological processes.

The oxidation mechanism of native and denatured CaM, at a glassy carbon electrode, was investigated using differential pulse voltammetry. Native and denatured CaM presented only one oxidation peak, related to the oxidation of tyrosine amino acid residues. Ca^{2+}-induced CaM conformational change and the influence of Ca^{2+} concentration in the electrochemical behaviour, was evaluated. Significant differences, in the peak potential and peak current of tyrosine amino acid residues, in the absence or presence of Ca^{2+} ions, were observed. The glassy carbon electrode surface modified with immobilized multilayer CaM film was characterized using electrochemical impedance spectroscopy. To demonstrate calcium aggregation by CaM, gravimetric measurements were also performed with a graphite coated piezoelectric quartz crystal with adsorbed CaM.

The changes Ca^{2+} induced in CaM conformational structure resulted in an alteration of Tyr amino acid residues electrochemical signals and may constitute the base for the development of a new generation of CaM based Ca^{2+} biosensors.

Quantitative Visualization of Molecular Delivery and Uptake at Living Cells with Scanning Electrochemical Probe Microscopy

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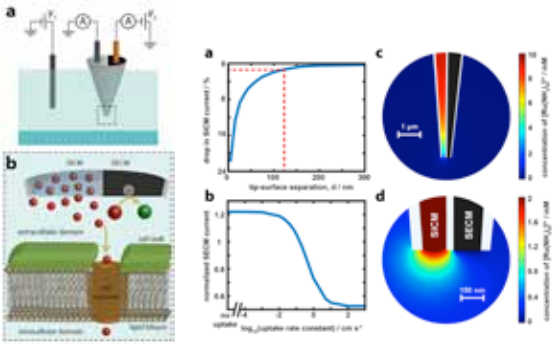
A multifunctional dual-channel scanning probe nanopipette that enables simultaneous scanning ion conductance microscopy (SICM) and scanning electrochemical microscopy (SECM) measurements is demonstrated to have powerful new capabilities for spatially mapping the uptake of molecules of interest at living cells. One barrel of the probe is filled with electrolyte and the molecules of interest and is open to the bulk solution for both topographical feedback and local delivery to a target interface, while a solid carbon electrode in the other barrel measures the local concentration and flux of the delivered molecules. This highly sensitive, self-referencing setup allows differentiation in molecular uptake rate across several regions of single cells with individual measurements taken at nanoscale resolution. Finite element method (FEM) modeling places the technique on a quantitative footing to allow the response of the carbon electrode and local delivery rates to be quantified. The technique is extremely versatile, with the local delivery of molecules highly tuneable via control of the SICM bias to promote or restrict migration from the pipette orifice. It is expected to have myriad applications across a wide variety of fields from drug delivery to neurotransmission as it is applicable to any electrochemically active molecule.



Electrochemical Nanostructured Biosensing Platforms for Small RNA Determination

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Electrochemical impedance spectroscopy has been used by our group, for the development and characterization of different format of nanostructured genosensors for small RNA determination.
In a first attempt, sensitive impedimetric detection of miR-222, a miRNA sequence found in many lung tumors, was investigated by using gold-nanostructured disposable carbon electrodes and enzyme-decorated liposomes. The proposed method was based on the immobilization of thiolated DNA capture-probes onto gold-nanostructured carbon surfaces. Afterwards, the capture-probes were allowed to hybridize to the target miRNAs. Finally, enzyme-decorated liposomes were used as labels to amplify the miRNA-sensing, by their association to the probe-miRNA hybrids generated onto the nanostructured transducer. By using this amplification route a limit of detection of 0.40 pM, a limit of quantification of 1.7 pM and an assay range spanning three orders of magnitude (1.7-900 pM) was obtained (RSD%=13). This limit of quantification was 20 times lower than that obtained using a simple enzyme-conjugate for the detection.
In a further approach, direct determination of small RNAs was described using a functional-polymer modified genosensor. The analytical strategy adopted involves deposition by electropolymerization of biotinylated polythiophene films on the surface of miniaturized, disposable, gold screen-printed electrodes, followed by the layer-by-layer deposition of streptavidin, and then biotynilated capture probes. A small RNA target was determined via the impedimetric measurement of the hybridization event in a label-free and PCR-free approach. Under optimized conditions, the limit of detection was 0.7 pM (15% RSD). The genosensor was applied for determination of miRs in total RNA extracted from human lung and breast cancer cell lines, discriminating between the cancer-positive and -negative cells, without any amplification step, in less than 2 h.

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Controlled Carbon Nanotube layers for Impedimetric Immunosensors: High performance label free detection and quantification of Anti-Cholera Toxin antibody

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An impedimetric immunosensor is developed by combining the advantages of a film of carbon nanotubes (CNT) with controlled thickess and morphology and the properties of a functionalized electrogenerated polypyrrole film bearing NTA [1]. The presence of this function on the surface of the molecular edifice allowed the immobilization, via the formation of a copper complex and the immobilization of the bioreceptor unit, cholera toxin B Subunit, modified with biotin entities. Copper is first complexed by the acid function of the polypyrrole-NTA film and then by the biotin function of the toxin [2]. The subsequent binding of the analyte antibody anti-cholera toxin is provided by antigen / antibody recognition. The system was monitored by impedance spectroscopy and cyclic voltammetry at each step of the building process. The thickness and homogeneity of the CNT films were examined using a laser microscope. After optimization, the resulting impedimetric cholera sensor shows excellent reproducibility, increased sensitivities, a very satisfying detection limit of 10^{-13} g mL^{-1} and an exceptional linear range for anti-cholera detection of 8 orders of magnitude (10^{-13}-10^{-5} g mL^{-1}) and a sensitivity of 24.7 ± 0.4 Ω per order of magnitude.

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Supercapacitive Photo-Bioanodes and Biosolar Cells for Conversion and Accumulation of Light Energy

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Photosynthesis is the only biological process that can harvest energy derived from the sun. Employment of the photosynthetic apparatus in photo-bioelectrochemical cells (PBCs) is one of the possible and promising prospects for green and self-sustainable energy harvesting.Among a number of biological components utilized in PBCs, thylakoid membranes combine benefits of keeping photosynthetic protein complexes in their native surrounding environment with low-cost and fast isolation procedure and well-established electron transfer to a various of conductive materials [1].
Consideringthe evidence described above and recently developed principle of simultaneousgenerationand storageof electric power [2] the concept of a supercapacitive photo-bioanodeand a biosolar cell (photo-biosupercapacitor) has been demonstrated [3,4]. The influence of the capacitanceof the charge storing component on the performance of the photo-bioanode was investigated and facilitated electron transfer processes occurring in the system were demonstrated [4]. Employment of the complete supercapacitive solar cell allows the achievement of a current density of 280 μA cm^{-2} in the pulse mode with a residual activity of 60% after 6 h of continuous illumination [4].

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A Systematic Study on the Response of Multiphase Vesicles to Electric Pulses

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Electroporation, permeabilising the cell membrane by applying electric pulses, is a popular method for drug/gene delivery. However, despite the wide use of this technique, the fundamentals of the electroporation mechanisms are not yet completely understood. In order to systematically investigate the electroporation mechanisms, giant unilamellar vesicles (GUVs) made from fluid-phase phospholipids have been used as simplified models of the cell membrane [1-3] [1-3]. However, these cellular models neglect the heterogeneity of the membrane of the real cell. Therefore, we have studied the role of gel phase lipids in membrane electroporation by preparing GUVs from both pure fluid phase and gel phase lipids, as well as mixtures of fluid and gel phase lipids in different ratios. After exposure of the GUVs to the pulsed electric field, the fluid phase lipids GUVs have shown size reduction while preserving spherical shape, as reported before [2, 3]. In contrast, the gel phase lipid GUVs exhibited buckling behaviour, which has not been observed in previous experiments [4] [4]. Moreover, it has been found that already a small percentage of gel phase lipids alters the morphological responses of the GUVs to buckling and that the phase separation of the two lipid phases affects the stability of the GUVs. It can be concluded that the results of the pure fluid and gel phase GUVs provide a better insight into the mechanism of the electroporation of GUVs, while the heterogeneous behaviour of the multiphase GUVs offers us a more realistic model of the cell.

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Electrochemical Mapping of the Surface Charge of Living Cells

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Scanning Ion Conductance Microscopy (SICM) has long been established as a powerful tool for non-invasive, topographical imaging of soft samples, most noticeably, living cells.[1] Part of a diverse family of scanning probe microscopy techniques, SICM utilizes changes in the ionic current between a quasi reference counter electrode (QRCE) in a single barreled nanopipette and one in bulk solution to sense the substrate and hence track the surface topography by maintaining a constant feedback value. We have recently introduced new potential control schemes into SICM that help to extend the functionality of SICM, making it a powerful nano-tool for surface charge mapping of extended substrates as well as for accurate recording of surface topography.[2, 3] By combining the results of SICM experiments with finite element method (FEM) simulations, it is demonstrated that through minimizing the bias between the QRCEs, the technique becomes a tool for non-convoluted topographical mapping, insensitive to surface charge. Upon extracting the topographical information, the bias can then be varied (pulsed or swept) making SICM sensitive to the local composition of the diffuse double layer at charged interfaces in a powerful, self-referencing approach. This technique is shown capable of distinguishing surface charge heterogeneities across the membrane of viable living cells (both plant cells and mammalian cells), even under physiological conditions, where the double layer is very thin.[4]

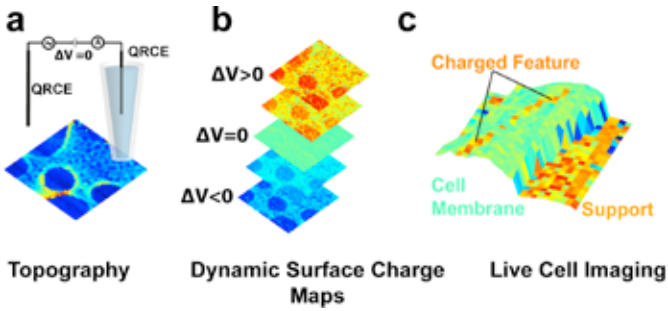

Figure 1: Bias Modulated SICM allows for precise probe positioning free from surface charge effects (a) and as a tool for surface charge mapping when a varying bias is applied (b). SICM is can be used to simultaneously map the topography and surface charge of viable living cells, identifying surface charge heterogeneities on cell membrane surface, with consequences for understanding the functioning of cells.

Pulsed Electric Fields induce cell-envelope damages on living bacteria

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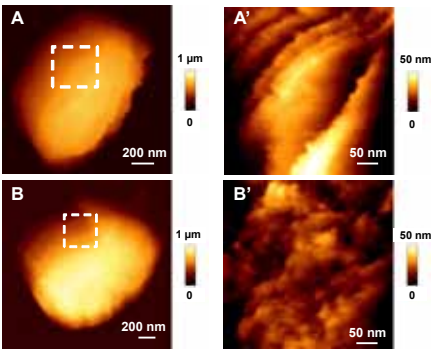
INTRODUCTION

The bacterial cell wall is the main barrier against the environment and also confers the bacteria their shape and prevents cell rupture and osmotic shock. However, the studies on cell-envelope disruption, induced by Pulsed Electric Fields (PEF) are limited. To fill this gap, we used multiparametric Atomic Force Microscopy (AFM) a technique which allows the nanoscale observation of live single cells. AFM provided, height maps, stiffness maps and hydrophobicity maps by Chemical Force Microscopy [1].

RESULTS

We obtained a high inactivation of bacteria PEF in exponential growth phase, with a loss of viability above 1 log10 with 1000 pulses (7.5 kV/cm, 5 µs) and almost 3 log10 after 10,000 pulses. The spores were the most resistant to PEF with only 1 log 10 of decrease with 10,000 pulses.

Then, we compared by AFM the morphological characteristics of bacteria before and after pulses. An example of results was shown on the spores.



Height images of full spores before (A) and after (B) 10,000 pulses with zoom images in A' and B' respectively.

CONCLUSION

We demonstrated here at the nanoscale, an alteration of spores after PEF [2]. Similar observations were observed on vegetative form and correlated with electron microscopy. The next step will be to understand the molecular mechanisms involved in these damages and study the influence of PEF on biofilm communities.

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Laccase-Catalyzed Bioelectrochemical Oxidation of Water Assisted with Visible Light

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Water splitting has been a top matter of research for the last decades because of its potential to provide H_2 while avoiding hydrocarbon sources. The biggest challenge to overcome is minimizing the energy barriers, which convey high overpotentials that increase the process' energy demand. Many catalysts have been researched for both water reduction and water oxidation, although few enzymatic biocatalysts have been. Hydrogenases' family have been matter of top research and many advances have been accomplished on this side whereas biocatalytic water oxidation process is more challenging and still demands more research on new biocatalysts. In fact, the only biocatalytic complex able to naturally oxidize water into O_2 is PSII, although recently was described the possibility to force a multicopper oxidase immobilized on a carbon electrode to oxidize water [1]. Herein we present the modification of FTO electrodes with In_2S_3 - a n-type semiconductor chalcogenide that absorbs visible light ($\lambda \geq 600$nm) and its further use as active scaffold for laccase-catalyzed oxidation of water. Irradiation of FTO-In_2S_3-laccase electrode yields O_2 production at much lower applied potential than the previous example where only electric energy was applied. The present system allows a diversification of the energy applied to accomplish the water splitting, taking a portion from the Sun.

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Clinical and Numerical Workflow for IRE Ablation

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Electroporation-based therapies (EPT) consist in applying high voltage short pulses to cells (typically several hundred volts per centimeter during about one hundred microseconds) in order to create defects in the plasma membrane. They provide interesting alternatives to standard ablative techniques, in particular for deep seated located tumors (near vital organs or important vessels). However their use and their evaluation are still controversial in clinics [Padia2016,Seror2017]. We present here a clinical workflow combined with numerical studies dedicated to IRE ablation. The clinical data consist on the one hand of a pre-treatment CT-scan, a C-arm Cone Beam CT with the electrodes and the post-treatment MR performed 3days after the IRE. On the other hand, chronograms of the electric intensity measured during the treatments are available.

Our multimodal image registration tool based on elastic deformations makes it possible to superimpose accurately the different image modalities to visualize the treatment effect. Moreover, we calibrate our dynamical tissue electroporation model on the chronogram, and compare the results with the post-treatment MRI directly on the image. We also compare the numerical simulation with the non-linear steady model of Garcia et al. [Garcia2014]. This study focused on one specific patient paves the way of augmented reality tools to evaluate a priori the IRE ablation efficacy thanks to advanced medical registration tool and appropriate electroporation models.

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Novel Hybrid Biofuel Cell Type APAP/O_2 Based on a Fungal Bioanode with a *Scedosporium dehoogii* biofilm

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A fungal biofilm has been successfully elaborated in a microbial fuel cell (MFC) device, *for the first time*, as a carbon felt colonized by a filamentous fungus denoted *Scedosporium dehoogii*. The elaborated biofilm was then used as bioanode in an APAP/O_2 novel hybrid MFC. The fabricated MFC showed APAP as an efficient fuel with highly stable output performances offering a power density at 6.5 mW m^{-2} with EMF +450 mV, under physiological conditions, as very recently reported [1]. The cathode was a carbon felt modified with poly-Ni(II) tetrasulfonated phthalocyanine (poly-NiTSPc) replacing advantageously the classical Pt/Air cathode, see [2].

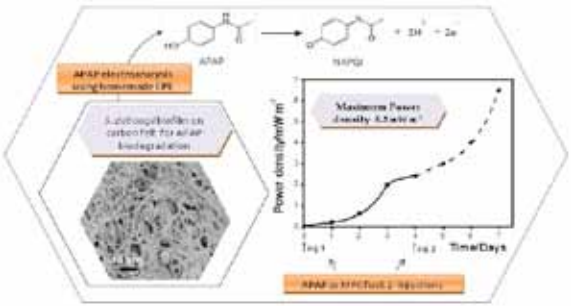

Fig. 1 : APAP/O_2 MFC power density and principle of "homemade" CPE for APAP monitoring

Keywords: APAP, Microbial fuel cell, *Scedosporium dehoogii*, Fungal biofilm, Carbon paste electrode, Bioanode; polyNiTSPc; ORR ; CPE

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Rapid and Quantitative Assessment of Redox Conduction across Electroactive Biofilms via Double Potential Step Chronoamperometry

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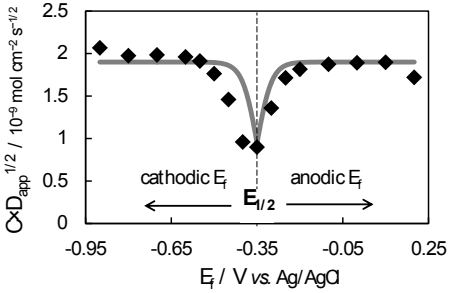
The mechanism of electron transport across electroactive biofilms (EABs) is of high interest and still a matter of debate. Quantitative assessments of their redox conduction take considerable time and require non-turnover conditions (absence of substrate), which can be detrimental to EABs. Here we measured the charge transport parameters of *Geobacter* spp. dominated EABs with double potential step chronoamperometry (DPSC) via Cottrell analysis. The DPSC measurement is simpler and much faster than usual techniques and allows the determination of the charge transport parameters even under turnover conditions. The electrochemical responses were well-described by a model of redox conduction only driven by electron diffusion within the EAB. The apparent diffusion coefficient for the electron (D_{app}) was measured at ~ 3.2×10^{-7} cm^2 s^{-1}, a value similar to those recorded for pure *Geobacter sulfurreducens* EABs, or for some redox polymers with comparable concentrations of redox centers. This method will be valuable for assessing the impact of EAB characteristics and environmental factors on charge transport ability of the biofilm, and for determining the rate-limiting step(s) for current production. (Xu Zhang *et al.*, ChemElectroChem, *accepted*).



Evolution of ($C \times D_{app}^{1/2}$) with the final step potential E_f.
The V-shaped grey line corresponds to the theoretical evolution of ($C \times D_{app}^{1/2}$) with E_f for a purely diffusive redox conduction involving a hypothetical single redox cofactor of $E_{1/2} = -0.35$ V vs. Ag/AgCl.

How to avoid hydrogen peroxide in horseradish peroxidase based sensing?

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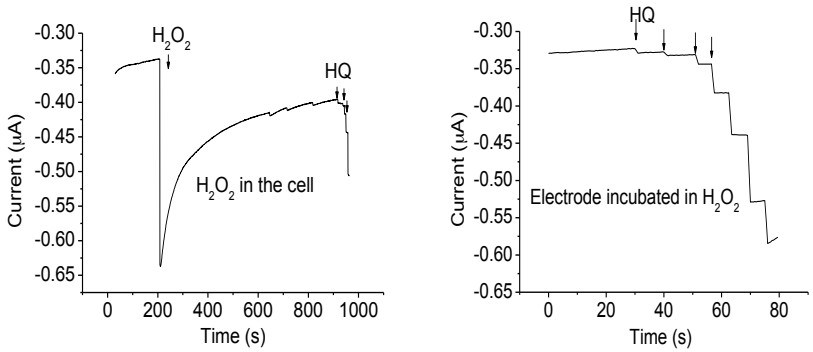
Horseradish peroxidase (HRP) is a heme enzyme, which is able to catalyze the oxidation of a broad range of substrates by the addition of hydrogen peroxide. It is one of the most useful and important enzymes that is used in bioanalytical applications, for example, as a label in immunoassays or redox enzyme in electrochemical biosensors. The last ones were particularly designed for the detection of phenols and its derivatives.[1-2]

We report a new strategy to avoid H_2O_2 in an electrochemical cell during the detection of phenolic compounds by an HRP-modified electrode. This strategy is based on pre-activation of the electrode by incubation in a H_2O_2 solution for a certain time. This activation is only possible when TiO_2 is employed as the support for the enzyme. Phenolic compounds such as 2-aminophenol, 4-aminophenol, catechol, phenol, and 2-chlorophenol are detected by our new sensing approach. Limit of detection in the optimum conditions is in the range of 40-80 nM.

This approach aims to solve some practical difficulties of using HRP in biosensors, including suppression and stabilization of the background, leading to a simple and rapid biosensor.



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Nanopiercing the Cell Membrane with Nanochannel Electroporation: A Theoretical View

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Exposure of a living cell to pulsed electric fields promotes creation of transient pores in the cell membrane. This phenomenon, called electroporation, allows enhanced delivery of exogenous molecules into the cell interior.[1] While cells are conventionally exposed to electric field in suspension, such bulk electroporation techniques still suffer from some limitations, particularly low efficiency and random transfection in gene delivery. Recently, a novel approach of cell electroporation has been proposed to overcome these limitations, which is based on a microchannel-nanochannel-microchannel configuration (referred to as nanochannel electroporation).[2] The biological agents to-be-delivered are added into the first microchannel, whereas the cell is placed into the second microchannel and positioned next to the nanochannel (Fig. 1). We have theoretically investigated cell membrane electroporation in such experimental configuration and have explored the reason why nanochannel electroporation, unlike conventional bulk electroporation, bypasses endocytotic pathways in delivery of large macromolecules including DNA and nanoparticles.[3]

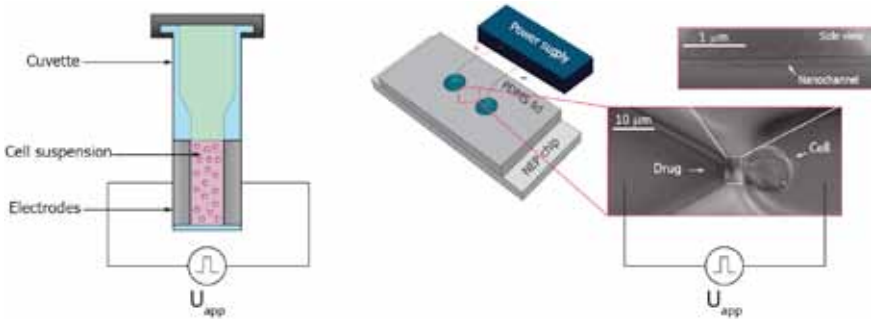

Fig. 1: Classical bulk electroporation (*left*) and nanochannel electroporation (*right*).

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Electrocatalytic H_2 evolution with a Poly-(cobaloxime)/carbon nanotube hybrid electrode

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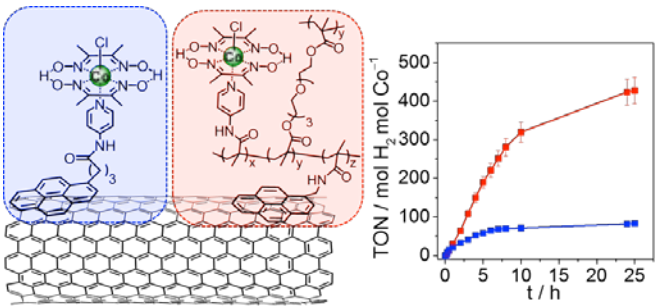
During the past ten years, many studies have focused on the use of earth abundant metal complexes to electrocatalytically evolve H_2, envisioned as a sustainable fuel. The structures of some of these catalysts are directly inspired by the active site of hydrogenases, natures' benchmark catalysts for H_2 evolution or oxidation.[1]
Branched polymers have only just recently been used to integrate molecular catalysts for H_2 evolution, in aqueous conditions.[2] This strategy is particularly interesting as it provides the possibility to incorporate, on the same molecular structure anchoring groups for the catalyst, surface modification, redox and proton relays, as well as a "protecting" matrix, similar to the amino acid scaffolding surrounding the active site in redox enzymes.
Recently, we showed that it was possible to enhance the catalytic activity of a cobaloxime catalyst, known for its inherent instability during catalysis, by incorporating it to a polymeric matrix.[3] Through non-covalent interactions the polymer has been successfully integrated to a carbon nanotube free standing electrode. While the "monomer" counterpart was rapidly deactivated, the polymer exhibited an enhanced (5-fold increase) activity and stability over time. This result demonstrated that the integration of molecular complexes with polymer chains is a promising strategy for the development of highly active and stable catalysts.



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How studying membrane and cell processes involved in electropermeabilization in relevant models may improve its development in cell biology and in clinics

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The use of electric field pulses to deliver cytotoxic drugs and nucleic acids in cells and tissues has been successfully developed (Yarmush, Golberg et al. 2014). Electropermeabilization is nowadays used in clinics to treat skin cancers and is promising for vaccination and gene therapy. But its safe and efficient use requires the entire knowledge of the electrotransfer process. Despite the fact that the pioneering work was initiated many years ago, the mechanisms underlying DNA electrotransfer remain to be elucidated (Rosazza, Meglic et al. 2016).
We will present here how using biological models with increasing complexities obtained by tissue engineering, in addition to imaging tools, can help to go further in the elucidation of the mechanisms involved in molecule delivery into cells in tissues. Small molecules can freely cross the electropermeabilized membrane and have a free access to the cytoplasm (Gibot, Wasungu et al. 2013), explaining why electrochemotherapy is so efficient. Plasmid DNA face physical and cell barriers that reduce transfection efficiency and engender a complex mechanism of transfer (Gibot and Rols 2013; Madi, Rols et al. 2015-2016). In addition to proposing a reliable alternative to animal experiments, tissue engineering produces valid biological tools for the *in vitro* study of cytotoxic drugs and gene electrotransfer mechanisms in human tissue.

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A Whole Cell Approach to Light-Driven H_2-Evolution and C=C or C=O Bond Hydrogenation by Non-Photosynthetic Bacteria

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New technologies which harness solar energy for the sustainable production of fuels and chemicals are needed to combat the harmful environmental effects of accessing fossil reserves. Solar-chemicals production can be achieved by combining light-harvesting reagents and enzymes. However, most systems reported to date require time-consuming enzyme purification and the isolated enzymes typically exhibit poor long-term stability [1].

The aim of this research is to develop a whole cell approach to solar-chemicals production where protein purification is not required and a single non-photosynthetic micro-organism can act as a multi-faceted catalyst. This concept has been demonstrated using *Shewanella oneidensis* MR-1 (MR-1) [2]. The approach uses methyl viologen to shuttle photo-excited electrons from water-soluble photosensitisers into MR-1 to catalyse four reactions: H_2-evolution by the reduction of aqueous protons, C=C bond hydrogenation by the reduction of fumarate to succinate, and C=O bond hydrogenation by the reduction of pyruvate to lactate or CO_2 to formate.

Microbial growth conditions were established which favour the simultaneous presence of enzymes key to all four of these reactions. A range of photosensitisers, including eosin Y and $Ru(bpy)_3^{2+}$, were then assessed for their ability to photo-reduce methyl viologen. In the presence of MR-1, photo-reduced methyl viologen was able to drive the desired chemical transformations. H_2 production was quantified using electrochemistry and gas chromatography. Light-driven reduction of carbon-based compounds was quantified using ^{1}H-NMR.

Overall, a versatile strategy for solar-chemicals production has been developed. In the future, the bacterium will be combined with bio-compatible photosensitisers such as carbon quantum dots to enhance longevity. Additionally, this system could be used as a platform for more complex chemical transformations with other species of bacteria or a genetically-modified MR-1.

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Bioanodes based on hydrogenases entrapped in viologen-modified polymers: protection and reactivation strategies

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Hydrogenases are nature's highly active catalyst for the H_2 oxidation and evolution reaction. Their active sites are based on earth abundant metals, i.e. Fe and Ni. Thus, they are considered as promising alternatives for noble metal based catalysts, which are scarce and costly. Besides their high activity, hydrogenases are highly sensitive towards O_2, which is a major drawback when it comes to technological applications. We recently demonstrated that even a highly sensitive [FeFe] hydrogenase can be protected under oxidative conditions by incorporation of the biocatalyst into a viologen-modified redox polymer, which is not only used for the electrical wiring of the enzyme but also for the reduction of incoming O_2. [1]
Here, we exploit additional protection mechanisms for various hydrogenases (e.g. [NiFeSe] and [NiFe] hydrogenases) based on bi-enzymatic O_2 removal systems that are immobilized in redox-silent polymer layers on top of the active H_2 oxidizing layer. Moreover, we show that the low-potential viologen based mediator can be used as a reactivation element for hydrogenases that have been deactivated upon exposure to O_2. We further demonstrate that the highly active [NiFeSe] hydrogenase from *Desulfovibrio vulgaris* Hildenborough can be used to set new benchmarks for H_2 oxidation currents on flat electrodes (1.7 mA cm^{-2}) when incorporated into a stabilizing viologen-modified redox polymer matrix. [2]

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Study of Biological Barriers by Electrochemistry: In-vitro Model of Inflammatory Condition

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Biological barriers, e.g., skin, lung and intestinal epithelium are vitally important for survival of animals including humans. These barriers are constantly challenged and damaged, e.g., by microbes and toxins. Each barrier possesses physical-physicochemical (dead tissue) and biological (living tissue) defense features. Knowing physicochemical properties of these barriers is important, e.g., in optimizing drug delivery, developing noninvasive bioanalysis methods. Treating the barrier diseases though requires knowledge about multitude of reactions involving powerful biological antioxidative, immune and repair systems, including surface microbiota. Recently, we have introduced an electrochemical setup, which consists of an electrode covered with a biological barrier and showed that the electrochemistry can be used to study the permeation of drugs through the barriers. In these experiments, the setup consisted of a skin membrane covered electrode [1, 2]. In this presentation, the setup, which comprises an intestine covered electrode, will be discussed in comparison to skin covered electrode. The electrodes, covered by these biological barriers, were used to investigate catalase activity present in skin and in intestine. It was found that these barriers contain a high activity of catalase enzyme. The proposed in-vitro setup was used to study the interaction of catalase present in tissue with polyphenols, plant extracts, and other antioxidants present in liquid formulations. These experiments and the data indicate that the proposed setup is an important electrochemical tool enabling studies of processes relevant to inflammation situation on tissue level [3].

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Development of Enzymatic Electrodes with Mutant Pyranose-2-Oxidase for Enzymatic Biofuel Cell Applications

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In this study, novel enzymatic electrodes with mutant pyranose-2-oxidase (P2O-T169G) were developed for enzymatic biofuel cell (EBFC) applications. Although glucose oxidase (GOx) is known to be the most widely used enzyme for glucose oxidation, it has several drawbacks such as forming hydrogen peroxide in the presence of oxygen, which can inhibit the enzyme. P2O-T169G, on the other hand, was reported to have low activity towards oxygen providing an advantage to be used in EBFCs compared to GOx.

A new immobilization approach incorporating multi-walled-carbon nanotubes within ferrocene (Fc)-Nafion film as a nanocomposite support for enzyme crosslinking was successfully implemented. The electrochemical tests demonstrate that the electron transfer can be achieved successfully from enzymes to the electrode via Fc mediator. Co-immobilisation of Fc with the enzymes improves the catalytic current values by 3.3 and 2.3 fold for P2O-T169G and GOx respectively compared to the system where enzymes used in solution. The electrodes modified with P2O-T169G is more stable than GOx at 0.15 V (vs Ag/Ag^{+}) for 12 h of operation. During the first hour of stability test, P2O-T169G maintains 70 % of its initial current whereas GOx loses 90 %. Single chamber EBFCs using P2O-169G or GOx at the anode and bilirubin oxidase at the cathode with air-saturated or de-aerated glucose solutions were tested. The performance of the EFC with P2O-T169G anode showed little change between air-saturated or de-aerated glucose solutions indicating that oxygen did not affect the performance. A maximum power density of 9.56 μW cm^{-2} was achieved which is ~25 % more than GOx.

An air-breathing cathode design is then employed to eliminate the mass transport limitation at the cathode with P2O-T169G at the anode resulting a maximum power density of 29.8±6.1 μW cm^{-2} at 0.318 V. It is shown that the developed enzymatic electrodes would have a potential to improve the performance and stability of EBFCs for a number of applications in micro-electronics.

Glucose Dehydrogenase on Flexible Carbon Fiber Arrays for Biofuel Cell Implanted in Small Insects

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An enzyme-based glucose/O_2 biofuel cell (BFC) operating as a micropower source in a live ant (*Atta sexdens sp*.) is reported. The BFC, consisting of two modified flexible carbon fiber (FCF) electrodes [1], was implanted in the ant's head, and its hemolymph supplied the BFC with glucose and molecular oxygen. One of our aims was the development of an experimental setup using FCF-modified electrodes inserted into glassy capillary microelectrodes for implantation in the ant head. We envisioned the possibility of operating biodevices on micro scale with high efficiency in small living systems, which would afford interesting paths for energy conversion and for sensing and monitoring devices in general. An FCF modified with poly(neutral red) and the glucose dehydrogenase (GDh) enzyme was used as the bioanode, and an FCF modified with bilirubin oxidase (BOx) served as the biocathode. Bioanode and biocathode exhibited high current densities, 2.4 and −3.6 mA cm^{-2}, respectively, leading to a BFC with a maximum power output of 268 μW cm^{-2} and open circuit voltage of 720 mV. Fueled by the hemolymph inside the ant's head, the implanted BFC can produce a voltage output of 500 mV.

Keywords: flexible carbon fiber, glucose dehydrogenase, biofuel cell

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Development of Nano-Sized Multi-Material and Multifunctional Bioelectronic Systems

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There is a pressing need to develop new strategies and technologies for bioelectronic applications. In order to advance bioelectronic systems we need techniques that allow for the merging of multi-material systems which convey bio/functionality. Towards this goal we combine wireless electrochemistry with three-dimensional (3D) printing and provide proof of concept that this could be used to fabricate novel bio/electronic functional systems. Initially omnidirectional microwires are grown wirelessly with diameters up to 200 nm by self-assembly of silver nanoparticles between inkjet printed electrodes, significantly reducing the diameter of 3D interconnects within circuits compared to 3D printing techniques alone. We demonstrate that by tailoring electrode geometry the electronic field dimension can be modulated which affects the growth of conductive microwires. This coupling of wireless electrochemistry with 3D printing was also used to grow wires *in situ* with a monolayer of Chinese Hamster Ovary cells forming a bio-interfacing mesh of conductive conduits. The combination of wireless electrochemistry with 3D printing techniques provides an alluring platform to fabricate novel cellular-bioelectronic systems of use in electroceuticals.

Utilization of Supercapacitive Feautures in Bioelectrochemical Systems

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Bioelectrochemical systems (BES) are interesting devices able to convert organic compounds into electricity through chemical, biological and physical reactions. Unfortunately, the power output is quite low and therefore, in order to utilize the output for practical applications, BESs have to be coupled with external supercapacitors and power management system in order to boost up power/current generated. Recently, the supercapacitive features of the anode and cathode electrodes of bioelectrochemical systems have been exploited. In this way, anode and cathode electrode work as negative and positive electrode of an internal supercapacitor. The two electrodes are self-charged by the red-ox reactions taking place at the anode (organic oxidation) and at the cathode (oxygen reduction). Discharges and self-recharges were shown at high current pulses [1]. An MFC with activated carbon (AC)-based cathode had a maximum power of 2.98 Wm^{-2} (5.36 Wm^{-3}) and the output was affected significantly by cathode ohmic losses. In order to further enhance the output, two strategies were adopted: i) increase the overall cell voltage using Fe-based or bilirubin oxidase (BOx)-based cathodes; ii) use an additional supercapacitive electrode (AdE). The combination of the two strategies brought to a P_{max} of 84.4 Wm^{-2} (152 Wm^{-3}) with BOx cathode, 62.2 Wm^{-2} (112 Wm^{-3}) with Fe-AAPyr and 26.7 Wm^{-2} (49 Wm^{-3}) with AC cathode. The same supercapacitive features were investigated in microbial desalination cells (MDC). High surface area electrodes are used as anode and cathode of the MDC and due to the red-ox reactions they were self charged. To circumvent high cathode high ohmic losses, a supercapacitive electrode (AdE) was added to the cell and short-circuited with the cathode. The effect was a significant increase of power output. A total of 7600 discharge/self-recharge cycles (equivalent to 44 h) were recorded for SC-MDC and SC-MDC-AdE. Wastewater treatment, water desalination and power/current pulses were achieved.

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Bile Acids and Cholesterol: Possibilities of Electrochemical Oxidation and Reduction at Bare Electrode Materials

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The direct redox activity of bile acids and cholesterol is limited to far positive/negative potentials under variety of conditions and electrode materials, as we reviewed recently [1]. In this contribution, examples on electrochemical reduction of bile acids at mercury-based electrodes and electrochemical oxidation of cholesterol and bile acids at selected solid bare electrodes will be presented. Reduction of bile acids. is enabled by the presence of the carboxylic group at C_{24} with the hydrogen as source of the catalytic signal [1], and was investigated in pH range 2-12 in aqueous or mixed methanol-aqueous media at hanging mercury drop electrode and mercury meniscus-modified silver solid amalgam electrode. Depending on their structure and pH values, the bile acids and their conjugates are in ionic/protonized form strongly influencing their electrochemical behaviour. The reductive process proceeds at far negative potential at ca −1350 mV to −1800 mV vs. Ag/AgCl and can be used for their quantitation using cyclic voltammetry and square wave voltammetry. Oxidation of bile acids and cholesterol was investigated at platinum, glassy carbon and boron-doped diamond electrodes. In acetonitrile their presence can be traced as earlier onset of anodic current compared to supporting electrolyte solution. Primary bile acids provide a well-developed voltammetric signals at reasonable potentials of around +1.2 V when using water content under 5% with C7α hydroxy group as the most probable site of the electrochemical attack. Factors influencing the stability of this signal and attempts on identification of reaction mechanism and its products using IR spectroscopy, mass spectrometry and electron paramagnetic resonance will be further presented.

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Enzyme and Yeast in a Cooperative Ethanol Biofuel Cell

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Microbial and enzyme biofuel cells (BFCs) have been extensively studied not only for electrical energy conversion, but also as promising biodevices to explore the use of fuels from biomass.[1] Here, we show a new type of BFC, named cooperative BFC, where the bioanode is composed by alcohol dehydrogenase enzyme (ADH) and *Saccharomyces cerevisiae*. Flexible carbon fiber (FCF) was used in the configuration of the electrodes. The yeast converts glucose (or other carbohydrates) to ethanol in anaerobic conditions, while the ADH catalyzes the oxidation of ethanol to acetaldehyde. We propose that ethanol (fuel) can be formed in a friendly environment via anaerobic fermentation, and then, ethanol can be oxidized in the presence of NAD^+. As result from the half-cell experiments (bioanode), it was observed a linear correlation between the oxidation current and the ethanol produced. For the unit cell (with bilirubin oxidase on FCF cathode), a maximal current density of 80.0 μA cm^{-2} and open circuit voltage of 0.700 V were obtained. Maximum power density about 10 μW cm^{-2} at 0.35 V was also observed. Thus, we demonstrate that cooperative bioanode can be successfully implemented in a BFC. Furthermore, we glimpse this approach allows a large variety organic molecules can be used for the ethanol bioproduction from yeast fermentation.

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Electrocatalysis of the Enzyme Nitrogenase: Insights into Mechanism

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We are using mediated electrocatalysis to probe the mechanism of the enzyme nitrogenase. Nitrogenase catalyzes the multi-electron/proton reduction of dinitrogen (N_2) to ammonia (NH_3), one of the most challenging reactions in biology. We are developing electrochemical methods that allow a unique way to probe the mechanism of this complex enzyme. Progress to date includes success in sequestering purified nitrogenase on an electrode, with demonstration of mediated electrocatalysis of a range of substrates.[1] Further, we have demonstrated that nitrogenase can be poised at defined potentials to access the potential-dependence of substrate and inhibitor binding.[2] Our electrochemical approach greatly simplifies the electron delivery to nitrogenase, removing the complexities that normally come from the involvement of the dinitrogenase reductase protein and ATP utilization. For example, we are using mediated electrocatalysis of the nitrogenase MoFe protein with H and D isotopes to examine steps in the formation of H_2 catalyzed by nitrogenase, revealing for the first time insights into this aspect of the mechanism. These and other electrochemical studies on nitrogenase will be presented.

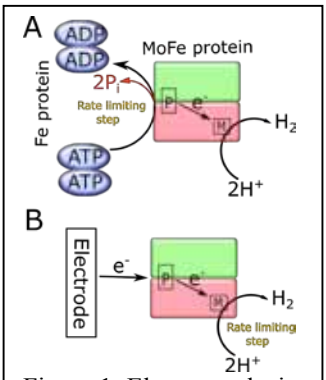

Figure 1: Electrocatalysis of nitrogenase. Nitrogenase catalysis with electron delivery from the Fe protein (panel A) or from mediated electro-chemistry (panel B).

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Photo-electrochemistry of FeFe hydrogenases

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FeFe hydrogenases are metalloenzymes that catalyze the oxidation and production of H_2. The active site of the enzyme, called H-cluster, is constituted by a 4Fe4S cluster bound via a cysteine to a 2Fe site, where each iron atom presents a CO and a CN ligand while an ADT group and a CO bridge the two irons.
The Fe distal to the 4Fe4S cluster is the binding site for the substrate but also for the inhibitors CO and O_2.
The photo-production of hydrogen by hydrogenases *in vivo* (by algae cultures[1]) and *in vitro* (by artificial mimic systems[2]) exploiting solar light, represents an interesting solution for the production of H_2 as an emission free solar-fuel to face the constant increase of energy need and the consequent necessity of "green" energy production approaches.
In this framework, recent papers dealt with the consequences of direct irradiation of FeFe hydrogenases with visible light, showing the photo-dissociation of the inhibitor CO[3] and the presence of light induced damage [4,5].
In this work we focus on these two aspects using photo-electrochemistry, studying the effect of irradiation on the enzyme under turnover conditions and after inhibition by CO, and performing DFT and TDDFT calculations to have a full picture of the photochemical processes.
We show the wavelength-dependence of the photo-dissociation of the inhibitor CO[6] and we describe the process at a QM level for the first time. Furthermore, we determine the mechanism of photo-damage on FeFe hydrogenase of *C. reinhardti*[7].

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Controlled delivery of plasmid DNA into skin

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Skin is an attractive target for gene therapy due to its easy accessibility and its immune properties. To enhance the efficiency and to ensure the safety of cutaneous gene therapy, the improvement of delivery methods is required. Gene electrotransfer is safe and efficient non-viral gene delivery method, widely used to deliver the plasmid DNA into different target tissues, including the skin. The effectiveness of skin gene electrotransfer relies on different parameters, such as the type of the electrodes, amplitude and duration of electric pulses and the design of the plasmid DNA. To provide optimal effectiveness of skin gene electrotransfer and to achieve the maximal therapeutic effectiveness of gene therapy, the main parameters of gene electrotransfer need to be optimized.

In the scope of the study, the main parameters of gene electrotransfer, such as type of the electrodes, plasmid structure and electrical parameters, were firstly characterized and optimized. The delivery of reference and therapeutic genes into skin cells was performed *in vitro* as well as *in vivo* in mouse skin. Gene expression levels, protein production and therapeutic effect was followed to determine the transfection efficiency. The optimization established safe, efficient and controlled method of skin gene electrotransfer. With optimized parameters of gene electrotransfer, the depth of transfection into skin and the level and the duration of gene expression could be controlled, as well as the protein distribution (local or systemic). The feasibility of the optimized method for the purposes of cancer therapy was proven on melanoma and sarcoma mouse tumor models. Peritumoral skin gene electrotransfer with plasmid DNA coding for the interleukin-12 resulted in prolonged growth delay of primary tumors and exerted the systemic effect on distant, untreated metastases.

Well characterized and optimized method of skin gene electrotransfer with anticancer action could facilitate the translation of the method into the clinics. Specifically, gene electrotransfer to skin as a target tissue is a promising approach for many medical applications, such as cancer gene therapy, vaccination and wound healing.

Conventional Biosupercapacitors

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Recently an entire new class of bioelectronics devices, *viz.* self-charging biosupercapacirors, has been disclosed [1]. These hybrid biodevices are able to convert different types of energies into electric energy and simultaneously store an electric power in the same volume used for conversion. Nowadays chemical [2,3] and solar [4,5] biodevices are known.

The fundamental concept of a conventional biosupercapacitor, *i.e.* an externally charged biodevice, was demonstrated in 2012 by Malvankar and co-workers using a half-cell, which was able to store electrical charges in the cytochrome *c* network of a bacterial biofilm [6]. However, neither a complete biodevice was designed nor purified redox proteins were used to store electrical charges in biomodified electrodes. Herein we demonstrate complete functional conventional biosupercapacitors built on redox proteins and enzymes.

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Engineering Oxidative Half Reaction of Redox Enzymes for the Biomedical Applications

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We have been engaged in the molecular engineering and application of varieties of redox enzymes, mainly FAD dependent oxidoreductases, such as glucose oxidases, glucose dehydrogenases, fructosyl-amino acid/peptide oxidases, cholesterol oxidase. These enzymes have been combined with artificial electron mediators to construct "second" generation type enzyme sensors, with the combination of variety of electrode platform, including disposable electrodes.

Recently emerged requirements in the development of accurate and precise sensing systems for the use medical diagnosis, including the need to meet the strict regulations while remaining competitive, have accelerated the demands in the improvement of conventionally utilized enzymes. The improvement of substrate specificity of enzymes or interference of enzymatic reactions should consider the possible existence of exogenically administrated or iatrogenically dosed chemicals. Therefore, the consideration should be taken not only for enzyme substrates, which are usually referred the substrate of reductive half reactions, but also the electron acceptor preferences, which are the second substrate of the redox enzyme reactions for their oxidative half reactions.

In this paper, we present our engineering approaches toward oxidative half reactions of several FAD dependent oxidoreductases, based on 3D structure analyses and predictions, combined with mutagenesis studies. The impact of single amino acid substitution was so effective that could alter preference of their electron acceptor, including oxygen for oxidases and artificial electron acceptors. These observations will provide future insight of the strategic approaches for the development of enzymes ideal for the electrochemical analyses dedicating for medical fields.

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Synthesis and characterization of genistein conjugated with gold nanoparticles for anticancer drug delivery application and therapeutic performance

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Gold nanoparticles (AuNPs) conjugated with drugs are used in diagnostics and therapies. For many years research has been carried out to form efficient drug carriers. Advantages of AuNPs as drug carries include: the use of drugs which are poorly soluble in water, facilitation of transport through cell membranes, targeted delivery of drugs, the possibility of macromolecular drug release, "multimodal" treatment – two or more drugs and addressing or imaging groups, securing faster mechanisms of action and improved efficiency of drugs. Apart from the combinations involving gold nanoparticles conjugated with drug substances through linkers, a direct bonding is also known. We have synthesized a new form of genistein conjugated with gold nanoparticles (AuNPs-GE) that could serve as drug carriers [1]. The MTT formation experiments indicate that genistein conjugated with AuNPs achieved the highest level of cytotoxicity compared with free genistein. AuNPs-GE synthesis is simple, clean and inexpensive. This conjugate was obtained in a one-pot synthesis and the formation of AuNPs-GE was monitored in terms of color change and UV-Vis spectroscopy. In creating AuNPs-GE, genistein acted as both reducer and stabilizer. The efficiency of the purification of the conjugate from free genistein was controlled by capillary electrophoresis. The size of the nanoparticles modified with genistein was obtained by the TEM and DSL techniques, respectively. The identity of genistein on the gold nanoparticles was proved by electrochemistry, NMR and Raman spectroscopy. The results obtained suggest that AuNPs-GE could enhance the anticancer effect of genistein.

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Calcium electroporation is efficient on rhabdomyosarcoma cells with less toxicity in normal muscle cells and in differentiated cells

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Rhabdomyosarcoma (RMS) is a malignant tumor derived from myoblasts that affects children and young adults and impacts also on the surrounding healthy muscle[1]. Increase in intracellular calcium level by electroporation (EP) may be a new approach to treatment numerous types of cancer [2]. Calcium ions influence on cell proliferation as well as cell death by apoptosis or necrosis [3].

We have studied the effect of EP combined with Ca^{2+} on cell survival *in vitro*. Two cell lines were used: C2C12 – mouse myoblast cell line and RD – human rhabdomyosarcoma cell line. Each experiment was performed on undifferentiated and differentiated cell lines. Cytotoxicity of EP (600V/cm-1000V/cm), and [Ca^{2+}] concentrations (0.25mM-5mM) was verified by MTS assay.

The RD viability was decreased by 10-50% and 50-70% after the following therapy (CaEP) parameters: 600V/cm+0.5-5mM and 1000V/cm+0.5-5mM respectively. The same parameters caused only 10-23% and 20-60% viability drop of C2C12. The differentiated cells viability was higher for both cell lines comparing to undifferentiated cells, however the RD cells were still more sensitive to CaEP therapy than C2C12 in the differentiated state.

These results confirmed that CaEP procedure can be an effective anti-cancer therapy for RMS, whilst relatively sparing normal muscle cells. These studies confirmed previous reports that CaEP is preferentially toxic to malignant cells and paves the way for further investigations of the use of CaEP in sarcoma treatment.

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Adaptive Response of Side-Chain Dynamics in Binding Site of Ribonuclease Inhibitor

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Conformational multi-states of polymer chain structure underlie fast and thermally robust modification of RNA metabolism required by the rapid conversion of the chemical population in short term memory of neural system to gene transcription and new protein synthesis for long term memory formation [1] and the understanding of which is essential for the application of the knowledge to neural disease [2, 3]. Ribonuclease inhibitor protein binds a broad range of endoribonucleases having vital roles in RNA metabolism. Structural response time in a binding process from an metastable association state formed by an applied electric field induced dissociation was analyzed for the binding states of the porcine ribonuclease inhibitor and the bovine ribonuclease A. Motion of surface side chains of the ribonuclease inhibitor in the water was analyzed for a full atom model simulated by a molecular dynamics (MD) method at 300 K for the complex of the porcine ribonuclease inhibitor and the bovine ribonuclease A (Protein Data Bank (PDB) code 1dfj). We analyzed the minimum distances between the side chain oxygen of the ribonuclease inhibitor and the counter oxygens of the ribonuclease for the TYR430 and TYR433 of the inhibitor after stepwise change of electric fields up to 10 MV/m application and removal of the field. Association processes of the contacting complex took place with jumps from metastable states to the other metastable states through the different path from the field induced dissociation process. Typical distance between nearest metastable states was 0.4 nm indicating twice of oxygen-oxygen hydrogen bond length. Transitions between nearest metastable states took place 1 ns time range after removal of the field. Major part of the dissociated states showed no transition indicating the waiting times for the transition were longer than the duration after the field removal. Orientation of TYR433 side chain of the ribonuclease inhibitor in dissociated ground states in crystal structure (PDB code 2bnh [4]) is “inward” for the binding association process. The orientation of the side chain is stably fluctuating around the “inward” ground state in the MD simulation [5]. The dissociated state was not complete ground state in this simulation based on the “outward” orientation of TYR433 side chain. The variation of waiting times indicates the distribution of the “excited states” due to the side chain conformation dynamics possible to yield the adaptivity.

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Reactive oxygen species action on cell membranes: Unraveling a potential mechanism of electroporation in the biological context using Molecular Simulations

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In this contribution we harness the capabilities of computational resources and the predictive power of advanced atomistic and quantum level molecular dynamics techniques to decipher key steps in several chemical and biophysical processes occurring during and following Electric field stimulations of cell membranes. We show that under low-voltage conditions, and predict that under nanosecond pulse electroporation conditions, peroxidation of model cell membranes by potent reactive oxygen species (OH• and OOH•) is significantly enhanced. We quantify then the permeability of the peroxidized membranes to a host of species including ions and mall molecules, to demonstrate that electrically mediated chemical effects may play a significant role in several processes experimentally evidenced following exposure of cells to high electric fields.

Proton transfer in outer-membrane flavocytochromes coupled with extracellular electron transport

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The iron-reducing bacterium *Shewanella oneidensis* MR-1 has an ability to transport respiratory electrons generated from cell inside to extracellular solid substrates via electron transfer integral *c*-type cytochrome complex, located at outer membrane (OM *c*-Cyts). This interfacial electron transport between OM *c*-Cyts and solid substrates is termed extracellular electron transport (EET). In recent years, we found that the rate of EET is largely enhanced by self-secreted flavin molecules associated with the formation of semiquinone (Sq) state as a binding redox cofactor in the OM *c*-Cyts[1]. However, the bound flavin Sq has more negative redox potential than the hemes in OM *c*-Cyts, which is energetically unfavorable for the kinetics of EET. Given that the redox reaction of Sq is coupled with the protonation/deprotonation reaction of nitrogen atom at 5-position in isoalloxazine ring (N(5)), proton transfer may limit the rate of EET. Here, in order to examine the effect of altering proton uptake capability of the N(5) on the rate of EET, we replaced flavin cofactor with other six redox active molecules possessing different redox potential and pK_a at the N(5). The current production from *S. oneidensis* MR-1 increased in the presence of the redox molecules with the N(5) to the similar extent with the bound flavin. As we expected, the pK_a at the N(5) in cofactors showed positive correlation with current production, while the redox potential did not. Because higher pK_a represents stronger proton acceptability in N(5), this finding suggests that the protonation reaction at N(5) in flavin is associated with EET and plays an important role to accelerate the rate of EET. In the presentation, we will show the observations for the solvent kinetic isotope effect on EET using partial deletion mutant of OM *c*-Cyts, and discuss about the proton transfer pathway associated with EET via flavin-bound OM *c*-Cyts.

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Electrochemical pH Dependency in Anode-Respiring Biofilms Suggests a Proton-Dependent Electron Transfer Reaction

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Anode-respiring bacteria (ARB) catalyze the complete oxidation of organic compounds (e.g. acetate, glucose) into electrical current and carbon dioxide. ARB naturally produce a biofilm at the electrode surface of up to 100 micrometers, where even cells on the outer part of the biofilm are participating in current production. Inside this biofilm a pH gradient form due to the production of protons from anode respiration. In this study, we first assessed the effects of pH and buffer concentration on current production and growth of biofilms of *Thermincola ferriacetica* – a thermophilic, Gram-positive, anode-respiring bacterium (ARB) – grown on anodes poised at a potential of -0.06 V vs. SHE in microbial electrolysis cells (MECs) at 60°C. *T. ferriacetica* generated current in the pH range of 5.2 to 8.3 with acetate as the electron donor and 50 mM bicarbonate buffer. Maximum current density was reduced by ~80% at pH 5.2 and ~14% at 7.0 compared to pH 8.3. Increasing bicarbonate buffer concentrations from 10 mM to 100 mM resulted in an increase in the current density by 40 ± 6%, from 6.8 ± 1.1 to 11.2 ± 2.7 A m^{-2}, supporting that more buffer alleviated pH depression within *T. ferriacetica* biofilms.

Biofilm anodes of the thermophilic ARB *Thermincola ferriacetica* were also established to determine if its thermodynamic response is proton-dependent. For all conditions, pH was a major contributor to altering energetics of ARB biofilms; resulting in a 48 ± 7 mV/ pH unit shift in their midpoint potential. Under turn-over conditions at current densities > 10 A/m^2 the midpoint potential shift measurement is affected by the pH gradient within the biofilm, resulting in a decreased apparent pH dependency of 32 ± 2 mV/pH unit. This phenomenon supports the presence of a proton-dependent rate-limiting process, which combined with reported pH gradients within the biofilm, can limit ARB growth and electric current generation.

Analyses of the electron transfer pathway of direct electron transfer type iron sulfur flavo cytochrome type glucose dehydrogenase complex ~enzyme for continuous glucose monitoring~

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We have been engaged in the study and application of the bacterial FAD dependent glucose dehydrogenase (FADGDH) complex isolated from *Burkholderia cepacia*. FADGDH complex is composed of three subunits: a catalytic subunit, a hitch hiker protein of catalytic subunit, and a multiheme electron transfer subunit[1-4]. FADGDH complex may represent several bacterial FAD dependent dehydrogenase complexes, which are also composed of three subunits. Thanks to this property, direct electron transfer principle based *in vivo* continuous glucose monitoring (CGM) is being developed using FADGDH complex. Recently, we have reported that catalytic subunit of the FAD dehydrogenase complex harbor an Fe-S cluster, and the Fe-S cluster is involved in the intra- electron transfer from FAD in the catalytic subunit, and also in the inter-electron transfer between catalytic subunit and electron transfer subunit[5]. These enzyme complexes are now categorized as the iron sulfur flavo cytochrome type dehydrogenase complexes. However, the electron transfer pathway within the electron transfer subunit as well as the mechanisms of the electron transfer to the external electron acceptors or electrodes are yet to be elucidated.

The spectroelectrochemical analyses of the electron transfer subunit of FADGDH, together with the mutational analyses of heme ligands suggested the sequence of the electron transfer among the three hemes of multi-heme cytochrome *c*. This information will lead further engineering studies of enzymes for direct electron transfer type CGM.

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Shock-sensors as a tool for online monitoring of Anaerobic Digestion process

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In the Anaerobic Digestion (AD) process, Volatile Fatty Acids (VFAs) have been widely accepted as reliable indicators of the metabolic imbalance caused by sudden changes in the operating parameters or partial inhibition of the microbial population. (Jin, Angelidaki, & Zhang, 2016). In ADs, VFAs concentrations are normally measured by operators with off-line and time consuming techniques. Currently, Bio Electrochemical Systems (BES), are attracting increasing interest as a tool for on-line VFAs monitoring system, based on the principle that electro-active microbial populations can produce current/voltage signals, in proportional extent to bioavailable organics (Chang, Moon, Jang, & Kim, 2005). Here we present a different approach based on the shock-sensor concept: the negative peaks in the current generation correspond to a sudden increase of inhibiting substances in the solution.

The bioreactor consisted in a Microbial Fuel Cell (MFC) with open-air cathode, fed in a batch cycle with four types of waste materials: cheese whey, kitchen waste, citrus pulp and fishery waste. During the acidogenic phases, MFC voltage trends over 100 Ω external load were proportional to the increase of VFA concentrations observed in the AD up to 1 gL^{-1}, for all the tested substrates. When the total VFAs concentration overcome 1 gL^{-1}, the MFC voltage evidenced an inhibition trend typical of a shock-sensor. Polarization curves pointed out that the anodic bacteria were subjected to the inhibition, while biocathode was not affected.

In order to analyse a more controlled system, a three-electrode set up sensor is currently being tested in a lab-scale anaerobic digester fed with digestate. The current production will be evaluated for cumulative additions of a mixture of VFAs with the working electrode polarized at +0,2V vs. Ag/AgCl.

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Anomalous, Low Diffusion Through Micropores: Implications for Mass Transport Control

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Microporous track-etched membranes have discrete, cylindrical holes enabling assessment of porosity and pore area through imaging without the added uncertainty of tortuosity factors. They serve as useful diffusion barriers for biosensors and also as permeable cell culture surfaces for bioreactors where bidirectional nutrient/O_2 transport at polarized cells is required. The assumption that solute mass transport through micron dimension pores is equivalent to that in bulk water, however, has not been tested. This baseline study measured solute diffusion coefficients for four electrochemically active solutes: H_2O_2, pyrocatechol, acetaminophen and ascorbic acid at 12μm thick track-etched membranes of pore diameter 0.4 and 8μm, variously formulated from polycarbonate and polyethylene terephthalate. Hydrodynamic amperometry and cyclic voltammetry were used for diffusion measurements. Both approaches demonstrated a major reduction in pore diffusion coefficients: $3.17x10^{-7}cm^2s^{-1}$ for H_2O_2 to $1.43x10^{-10}cm^2s^{-1}$ for ascorbate, representing orders of magnitude reduction ($x10^2$ - 10^4 *vs* bulk water). Diffusion retardation at polycarbonate was also greater than at polyethylene terephthalate ($x10^2$). This suggests a material surface effect on pore diffusion, but one that cannot be explained by an established nanometer dimension double layer model. The radical possibility remains that the water structure itself was different [1] within the pores. If so, this would have implications beyond sensor membranes. Further studies of these membranes coated with various crosslinked biolayers of Type I collagen, collagen-fibronectin, collagen-heparin, collagen-chondroitin sulphate and Type IV collagen as tissue matrix analogues showed predictable moderate, increases in diffusional resistance, with the greatest effect seen for ascorbate at the anionic matricies. With natural connective tissue, tendon and cartilage, however, diffusive resistance was considerably greater, but predictable. Diffusion coefficients here of $1.09x10^{-8}cm^2s^{-1}$-$8.33x10^{-6}cm^2s^{-1}$ could be rationalized as reflecting either close packed collagen (tendon) or cell loading (cartilage), a significant challenge for cell viability in tissue engineering.

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Nanotech-Based Biosensors and Bioanalytical Systems

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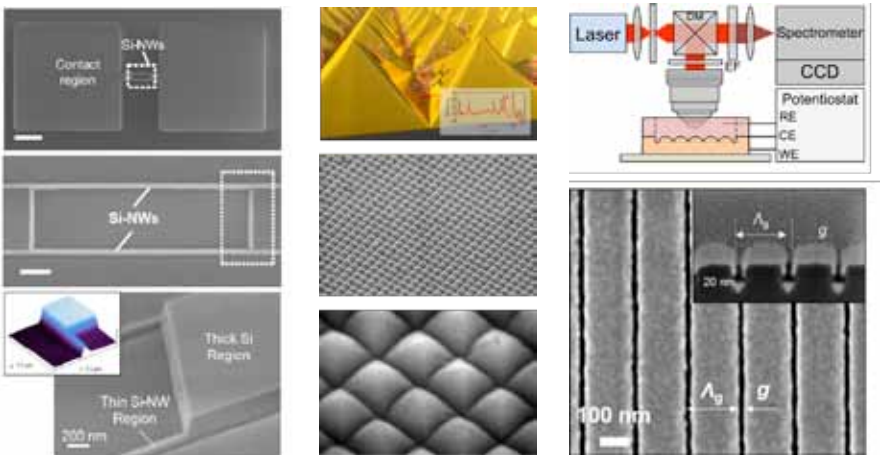
We present nanostructures for DNA and biomolecular biosensing. Silicon nanowires are fabricated by a combination of anisotropic etching techniques to realize <10 nm, triangular <111> shaped sensors for detection of hyper-methylated DNA as early detector for cancer. As an alternative we realized nanosized gold covered pyramids allowing for SERS measurements and biomolecular identification providing a high average enhancement factor (AEF) An even more homogeneous high AEF was obtained by creation of very narrow nanoslits between gold strips. The individually addressable interdigitated strip are used to perform electrochemical oxidation of biomolecules and simultaneous SERS detection. Finally we will present a sandwich assay using darkfield imaging of DNA coated nanoparticles for ultrasensitive DNA detection.



Silicon nanowires (left), gold nanopyramids (middle) and nano-interdigitated electrodes for electrochemical SERS (right)

Beyond the "Standard Model" (of Electroporation) — Cells Fight Back!

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The "standard model" of electroporation describes transport through pores formed when the transmembrane voltage exceeds a critical value. The model has evolved from the transient, stochastic pore model proposed over 30 years ago, but despite many improvements it does not predict key features of electropermeabilized cells, including: pore lifetimes; dynamics of membrane potential, electrical conductivity, and permeability to small molecules; multiple-pulse protocol outcomes; effects of cell size and medium conductivity.

To be predictive beyond the initiation of permeabilization, electroporation models must integrate the immediate physics of electropore formation with the subsequent physical, chemical, and biological responses of cells to the stress of membrane disruption. Long-lived permeabilizing structures (electropermeasomes) must be identified from biomolecular models and from experiments. Transport-related stress and damage responses must be represented: restoration of ion concentration gradients (Ca^{2+}, K^+, Na^+), osmotic and volume regulation, membrane repair, and metabolic adjustments, including recovery of ATP. Contributions from electromodulated membrane proteins must be incorporated. These structures and processes comprise the regime of the new model.

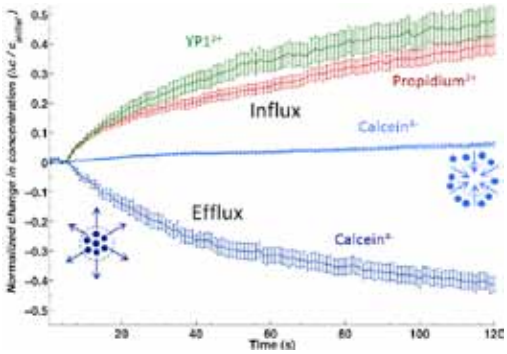

Figure 1. Quantitative electroporative influx and efflux of fluorescent indicator dyes.

We present data exemplifying two key limitations of models in use today (Fig. 1), and we outline experimental and analytical strategies for continuing the extension and expansion of the "standard model" to a biological system model — the electropermeome.

Adhesive Polydopamine-based Electrodes for Laccase and Magnetite Nanoparticles Immobilization

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Inspired by the adhesive properties of mussel foot proteins, polydopamine (PDA) spontaneous coatings have emerged as a simple surface modification approach able to cover virtually any organic or inorganic material [1]. PDA is rich in catechol, amines and imines, and its excellent biocompatibility has stimulated its application in different areas, e.g. biosensors. PDA quinone groups display latent reactivity towards primary amines allowing covalent binding of target biomolecules, such as Laccase (Lac), a well-known phenol oxidase, through a Schiff base formation or Michael type addition.

The aim of this work is to optimize the catalytic activity of immobilized industrial Lac on PDA films, chemically and electrochemically grown at carbon surfaces. Magnetite nanoparticles (Fe_3O_4-NPs, *ca.* 40 nm) were also co-immobilised with Lac envisaging the enhancement of the amperometric signal [2]. PDA films with different thicknesses were grown and their electrochemical properties evaluated. Ellipsometric and AFM measurements allowed to correlate the film optical thickness and morphology, respectively, with the deposition time or growth charge. Chemically or electrochemically synthesized PDA films reveal similar properties, including hydrophilicity, however, electrochemical growth offers a better control over PDA oxidation state. A simple and effective co-immobilization of Lac, PDA and Fe_3O_4-NPs results in high sensitive responses towards the detection of diammonium 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate) (ABTS), displaying a great potential to be used in portable phenolic biosensors.

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Fe(II)-oxidizing super-complex from extreme acidic environment reconstruction on electrode

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Acidithiobacillus ferrooxidans, a chemolithoautotrophic Gram-negative bacterium, thrives at extremely low pH (pH 1.6-2). It uses the energy from the oxidation of metals or sulfur compounds for growth. Despite its important role in bioremediation and bioleaching, thorough exploration of its physiology is limited, and information at the molecular level is even less. A vertical Fe(II)-oxidizing respiratory chain which spans the outer and inner membranes of the bacterium has been identified. The properties and physiological functions of each protein involved in this pathway and the biochemical requirements for this chain to operate at pHs as low as pH 2 need yet to be fully understood. For that goal, three potentially interacting metalloproteins have been purified, i.e. a diheme cytochrome (Cyt c_4), a copper protein (AcoP) of unknown function, and the soluble part of a cytochrome c oxidase (CoxB). They were immobilized on different electrode surfaces, able to mimic the physiological environment and/or the partner. The redox potentials were determined as 131 mV&262 mV, 340 mV and 120 mV for Cyt c_4, AcoP and CoxB respectively, underlining the high potential value of this redox chain. By varying the solution pH and ionic strength, molecular basis for electron transfer between each protein and either graphite or SAM-modified gold electrodes were defined. The electrochemical data were validated by examination of both the crystal structure and the protein dipole moment as a function of pH. They especially highlight the physicochemical and biochemical determinants for the proteins to transfer electrons in high acidic environment. Protein-protein interaction was further studied by Bio-layer Interferometry which allowed to determine binding constants. Protein-protein interactions were also studied by electrochemical tools, according to a procedure where part of the physiological chain is reconstituted at the electrode surface. Notably, significant redox potential shifts were observed in case of interacting proteins. From these results a scheme of how the proteins interact between them *in vitro* is proposed, which can help to the elucidation of *in vivo* mechanism of electron transfer from the external membrane to the final electron acceptor situated in the inner membrane.

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An energy-harvesting device based on supercapacitive enzyme-modified nanoporous gold electrodes: an autonomous pulse generator

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Integration of biological fuel cells (BFCs) with electrochemical capacitors (known as supercapacitors), can be used to take advantage of the features of high specific power density combined with high levels of durability[1-3]. These hybrid devices can generate cyclic, scaled up power pulses from the discharge of the supercapacitor, which is then recharged via the BFC in open-circuit mode. We describe a supercapacitor/biofuel cell hybrid device obtained by the facile, one-step electrodeposition of PEDOT/Os polymer/enzyme onto dealloyed nanoporous gold electrodes[4]. The device can generate a pulsed current density of 2 mA cm^{-2}, with an instant maximum power density of 608.8 μW cm^{-2}, 468 times higher than that of the BFC alone. The modification layer shows reasonable stability without visible leakage of the redox mediators over 50 cycles of operation at 0.2 mA cm^{-2} for approximately 7 hours. A proof-of-concept pulse generator, simulating the requirements of a pacemaker (0.02 Hz pulse at 10 μA for 0.5 ms), has been successfully demonstrated using a series of connected cells. Replacing the enzymatic cathode with an abiotic MnO_2 electrode yields equivalent power and current densities, resulting in an oxygen-independent biobattery/supercapacitor hybrid device.

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Electrochemiluminescence Ratiometry for Bioanalysis

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Electrochemiluminescence (ECL) is a luminescence excitation process triggered electrochemically. Combining the advantages of zero optical background and easy reaction control by applying electrode potential, ECL has been proved to be a highly sensitive and enhanced selective method attracted much attention in the areas of biological, environmental and food analysis. Through monitoring the change in ECL intensity, species that directly take part in ECL reaction or indirectly influence the reaction could be quantified. However, other factors such as environmental conditions can interfere with the signal output, especially during trace analysis, which may cause false positive or negative errors.Ratiometric detection is an ideal strategy to limit the interference factors via normalizing environmental variation by self-calibration, which has been widely developed in fluorescence. Inspired by the dual-wavelength ratiometry, one can create ECL ratiometry to make the detection more convincing. The major challenge to carrying out ratiometric ECL measurement is to create ECL report units with two emitting states that have the potential-dependent properties or wavelength-dependent properties upon the substrate concentration. Here, I will report some examples based on ECL ratiometry.

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Liquid-junction-free substitutional stripping voltammetry based on a closed bipolar electrode system and its application to endotoxin sensor

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A liquid-junction-free substitutional stripping voltammetry (SSV) was developed and applied to endotoxin sensing. SSV is a type of stripping analysis that changes the redox current to metal deposition (Fig. 1A)[1]. The amount of the deposited metal is detected by anodic stripping voltammetry after the deposition process. Generally, SSV requires a liquid junction to maintain electrical conductivity between separated cells. This is a critical problem to fabricate a chip-type sensor integrated a SSV system. In this study, a couple of driving electrodes connected to a potentiostat were used to provide the electrical conductivity for the two cells (Fig. 1B). This system is structurally same as closed bipolar system (cBPES). We demonstrated quantification of *p*-aminophenol (pAP) using this liquid-junction-free SSV[2]. A linear relationship between pAP concentration and SSV electrical charge was successfully obtained. Then we applied this method to endotoxin sensing. We used Boc-Leu-Gly-Arg-pAP as a substrate for *Limulus* regent[1,3]. We successfully detected 10 EU/L endotoxin with a chip-type electrode device integrated a liquid-junction-free SSV system.

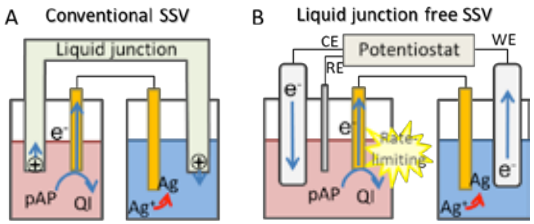

Fig. 1. Schematic illustration showing (A) conventional system and (B)liquid-junction-free system for deposition process of SSV.

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Electrochemical Properties of Glucoside 3-Dehydrogenase, an Enzyme for 1,5-Anhydro-$_D$-Glucitol Sensing

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Glucose 3-dehydrotenase (G3DH) catalyzes the oxidation of the third hydroxy group of pyranose. Our group reported isolation, characterization and their application of bacterial G3DHs[1, 2]. The G3DH can be applied for the monitoring of 1,5-anhydro-$_D$-glucocitol (1,5AG) measurement. 1,5AG is a serum polyol, and has been utilized clinically to monitor short-term glycemic control. Since 1,5AG is the 1-deoxy form of glucose, it is not the substrate of common glucose-1-oxidoreducatases. Recent genomic information provided several putative G3DH structural genes. We cloned and recombinantly prepared one of those candidates, FAD-dependent G3DH gene from *Rhizobium radiobacter*. The G3DH operon composes of three ORFs, a catalytic subunit (α subunit), a small subunit (γ subunit) and a protein predicted to encode cytochrome c. The α subunit contains conserved FAD-binding motif and conserved cysteine-rich region in common with FAD-dependent glucose dehydrogenase that has [3Fe-4S] clusters in catalytic center [3].

In this study, we attempt to clarify the electrochemical properties of *Rhizobium radiobacter* G3DH. By co-expression of α and γ subunits in *E.coli* BL21 (DE3), G3DH γα complex was prepared. G3DH γα complex showed dye mediated G3DH activity. Electron paramagnetic resonance (EPR) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis revealed that G3DH catalytic center possesses an iron-sulfur cluster. These results suggested that cysteine-rich region of *Rhizobium radiobacter* G3DH constitutes an iron-sulfur cluster that may play an important role in the electron transfer.

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A Microfluidic Approach for Determining the Combined Effects of Nutrient Concentration and Hydrodynamic Conditions on Electrical Output of *Geobacter Sulfurreducens* Biofilms

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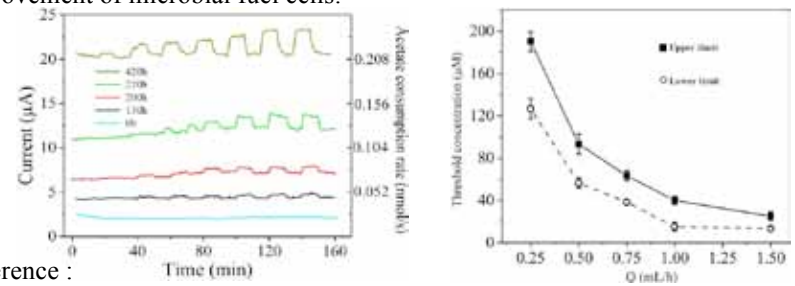
During the last 10 years research in the field of microbial electrochemistry and electroactive bacteria many achievements have been accomplished. To date, however, a deep study on hydrodynamic effects on electricity production is lacking.

Here we present a three-electrode microfluidic device and methodology for obtaining *in situ* electrochemical measurements under different hydrodynamic and acetate concentrations. [1] *Geobacter sulfurreducens* was used as electroactive bacteria, which was grown in an anaerobic environment on a graphite electrode with electrochemical potential controlled technique. Flow rate was modulated in the range of 0.2 to 4 $mL \cdot h^{-1}$ (6.9×10^{-3} to 0.14 $cm \cdot s^{-1}$ velocity) during different growth times (0 to 420 h) of the *Geobacter* biofilm. Increases to flow rate showed an increase to electrical current from the electrode adhered biofilm. This was conducted on mature biofilm at several different acetate concentrations and the results were the same. The net current increase became higher with biofilm age but, surprisingly, the percentage increase over the background current remained constant at all times. At extremely low nutrient concentrations, we succeed in finding threshold conditions between respiratory and non- respiratory behavior. We will present this data along with explanations related to kinetics of bacterial respiration. Finally, we will discuss how these finding can lead to new avenues for improvement of microbial fuel cells.



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Lipidic Cubic Phase-Modified Electrochemical Sensor for ATP-hydrolyzing Enzyme Activity Monitoring

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More than one third of the human proteome is attributed to membrane proteins (MPs). Their presence is highly significant in cell events such as the transport of molecules and signal transduction. Therefore, new methods are under development to enable better characterization of MPs. Lipidic cubic phase (LCP) is one of the most suitable membrane-mimetic matrices for the stabilization and crystallization of MPs. However, only few MPs have been studied using LCP. The goal of our work was the incorporation of Na^+/K^+-ATPase transmembrane protein into monoolein-based LCP with subsequent verification of the protein activity.
To identify the structure of LCP, small-angle X-ray scattering (SAXS) measurement was performed. SAXS data for LCP without and with incorporated protein exhibited exactly the same Pn3m symmetry. Thus, Na^+/K^+-ATPase incorporated in the cubic phase does not affect the structure of the lipid phases.
The Na^+/K^+-ATPase activity in LCP was studied spectroscopically [1] and using electrochemical methods and LCP-modified glassy carbon electrode. The enzyme activity evaluation is based on the ability of Na^+/K^+-ATPase to hydrolyze ATP to form ADP and free phosphate, the latter reacting with ammonium molybdate under acidic conditions. The detection was performed in the presence of ascorbic acid, where the voltammetric response at around +0.2 V *vs.* Ag/AgCl/3M KCl was monitored in the presence or absence of ouabain, a Na^+/K^+-ATPase specific inhibitor. In addition to ATP-hydrolyzing activity, the charge and current passing through the LCP film was monitored in the absence and presence of ATP using chronocoulometry and chronoamperometry.

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Charge Recombination in Biophotovoltaics based on Reaction Centers in Redox hydrogels and on Ubiquinone as Charge Carrier

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Bacterial reaction centers (RCs) are photosynthetic proteins which convert solar energy into chemical energy by absorbing infrared light and reducing quinones to quinols. Because of the high quantum efficiency of these processes, RCs have been of great interest for bioelectronics, biosensors and biophotovoltaics[1]. However, the performance of the device is limited by the charge recombination between the charge carriers and the photoelectrode, which is a process that decreases both the photocurrent and the open circuit voltage (OCV). Here we demonstrate that the reoxidation of the reduced quinone at the photoelectrode surface contributes to charge recombination and this process can be limited by modifying the Au surface with a blocking layer. We exploit Os complex based redox hydrogels to immobilize and electrically contact the RCs to the gold electrode and ubiquinone is used as electron acceptor for the RCs. 11-mercaptoundecanoic acid (MUA) is particularly well-suited as blocking layers because the reoxidation of the reduced quinone on the MUA modified Au is significantly impeded, while the electron transfer between the Os complex and the MUA modified Au is mostly unaffected. As a result, the blocking layer significantly increases the OCV by 150 mV. This approach is even successful for photoelectrodes that have a large driving force for charge recombination as demonstrated by the photocurrent obtained from electrodes modified with a combination of Os complex modified redox hydrogels and RCs mutants which have more positive redox potentials. This concept opens up the possibility to build photovoltaic cells free of semi-conductor materials able to compete with their semi-conductor based counterparts in terms of OCV.

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Photosystem 1-based light-induced H_2 evolution and in-situ detection by a hydrogenase-based microbiosensor

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Most of the energy we consume today comes from fossil fuels, which rely on photosynthesis-driven biomass accumulation for millions of years. With an increased understanding of the structures and processes accomplished by the photosynthetic protein complexes, there is a hope to harness solar energy by tuning the properties of the natural light harvesting units thus shortening the time required for creating fuels such as H_2 [1].

Photosystem 1 (PS1) is one of the key protein complexes that drive the photosynthetic process. Its abundance, robustness and nearly perfect quantum yield make PS1 particularly suitable for energy conversion purposes [2]. In our work, isolated PS1 is integrated within an Os complex-based redox polymer and the conversion of visible light into electricity is realized [3]. Furthermore, the low negative potential of the light-induced F_B^- cofactor in PS1 is used to reduce protons into H_2, using a suitable catalyst tethered in close proximity to the F_B site [4].

On demand of the local detection of evolved H_2, a novel enzymatic micro-biosensor is demonstrated. The miniaturized biosensor based on a hydrogenase embedded within a viologen-based redox polymer allows the detection of H_2 with high sensitivity. Implemented in a SECM set-up, this versatile biosensor shows its ability to monitor local electrocatalytic activity of a diversity of (photo)catalysts for hydrogen evolution.

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Abstracts of Poster Presentations

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Photoelectrochemical response of nanostructure diamond in saline solution and its application for neuronal stimulation

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Owning to its high charge injection capability, as well as a number of other key properties, nitrogen-doped ultrananocrystalline diamond (N-UNCD) has emerged as a candidate for electrically driven neuronal stimulation electrode. This work investigates the photoelectrochemical activity of N-UNCD in response to sub-bandgap illumination, to assess its potential for use as an optically-driven stimulation electrode. Whilst N-UNCD in the as-grown state exhibits a weak photoresponse, the oxygen plasma treated film exhibits two orders of magnitude enhancement in its sub-bandgap open circuit photovoltage response. The enhancement is attributed to the formation of a dense network of oxygen-terminated diamond nanocrystals at the N-UNCD surface. Electrically connected to the N-UNCD bulk via sub-surface graphitic grain boundaries, these diamond nanocrystals introduce a semiconducting barrier between the sub-surface graphitic semimetal and the electrolyte solution, leading to a photovoltage under irradiation with wavelengths of $\lambda = 450$ nm and shorter. Within the safe optical exposure limit of 2 mW mm^{-2}, charge injection capacity of 0.01 mC cm^{-2} is achieved using a 15×15 μm electrode, meeting the requirements for extracellular and intercellular stimulation. The nanoscale nature of processes presented here along with the diamond's biocompatibility and biostability open an avenue for the use of oxygen treated N-UNCD as optically driven stimulating electrodes.

One electrolyte based glucose/oxygen electric power biodevice with an operating voltage exceeding 1.24 volt

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Rapid technological developments in recent years have opened up many possibilities to construct personal electronic/electromechanical devices, including those for biomedical applications. However, such devices need to be powered by efficient electric power sources [1] that, in the case of implanted biomedical devices [2], also need to be long-lasting, safe, stable, and biocompatible. While fuel cells have the potential to address this problem, and have been researched for several decades, one of their main drawbacks is a low operating voltage, which is thermodynamically limited to 1.24 V in the case of glucose/oxygen devices [3]. Here we report a proof-of-principle demonstration of a high-voltage biodevice that is capable of generating sustained electric power at voltage, which exceeds the thermodynamic limit. Due to the interplays between faradaic and non-faradaic electrochemical processes, as well as between ionic and electronic conductivities, the biodevice, consisting of several self-charging biosupercapacitors connected in series and operating in the same electrolyte in a pulse mode, provides apparently unreachable open-circuit and operating voltages. Owning to redox enzymes, biocatalysts par excellence, individual biosupercapacitors are self-charged, and after that, the complete biodevice is externally discharged. Our results demonstrate proof-of-principle operation of a high-voltage biological power source working in an authentic human blood stream, which we anticipate to be a starting point for the design of high performance implanted power sources.

This work was financially supported by the Swedish Research Council (project 2013-6006).

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Micro(bio)sensing tools for nitrite detection in the brain

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Nowadays, the role of nitrite (NO_2^-) and its precursor, nitrate (NO_3^-), in human physiology assumes a great relevance, placing them at the forefront of nitrous oxide (NO) biology. The so-called nitrate-nitrite-nitric oxide pathway might represent an important alternative source of NO, especially under conditions of neuronal hypoxia and ischemia.[1] This work aims at assessing whether nitrite is a brain mediator of microcirculation via its reduction to NO upon localized glutamatergic stimulation. When associated with fast electrochemical techniques, microelectrode-based sensors allow studying the role of neurotransmitters and neuromodulators in brain function and the pathophysiology of central nervous disorders. Therefore, we proposed the development of novel (bio)sensing tools for nitrite measurement in Wistar rat brain extracellular space using real-time fast-scan cycling voltammetry (FSCV). Particularly, homemade carbon fiber microelectrodes (CFM) were used as working electrode platforms to perform FSCV nitrite sensing, while a platinum microelectrode array (MEA)[2] modified with cytochrome *c* nitrite reductase[3], glutaraldehyde and multi-walled carbon nanotubes was used as the biosensing tool for nitrite detection. The CFM sensor was able to detect reproducible transient signals *in vivo* upon pressure injection of exogenous nitrite, with a peak concentration and rise time of 57 µM and 27 s for 38 nL, and 129 µM and 24 s for 125 nL of injected solution. The analytical performance of the MEA nitrite biosensor was analyzed by amperometry *in vitro*, over the course of ten days, and showed an average linear range of 20–400 µM, while the sensitivity decreased from 29 ± 9 nA/µM to 11 ± 4 nA/µM.

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Needle type graphene-based field effect transistor sensors

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Field effect transistors (FETs) are very sensitive tools for the detection of surface potential modulations showing high sensitivity for the detection of a large number of different analytes [1]. Recently, we show the fabrication of needle type FET using double barrel carbon nanoelectrodes (dbCNEs) as source (S) and drain (D) electrodes and polypyrrole as channel between S and D. This type of sensors were used to detect local pH changes or the release of ATP at single cells [2]. Graphene and graphene-related materials are presently suggested as the materials of the future. They show switchable charge-carrier mobility being considered an ideal material for FET fabrication.

Graphene-based needle type FET sensors were fabricated using dbCNEs. Reduced graphene oxide (rGO) was deposited as channel between the two carbon nano-electrodes by electrodeposition and simultaneous reduction of the graphene oxide on the dbCNEs. The functionality of this type of devices for the detection of different analytes, such as protons, dopamine or glutamate will be shown.

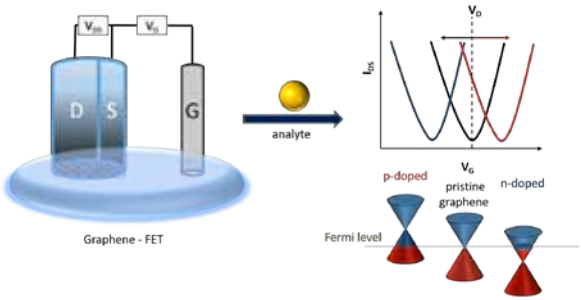

Figure 1: Needle type graphene-FET architecture used for the detection of different analytes

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Process for simultaneous immobilization of two enzymes inside PMMA microchannels

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Simultaneous immobilization of two different enzymes inside poly(methyl methacrylate) (PMMA) microchannels was made for the first time. A simple and rapid method, involving only two steps to immobilize both enzymes in less than one hour is presented. *Glucose oxidase* (GOx) and *horseradish peroxidase* (HRP) were select as model for this purpose. The resulting immobilization was followed by photometry, based on the classical Trinder reaction, developed for quantification of glucose in human serum. To determine the best immobilization condition, a multiparametric study was performed, exploring a 3^3 Box–Behnken design. The optimization studies established that the best injection volume was 20.0 μL and the optimum flow rate was 60.0 μL min^{-1} for the dimensions of the microreactor utilized. Linear responses in the range of 20.0 μmol L^{-1} to 1.00 mmol L^{-1} glucose was obtained using these conditions. The detection and quantification limits were evaluated as 0.27 μmol L^{-1} and 0.88 μmol L^{-1}, respectively. The stability of a so prepared micro-reactor decreased slowly with its utilization. A human serum sample was analyzed by the proposed methodology and the results compared with the classical (cuvette) photometric analysis. The experience acquired in our previous studies [1-3] was of great importance for present challenge. The immobilization process was performed under flowing conditions and all the process was reduced to only two steps. For each reactor sealed in the lab, polyethyleneimine solution (5% w/w in DMSO) was passed through its main channel for 20 min at 15.0 μL min^{-1} flow rate. In sequence, to immobilize the enzymes, a 5 mL final volume mixture of the two enzymes and glutaraldehyde, in 0.10 mol L^{-1} phosphate buffer (pH 7.0), was circulated for 30 min through the reactor. The enzymes amount, so as the glutaraldehyde concentration were optimized through a 3^3 Box-Behnken planning design. A series of 15 reactors modified with different amounts of enzymes were prepared to evaluate the effect of one enzyme on the other. The best combination was extensively tested and after 1000 injections, the signal decreased to 36% of the original signal.

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Development of an Electrochemical Immunosensor from Poly (3,4-ethylenedioxythiophene) / Polydopamine

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The detection of biological and chemical pathogens, contaminants and other important analytes plays a crucial role in the prevention of disease spread, infections and pathologies. Immunosensors are biosensors based on the antigen-antibody interaction, which is responsible for eventually generating the analytical signal [1]. In principle, high specificity and low limit of detection are thanks to the extreme affinity that antibodies have for their antigen.

In this works, a glassy carbon electrode (GCE) modified with a hybrid film of poly(3,4-ethylenedioxythiophene)/polydopamine (PEDOT/PDA) was obtained [2]. . This GCE / PEDOT / PDA electrode will present functional groups which will provide a platform with quinone/catechol groups capable of immobilizing an IgG antibody by the Michael reaction [3]. The obtained GCE/PEDOT/PDA is incubated with human IgG antibodies and then interacted with human IgG antigens. These electrodes should be interference-free with respect to sample, cheap, useful for fast detection in aqueous solution and thus usable in various applications of interest.

Electrochemical techniques such as cyclic voltammetry and electrochemical impedance spectroscopy are performed versus of red-ox couple (cationic and anionic) probes and the presence of the antigen is detected through binding to its antibody.

Finally, the GCE/PEDOT/PDA electrode could be used in the determination of human IgG antigen by performing experiments at different concentrations of antigen.

Acknowledgements
This research was financially supported by Fondecyt (Chile) 1150228.

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Hybrid system: Ag nanoparticles/carbonic anhydrase for CO_2 electroreduction.

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Solar fuels production from CO_2 is a modern time challenge; electrochemical reduction of CO_2 rises as an option for this purpose. However, selectivity of CO_2 catalysis still needs to be optimize, formic acid (FA) synthesis from CO_2 is an attractive alternative due the multiple uses of FA no just as a energy storage molecule also as carbon source for biotechnological process [1,2]. In order to obtain FA at low potentials, nobel metal particles like Ag nanoparticles (Ag NP) could be used for this purpose yet an interfacial supply of HCO_3^- must be kept [3]. The pH control of this reaction is fundamental for that carbonic anhydrase is a fast stable enzyme which can be conjugated to Ag NP maintaining is catalytic properties. In the present work silver nanoparticles (Ag NP) conjugated with carbonic anhydrase (Ag NP/CA) triggers the production of FA from CO_2 by keeping interfacial HCO_3^- available in this way Ag NP turn it into FA at low cathodic potentials (Fig. 1). The electrocatalytic activity of Ag NP and Ag NP/CA hybrids were studied by long term electrolysis and Scanning Electrochemical Microscocopy allowed the *in situ* detection of FA, also differences depending of the nanoparticles size were analyzed. The proposed hybrid catalysis is a brand new option for CO_2 fixation and energy vector production.

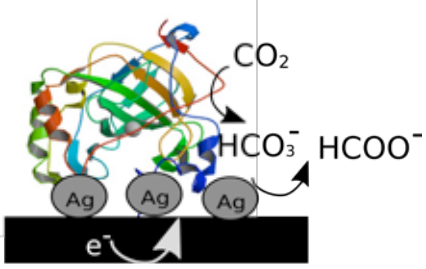

Figure 1. Ag NP/AC hybrid catalyzes CO_2 reduction into $HCOO^-$.

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The [FeFe] hydrogenase Hnd from *Desulfovibrio fructosovorans*

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The versatility of the *Desulfovibrio* H_2 metabolism is due to a complex hydrogenase system consisting of several different enzymes located in different cellular compartments. In *Desulfovibrio fructosovorans*, our model organism, these enzymes are cytoplasmic, periplasmic and membrane-bound [FeFe] and [NiFe] hydrogenases. This diversity makes the role of these various hydrogenases difficult to determine. [1-3]
This study focuses on the tetrameric hydrogenase Hnd. Sequence alignments of Hnd with other hydrogenases reveal strong similarity with multimeric [FeFe] hydrogenases that can perform electron bifurcation [4]. In order to characterise Hnd at the molecular level, we have cloned the *hnd* operon encoding the four subunits of the complex for homologous production of a Strep-tagged recombinant hydrogenase. This enzyme was purified by affinity chromatography. We present here the first overexpression of this type of hydrogenase and preliminary results of the biochemical and physico-chemical characterisation, included electrochemical studied of this complex enzyme.

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Nickel Nanoparticles as a New Tool in the Sensitive Sensing of Biomolecules of Interest

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The importance of nanostructured materials in obtaining new (bio)sensing devices with superior analytical properties has been demonstrated and it is still investigated [1]. Among the nanostructured materials, diverse metal nanoparticles are synthesized and find application in solar and oxide fuel batteries for energy storage, and can be incorporated in diverse ways on electrode surfaces to improve their sensing ability [2]. In this work nickel nanoparticles (NiNP) were chemically synthesized in the presence of polymer stabilizers and characterized by several surface techniques, i.e. scanning and transmission electron microscopy, X-ray photoelectron spectroscopy.

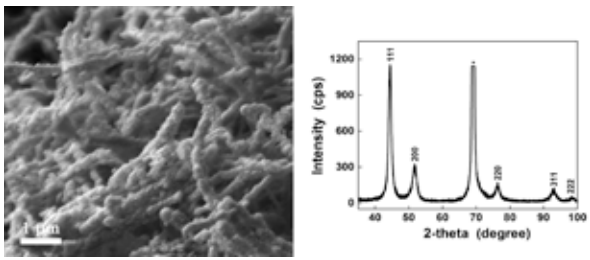

Fig.1. SEM and XRD characterization of NiNP

The modification of electrodes by the NiNP was done by using different immobilization techniques, and the electrochemical response of the NiNP modified electrodes compared with the ones of Ni film electrodes, the latter being obtained by electrochemical Ni deposition. Cyclic voltammetric and electrochemical impedance spectroscopy profiles of both NiNP modified electrodes and Ni film electrodes were investigated in different electrolyte solutions.

After the optimization of the NiNP modified electrode construction, the electrodes were applied for the detection of key analytes, and the catalytic properties were investigated. The NiNP modified electrodes were also used as substrates for enzyme immobilization, for the development of new enzyme electrochemical biosensor.

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Rhenium Complexes Based on 2-Pyridyl-1,2,3-Triazole Ligands: a New Class of CO_2 Reduction Catalysts

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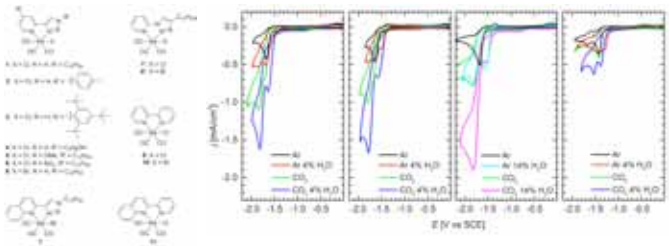
A series of [Re(N^N)$(CO)_3$(X)] (N^N = diimine and X = halide) complexes based on 4-(2-pyridyl)-1,2,3-triazole (pyta) and 1-(2-pyridyl)-1,2,3-triazole (tapy) diimine ligands have been prepared and electrochemically characterized. The first ligand-based reduction process is shown to be highly sensitive to the nature of the isomer as well as to the substituents on the pyridyl ring, with the peak potential changing by up to 700 mV. The abilities of this class of complexes to catalyze the electroreduction of CO_2 were assessed for the first time. It is found that only Re-pyta complexes that have a first reduction wave with a peak potential at around -1.7 V vs SCE are active, producing CO as the major product, together with small amounts of H_2 and formic acid. The catalytic wave that is observed in the CVs is enhanced by the addition of water or trifluoroethanol as a proton source. Long-term controlled potential electrolysis experiments gave total Faradaic yield close to 100%. In particular functionalization of the triazolyl ring with a 2,4,6-tri-tert-butylphenyl group provided the catalyst with a remarkable stability.



(Right) : Rh complexes investigated in this study. (left) : cyclic voltammograms of 1 mM of compounds 1, 2, 3, and 1' (left to right) in MeCN with 0.1 M Bu_4NPF_6 under argon or CO_2 and with or without water

We acknowledge PSL Research University ("PSL-Chimie" Project CATACARB) for funding.

Biophysical Characterisation of the CymA Quinol-Oxidase from *Shewanella oneidensis*.

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The metal reducing bacteria *Shewanella oneidensis* is a facultative anaerobe able to use a variety of terminal electron acceptors (TEAs). These include soluble substrates such as molecular oxygen, nitrate, nitrite and fumarate and insoluble metal oxides. Electrons produced from catabolism of carbon rich nutrients enter the periplasm via quinols in the periplasmic membrane. These electrons are shuttled to cytochrome rich terminal electron acceptor reductases and electron transfer proteins in the periplasm. The process of electron transport is not well understood but a key component of the process is the periplasmic membrane associated quinone oxidoreductase CymA. This protein is a member of the NapC family of proteins which are attached to the membrane via an N-terminal α-helix and contain four *c*-type haem cofactors. Members of the family are thought to exist as homodimers and some are also attached directly to a specific TEA reductase partner protein. CymA does not have a specific binding partner and appears to be promiscuous. Indeed, CymA has been shown to interact transiently with at least two different periplasmic multihaem cytochromes; small tetrahaem cytochrome and fumarate reductase.

Previous work has characterised CymA electrochemically. The electrode potential of each of the *c*-type haem cofactors was measured, menaquinone-7 was proposed as a co-factor of the protein and, like other members of the NapC family, CymA appears to be a dimer.

This work expands upon previous studies using an N-terminal his-tagged CymA construct that can be purified in two quick steps. The oligomeric state and kinetics of quinol oxidation have been investigated for wild-type CymA and single-site variants.

Optimization of Mediated Electron Transfer of Glucose Oxidase *via* the Study of New Redox Probes

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Exploiting the ability of enzyme to convert chemical energy to electrical energy is emerging since the last 2 decades. Enzymatic biofuel cell H_2/O_2 or glucose/O_2 have been developed and allow power density of tens of $mW.cm^{-2}$.[1] For biofuel cell glucose/O_2, at the anode it is necessary to employ a redox mediator to transfer electron from the enzyme to the electrode (mediated electron transfer). In comparison to direct electron transfer (without a redox probe), the use of a redox molecule decreases the open circuit voltage in the case of biofuel cell devices. One of the most common redox mediators for glucose oxidase is the 1-4 naphthoquinone with a redox potential of -0.150 *vs.* Ag/AgCl corresponding to a potential difference of 310 mV between the redox moiety and the active site of enzyme (glucose oxidase or FAD-GDH).

We screened a large variety of molecules with a redox potential lower than the 1-4 naphthoquinone to increase the open circuit voltage of the biofuel cell and maintaining a good electron transfer rate. Due to a lowest redox potential, some anthraquinone derivatives appear to have promising redox potentials for mediated electron transfer with FAD dependent enzymes. In this context, different anthraquinones have been studied with different functional group modulating the redox potential varying from -0.670 to -0.350 V *vs.* Ag/AgCl. These different targets have been immobilized on surface modified with carbon nanotubes. Then FAD-GDH enzyme has been grafted on the modified electrodes and studied toward glucose oxidation. This study allows screening different molecules and discriminates the redox probe able to mediate the electron transfer.

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Liquid-junction-free substitutional stripping voltammetry based on a closed bipolar electrode system and its application to endotoxin sensor

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A liquid-junction-free substitutional stripping voltammetry (SSV) was developed and applied to endotoxin sensing. SSV is a type of stripping analysis that changes the redox current to metal deposition (Fig. 1A)[1]. The amount of the deposited metal is detected by anodic stripping voltammetry after the deposition process. Generally, SSV requires a liquid junction to maintain electrical conductivity between separated cells. This is a critical problem to fabricate a chip-type sensor integrated a SSV system. In this study, a couple of driving electrodes connected to a potentiostat were used to provide the electrical conductivity for the two cells (Fig. 1B). This system is structurally same as closed bipolar system (cBPES). We demonstrated quantification of *p*-aminophenol (pAP) using this liquid-junction-free SSV[2]. A linear relationship between pAP concentration and SSV electrical charge was successfully obtained. Then we applied this method to endotoxin sensing. We used Boc-Leu-Gly-Arg-pAP as a substrate for *Limulus* regent[1,3]. We successfully detected 10 EU/L endotoxin with a chip-type electrode device integrated a liquid-junction-free SSV system.

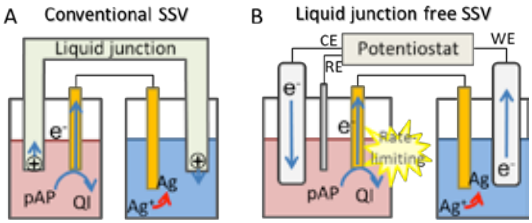

Fig. 1. Schematic illustration showing (A) conventional system and (B)liquid-junction-free system for deposition process of SSV.

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The Influence of Divalent Cations and Ionic Strength on Enzymatic Activity, Structure and Catalytic Current of Fructose Dehydrogenase (FDH)

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In the last decades, many redox enzymes and proteins have been investigated for the possibility of a direct electron transfer (DET) reaction between the active site of the enzyme and an electrode. DET reactions have received considerable attention for construction of biosensors and biofuel cells [1]. *Gluconobacter japonicas* fructose dehydrogenase (FDH) is a heterotrimeric-membrane-bound enzyme with DET characteristics. FDH catalyzes the oxidation of D-fructose to 5-keto-D-fructose. Subunit I (67 kDa) and II (51 kDa) comprise the substrate active flavin adenine dinucleotide (FAD) and additionally three heme *c* moieties as redox prosthetic groups, whereas subunit III (20 kDa) contains no cofactor and is presumed to be related to the stability of FDH [2]. In this work, the effect of Ca^{2+} and Mg^{2+} as well as ionic strength on the catalytic current of FDH modified electrodes was investigated by flow injection amperometry drop-casting FDH onto graphite electrodes. Additionally, the activity of FDH was spectrophotometrically assayed by using both mono- and di-electron acceptors (ferricyanide, cytochrome *c*, and 2,5-dichloroindophenol) in the absence and the presence of Ca^{2+} and Mg^{2+} and tuning the ionic strength. Since FDH is a membrane-bound enzyme, so far impossible to crystalize, circular dichroism spectra were carried out under the same conditions in order to verify possible structural changes in the secondary structure of FDH [3], allowing further considerations on the mechanism of DET.

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Electrochemical signal enhancement with highly labeled nanoparticles for the sensitive detection of bacteria in platelet concentrates

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Transfusion-transmitted bacterial infection (TTBI) is still one of the most feared complications of blood transfusion. From all blood extracts, platelet concentrates (PCs) represent one of the highest risks for bacterial infection due to their storage conditions at room temperature. The methods currently available for bacteria detection in PCs either require a preliminary culture step for detecting viable cells or Real-Time PCR method for detecting bacterial 16S rDNA. The existing rapid tests are combined with the late sampling of the PCs because of their lack of sensitivity. In this context, we aimed at developing a sensitive and rapid assay for bacterial detection in PCs. The required specifications were: 1) a short hands-on time, 2) a rapid response, 3) a minimum requirement for laboratory instrumentation and 4) a high sensitivity to provide the opportunity for a point-of-issue bacterial detection test combined with early sampling and immediate transfusion of PCs.

An electrochemical assay was developed with the use of methylene blue (MB)-DNA nanoparticles for signal amplification. Thanks to their high surface coverage, nanomaterials have emerged as a research field of great interest for improving sensitivity of Point-of-Care diagnostics (POC). To elaborate highly labeled nanoparticles, we developed an original technology of NP DNA functionalization based on a combinatorial solid-phase synthesis. 50 nm silica nanoparticles were covered with 6000 to 7000 labeled-DNA strands. It was shown that the technology was versatile and reproducible for DNA loading. The POC assay was developed with PC mixtures and proved to be specific for *E coli* detection. After dehybrization, the electrochemical detection of MB-DNA single strands on BDD microelectrodes leads to a detection limit of 10 CFU/mL, which is three orders of magnitude lower than the control without NP amplification.

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High Power Glucose/O_2 Biofuel Cell Constructed from Redox-Embedded Carbon Nanotube Buckypapers

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Enzymatic biofuel cells (EBFCs) using glucose and oxygen as the fuel and oxidant are very attractive due to their high selectivity and low environmental impact[1]. For enzymatic fuel cells to become competitive, major improvements in electrode design are required to enhance power density, voltage output and stability. Carbon nanotube buckypaper formed by vacuum filtration is rapidly emerging as an electrode material[2]. It is thin, lightweight and flexible, which is advantageous for construction of miniaturised power sources for implantable and wearable electronic devices. Here we have developed a freestanding paper biofuel cell comprising redox-molecule embedded multi-walled carbon nanotube buckypapers for electrical wiring of enzymes. A quinone derivative is used as a new electron mediator for fungal-derived FAD-dependent glucose dehydrogenase. Buckypaper functionalised with this mediator shows well-defined electrochemistry and very high steady-state current densities for glucose oxidation of J_{max} = 5.38 ± 0.54 mA cm^{-2} at 0.15 V vs SCE at neutral pH. For the cathode, buckypaper was functionalised with protoporphyrin IX to enable oriented immobilisation of bilirubin oxidase from Myrothecium verrucaria. The cathode also exhibited very high catalytic currents which outperform the best performing paper biocathodes in the literature. The resulting glucose/O_2 fuel cell delivered a maximum power density of 0.66 ± 0.1 mW cm^{-2} or 24.1 ± 4.7 mW cm^{-3} at a cell voltage of 0.5 V.

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Different electrochemotherapeutical sensitivity of human adenocarcinoma cell lines – an in vitro study

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Electrochemotherapy (ECT) is an effective way of treating cutaneous and subcutaneous tumors, irrespective of their origin. Electrochemotherapy requires a very low dosage of chemotherapy drug. The application of the cell membrane electroporation in combination with cytotoxic drugs could increase their transport into cells. High electric field applied to the plasma membrane affects organization of the lipid molecules, generating transient hydrophilic electropores. Permeabilization cell membrane depends on the pulse duration, pulse amplitude and the numbers of delivered pulses.

We investigated human breast adenocarcinoma cells (MCF-7/WT) and doxorubicin resistant cells (MCF-7/DOX). Cells were incubated with doxorubicin 5 minutes before electropermeabilization. The concentration of doxorubicin was 1.7 μM. Electroporation parameters were: 100÷2000 V/cm, 50 and 100 μs, 5 and 8 pulses. Finally for ECT-Dx were selected: 1000 V/cm, 100 μs, 8 pulses. As electrodes we used thin stainless-steel parallel plates (4 mm gap). The cloning efficiency test was used to planting efficiency evaluation after ECT-Dx. The cellular morphology (CellMask Deep Red) and doxorubicin distribution was analyzed by confocal laser scanning microscope (CLSM) method.

The proposed therapeutic protocol ECT-Dx efficiently decreased cells proliferation simultaneously with increasing voltage values. Also CLSM method indicated disturbed cell membranes after treatement. As was observed resistant cells were equally sensitive to electrochemotherapy with drugs as their sensitive counterparts.

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Mechanisms of antimelanoma effect of oat β-glucan supported by electroporation

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There are still not specified mechanisms how beta-glucan molecules are transported into cells. Supposing, beta-glucan toxicity against tumor cells may be related to the overexpression of the transporter responsible for the transport of glucose molecules in the cells. In this case, glucans - polymers composed of glucose units are much more up-taken by tumor than normal cells. Increased GLUT1 (Glucose Transporter Type 1) expression has been demonstrated earlier in malignant melanomas (Angadi et al., 2015). GLUT1 expression promotes glucose uptake and cell growth in that cells (Ayala et al., 2010). Also in human melanoma tissues a significant correlation between GLUT1 expression and mitotic activity was found (Angadi et al., 2015). Overexpression of GLUT1 protein confers poor prognosis in a wide range of solid tumors (Koch et al., 2015, Kunkel, et al., 2007).
The aim of our study was to examine the effect of oat β-glucan (OβG) and OβG delivered with electroporation (EP) on viability and the level of GLUT-1 expression in human primary and metastatic melanoma cell lines (MeWo and Me45). There were used standard electroporation parameters: eight 100μs pulses and 1000 and 1200V/cm. The viability evaluation was performed by MTT assay. Immunofluorescent method was applied for GLUT-1 determination in melanoma cells. Moreover, the effect of OβG and OβG-EP was assessed after GLUT-1 blocking. The obtained results bring us to elucidate the mechanism of transport of the oat β-glucan into the cells.

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Low-Energy Pulsed Electron Beams Kill Bacterial Spores by Inducing Damages on DNA

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INTRODUCTION

The mechanism by which Low-energy pulsed electron beams (LEPEBs) lead to bacterial death remains poorly understood. The purpose of this study is to investigate the effects of low-energy pulsed electron beams on spore DNA.

METHODS AND RESULTS

Bacillus pumilus spores were spotted onto the surface of a Petri dish and air-dried at room temperature. After irradiation spots were recovered in water for spore coat removing, as described by Douki *et al*. [1]. Genomic DNA was then extracted *in situ* in agarose, restricted by *NotI* and digested by S1 nuclease before pulsed-field gel electrophoresis (PFGE) analysis.

As shown in the Figure 1, double (lane 3) and single (lane 4) strand breaks are generated on DNA by LEPEBs, resulting in a smear profile.

CONCLUSION

We demonstrate here that LEPEBs lead to spore death by inducing single and double strand breaks on DNA.

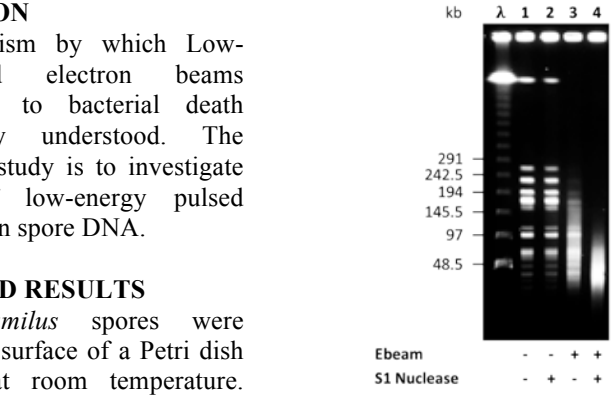

Figure 1: PFGE patterns of genomic DNA from non-irradiated (lanes 1 and 2) and irradiated (lanes 3 and 4) *B. pumilus* spores.

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Perspectives of Nanostructured Silver Amalgam in Bioanalysis

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Silver amalgam (AgA) represents one of the most suitable alternative electrode material with its electrochemical behavior very close to mercury electrodes [1], which are very useful in bioanalysis. [2] We presented a novel possibility to prepare micro and submicro particles of AgA by its direct electrodeposition on ITO from solution containing soluble Ag^{+} and Hg^{2+} inorganic salts. Chemical composition and geometric parameters are tunable by proper selection of solution composition, absolute metal concentration and individual parameters of electrodeposition. AgA particles at ITO proved enhancement in electrochemical reduction of model organic nitro-compound and thus confirmed their possibility to be electrochemically deposited at almost any conductive material what may change and potentially enhance its electrochemical properties. This approach of AgA electrodeposition has a big potential to be easily automatized and used for large scale preparation of micro/nano-electrodes or arrays of such electrodes, which could be prepared even inside of small portable sensors or devices like "lab-on-chip". [3] Moreover, AgA particles electrodeposited at ITO transparent electrode may be used in optical (e.g. elipsometry, surface plasmon resonance, UV/Vis and/or fluorescent spectrometry) and spectro-electrochemical methods. Since silver solid amalgam electrodes were successfully used in bioanalysis of not only nucleic acids and proteins, taking advantages of strong interaction of these biopolymers with the electrode surface [4], further electrochemical, spectroelectrochemical and/or optical studies of the biopolymers may obviously bring novel basic information about their interaction with AgA nanostructured surfaces, structural changes on electrically charged surfaces and applicable electrochemical methods.

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Development and Evaluation of Novel Sensitive Tyrosinase-based Biosensor for Selective Detection of Dopamine

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Detection of dopamine, an important neurotransmitter, in physiological media is linked to a large variety of medical conditions. Therefore, selective identification of dopamine concentrations allows the monitoring of Parkinson or Alzheimer diseases, attention deficit hyperactivity disorder and schizophrenia, being accepted as general diagnosing biomarker.

In this work gold electrodes previously modified with thin film of cobalt(II) compound (CoP) were employed towards sensitive and selective detection of dopamine by developing a novel tyrosinase-based biosensor. Voltammetry, electrochemical impedance spectroscopy and amperometry were used for electrochemical evaluation and characterization of biosensor behavior and selective dopamine detection in the presence of ascorbic acid - main interferent in biological samples. The tyrosinase-based biosensor reached a very good sensitivity up to 1.83 $\mu A\ cm^{-2}\ \mu M^{-1}$ and a detection limit of 0.59 μM, showing considerably better results in comparison to the non-enzymatic CoP sensor.

The biosensors performances were tested in the presence of real dopamine pharmaceutically samples, with satisfactory results in terms of recovery and relative standard deviation values. These results suggest the biosensors applicability in real samples such as human urine and blood serum.

Cell membrane depolarization and permeability of three cell lines of different excitability

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In electroporation-based medical treatments, excitable tissues are treated, either intentionally for example in irreversible electroporation of brain cancer, gene electrotransfer or ablation of the heart muscle, and gene electrotransfer of skeletal muscles, or unintentionally when excitable tissues are in the vicinity of the target treated area. We investigated 1) if excitable and non-excitable cells respond similarly to electroporation pulses, 2) if electroporation could be an effective treatment of the tumour of the central nervous system and 3) if the surrounding excitable tissue could be damaged during the treatment. We used three cell lines: mouse hippocampal neurones HT22, human glioblastoma U87, and Chinese hamster ovary cells CHO. First, we determined a strength-duration curve for all three cell lines. The electric field needed for depolarization decreased with increasing pulse duration. Second, we exposed all three cell lines to pulses of standard electrochemotherapy parameters. All three cell lines reached the threshold of electroporation at a similar electric field. The tumour cell line U87 resealed faster than other two cell lines. The time for depolarization coincided with the resealing time in the permeability experiments which could indicate that during depolarization and electroporation, ions and molecules enter through ion channels as well as through pores in the plasma membrane. Thus, electroporation is a feasible means of treating excitable and non-excitable cells. In future, it should be established to what extent the in vitro depolarization correlates to nerve-muscle stimulation and actual pain sensation.

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Fabrication of Hydrogel Embedded Wearable Sensors for Sweat Analysis

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Continuous monitoring of chemical and physiological processes is a key factor in preventive healthcare and efforts are dedicated to the development of wearable sensors and medical devices. The present work aims at development of flexible sensors as wearable and non-invasive devices at epidermal level for quantification of biomarkers in sweat. An electrochemical sensor for quantification of H^+ ions, an important parameter useful for monitoring biological processes or disease diagnosis and treatment, is described.

Electrodes were fabricated by thermal attachment of gold-coated poly(methyl methacrylate) electrospun fibers on polyethylene terephthalate flexible supports, **Fig. 1(a)**. Pd/PdO was electrochemically deposited and used as sensing interface.

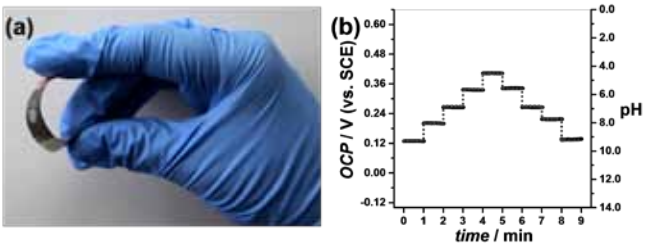

Fig. 1- **(a)** Photographs of hydrogel embedded pH-sensor. **(b)** OCP of PdO-Pd/Au/PMMA/PET function of time recorded in electrolytes with different pH values.

Surface characterization was performed by scanning and transmission electron microscopy while the chemical composition of the sensing layer was investigated by X-ray photoelectron spectroscopy upon basic and acid treatment. Also, the response to H+ ions was investigated by cyclic voltammetry and electrochemical impedance spectroscopy. The sensitivity of the electrodes was aprox. -60 mV per pH unit by measuring the open circuit potential in different media and in artificial sweat with pH values between 4 and 10, **Fig. 1(b)**. In order to demonstrate the functionality of the pH-sensor at epidermal level, Ag/AgCl reference electrodes were also fabricated and integrated with the Pd/PdO electrodes within a matrix of polyacrylamide hydrogel for measurements in artificial sweat.

Highly Sensitive Electrochemiluminescence Signaling of Cardiac Troponin I based on Bipolar Electrochemistry

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Acute Myocardial infarction (AMI) remains one of the major causes of death in people with cardiovascular disease [1]. Development of fast, sensitive and low cost detection strategies are of utmost importance when dealing with ultra-low level concentrations of analyte during early stages of disease progress.
The present study focused on the development of a wireless detection strategy using electrochemiluminescence as signal readout. Here a sandwich-type immunoassay for troponin, an important biomarker for AMI [2,3], was fabricated by modification of the anodic pole of indium tin oxide (ITO) bipolar electrode with a capture antibody, antigen and a secondary antibody conjugated to a synthesized ECL luminophore. The modified ITO bipolar electrode was placed between two driving electrodes and an external voltage was applied. The proposed wireless system showed excellent performance with ECL readout at very low troponin levels. This detection strategy has much promise as a developing tool for multi-analysis as well as real time diagnosis in clinical samples.

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Corrosion Characterization and in Vivo application of the new dental alloy: CoCrNbMoZr

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Anticorrosive and mechanical properties and also the microstructure of the new CoCrNbMoZr alloy were studied in comparison with Heraenium CE (the commercial CoCrMo alloy in use today). A structural and microstructural characterization was performed by SEM and XRD. The main electrochemical parameters were determined using the cyclic potentiodynamic polarization curves; the main corrosion parameters were estimated from the linear polarization measurements; electrochemical impedance spectra (Nyquist ad Bode) were displayed at open circuit potential; monitoring of open circuit potentials was done over a period of up to 1000 immersion hours in Carter-Brugirard artificial saliva of different pH values.

The new alloy is characterized by a multicomponent fine dendritic structure with typical interdendritic distances of a few microns. The EDS spectra detected the alloying elements and the EDS elemental analysis quantified that Mo, Nb, Si and C are enriched in the interdendritic regions.

The XPS spectrum survey for the new alloy showed a complex feature that included peaks for Co 2p, Cr 2p, Nb 3d, Mo 3d, Zr 3d, O 1s. The film is composed of the aforementioned elements` oxides and is thicker (6,5-8,0 nm) more compact and enhanced with oxides as compared with Hearaenium CE.

An increase in the contact angle value for the new Co-Cr-Nb-Mo-Zr alloy has been observed as compared to the commercial Heraenium CE alloy.

The quantity of ions released in artificial saliva for both alloys is relatively small, but the amount is significantly lower for the new alloy. (determined by ICP-MS)

The properties of this new dental alloy carry good improvements on biocompatibility and a high tolerance toward neighboring oral tissues. Thus the risk of oral mucosal clinical lesions noticed near alloy interface is minimized.

Gold nanoparticles modified with folic and lipoic acid derivatives as drug delivery system

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Targeted therapy uses antineoplastic drugs attached to biologically active molecules which can then selectively accumulate in the diseased tissue. Unique advantage of targeted therapy is the ability to destroy diseased tissues without damaging healthy ones. This is particularly important for small tumors at an early stage of development and small metastases[1]. A limitation of the use of targeted therapy is the necessity of finding appropriate receptors on tumor cells and biologically active molecules having affinity for these receptors.

Gold can bind compounds containing a thiol functional group (SH), giving the possibility of functionalization of the obtained nanoparticles and of their use in electronic devices, catalytic surfaces, biofuel cells, biosensors and drug delivery systems[2]. Gold nanoparticles (AuNPs) could contain on their surface a plurality of molecules of the drug or radionuclide atoms. Targeting biomolecule anchored to a thiol group on the gold nanoparticle directly guides it to the appropriate receptors on the tumor cells.

In the project we describe the synthesis of water soluble gold nanoparticles modified with lipoic acid and folic acid derivatives as potential targeted drug carriers for antitumor therapy. The synthetic approach relies on AuNPs phase-transfer step and then covalent coupling of doxorubicin. Physical properties of modified gold nanoparticles were characterized by UV-Vis spectroscopy, transmission electron microscopy, dynamic light scattering method, as well as electrochemical measurements i.e. Square Wave Voltammetry and Cyclic Voltammetry. Their ability of cell membrane penetration was monitored by confocal microscopy. Cytotoxicity was examined by the biological methods: MTT, Neutral Red Uptake or Anexin V affinity assays.

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Mediated enzyme electrodes for application to enzymatic fuel cells

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Use of substrate specific enzymes as catalysts to oxidize glucose at an anode and to reduce O_2 at a cathode, that when combined act as a glucose|O_2 enzymatic fuel cell (EFC), shows promise as a technology for conversion of in vivo available chemical energy to electrical power. This specificity eliminates the need for packaging and ion-exchange membranes and opens up the possibility of device miniaturization and potentially implantable or portable, membrane-less EFCs operating under moderate ambient conditions and on sugars as fuel. [1, 2] There remain, however, significant issues with the proposed technology, such as low power/current outputs, instability of enzymes and incomplete oxidation of fuels, which have thus served to instigate a range of studies to improve the performance of EFCs. [3] For example, we have shown that addition of multi-walled carbon nanotubes (MWCNT) to the enzyme electrode preparation step results in increased surface area leading to improved operational output and stability under pseudo-physiological conditions. [4] These nanostructures provide a support which acts as a scaffold for improved retention of enzymes and electron-shuttling mediators. [5] Here we present a comparison of glucose oxidation by enzyme electrodes prepared using MWCNT and a range of conductive and non-conductive particles as alternate supports to probe whether the size and property of the supports can improve current density and/or stability for these electrodes. In addition a design of experiments approach, previously used to optimize glucose oxidation by enzyme electrodes, [6] has been used to provide enhanced current production for an oxygen reducing cathode enzyme electrode.

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Gold Nanostructured Materials in Highly Sensitive Aptasenor Design

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Nanostructured materials possess many appealing features such as a high surface area/volume ratio and size-dependent optical and electrical properties, which are highly desirable for designing (bio)sensors with optimized properties [1]. The properties of gold nanomaterials are particularly of interest to many researchers, since they show unique physiochemical properties such as optical adsorption of specific wavelength of light, high electrical conductance with rich surface electrons, and facile surface modification with sulfhydryl groups. These properties have facilitated the use of gold nanomaterials in the development of various hybrid systems for biosensors and molecular diagnostics [2].
Reliable, cheap, and sensitive portable (bio)sensors have been highly pursued for applications in biomedial analysis. Herein, we present the development and optimization of a gold nanostructured platform, which was further employed in the fabrication of an electrochemical aptasensor for dopamine detection. To obtain a high surface area for the immobilization of aptamers specific for dopamine, polystyrene beads were firstly deposited on the electrodes. The gold nanostructured platform was electrochemically achieved by cyclic voltammetry, followed by removal of the polystyrene beads using an organic solvent. The resulting gold platform with high surface was further functionalized with thiolated aptamers specific for dopamine. Electrochemical Impedance Spectroscopy measurements were performed to confirm the immobilization of the aptamer on the surface of the electrode and the formation of the affinity complex by the interaction of the aptamer with different concentrations of dopamine. Experimental parameters, such as concentration and incubation time with aptamer and incubation time with dopamine, were optimized in order to find the best conditions for the aptasensor.
This work provides a foundation for developing simple, rapid and easy to use devices that are able to detect various neuromediators in biological samples.

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Study on cytochrome c self exchange in solution and within multilayers on modified gold electrodes

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The redox behavior of proteins plays an important role in the design of bioelectronic systems. We have shown several functional systems exploiting the electron exchange properties of the redox protein cytochrome c (cyt c) in combination with enzymes for sensorial use [1,2] and photoactive proteins for bioenergetics applications [3]. The operation is based on an effective heterogeneous electron transfer reaction at modified electrodes but also to a large extent on the self-exchange properties of cyt c molecules immobilized in multiple layers on electrodes.

In this study different alanine mutants of human cyt c have been examined here with respect to the influence of the mutation site on the heterogeneous electron transfer rate in a monolayer on electrodes as well as on the self-exchange rate while being assembled in multilayer architectures. For this purpose 5 variants of the wild type protein have been prepared to change the chemical properties of the surface area near the heme edge. The structural integrity of the protein mutants has been verified by NMR and UV/Vis measurements. It can be demonstrated first, that the single point mutations influence the heterogeneous electron transfer rate constant at thiol-modified gold electrodes. Second, it is shown that electro-active protein/silica nanoparticle multilayers can be constructed with all forms of human cyt c prepared. The scan rate dependent voltammetric behavior for the mutant proteins in comparison to the wild type is altered in some multilayer arrangements. Higher self-exchange rate has been found for e.g. K79A. The results demonstrate that the position of the introduced change in the charge situation on the surface of cyt c has a profound influence on the exchange behavior. In addition, the behavior of the cyt c proteins in assembled multilayers is found rather similar to the situation of cyt c self exchange in solution verified by NMR.

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Transparent chemical and solar biological power sources

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We present transparent biological power sources, namely conventional and charge-storing bio-fuel cells as well as bio-solar cells.

The very first solar biosupercapacitor is built from a dual-feature photobioanode combined with a double-function enzymatic cathode. The self-charging biodevice, based on transparent nanostructured indium tin oxide electrodes modified with biological catalysts, *i.e.* thylakoid membranes and bilirubin oxidase, is able to capacitively store electricity produced by direct conversion of radiant energy into electric energy. When self-charged during 10 min, using merely ambient light, the biosupercapacitor provided a maximum of 6 mW m^{-2} at 0.20 V [1].

In contrast to solar biosupercapacitors, chemical biodevices convert chemical energy from different biofuels into electric energy. In the case of an enzymatic fuel cell based on transparent nanostructured indium tin oxide electrodes a power output of 1.4 μW cm^{-2} at 0.67 V was registered when working in a continuous mode. Since chemical biosupercapacitors are able to accumulate electric power, the same type of electrodes was used to develop a self-charging chemical biosupercapacitor that was able to increase the power output up to 320-fold when operating in a pulse mode.

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Growth of bacterial biofilm on novel polycation/gold substrates

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Bacterial bioelectrodes can be utilized e.g., in microbial fuel cells (MFCs), in which chemical energy from organic compounds is converted to electrical energy by microorganisms. Performance of bioelectrodes is an important factor which influences not only current efficiency, but also the growth of biofilm on the surface of bioanode. Bacteria species like *G. sulfurreducens* and *E. coli* are known to form biofilm on electrode material [1]. However, many of those microorganisms might be virulent [2]. Therefore, there is a need for finding more human friendly bacteria species, that may be used in MFCs systems. Herein, we present a novel polycation/gold electrode with a biofilm formed by human friendly lactic acid bacteria (*Lactobacillus rhamnosus GG*), which can be find in our digestive system [3]. Normally, this kind of microbes do not form biofilm on metallic materials. However, there are substances like natural or artificial polymers that may enhance bacterial adhesion to the metallic surface [1]. In our experiment, a gold deposited copper foil and pure copper foil were covered with cationic derivative of dextran and investigated as a potential base for *Lactobacillus rhamnosus GG* biofilm formation. SEM images for the polycation/gold substrates confirmed the presence of bacterial biofilm. What is more, only a few bacteria were observed on bare gold. In case when the copper foil was used, no formation of the biofilm was observed, even in the presence of polymer layer. Moreover, dead bacteria cells were clearly visible. In order to study electrochemical properties of bacteria/polycation/gold system, an open circuit potential and cyclic voltammetry experiments were performed. The results showed that our system exhibits electrochemical activity, which may be beneficial for its potential use in MFCs.

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CO_2-conversion to Acids and Alcohols by Microbial Electrosynthesis

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The national project CO2TRANSFER aims the synthesis of butanol by using CO_2 and electrons in a microbial electrosynthesis cell (MES). The new technology offers a possibility of storing electricity from renewable energies like wind, water and solar energy in an environmentally friendly way. For the reduction of CO_2 to butanol 24 electrons are needed. In the first project stage the best suited microorganisms for microbial electrosynthesis will be identified. Bacteria such as *Clostridium carboxidivorans* as well as microorganisms such as *Clostridium acetobutylicum*, which are known for acetone-butanol-ethanol fermentation will be tested for electron uptake. Figure 1 shows the first results with *C. acetobutylicum* in a MES with CO_2. If the direct approach turns out not being possible, it will be tried to produce butanol via an intermediate (acids) using a co-culture. *Sporomusa ovata* produced acetate via direct electron transfer, which was proved by Nevin [2].

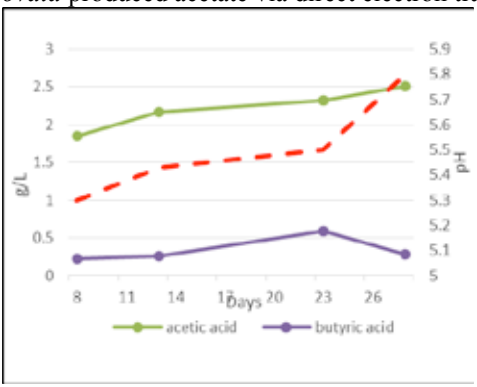

Fig 1. Acid production in a MES with *C. acetobutylicum*

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Electrochemical DNA-Biosensors Based on Long-Range Electron Transfer: Amperometric Detection Using Ultramicroelectrode and a Two-Electrode Setup

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MicroRNAs are RNAs from 18 to 23 bases involved in many biological processes. They were recently discovered and they are the last biological class of molecules making up the genetic code. As they can be found in different biological liquids, they enable the diagnosis of several pathologies[1] and can be considered as excellent biomarkers[2].

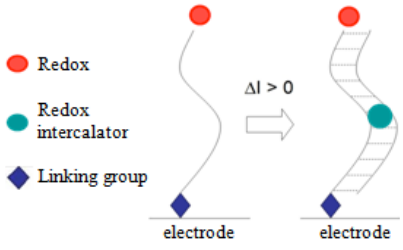

Figure 1: Scheme of a long range electron transfer RNA biosensor with redox intercalator

However quantification of microRNAs, from 10^{-12} to 10^{-18} M in complex matrixes is a real challenge since it requires the development of a specific and very sensitive measurement tool. Electrochemical biosensors and particularly the catalyzed long-range electron transfer DNA-sensors offer an efficient way to do so[3].

In this study, we develop an ergonomic DNA-biosensor based on a two-electrode electrochemical setup using a microelectrode and combining two different grafting strategies for the probe: *(i)* a self-assembled monolayer (SAM) constituted of a one (or three) dithiol(s) labeled probe at the surface of a gold electrode; *(ii)* the non-labeled DNA probe is deposited at the surface of the electrode by electrostripping. In both cases target hybridization is detected using Fe^{II}/Fe^{III} oxydo-reduction catalyzed by methylene blue (MB). Detection of complementary target as well as detection of unique mismatch is performed in a 35 minutes protocol.

New Pyrene-Diazirine Photoactivable Electrode Materials for Biosensing Applications

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By combining electrochemical and photochemical properties, a versatile tool for biomolecules photografting (eg. enzymes, antibodies, aptamers) onto nanostructured surfaces can be developed. Diazirines are of particular interest and often preferred to other photoreactive crosslinking reagents as they exhibit more advantages, including its small size, short lifetime upon UV irradiation and higher subsequent reactivity [1], than benzophenone.
The development of a new immobilization platform based on the use of a new diazirine derivative (pyrene-diazirine) for proteins binding was achieved. Upon UV irradiation, diazirines form highly reactive carbene intermediaries, which can react with C-H, N-H, and O-H bonds, making them excellent photo-reactive crosslinking reagents [2].
To get this purpose, a new compound has been synthesized allowing its π-stacking onto multi-walled carbon nanotubes (MWCNT), and its electropolymerization at both Platinum (Pt) and MWCNT/Pt electrodes.
The electropolymerization parameters were optimized and the electrogenerated films of poly(pyrene-diazirine) were characterized by cyclic voltammetry. Different modified electrode configurations are described. In order to prove the photoreactivity of these electrogenerated architectures, glucose oxidase as protein model was photografted by irradiation, and tested by amperometry for the detection of glucose. The proposed glucose biosensor has been tested and the obtained results have proven its high efficiency on enzyme photografting showing their potential use as a photoelectrochemical approach for biomolecule immobilization.

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New Sensing Platforms for Enzyme Immobilization based on Hybrid Films of Poly(Methylene Blue) from Deep Eutectic Solvents and Carbon Nanotubes

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Hybrid materials based on conducting/electroactive redox polymers and carbon nanotubes (CNT) have received considerable attention in recent years in the bioanalytical field, especially for biosensor development, due to their capability of tailoring their structure and enhancing the sensitivity and electrocatalytic activity of the corresponding biodevices [1]. Usually, the enzyme redox centre is surrounded by hydrophobic polypeptides, limiting the rate of electron transfer. Therefore, there is increased interest in using highly conductive materials for developing new biosensor architectures. Deep eutectic solvents (DES) are a new class of "green" designed solvents that offer an inexpensive, biodegradable and robust alternative as innovative solvents for polymerization, in the synthesis of electroactive/conducting films for the development of biosensors [2].

A new strategy to develop highly conductive platforms for biosensing applications is reported. Hybrid composites consisting of poly(methylene blue) films made by electropolymerisation in DES and carbon nanotubes were developed. The DES synthesized polymer shows nanostructured features which increased its electronic conductivity/ redox activity compared to the aqueous analogue, and together with CNT enabled the construction of highly performance electrochemical sensors. The applicability of the developed sensor was demonstrated using ascorbic acid and acetaminophen. The new sensors showed good sensitivity, stability, reproducibility, repeatability and high recovery factors when used in pharmaceutical formulations, providing a new method for simple and highly sensitive detection of key analytes in complex matrices. Moreover, it broadens the use of green solvents in electrochemical sensor construction, to develop improved enzyme-based biosensors.

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Electrochemical Impedance Spectroscopy of the Charge Transfer Processes between NAD and Phenol Hydroxyl Group Containing Styrylquinolinium Dye

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Recently, the influence of the dye 4-{(E)-2-[4-(dimethylamino) naphthalen-1-yl]ethenyl}-1-methylquinolinium iodide (DANSQI) on cellular processes was investigated in *Candida melibiosica* 2491 yeast-based biofuel cells.
In this study, the charge transfer processes between NAD and recently synthesized hydroxyl-group containing styrylquinolinium dye ((E)-1-ethyl-4-(2-(4-hydroxy-naphthalen-1-yl)vinyl) quinolinium bromide, shortly D-OH) was investigated by means of EIS. Although the reduced and oxidized forms of NAD differ only with two electrons and a proton, in solution they showed different impedance spectra (Fig.1a). The charge transfer hindrances in NADH sample were 5-times lower than those of NAD^+. In the presence of the dye (Fig.1b), however, both real and imaginary impedance of the NAD^+ sample significantly decreased, indicating an electron exchange between the dye and NAD^+.

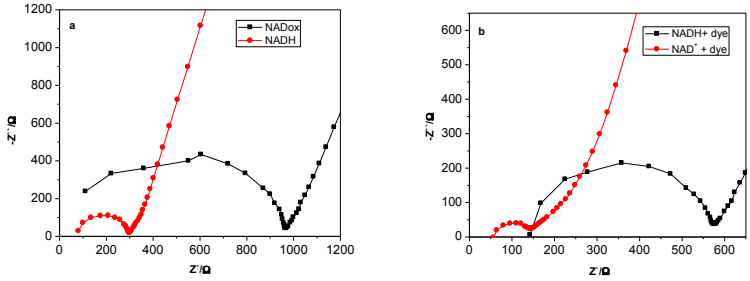

Fig. 1. Impedance spectra presented as Nyquist plots of: a) 500 μM NAD^+ or NADH in 10 mM NaOH; b) NAD^+ or NADH mixed with 50 μM dye D-OH.

Biosystems Immobilization on Gold Nanoparticles (AuNP). A Versatile Support Material for Biosensor Design

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The development of new materials for studies on biosystems/electrodes interaction leads to the use of metal nanoparticles [1]. Gold nanoparticles (AuNP) allow the design of electrode surfaces suitable for their use as current collector for biosensors. Studies with Au NP demonstrate that having good dispersion control generates multiple electrodes on the same surface, thus, increasing the sensitivity on the electrochemical response [2]. Also, due to the size of macromolecules like enzymes or nucleic acids, the molecular architectures needed for anchoring, must be simpler, cheaper and favored towards charge transfer phenomena [3]. In this work AuNP electrodeposited on Glassy Carbon electrode (GC) were functionalized with 4-Aminothiophenol (AMTP) to obtain a platform for biosystem immobilization. By controlling the dispersion and size of the Au NP it was possible to covalently immobilize and evaluate the electrochemical responses of several biosystems like an enzyme; Horseradish peroxidase (HRP), an antibody; Anti PilA and a bacteria; *Geobacter sulfurreducens*. The versatility of the presented GC/Au NP/AMTP electrode is a good alternative for biosensor design since the dispersion allows good stability of the biosystems and avoids pasivation of the electrode.

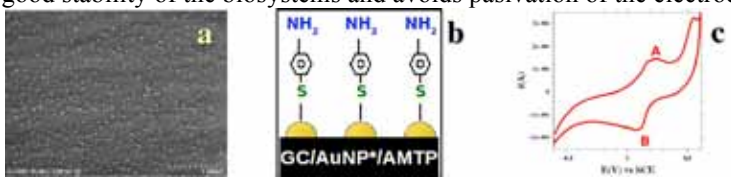

Figure 1. Evaluation route: a) Au NP dispersed on GC, b) Molecular functionalization of AuNP whit AMTP and c) response of HRP immobilized covalently on GC/Au NP/AMTP.

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Analytical Platform for Multiscale Biodetection on 2D Pores network

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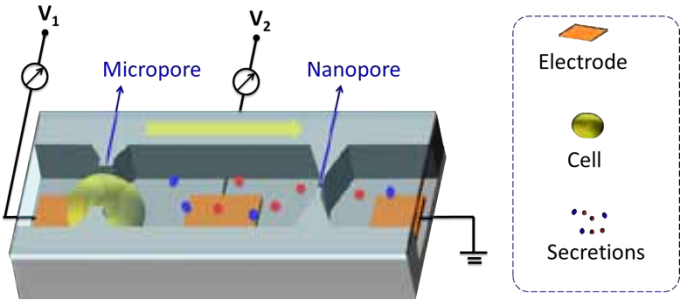
Lymphocytes play an important functional role in immunity by producing a panel of secretions. Different types and concentrations of cytokines are secreted depending on the subtype of lymphocytes, their functions and on their actions in diseases. Currently, the secretion profiles are obtained on a cell population and therefore averaged. Thus, it is of great interest to create a tool able to analyze each cell individually and attribute to each one its profile of secretions. In order to achieve this goal, the PLANARPORE project was launched and consists in conceiving a microsystem combining planar functionalized micro- and nano-pores, to respectively capture cells and analyze their secretions (figure), using fluorescent microscopy or electrical detection. The CLEF technology, already developed in our team and allowing specific functionalization of 3D micro- and nano-holes, will be investigated for localized functionalization of the 2D planar pores. Up to now, silicon-PDMS microchips containing one micropore were fabricated. Characterization of the pore by impedance spectroscopy and simulation of the distribution of the potentials inside the channels brought information to elaborate the best geometry to allow CLEF grafting and cell circulation. Monitoring of circulations of microparticles and of cells (macrophages and lymphocytes) through the pores is in progress.



Dendrimers as a Platform for Designing Biologically Active Carriers

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Dendrimers are a special class of polyelectrolytes, which are characterized by their densely branched and well-defined spherical geometry. Amino-terminal dendrimers resemble spheres, whose uniform surface charge densities can be continuously modulated by pH or ionic strength. Dendrimers have a wide range of applications, including biomedical applications such as drug delivery, gene delivery, cancer therapy and diagnostic agents. Their unique properties originate in their macromolecular structure. It is known that dendrimers can undergo irreversible swelling, and this process is directly connected to the degree of protonation of dendrimer molecules. The hydration of dendrimer films seems to be a crucial aspect in their implementation. In all biological systems, the conformational stability is intrinsically connected with natural hydration.

The present study focuses on the structure of dendrimer monolayers on gold surfaces. We used multi parametric surface plasmon resonance (MP-SPR) and a quartz crystal microbalance with dissipation energy monitoring (QCM-D) to investigate the conformational behaviour of the 6^{th} generation of PAMAM molecules. The combination of MP-SPR and QCM-D measurements allows the investigation of factors that govern the immobilization of PAMAM dendrimers on a gold coated sensor surface. Changes in pH have a strong effect on the binding affinity of the polymer to the surface and additionally, indicate very different conformation behaviours. The trends in the binding affinity and the surface saturation amount correspond well with the degree of change of protonation of the adsorbed molecules. This data very clearly indicates how the structure of the dendrimer can influence the properties of the polymer film formed on the gold surface. PAMAM films on gold surfaces are composed of 80% water. This is a particularly large value compared to the amount of water associated with the dendrimer molecules during the swelling process, which was estimated at 25-30%. This is one of the additional advantages of dendrimeric systems.

These investigations lead to a more profound understanding of the self-assembling behaviour of branched polyelectrolytes, which are interesting candidates for reversibly tuneable swelling materials for drug delivery or smart molecules for surface functionalization.

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Self-Assembling Behaviour of Proteins: from Solution to Surface

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The use of systems that control the delivery of active substances, has led many researchers to focus on the development of nanocarriers based on inorganic particles, synthetic polymers and biopolymers. Great progress has been made, in particular, through the development of methods for the synthesis of new materials with controllable/dedicated physicochemical properties. With the use of the nanoparticle platform as a drug delivery system, it is necessary to develop knowledge of their behaviour in biological systems. Unfortunately, despite careful selection of many favorable physicochemical parameters of nanocarriers, they are often eliminated from the biological system as a result of rapid deactivation in the process of opsonisation. The use of protective layers on the carriers is a way to trick the immune system and thereby modify the pharmacokinetic profile of drug delivery.

Nanoparticles entering biological systems are almost always covered with biofluids. Thus, to develop selective delivery of nanoobjects to particular compartments of the body it is crucial to understand phenomena involved in conformational changes and the displacement of proteins at the interface. From the nanomedical viewpoint, the phenomena of competitive binding and protein displacement – defined as the Vroman effect – are also extremely important because they determine selective delivery of nanoscale objects to specific compartments of the body.

The main scientific objective of this project is to develop a complete, quantitative description of the mechanisms governing the phenomena of protein adsorption on the surface. Multidimensional research using advanced in-situ measurement techniques and molecular dynamics (MD) simulations allows conducting multifaceted study of the protein structure formation. The mechanisms of interaction of functional materials with different types of proteins present in the plasma, together with the analysis of conformational changes and reorganization of protein structures on the functional surfaces has great cognitive value. It will also contribute to a better understanding of the physicochemical mechanisms of creating protein layers on the polymer surface.

The results obtained in this project will contribute to a better understanding of the physicochemical mechanisms of the formation of protein layers with controlled architecture and functionality at interfaces.

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Redox Properties of Thiosulphate Dehydrogenase (TsdA) Enzymes

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The thiosulphate dehydrogenase (TsdA) enzymes are a recently discovered family of periplasmic diheme *c*-type cytochrome oxidoreductases widespread in bacteria. The purple sulphur bacterium *Allochromatium vinosum* (*A.v.*) uses TsdA *in vivo* to oxidise thiosulphate to tetrathionate as part of photosynthesis, whereas the mammalian gut pathogen *Campylobacter jejuni* (*C.j.*) reduces tetrathionate to thiosulphate with its own TsdA in times of anaerobic stress.

Magnetic circular dichroism (MCD) in the near-infrared region has probed the axial heme ligation of the oxidised enzymes. Both *C.j* and *A.v.* TsdA unusually have His/Cys^- as ligands to their active site hemes. The second heme has His/Met ligation in *C.j.*TsdA but His/Lys ligation in *A.v.*TsdA. MCD of the ascorbate equilibrated proteins (sample potential +60 mV vs SHE) shows no reduction of the His/Cys^- ligated hemes whereas the second heme is substantially reduced in both cases. To provide further insight into the redox properties of these proteins their cyclic voltammetry is being studied. The non-turnover peaks describing reduction and oxidation of both proteins are complex and different from one another. Taken together the results suggest that gated electron transfer between the hemes may be a feature of this class of enzyme.

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Synthesis, Characterization and Application of Organometallic Complexes for the Development of Electrochemical Affinity Biosensors.

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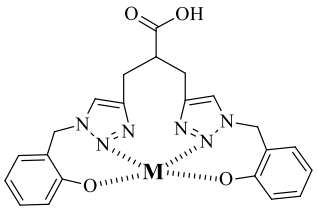
Affinity Biosensors based on antibodies and aptamers appear as promising alternative analytical tools for the detection of a wide variety of bioactive molecules that do not display any electrochemical and enzymatic activity. Usually, target-antibodies or target-aptamers interactions are revealed using enzymatic labeling, which can be sensitive to complex matrices and need different incubation and washing steps.

To avoid the use of these labile enzyme markers, we present in this work an alternative labeling method based on the application of organometallic complexes detectable with different electrochemical techniques. More specifically, we have focused on the synthesis of bis-1,2,3-triazole ligands and their corresponding metal complexes (scheme 1). A great coordination sphere allowing the complexation of copper and oxovanadium was proved. The complexation reaction has been controlled by spectrophotometric titration study, which showed an intense absorption around 400 nm, corresponding to ligand metal charge transfer. Additionally, the X-Ray Structures of bis-triazole ligand and their corresponding metal complexes showed the co-existence of dinuclear coordination complex, which can explain the important amplification of electrochemical signal. A good stability and solubility, up to 1 mM of metal complexes in phosphate buffer at pH 7 has been demonstrated.



Scheme 1. Structure of metal complex based on bis-1,2,3-triazole ligands.

Enzymatic Sensors as a Tool for Monitoring Cell Culture Growth

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Determination of cell growth and viability is crucial to monitoring bioprocesses. There are two group of methods: direct methods which include microscopic counting, electronic particle counting, image analysis, etc, and indirect methods, based on the chemical analysis of a culture component or through the measurement of metabolic activity. Glucose and lactate are two important parameters because they can be related with the cell growth. Commercially available kits and instruments are available to quantify glucose and lactate levels within cell culture media. These kit detection methods are usually colorimetric or fluorometric and are compatible with standard lab equipment such as spectrophotometers.

In this work two enzymatic sensors based on screen printed electrodes are presented as alternative tools to monitor in an easy way the glucose and lactate levels in culture media without the needs of the addition of any reagent. Using the glucose sensor, changes of the glucose levels in culture can be detected after 24 hours whereas in the case of lactate sensor detectable change occurs after 48 hours. Different cell cultures, with different number of cells, were tested and the results revealed that the levels of both glucose and lactate detected with these enzymatic sensors are related with the number of cells presents in the culture.

Finally, a dual enzymatic sensor has been developed to assess both parameters simultaneously. In this case the quantification range was 0.1-1 mM and 0.1-0.5 mM for glucose and lactate, respectively without any detectable cross-talking signal between neighboring electrodes. The performance of these device is tested in cell cultures showing that the increase of lactate level is two times the decrease of amount glucose after 48 hours of culture time according with typical metabolic mechanism

Acknowledgments
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Highly Sensitive Electrochemical Biosensors Based on Lithographically Patterned Electrodes

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In these days, it is very important to develop efficient biosensors with high sensitivity and selectivity for clinical purposes. Especially, electrochemical biosensors have been widely utilized because their own advantages: simple, cheap, reliable, and miniaturized system. To enhance the resolution of electrochemical biosensors, one of the best strategies is to use nanoscale electrodes. In this study, lithographically patterned electrodes were prepared as nanoscale electrodes using photolithography, e-beam lithography, focused-ion beam, and so on. DNA sensors and immunosensors based on electrochemical detection methods were tested on these electrodes, respectively. In addition, to get lower detection limit, signal amplification methods were tried. This study shows that it is possible to get ultrasensitive electrochemical biosensors through combination of nanoscale electrodes and signal amplification methods.

Bacterium based conductometric biosensor for phenol Detection

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Phenol, as one of the 129 specific priority chemicals, is the most common toxic environmental pollutants and mainly originates from four industrial processes comprising refineries, coking operations, coal processing and manufacture of petrochemicals. Bioaccumulation of phenol in human and animal tissue can lead to severe consequences. In this survey a *Pseudomonas* sp., with *Catechol 1, 2-dioxygenase* (1, 2-CTD) gene - from polluted soils of Tehran oil refinery region-Iran - was deposited on the sensitive area of a conductometric transducer made of gold interdigitated electrodes (IDE)s. The bacteria were immobilized on IDEs through cross-linking in the presence of glutaraldehyde vapor [1, 2]. The performance of this biosensor was evaluated, in terms of sensitivity, reproducibility and specificity, for phenol detection. The conductivity variations in presence of different concentrations of phenol (0.01-3mM) were reported whereas no signal variation was measured for 4-chlorophenol. Designing of phenol biosensors based on bacteria having 1, 2-CTD gene, that plays an important role in cleaving the bond between the phenolic hydroxyl groups, would be one of the promising methods for the on-site measurement of phenol.

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Single Compartment Microbial Fuel Cells with Air-Breathing Cathode and Platinum-Modified Bioanode

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Among the new alternative sources of energy, electrochemical energy conversion is now a prime consideration in the research of electricity generation devices. Microbial fuel cells transform chemical energy into electrical energy by redox reactions catalyzed by microorganisms. Some bacteria, so-called electro-active, are be able to do these reactions and can transfer the electrons to an electrode. This transfer may be direct with nanowire-type conductive located on the membrane wall or indirectly via a redox mediator excreted by the cell which transfers electrons from the intracellular molecules to the electrode. The generation of bacterial electricity is a rapidly growing area of research as it is a clean process using renewable fuels [1]. However, the optimization of operating conditions and electrode materials is an essential issue for the improvement of the process, which is limited in terms of power. The use of air-breathing cathode [2] partially removed the kinetic limitations associated with the cathodic reaction of the cell by the use of oxygen from the air and not dissolved oxygen in solution. On the anodic side, the modification of the electrode aims to promoting the electrical connection and the growth of the bacterial biofilm. In particular, the addition of platinum [3] has a remarkable result. We present in this work the contribution of platinum particle generated in situ on the electrode associated with an air-breathing cathode for the construction of a microbial fuel cell based on compost garden.

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Studies of new cyclodextrin derivative as a drug carrier

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Cyclodextrins (CDs) are cyclic oligosaccharides composed of α-(1,4) linked glycopyranose units. Their spatial structure resembles the shape of a truncated cone with a hydrophobic inner cavity and hydrophilic outer surface. CDs are able to form host-guest inclusion complexes with a hydrophobic molecules as a guest [1]. The capability of cyclodextrins to form inclusion complexes allows to use them as carriers for drugs. In our study, drugs containing anthraquinone moiety: doxorubicin (DOX), daunorubicin (DNR), 9,10-anthraquinone sulfonic and carboxylic acids (AQ2S, AQ2CA) are used as the cyclodextrin guest molecules.
DOX and DNR are anthracycline antibiotics widely used as anticancer drugs. The primary factor limiting full use of the anthraquinone derivatives are their toxic effects on the myocardium. The specific toxicity is due to generation of excess reactive oxygen species (ROS). Creating an inclusion complex between the anthracycline molecule and cyclodextrins reduces this effect. Native cyclodextrin complexes have the disadvantage of low stability constants, however modification of CD with appropriate aromatic groups can increase the stability constants of the CD-drug complex [2,3].
The aim of this study was to investigate the electrochemical and spectroscopic behavior of the complexes formed between newly synthesized βCD derivatives and the selected 9,10-anthraquinone compounds. Using the modified Osa equation we determine the stability constant of these complexes in physiological pH, 7.4, and at more acidic pH, 5.5 corresponding to the pathological conditions in the living organism.

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Double emulsion process in co-encapsulation of hybrid multifunctional agents: Influence of the surface functionalization on the nanosystem stability and electro-photodynamic treatment

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Effective nanocarriers (NCs) for cancer treatment need both, passive and active targeting approaches to achieve highly specific drug delivery to the target cells while avoiding rapid clearance by the Mononuclear Phagocyte System (MPS) and cytotoxicity to normal (not tumour) cells (*Mol Pharm. 2008, 5:505*). Recently the field of drug encapsulation has also raised much interest in part due to the advancement of the biomaterials used to elaborate the capsules with novel functional properties obtained by nanoemulsion structural design (*Curr Op Colloid Interface Sci. 2012, 17:235*). Among those approaches, the double emulsion method known to the embedding process, is considered to be the most promising because it permits the encapsulation both hydrophilic and hydrophobic cargo (*Soft Matter 2012,8:2554*). The biological effectiveness of those multifunctional nanosystems in combined anticancer therapy, may be also intensified by increasing their intracellular transport by electroporation approach.

Thus in the present study polyester-origin nanocarriers with various functional properties, i.e., coated with PLGA, PLGA-PEG or PLGA-FA were obtained by double emulsion (w/o/w) evaporation process for co-encapsulation a hydrophobic porphyrin photosensitizing dye - verteporfin in combination with low-dose cisplatin - a hydrophilic cytostatic drug, to applied them as multifunctional hybrid agents for bioimaging and synergistic anticancer activity assisted by electroporation purposes on human ovarian cancer cells (SKOV-3) and control CHO-K1 cell line. The influence of the nanocarrier charge and the polymeric shield variation was tested upon the colloidal stability measured by microelectrophoretic and turbidimetric methods as well as the cellular internalization and synergistic anticancer activity of the electro-photodynamic treatment (EP-PDT).

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Optimization of synthesis conditions of poly-L-lysine-graphene oxide composite for application in electrochemical biosensing

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Poly-L-lysine (PLL) has a number of applications in medicine and research in various forms such as a regular polymer, a protein carrier, nanoparticles, a hydrogel, an enzyme inhibitor etc. [1]. PLL is also applied in electrochemical biosensing and it has two main tasks: i) entrap biological molecules or nanoobjects; ii) ensure biocompatibility of nanoparticles, which enhance the response signal of biosensors. Technically, it can be used as cationic polyelectrolyte in cytopathology as well [1,2].

PLL is used also to form carious nanocomposites with graphene or graphene oxide (GO) [3], however, too little attention is paid to processes going during LL polymerisation and PLL-GO composite formation.

Characterisation of such a composite in each state is performed by vibrational spectroscopy and electrochemical methods. The optimisation parameters as well as performance as a sensor will be presented and discussed.

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Mediated and light-triggered electron transfer between quantum dots and enzymes as basis for photocatalytic biohybrid systems

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Quantum dots (QDs) are small colloidal semiconductor nanoparticles which have been studied with growing interest during the last decade because of their interesting optical properties.
This study reports on mediator-based biohybrid approaches for the connection of QDs with enzymes for the light-driven oxidation of sugars.[1] In the focus are CdSe/ZnS QDs which can be attached to an electrode allowing light-induced charge carrier generation and subsequently electron transfer reactions between the electrode and the nanoparticles. The QDs can not only exchange electrons with the electrode, but can also interact with donor or acceptor compounds in solution, providing access to the construction of signal chains starting from an analyte molecule in solution and ending in a read-out by spatially focused illumination.[2] The QDs are chemically synthesized and immobilized via a dithiol linker on gold electrodes. Stable and well-defined photocurrents have been found over a wide potential range. The functionality of the prepared QD electrodes have been investigated by using small redox molecules such as ferrocyanide and ferrocenecarboxylic acid resulting in a concentration-dependent increase of the photocurrent. This gives access to the construction of mediator based light-controllable signal chains. For example PQQ-dependent glucose dehydrogenase and fructose dehydrogenase have been coupled with QD electrodes for photoelectrochemical glucose and fructose oxidation. Finally, it is also shown that by covalent coupling of ferrocenecarboxylic acid to (PQQ)GDH conditions can be found to assemble the whole system on top of a QD electrode for biosensorial glucose detection. These results provide the basis for light-switchable biosensing and bioelectronic applications, but also open the way for self-driven point-of-care systems by combination with solar cell approaches.

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Cyclic lipopeptide antibiotic daptomycin oxidation mechanism at a glassy carbon electrode

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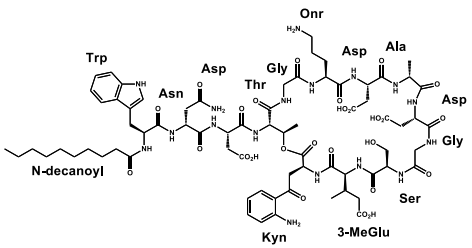
Daptomycin (DPT), was the first approved drug from a new class of antimicrobials, the cyclic lipopeptides, Scheme 1.
The electrochemical oxidation of DPT, by cyclic, differential pulse and square wave voltammetry, in a wide pH range, at a glassy carbon electrode, was investigated. The DPT oxidation was a diffusion-controlled process, occurred in two irreversible pH-dependent steps, and a mechanism was proposed. Among the 13 amino acids present in the DPT composition, only tryptophan (Trp) amino acid is electroactive, in aqueous solutions.



Scheme 1. Chemical Structure of daptomycin.

The effect on the DPT electrochemical oxidation of denaturing agents, urea, which affects the secondary and tertiary structure without affecting the primary structure, detergent SDS that dissolves hydrophobic proteins, and the reducing agent DTT that disrupts disulphide bonds, which are essential for qualitative and quantitative analysis in proteomic mechanism investigation, did not show the occurrence of any new peaks.
The electroanalytical quantification of DPT, for the first time in fetal bovine serum biological fluid, was performed.

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Electrochemical strategy for in situ identification of Pseudomonas Aeruginosa strains

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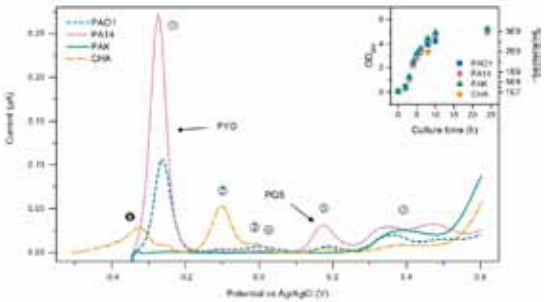
Detection of *Pseudomonas Aeruginosa* (PA) is of the first importance in clinical diagnostic since this bacteria is one of the main pathogenic agent involved in nosocomial infections. Among all the methodologies proposed for the detection and identification of PA, electrochemistry appears as a valuable tool owing to its ability at working *in situ*. Moreover, PA, as other bacteria, is known to produce a complex cocktail of (bio)molecular species. Thereby, its *in situ* electrochemical detection using electrochemical methods appears as an elegant alternative to detect and further characterize bacteria in media of interest without any requirement for surface functionalization or sample preparation[1]. The present work reports the direct electrochemical characterization (SWV) of PA growth supernatant. Since different bacteria strains generate different secretome due to their different genome and metabolic pathways, we determined the electrochemical fingerprints for 5 different PA strain supernatants (c.f. figure below) and their evolution overtime on glassy carbon electrodes. To amplify the recorded electrochemical response, accumulation strategies were developed on electrodes that are modified with electronically conducting polymer films and 3D macroporous scaffolds.



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Potentiation of electroporation by conductive nanoparticles

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Electroporation, a combination of short-duration high-intensity electric field pulses, has been demonstrated to be highly effective in the introduction of various molecules, included drugs, in the cell cytoplasm. In particular, electrochemotherapy has been successfully used *in vivo* in the treatment of cutaneous and subcutaneous tumors in humans. To advance the applicability of electroporation, innovative approaches to increase its efficiency are necessary.

In this context, we aim to assess the possibility of reducing the external electric field applied. To perform this goal, the use of conductive nanoparticles (NPs) is proposed as a method to amplify locally the intensity of the external electric field applied. In this way, the NPs could act as *nanoamplifiers* [1] and would consequently lead to a drastic reduction of the external electric field intensity required to achieve effective cell electropermeabilization.

Among conductive NPs, gold NPs are of great interest for varied biomedical applications, notably thanks to their high colloidal stability, remarkable biocompatibility and good renal clearance. Our first *in vitro* results of this study indicate that the presence of gold NPs at the cell membrane enhance both the proportion of permeabilized cells and the level of permeabilization itself.

This innovative study highlights the potential of gold NPs for enhancing the efficiency of electroporation *via* the amplification of local electric field, which may pave the way for a promising non-invasive therapy.

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Fluorinated xerogel as a screening layer for brain nitric oxide detection on carbon fiber microelectrodes.

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NO is an important free radical synthetized and released by brain cells. It can modulate synaptic transmission and neuronal network activity but also mediate neuronal injury through oxidative stress. Detection in the living brain is an important challenge to understand its role in normal and pathological brain functioning. Because NO is extremely labile and reactive, in situ electrochemical detection is particularly well-suited. NO can be readily oxidized on a carbon or platinum microelectrode. However, its oxidation potential is close to that of nitrite and other endogenous brain molecules like ascorbic acid (AA) or serotonin (5-HT), which poses a selectivity problem. Recently, fluorinated xerogels were proposed for NO detection based on their excellent properties for NO diffusion and rejection of interfering species. However, this approach has never been fully characterized in vivo. Here, we used 7 µm diameter carbon fiber microelectrodes first coated with a layer of nickel-porphyrin (Ni-P) and a screening layer of trimethoxymethylsilane (MTMOS) and heptadecafluoro - 1,1,2,2- tetrahydrodecyl trimethoxysilane (17FTMS). Ni-P lowered NO oxidation potential by 50 mV compared to bare carbon. Most importantly, the fluorinated xerogel provided improved selectivity compared to Nafion, a fluoropolymer commonly used to block interfering molecules. Nitrite, 5-HT and AA amperometric detection was significantly reduced by the silane layer and NO detection was stable over 7 days of storage or throughout a 3h in vivo experiment. NO detection limit in vitro was 16 nM. In vivo, these electrodes could detect brain NO release evoked by a local microinjection of the glutamatergic agonist N-methyl-D-aspartate, and the amperometric signal was almost completely blocked by the NO-synthase inhibitor 7-nitroindazole. Fluorinated xerogel therefore provides excellent stability and selectivity over endogenous interfering molecules for brain NO detection.

The influence of electroporation with calcium ions on model neural cells – in vitro study using model pheochromocytoma cells

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In recent years, particularly in developed countries, the occurrence of neurodegenerative diseases that are amyloid-based has increased significantly. These include Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis (ALS), and many others. There are several hypotheses providing feasible mechanisms for development of fully symptomatic neurodegenerative diseases. The recent studies indicate that the neurodegenerative processes may correspond to incorporation of amyloid oligomers into the cell and organelle membranes, creating weakly cation-selective ion channels that allow uncontrolled influx of calcium into nerve cells.

We introduce a new protocol for studying the effect of calcium on neural cells. Since amyloid channels have not been established yet, another method of increased membrane permeabilization needs to be proposed. Here, calcium transport to model pheochromocytoma cells (PC-12 and PC12-differentiated with NGF) is enhanced utilizing electroporation with microsecond pulses. Depending on the pulse length, temporary pores are created in the cell or intracellular organelle membranes. We studied the effect of the electric pulses with Ca^{2+} and alone on the cells viability (MTT and clonogenic assay) and membrane state (Cell Mask staining). Additionally, the expression of voltage-gated calcium channels and F-actin was determined. We observed that Ca^{2+} channel expression strongly depends on the type of the cells, the electroporation parameters and the calcium concentration.

Does catechin increase sensitivity of pancreatic cancer cells to electrochemotherapy? In vitro studies on primary cell culture

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The understanding of biology and pathogenesis of pancreatic cancer has increased in recent years, however contemporary medicine is still facing the strikingly low overall survival rates. Lack of significant clinical response to chemotherapy is mainly attributed to multidrug resistance (MDR) of pancreatic cancer cells and disturbances in the route of drug administration. According to recent studies catechin - flavonoid present in green tea - may influence the drug resistance by acting on ATP-binding cassette transporters, involved in detoxification. Blocking of these proteins could potentially increase the efficiency of electrochemotherapy with cisplatin. The aim of the study was to evaluate the effect of catechin preincubation on the sensitivity of pancreatic cancer primary cells to electrochemotherapy with cisplatin.

The research material was derived from pulmonary metastasis from pancreatic cancer. Cells were maintained in standardized conditions and only early passages were used for experiments. The cells were preincubated in medium containing catechin concentration of 10 μM for 72h. Following chemotoxicity tests we performed electroporation with EP buffer with 5 and 10 μM concentration of cisplatin (CisEP), using the electric field strength in range 600÷1000V/cm. Cell viability was measured via MTT assay after 24 and 72 hours of incubation. The expression of ABCB1 protein was evaluated with immunocytochemical approach. MDR-1 conjugated with allophycocyanin (APC) was estimated by flow cytometry method. Obtained results indicate that catechin preincubation can sensitize cells derived from metastatic pancreatic cancer to cisplatin and CisEP. The improvement of response to therapy via catechin application may result in decreasing the dosage and limiting side effects.

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Electricity production in electrochemically assisted constructed wetland systems: Continuous flow *versus* intermittent flow

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The biomass used in a fuel cell is an option as an alternative energy source. Among the many ways in which biomass can be used, the plant microbial fuel cells produce electricity *in situ* from the biological activity of microorganisms and plants. Furthermore, artificial wetlands can take advantage of its own processes for simultaneously to treat wastewater and to produce electricity, implementing the plant microbial fuel cells components into it. The metabolic activity of microorganisms represents one of the main mechanisms for pollutants removal in artificial wetlands, releasing electrons to the medium, where they can be captured by means of an electrochemical system to obtain a usable electric current. The aim of this research was to determine which reactor, whether a continuous or intermittent flow reactor has a better performance on power generation and organic matter removal. The variable factor that determined the experimental stages was the electrical resistance value in the external circuit (open circuit, 5600, 1000, 560 and 10 ohm). Electrical parameters were monitored in the experimental system such as electrical conductivity, open circuit voltage, current and electrical power; they were considered as response variables. The influence of water quality indicators at the input and output of reactors was evaluated according to: organic matter measured as chemical oxygen demand, total nitrogen, sulfates, pH, temperature, electrical conductivity, and dissolved oxygen. The results indicate that reactor operating in batch mode throughout the experimental stages render a higher organic matter removal (average 94%), while the reactor operated in continuous mode with plant species has a power output of 18.12 mW/m^2, which is 1.6 times more than the power produced by the batch reactor. The highest generation stage of power density occurred during the 5600 ohm of electrical resistance connection, suggesting that electroactive bacteria were hardly adapted to the electrical resistance changes, even more the highest drop resulted at the 10 ohm resistance connection. However, operating in continuous mode leads to stable organic matter removals and power production. In contrast, operating in batches destabilizes the system when the reactor is emptied and filled.

Influence of the Light Irradiation on the Sediment Microbial Fuel Cell Electrical Outputs

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In this study, the influence of the light irradiation on the performance of lab-scale Sediment Microbial Fuel Cells (SMFCs) was investigated. For this purpose, a special design of SMFC was developed, allowing the whole sediment and water layers to be illuminated. The constructed SMFCs were operated for a month at open circuit mode for acclimation and re-distribution of the bacterial species along the sediment column, after which they were loaded with 1 kΩ external resistor. During the whole experimental window the explored SMFCs were exposed to natural sunlight or artificial illumination. The obtained results showed a distinctive oscillating current response, following the day-night cycle both at natural and artificial illumination. The contribution of the different bacterial types, colonizing the sediment layer, to the SMFC electrical outputs is under ongoing evaluation.

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Formate Oxidation and CO_2 Reduction by Immobilized Formate Dehydrogenase from *Rhodobacter capsulatus*

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The metal containing formate dehydrogenases (FDH) have drawn a lot of interest in the past several years due to their involvement in the reversible reduction of CO_2. The ability to sequester CO_2 from the atmosphere and produce formate makes these enzymes an interesting system for elucidating the mechanism of CO_2 reduction and their future use in the field of biofuel cells.
Formate dehydrogenase from *R. capsulatus* (*Rc*FDH) is a cytoplasmatic, mononuclear molybdenum containing enzyme. The active center, which contains the bis-molybdopterin guanine dinucleotide (MGD) cofactor, catalyses preferentially formate oxidation to CO_2. The electrons from the reduced active center are then transferred through a relay of several iron sulfur clusters to a FMN, where concomitant NAD^+ to NADH reduction occurs. The back reaction, reduction of CO_2 to formate can also be performed by this enzyme [1,2].
Here we report a study of the electrochemical behavior of the immobilized *Rc*FDH and its diaphorase (FdsGB) subunit using cyclic voltammetry (CV) and square wave voltammetry (SWV). The enzyme was immobilized on a graphite rod electrode with a cationic surfactant as a modifier. From SWV two redox waves indicated a direct electron transfer between the enzyme and the electrode surface and were assigned to the FMN and to one of the iron sulfur clusters present in the FdsGB subunit. The direct bioelectrocatalysis of formate oxidation was also detected and the immobilization of the enzyme in its active state was further proven by the evolution of bioelectrocatalytic currents for all the other substrates (NAD^+, NADH and CO_2). The possibility for studying the electron transfer pathway within the enzyme and between the electrode and the immobilized enzyme was also exploited.

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An Ultrasensitive Electrochemiluminescence based assay for the label-free detection of miRNA biomarkers associated with Neuroblastoma disease progression.

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Currently in clinical settings, there is a growing requirement to tailor the aggression of a treatment plan to disease severity at an individual level in a bid to reduce the instances of long term side effects of aggressive drug therapy in cases where milder treatment is adequate. As miRNA expression is one of the controlling factors of gene expression and thus phenotype, expression profiles of miRNA may be used to not only identify disease, but also give specific information about the disease state and therefore allow the tailoring of treatment plans to individual cases for efficient disease management. Recently, miRNA based biomarkers have been identified that can indicate the severity and possibility of spontaneous regression in Neuroblastoma[1], the most common form of extracranial solid tumour in infants. Therefore, the detection and accurate quantification of these miRNA from circulating tumour cells could potentially result in improved disease treatment and long term clinical outcomes.

Here, we present an electrochemiluminescent based assay for the ultra-sensitive and ultra-selective detection of a miRNA target associated with neuroblastoma. In this body of work we discuss the use of self assembled monolayer of oligonucleotides capable of immobilising target miRNA to the surface of an electrode, whereby a luminescent probe labelled miRNA strand can hybridise to the immobilised miRNA target and then be detected through electrochemiluminescence. Furthermore, we discuss the use of nanotextured electrode surfaces to increase the sensitivity of this assay[2] in a bid to further improve the dynamic range of this assay and provide improved signal and lower limits of detection.

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Electrochemical Studies of Membrane Protein Reconstituted in Lipidic Liquid Crystalline Cubic Phase as the Biomimetic Environment

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Retained activity of proteins incorporated in the artificial membranes is essential for their use in membrane - based sensors or biofuel cell devices. Direct adsorption of enzymes at electrode surfaces results often in partial loss of the activity. Membrane proteins, generally are not stable outside the cell membranes, however, they can be stabilized by entrapment in a matrix that resembles natural lipidic environment. A chloride-conducting ion channel from *Escherichia coli* (EcClC) was incorporated into the monoolein (MO) based liquid crystalline phase (LCP) and was studied with electrochemical methods. To identify the structure of LCP, small-angle X-ray scattering (SAXS) measurement was performed. Since ecCLC is a potential dependent protein chronocoulometry was used to study the charge passing through the LCP film as a function of potential applied to the electrode. Charge transients were obtained by jumping from $E_i = 0$ V to final potentials varying from −300 to + 300 mV. From the charge vs. potential graph the difference between charge passing through the LCP and LCP with ecClC channel protein was evaluated. Results indicate that more negative charge is passed through the electrode covered with LCP with ecClC which confirms that ecClC acts as Cl^- ions transporter. More negative charge passes at negatively, charged electrode protein contribution seen at -50 to -150mV (more negative charge) and at 0- to +100mV (positive charge flow is compensated by negative charge passing through protein). The difference of charge passing through the LCP and LCP with ecClC channel protein was calculated and more negative charge was found to flow through the LCP covered electrode loaded with ecClC at pH 4.5 than at pH 7.4. These results demonstrate that ecCLC, the membrane protein can be reconstituted in the lipidic cubic phase, and preserves its functionality and activity in the transport of chloride ions.

Constant current chronopotentiometric analysis of non-conjugated proteins

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Development of a label-free electrochemical method for analysis of practically all proteins represents a great challenge for electrochemistry to enter wide fields of proteomics and complement standard methods. Last decades we have been developing label-free and reagent-free electrochemical methods for protein [1], protein-nucleic acid [2] and protein-protein interaction analysis [3]. Almost 15 years we studied peptides and proteins using constant current chronopotentiometric stripping (CPS) analysis at mercury-containing electrodes. Well-developed peak H, due to the catalytic hydrogen evolution, displays sensitivity to local and global changes in protein structure at bare and thiol-modified mercury electrodes [1]. The method was applied in the analysis of tumor suppressor p53 protein, α-synuclein and peptides involved in neurodegenerative diseases, membrane proteins and for studies of sequence-specific p53-DNA binding [1]. CPS analysis was used also for detection of protein damage due to singlet oxygen ($^1O_2$) oxidation [4]. Our results obtained using the CPS method were in good agreement with results generated by other methods such as fluorescence, dynamic light scattering, gel electrophoresis, H/D exchange mass spectroscopy etc. [1,3,4]. These results together with previous ones obtained with oncoproteins [1], such as tumor suppressor p53 and anterior gradient protein AGR-2 showed interesting properties of surface-attached proteins and offer simple and inexpensive tools for protein research important in present proteomics and biomedicine.

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Photoreduction of an Extracellular Cytochrome

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The mechanisms of photosynthesis allow almost all life on earth to exist. Capturing the energy of sunlight within chemical bonds requires a specialized set of proteins and cofactors in set arrangements that in plants and algae occur within chloroplasts. These proteins sit in the thylakoid membrane and use sunlight produce high energy electrons from water and inject them into an Electron Transport Chain. Photosynthesis does have its shortcomings: it is an inefficient process, wasting much of the light available, also photosystem II is susceptible to photoinhibition and must constantly be replaced at great cost to the cell. By replicating the fundamental principle of light-driven electrochemical gradient formation but increasing efficiency, durability and versatility it may be possible to create a system capable of performing a vast array of green chemistry.

Motivated by these ideas, we are investigating the possibilities for using the porin:cytochrome complex MtrCAB from *Shewanella oneidensis* MR-1 as a means to inject photoenergised electrons into a bacterium. The MtrCAB complex spans the outermembrane and enables MR-1 to exchange electrons between the intracellular and extracellular environments. Here we show that the extracellular cytochrome, MtrC, can be engineered to contain a surface exposed cysteine to which a Ru(II)tris-bipyridyl based photosensitizer can be covalently attached. The labelled protein undergoes photoreduction at much faster rates than achieved when unlabeled MtrC[1]is illuminated with water-soluble $Ru(bpy)_3$ dyes. In addition, the labelled protein transfers electrons to the purified MtrAB complex in detergent solution. Preliminary data investigating the ability of MtrCAB to support light-driven transmembrane electron transfer will be presented.

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The Scaling of Gene Electro-Transfer: Experimental and Theoretical Analysis

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The permeability of the cell membrane can be transiently increased by applying electric field pulses to cells, a process known as electroporation or electro-permeabilization. This technique is also used to transfect cells with DNA molecules for therapeutic gene delivery applications. However, the mechanism of DNA translocation across the membrane during electroporation is still under debate and this prevents the wide-spread use of electroporation mediated gene delivery for clinical purposes. Only recently, it has been shown that endocytosis might be the dominant mechanism during transfection of cells with DNA molecules using conventional electroporation techniques [1]. In order to better understand the mechanism of gene delivery via electroporation, double stranded linear DNA molecules of different sizes (base pairs) have been used to establish a scaling between the size of the DNA molecules and the translocation efficiency during electroporation of Giant Unilamellar Vesicles (GUVs). The translocation efficiency was determined using fluorescence microscopy by measuring the amount of DNA molecules loaded inside the GUVs during/after pulse application. The experimental results were compared with existing models/theory to interpret the mechanism of DNA translocation in vesicles by electroporation. Such a scaling is also useful to test the applicability of GUVs as model systems to study gene delivery via electroporation.

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Label Free Electrochemical Immunosensor for Sensitive Detection of Mucin4

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Mucins are large O-glycoproteins secreted or membrane-bounded in cancer cell development and cell signaling pathways associated with epithelial tumorigenesis [1]. Mucin 4 (MUC4) is normally expressed in airway epithelial cells, in breast milk, saliva, ear and eye fluids, while its aberrant expression has been reported in pancreatic, breast, lung, ovarian, colorectal, and bladder carcinomas [2]. There are many studies that support MUC4 role as a candidate for diagnostic and therapeutic applications in cancer and other pathologies, thus its level in patient sera has the potential to function as a diagnostic and prognostic marker for early stage cancer diagnoses. To our knowledge only one immunoassay for MUC4 is reported in literature based on surface-enhanced Raman scattering [3]. The development of an electrochemical immunosensor for MUC4 detection, using electrochemical reduction of diazonium salts for the covalent attachment of the antibody is presented. The sensing platform was obtained by electrochemical grafting of *in-situ* generated aryl diazonium ions from *p*-aminophenyalcetic acid onto the graphite-based electrodes. The nanoscale film was then functionalized by amino-terminated antibody using standard peptide chemistry. The influence of grafting conditions on over the layers' generation was studied using electrochemical measurements and the sensor analytical performances are presented. The novel label-free immunosensor allows the sensitive and selective electrochemical detection and quantification of MUC4 in spiked human serum samples proving its potential for biomedical and clinical applications.

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Electrochemical and Fluorescent Studies of Tetrazines based on Host-Guest Complexation both in Solution and Films

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Tetrazines are electroactive six-membered aromatic heterocycles with a very high electron affinity. They display interesting fluorescence properties that can be electrochemically monitored. These aspects make them especially attractive for sensing applications [1].

Four tetrazines substituted by linear 2,3-naphtalimide antennas and/or adamantane groups were analyzed in organic and aqueous media. Their solubilization in aqueous solutions was successfully achieved by using β-cyclodextrin (β-CD) and gold nanoparticles modified with β-CD due to the formation of inclusion complexes between the tetrazine ring and/or the organic groups and the β-CD cavity. These redox supramolecular assemblies were characterized in water by electrochemical and fluorescence measurements [2].

Tetrazine derivatives were also immobilized onto electrogenerated films of polypyrrole functionalized with β-CD indicating that the resulting supramolecular material kept the electrochemical and fluorescent properties of tetrazines. The stable inclusion of the tetrazine compounds within the electropolymerized β-CD film was analyzed by using fluorescence microscopy and cyclic voltammetry. These inclusion complexes were then used as a "bridge" to immobilize the β-CD-tagged glucose oxidase proving the efficiency of this method to build new supra-biomolecular architectures [3].

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Electrochemical Detection of Cellular Iron Reduction in Epithelial Cancer Cell Lines

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Iron is an essential nutrient required for the functionality of many living organisms. In mammals, iron plays a key role within a variety of processes. These include the transport and storage of oxygen, as well as the catalysis of key reactions throughout the body. Extracellular iron has been shown to be reduced via transplasma membrane electron transport systems (TPMETS). We have utilised the electrochemical mediator ferricyanide as a tool to measure the reduction capacity of three epithelial cancer cell lines (A549, Calu-3 and H1299) toward iron. Linear sweep voltammetry was used to monitor the magnitude of oxidation current which could be correlated to the concentration of iron reduced. Viability studies were also carried out to ensure our iron compound, potassium ferricyanide, did not cause cytotoxicity upon incubation. The capability of both cell lines to reduce iron hints at the presence of ferrireductase enzymes present in the membrane of the cells. Our further work will seek to confirm and quantify these enzymes, with further implications for understanding the underlying biochemical modulation of iron transport via transplasma membrane electron transport systems. This work is important for the understanding TPMET systems in more detail, and in particular adsorption of ions onto membranes.

Paper-based Wearable mW class Biofuel Cells Powered by Glucose and Lactate

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Printable Electrochemistry has attracted attention for the development field of a disposable biosensor and biofuel cell. Paper-based BFCs (PBFCs), which are based on printing technologies, are attracting increasing attention as new energy harvesting systems for sensor tags, wearable biomedical devices, and small electrical devices [1-3]. In the present study, we introduce a high power glucose PBFC, which was created by a 4-series, 4-parallel array structure. The single PBFC was composed of a bioanode and a biocathode that were based on porous carbon electrodes constructed using porous carbon inks. The present PBFC, which is based on a new array structure, exhibited an output power of about 1 mW, and an open circuit voltage of about 2.5 V. The present PBFC was able to light an LED directly, without the requirement of a step-up circuit board.

A disk-typed glucose biofuel cell, which assumes to generate electric power from glucose in urine, was also developed. We achieved a 0.7 mW maximum output power from an artificial urine containing 100 mM glucose solution.

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Entrapment of Biomolecules in Tunable Nanoporous Gold Networks: A Strategy for Improved Rates of Electron Transfer

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Nanoporous gold (NPG) is a material of emerging interest for the immobilization of enzymes. NPG materials provide a high surface area onto which biomolecules can either be directly physisorbed or covalently attached after first modifying the NPG with a self-assembled monolayer. The immobilization of enzymes on NPG is of interest for applications in sensors[1], assays, catalysis and biofuel cells[2]. By controlling experimental parameters such as the composition and thickness of the alloy and the dealloying conditions, the surface area and morphology[3] of NPG electrodes can be tailored to the desired application. The entrapment of enzymes, such as glucose dehydrogenase, bilirubin oxidase[2], laccase and fructose dehydrogenase[1], in the porous network can lead to enhanced protein loadings and favourable orientation of the active site of the enzyme, resulting in significantly improved current densities. In addition, rates of direct electron transfer can be enhanced removing the need for redox mediators, paving the way for implantable biodevices such as sensors and fuel cells. Comprehensive studies have been performed on fructose dehydrogenase modified NPG electrodes. The biosensor was used to detect D-fructose in a wide range of natural sweeteners and beverages. In a biofuel cell, glucose dehydrogenase and bilirubin oxidase modified NPG electrodes showed improved stability and significantly increased power outputs in comparison to planar electrodes. The response of a wide variety of bilirubin oxidases immobilized on NPG electrode has been examined in detail.

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Amperometric Biosensor Based on Gold Nanoparticle Doped-Poly (8-anilino-1-napthalene sulphonic acid) and Tyrosinase for Detection of Tyramine

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Tyramine is an indirectly acting sympathomimetic amine which releases norepinephrine from a sympathetic nerve ending, and it has been reported that tyramine-containing foods can cause unnatural and toxic effects, when ingested in large quantities. For these reasons, the development of a fast and accurate method to measure tyramine concentrations in foods is important [1]. Electrochemical enzyme biosensors based on polymers and gold nanoparticles (AuNP) have been considered as the best choice for the in situ monitoring of biogenic amines owing to their high sensitivity, simple instrumentation, low production cost and promising response time. In addition to properties common to all nanoparticles, the attachment or incorporation of (AuNP) into thin films enhances the electron transfer between redox centres and electrode surfaces and make them an ideal material for sensors [1,2].

The aim of the present work was to develop an amperometric tyramine biosensor by immobilization of tyrosinase on poly (8-anilino-1-napthalene sulphonic acid) (PANSA) synthesized by a green synthesis method, modified with AuNP and deposited on a glassy carbon substrate. The electrochemical performance of the biosensor was evaluated and the biosensor characteristics including enzyme kinetics, influence of scan rate, pH, sensitivity and limit of detection for the detection of tyramine determined. Selectivity with respect to potential interferences in foods was investigated. The novel platform showed a good response due a strong interaction between tyrosinase and the nanocomposite, which makes it a promising biosensor for quantification of tyramine.

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Electrochemical Study of CoQs and Their Hydroxylated Derivatives

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Coenzymes Q constitute a family of compounds containing a benzoquinone ring (the hydrophilic head) to which an isoprenoid chain (hydrophobic part) is attached. They are redox active lipophilic compounds that are found in various organelle membranes of all living organisms. Importantly, ubiquinone Q_{10} is an essential component of the electron-transport chain and plays key role in mitochondrial energy production. It functions as an electron carrier and transfers two electrons from complexes I and II to complex III in the inner mitochondrial membrane, a crucial role that no other molecule can perform. In its reduced form, Q_{10} can easily give up one or two electrons and thus acts as an antioxidant or prooxidant.

Since Q_{10} is a fat soluble, hydrophobic compound, its redox chemistry is investigated mainly in organic solvents. It is questionable if this approach reflects the membrane conditions, where Q_{10} is incorporated in a lipid environment in contact with aqueous solution. Here, we report on a novel approach for studying the redox properties of Q_{10} by using functionalized multiwalled carbon nanotubes (MWCNT) deposited on glassy-carbon electrodes. MWCNTs were treated with Q_{10} and added lipids to mimic the biomembrane matrix. With a set of different electrochemical experiments, the kinetics and thermodynamics of the redox reaction of Q_{10} in buffered solutions and the pH dependence were analyzed.

Recently, we synthesized novel hydroxylated derivatives of CoQs. We found that CoQs, when exposed to high pH, undergo complex chemical transformations. The hydroxyl group attacks the ring substituting one methoxy group at ring position 2 or 3. As important biological aspects, hydroxyl-Q_{10} was also generated in presence of cytochrome P450 enzyme, and is present in the quinone pool of beef heart mitochondria (ca. 1%, measured by HPLC-MS).

The novel hydroxylated derivatives have different properties compared to native CoQs. The voltammetric analyses show that these compounds have more negative redox potentials which make them stronger antioxidants. They have high affinity to bind Ca^{2+} that may be of great importance for their function in cell membranes. Our research was extended to other analogues of biologically relevant quinones, such as mitoQ and decylubiquinone, to further study the physico-chemical and functional properties of these hydroxylated forms.

Monoolein Cubosomes Doped with Magnetic Nanoparticles – a Hybrid Material for Drug Release

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Lipidic liquid crystal materials can be prepared in the form of cubic phase or cubosomes - dispersed LCP into nanoparticles. Both forms have the same properties which make them highly interesting as the matrix for drug delivery - they are non-toxic, bioadhesive, biodegradable in the body and have a large inner surface and can accommodate the appropriate amount of active molecules of any polarity. But cubosomes have an advantage over cubic phase because they have lower density. Cubosomes doped with nanoparticles are of interest as the drug delivery systems and as contrast agents for magnetic resonance imaging (MRI). [1, 2]
We prepared hybrid cubosome dispersions – magnetocubosomes, loaded with magnetic NPs and the drug. The TEM and Cryo-SEM images shows the spherical morphology of the cubosomes and confirmed that the nanoparticles are inside the cubosomes. The size of the LCP particles was obtained from the analysis of DLS measurements. The cubic nature of the LCP dispersion was characterized by SAXS. The drug release profiles from the magnetocubosomes were established using SWV. The experiments were performed in the absence/presence of external magnetic field. We received LCP nanoparticles with average diameter of 150nm and $Im\bar{3}m$ cubic structure, which exhibit magnetic properties and move in the magnetic field. We also show that the use of the magnetic field accelerates the elution of the drug.

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Optimization of Microbial Hydrogen Peroxide Producing Cells (MPPCs) for Wastewater Applications

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We investigated the use of a microbial electrochemical cell to produce hydrogen peroxide (H_2O_2), named a microbial hydrogen peroxide producing cell (MPPC). H_2O_2 is a powerful oxidant that can be used for on-site tertiary treatment, pre- or post-treatment of sludge, or cleaning membranes. Its production in MFCs is possible by allowing the 2-e^- oxygen reduction reaction (ORR) to occur at the cathode. Through various studies on materials for cathodes and membranes, we optimized an MPPC and have shown consistent H_2O_2 production in MFCs fed with acetate on the anode to concentrations of ~ 3 g H_2O_2/L. These concentrations were possible by optimizing operational conditions, including the cathodic hydraulic retention time and the cathode salt solution. These concentrations are significantly higher than those needed for many of the possible onsite applications of H_2O_2, for e.g. disinfection.

In an MPPC fed with primary sludge (PS) at the anode, a maximum H_2O_2 concentration of ~230 mg/L was achieved in 6 hours of batch cathode operation. We observed the H_2O_2 gradually decayed with time due to the diffusion of H_2O_2-scavenging carbonate ions from the anode, which could explain the lower concentrations obtained. We also evaluated a single-chambered MPPC (sMPPC), where we removed ion-exchange membranes so that H_2O_2 can move into the anode chamber for enhancing sludge stabilization, and rather used a polyester fabric as separator between anodes and cathodes. Even though we increased PS loading rates in the sMPPC by ~5-fold than that of dual-chambered MPPC, we achieved higher Coulombic recovery, 42%. We achieved the effluent sludge quality similar to Class B biosolids having 52% VS removal and 1.2 x 10^5 MPN per g dry-weight PS. Our results should pave the way for further development and scale-up of MPPCs and their application for treatment and/or H_2O_2 production.

Electrochemistry of Sarco/endoplasmic Reticulum Ca-ATPase

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Sarco/endoplasmic reticulum Ca-ATPase (SERCA) is a transmembrane protein that is able to transfer calcium ions from the cytosol of the cell to the lumen of the sarcoplasmic reticulum at the expense of ATP hydrolysis during muscle relaxation. Here we report the electrochemical investigation of SERCA1 isoform isolated from rabbit muscle using mercury and carbon electrodes. Two types of samples were analyzed, *i.e.* solubilized protein and protein-membrane complex (sarcoplasmic reticulum vesicles). Both samples were studied using a previously developed methodology proposed for the analysis of membrane proteins [1-4]. The electrochemical reactions of SERCA based on oxidation or reduction of surface-localized amino acid residues were studied. In addition, the interfacial behaviour of SERCA was observed by impedance spectroscopy. The methodology presented here could be used in further studies of the structural integrity and intermolecular interactions of solubilized and reconstituted samples of SERCA.

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Utilization of Protein Electrochemical Testing in Medicinal Chemistry Research: Concept of Artificial Modifications

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The paper will focus on the electrochemical investigation of water-soluble globular proteins and selected poorly water-soluble (membrane) proteins. Special attention will be paid to the electroanalysis of protein chemical modification (oxidation), post-translational modifications and protein interactions with drugs and highly reactive species and metabolic by-products.
The general approach for our electrochemical sensing platform is based on protein intrinsic electroactivity monitoring, *i.e.* the electrochemical reactions of amino acid residues of the investigated proteins using chronopotentiometry and voltammetry [1]. To be more specific, the modification of human serum albumin and the sodium-potassium pump by methylglyoxal (metabolic side product) will be described and discussed [2,3]. The electrochemical data will be supported by electrophoretic and immunochemical results.
Finally, the experimental concept of the artificial chemical modification of proteins by reactive carbonyl compounds will be shown here for the first time. The results presented are fully applicable for evaluating the surface modification, stability and reactivity of proteins isolated from human subjects. These open up new possibilities in the study of blood proteins in healthy subjects and subjects with pathologies, mainly those associated with metabolic syndrome.

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Permeability studies of Acrylate Bone Adhesives and their Correlation with Adhesive Strength

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Rapid set polymeric adhesives offer the prospect of injectable, biodegradable fracture fixators as a minimally invasive alternative to surgical metal fixation. However, little consideration has been given to their solute permeability, likely to compromise biosolute exchange between healing fracture surfaces. There is, moreover, no information available on permeability changes during adhesive ageing.

We determined age dependent permeability changes for a set of simple, readily measurable probe molecules (acetaminophen, catechol, hydrogen peroxide, oxygen) for a family of UV curable acrylic adhesives. Photoinitiator generated free radical polymerization allowed rapid curing based on chain extension through acrylate double bond opening. Adhesive films were used for permeability determination using a combination of CV [1] and hydrodynamic amperometry using a solute jump technique and an in-house diffusion model [2]. Correlation between adhesive strength and permeability was examined. Bone adhesion shear strength was 3.0 - 4.4 MPa for the range of acrylics. Ageing in buffer demonstrated strength loss starting from Day 9 with 50% loss by Day 18. Corresponding with this, adhesives were impermeable up to Day 6. At Day 9 diffusion coefficients of $2.5x10^{-8}cm^2/s$ (O_2) and $1.9x10^{-9}cm^2/s$ (ascorbate) were found, and increased to $2.2x10^{-5}cm^2/s$ and $1.0x10^{-6}cm^2/s$, respectively, at Day 19. This increase in permeability was molecular weight dependent with ascorbate/acetaminophen showing the smallest increment, and H_2O_2/O_2 showing the highest; no charge dependence was observed. Electrochemical permeability determination provides a novel, non-destructive means for assessing adhesive degradation which could assist with their optimisation as permeable joining materials.

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Simultaneous measurements of photocurrents and hydrogen peroxide evolution of PS2 complexes

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The conversion of photon energy into chemical energy is the most important natural energy conversion reaction on the planet performed by plants and algae. Coupling photosynthetic proteins with electrochemical systems holds a great potential to gain deeper insights into the photosynthetic light harvesting and electron transfer processes. Of particular interest is the elucidation of photodegradation processes. Photosystem 2 (PS2) for example is known to produce reactive oxygen species (ROS) like singlet oxygen or hydrogen peroxide which may induce damages of the proteins.
Here, we present the immobilization of isolated PS2 complexes in a monolayer on the surface of a gold electrode modified with PAMAM dendrimers. With this setup it is possible to monitor the photocurrent of PS2's photocatalytic water splitting activity and the generation of reaction products like ROS by combining chronoamperometry and fluorescence microscopy. We select the fluorescent probe Amplex UltraRed which is specific for H_2O_2. We reveal that H_2O_2 formation during the reaction is reduced by optimizing electron mediator concentrations highlighting the importance of fast electron release from the photosynthetic protein. This novel experimental setup is particularly useful in studying the influence of solvents on the activity of photocatalytic enzymes.

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Voltage monitor on FTO for the observation of ovarian cancer cells

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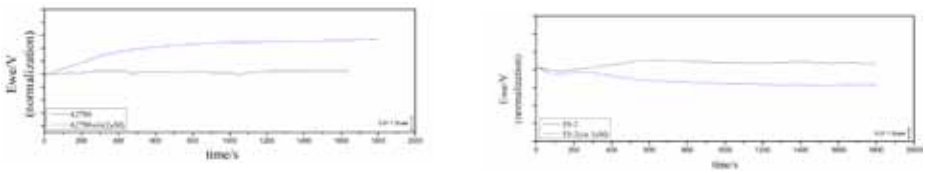
Ovarian cancer is the fifth most common cancer, affecting women in recent years. In addition, ovarian cancer has high lethality compare with other types of female reproductive cancers. In 2015, the American cancer society counts that more than 20,000 women were diagnosed and approximately 14,180 women died by the ovarian cancer [1,2]. Over 60% of patients have metastatic cancer when they are diagnosed, and the overall 5- year survival rate is less than 25%. In contract, the patients with early stage cancer at diagnosis have better prognosis with 5-year survival rate is more than 70% [3,4]. According to the results, the early detection for ovarian cancer is very important for cancer diagnosis.

In this research, a simple and rapid methodology has been developed. A FTO glass, platinum wire, and the Ag/AgCl reference electrode were fabricated as an electrochemical device. Different kinds of cell medium were added into the beaker for further testing. The monitor of open circuit voltage shows that the two cells demonstrate different behavior. In Fig. 1a, the electrode coated with the normal caner cell (A2780) shows a straight line, indicating the voltage different is not changed. However, in case of A2780 cancer cell with cisplatin shows the voltage curve increases gradually. In addition, Fig. 1b displays the difference between normal cancer cell and the case within cisplatin. The tendency result indicates that the case within cisplatin was declined.



Through this research, this voltage monitor methodology not only distinguishes drug resistance influence but also discriminate different kinds of cancer cells. We look forward to this study that is able to apply to the medical field and other applications.

Figure 1 the voltage change profile on (a) A2780 and (b) ES-2 cancen cell.

Ultra-sensitive Electrochemical Biosensor for CA125 Detection and Its Application in Ovarian Cancer Diagnosis

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In common cancer detection, the enzyme-linked immune-sorbent assay (ELISA) is the most popular and traditional clinic analysis. The CA125 ELISA is a solid phase immobilization of enzyme-linked immune-sorbent assay, which utilizes monoclonal antibody against distinct antigenic determinant on the intact CA125 molecule. The testing sample reacts simultaneously with two antibodies, indicating the CA125 molecules are sandwiched between the solid phase and enzyme-linked antibodies. The minimum detectable concentration of CA125 in ELISA is estimated to be 5 U/mL. According to clinical cases [1], this detection limit is not enough for health alarm. In our research, decreasing the detection limit and increasing analytical sensitive are our goals.

In this study, an electrochemical immunosensor fabricated on reduced graphene oxide (rGO) sheet has been created. Figure 1a shows the fabrication procedure of the immunosensor. The rGO is used to immobilize the receptor thionine (Thic) and secondary anti-CA125 antibody (Ab_2). The resulting rGO/Thic/Ab_2, namely nanostructure, is able to use as the label of the immunosensor. Primary anti-CA125 antibody (Ab_1), gold nanoparticles (AuNPs), bovine serum albumin (BSA), and cancer antigen 125 (CA125) construct the nanostructure on the glassy carbon electrode (GCE) becomes working electrode. Ag/AgCl electrode is the reference electrode and platinum wire is the counter electrode in this research [2].

In this research, the differential pulse voltammetry (DPV) has been applied in order to detect the varying concentrations of human serum on the sensor. According to the DPV results, the detection limit of this sensor is calculated to be $4.096*10^{-11}$ (g/mL), which is much sensitively than the previous studies [3-5]. In addition, the correlation coefficient of the calibration plots for thionine reaction integral area was 0.9489 (Fig. 1b). This novel electrochemical biosensor sensor shows outstanding performance such as accurate sensitively detection, easy fabrication, and quick analysis. We believe this novel sensor is able to the next generation of Home Care tool.

Role of Organic Solvents in Immobilizing Fungus Laccase on Single-Walled Carbon Nanotubes for Improved Current Response in Direct Bioelectrocatalysis

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Tuning interactions between proteins and solid surfaces is important for rationalizing molecular orientation of redox enzymes immobilized on the electrode to favor an efficient direct bioelectrocatalysis. Supramolecular docking calls for specific "linker" molecules to direct the position of enzyme redox active sites on surfaces of electrodes or conductive nanomaterials, for example, carbon nanotubes (CNTs). In this work we focus on a high-potential fungus laccase, a multi-copper oxidase that catalyzes electroreduction of oxygen, and investigate the regulative effects of organic solvents on laccase immobilization on single-walled CNTs (SWCNTs) and its direct bioelectrocatalytic activity. Herein the maximum reductive current response is dramatically improved by 600% through ethanol-assisted enzyme immobilization, which is a result of ethanol-promoted laccase-SWCNT contact and favorable enzyme orientation on SWCNTs from conformation analysis. Extended investigation on more organic solvents with distinct physiochemical properties show that organic solvents with lower polarity, weaker denaturing capacity and higher vapor pressure are well-suited for assisting DET. This study reveals that organic solvents regulate laccase immobilization for direct bioelectrocatalysis by balancing surface wetting and protein denaturing. It further solidifies our fundamental knowledge of bioelectrochemistry of laccase and may inspire the design of electrode-enzyme interfaces with tunable surface wettabilities.

Keywords: Bioelectrochemistry, Laccase, Direct Electron Transfer

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Development of a New Microfluidic Electrochemical Cell for Studies at the Biointerface: Towards *in situ* Studies of Electroactive Biofilm Anchoring Structure

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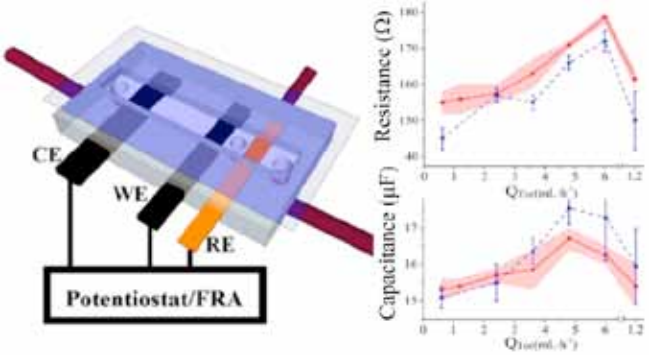
Electrochemical impedance spectroscopy (EIS) has been previously applied to verify the trend of bacterial cells attachment and biofilm formation. Various bioelectrical modeling for electroactive [1] and non-electroactive [2] biofilm were used to identify the electrode/electrolyte and the electrode/bacteria/electrolyte interfaces. The anchoring biofilm layer is expected to exhibit a different response to environmental stresses than for portions in the bulk, due to the protection from other strata and the proximity to the attachment surface.

In this project, *in situ* EIS measurements of biocapacitance and bioresistance of Pseudomonas sp. biofilms were conducted during the growth phase and under different shear flow conditions. Distinct, but reversible changes to the amount of biofilm and its structure at the attachment surface were observed during the application of elevated shear stress. This robust, precise and accurate microfluidic electrochemical device and responsive to shear [3] provides a perfect opportunity to pass from the first phase of development of a microfluidics electrochemical cell to implementation of electro active biofilms.



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A Method for Detecting Non-protein and Protein Moieties of Cytochrome c in a Single Electrochemical Scan

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Cytochrome c is one of the most studied conjugated proteins due to its electron-transfer properties and ability to regulate the processes involved in homeostasis or apoptosis. Here we report an electrochemical strategy for investigating the electroactivity of cytochrome c and its analogs with a disrupted heme moiety, *i.e.* apocytochrome c and porphyrin cytochrome c. The electrochemical data are supplemented with electrophoretic and immunochemical tools. The main contribution of this report is a complex evaluation of cytochrome c reduction and oxidation at the level of surface-localized amino acid residues [1] and the heme moiety [2] in a single electrochemical scan. The electrochemical pattern of native cytochrome c is substantially different from both analogs apocytochrome c and porphyrin cytochrome c, which could be applicable in further studies on the redox properties and structural stability of cytochromes and other hemeproteins.

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Design and Construction of an Enzymatic Fuel Cell with Molecular Oriented Bioelectrodes

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In order to improve the direct electron transfer in enzymatic biofuel cells, a rational design of a laccase cathode and glucose oxidase or glucose dehydrogenase anode is presented. The rational approach is based in the molecular orientation of the enzymes. The molecular orientation is made to induce the direct electron transfer between the redox active-site of the enzyme and the electrode surface. The ultimate goal of developing this enzymatic fuel cell is to reach remarkable efficiencies, limited only by thermodynamics and not by engineering issues. The cathode were first functionalized with different laccase substrate-like compounds, then the enzyme was allowed to interact through the T1 site that recognize the organic substrate, and then the complex was covalently bonded using the carbodiimide chemistry. On the other hand, the anode was functionalized with the FAD or PQQ cofactor bonded to different aromatic cross-linkers and then the apo-enzymes were reconstituted on the functionalized electrode.

Direct electron transfer of both enzymatic electrodes was successfully achieved and their performance was evaluated. The molecular oriented enzymatic electrodes showed a higher current density and power density than randomly bound enzyme electrodes. The operational stability was measured by chronoamperometry.

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