

# **1<sup>st</sup> PolTur Workshop on Biohybrid Graphene Solar Cells**

**Centre of New Technologies  
University of Warsaw  
Banacha 2C, Warsaw**

**20-22 November 2016**

**Organised by GraphESol Consortium:**

Joanna Kargul

Sebastian Maćkowski

Kasim Ocakoglu



# Programme



## Sunday, 20th November

### Arrivals

Informal Dinner 19.00, Stary Dom Restaurant, Puławska 104/106, Warsaw

## Monday, 21<sup>st</sup> November

08:00 – 08:55     **Registration (CeNT Atrium)**

**Venue:**            **Lecture Hall 1130**

09:00 – 09:10     **Welcome:** Joanna Kargul

**Session 1:**        *Biohybrid photoelectrodes and plasmonic structures*

**Chair:** Renata Bilewicz

09:10 – 09:40     **Joanna Kargul** (Centre of New Technologies, University of Warsaw, PL)  
*Tackling the grand challenge of solar energy conversion using extremophilic photosystem I*

09:40 – 10:10     **Kasim Ocakoglu** (Mersin University, TR)  
*Design of biohybrid electrodes for efficient light-to-current conversion based on photosystem I on graphene*

10:10 – 10:40     **Sebastian Maćkowski** (Nicolaus Copernicus University, PL)  
*Hybrid photosynthetic assemblies with plasmonically enhanced function*

10:40 – 10:45     **Piotr Węgleński** (Director of CeNT UW) – **Welcome**

10:45 – 11:15     **Coffee break**

**Session 2:**        *Interfacing electroactive proteins and modelling electron transfer at the interfaces of the biohybrid electrodes*

**Chair:** Kasim Ocakoglu

11:15 – 11:45     **Sławomir Sęk** (Faculty of Chemistry, University of Warsaw, PL)  
*Nanoscale molecular junctions incorporating peptides and peptidomimetics*

12:15 – 12:45 **Bartosz Trzaskowski** (Centre of New Technologies, Univ. of Warsaw, PL)  
*New hybrid materials and biomaterials - design and modelling of properties*

12:15 – 12:45 **Dominik Gront** (Faculty of Chemistry, University of Warsaw, PL)  
*Practical application of biophysical and bioinformatics methods for protein modelling*

12:45 – 13:45 **Lunch**

**Session 3: *Young Investigator Presentations***

**Chair: Sebastian Maćkowski**

13:45 – 14:00 **Patrycja Haniewicz** (Centre of New Technologies, University of Warsaw, PL)  
*Preparation of perfect biological material for constructing truly 'green' viable biohybrid solar-to-fuel nanodevices*

14:00 – 14:15 **Gleb Andryianau** (Centre of New Technologies, University of Warsaw, PL)  
*Towards well defined, highly organized nanoarchitectures for molecular engineering of bioelectrodes based on carbon materials*

14:15 – 14:30 **Ersan Harputlu** (Mersin University, TR)  
*Fabrication and characterization of graphene hydrogel and flexible graphene oxide films*

14:30 – 14:45 **Grzegorz Bubak** (Centre of New Technologies, University of Warsaw, PL)  
*Biohybrid electrodes based on positively and negatively charged reduced graphene oxide*

14:45 – 15:15 **Coffee break**

**Session 4: *Young Investigator Presentations***

**Chair: Joanna Kargul**

15:15 – 15:30 **Dorota Kowalska** (Nicolaus Copernicus University, PL)  
*Plasmon-induced absorption of blind chlorophylls in photosynthetic proteins assembled on silver nanowires*

15:30 – 15:45 **Marcin Szalkowski** (Nicolaus Copernicus University, PL)  
*Unusual energy transfer from photosynthetic antennae to graphene*

- 15:45 – 16:00    **Grzegorz Rotko** (University of Wrocław, PL)  
*Electrografting as an attractive way for covalent modification of graphene-based materials*
- 16:00 – 17:00    **Poster session (CeNT Atrium)**
- 17:00 – 18:00    **GraphESol Consortium Meeting**
- 19:00 – onwards    **Conference Dinner, Dom Polski Restaurant, Belwederska 18A, Warsaw**

## Tuesday, 22<sup>nd</sup> November

**Centre of New Technologies, University of Warsaw, Banacha 2C**

**Venue:**            **Lecture Hall 1130**

**Session 5:**        *Invited Lectures*

**Chair: Kasim Ocakoglu**

09:30 – 10:00    **Johannes Binder** (Faculty of Physics, University of Warsaw)  
*In-situ Raman studies of solution gated graphene*

10:00 – 10:30    **Barbara Pałys** (Faculty of Chemistry, University of Warsaw, PL)  
*Biosensing applications of electrochemically reduced graphene oxide*

10:30 – 11:00    **Coffee break**

**Session 6:**        *Young Investigator Presentations*

**Chair: Sebastian Maćkowski**

11:00 – 11:15    **Valentina Grippo** (Faculty of Chemistry, University of Warsaw, PL)  
*Lipidic cubic phase for hosting enzymes and improving their catalytic activity*

11:15 – 11:30    **Michał Kizling** (Faculty of Chemistry, University of Warsaw, PL)  
*Application of monomethyl ether methacrylate and ethylene glycol methacrylate phosphate copolymer as hydrogel electrolyte in enzymatic fuel cell*

11:30 – 11:45    **Maciej Dzwonek** (Faculty of Chemistry, University of Warsaw, PL)  
*Graphene – gold clusters hybrid matrix for enzyme immobilization*

11:45 – 13:00     **Lunch**

13:00 – 14:00     **Poster session (CeNT Atrium)**

14:00 – 15:00     **GraphESol Consortium Meeting**

15:00 – 16:00     **Solar Fuels Lab tour**

# Abstracts



# **Tackling the grand challenge of solar energy conversion using extremophilic photosystem I**

Joanna Kargul

*Solar Fuels Laboratory, Centre of New Technologies, University of Warsaw, Banacha 2C, Warsaw, Poland*

The amount of energy captured in one hour of sunlight that reaches planet Earth equals the total amount of energy produced by human population per year. To efficiently convert the effectively inexhaustible solar energy into high energy density solar fuels provides one of the main challenges for mankind faced with dwindling fossil fuel reserves. Natural photosynthesis sustains life on Earth as it provides nearly all the oxygen in the atmosphere, the food we consume and fossil fuels that we burn to run our present day economies. Imitating the reactions that occur at the early stages of photosynthesis represents the main challenge in the quest for construction of a viable ‘artificial leaf’. In this talk I will present our recent efforts on construction of the biohybrid solar-to-hydrogen biophotocatalytic cells and dye-sensitised solar cells that employ a highly efficient molecular machine of photosystem I (PSI), serving as light harvesting/charge separating biological module. I will also introduce our new bilateral initiative PolTur/GraphESol on applying graphene in conjunction of highly robust extremophilic PSI to construct a viable biophotocatalytic electrode.

Research is supported by the grant no. DZP/POLTUR-1/50/2016 funded by the Polish National Centre for Research and Development.

## **Design of biohybrid electrodes for efficient light-to-current conversion based on photosystem I on graphene**

Kasim Ocakoglu <sup>a,b</sup>

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The construction and development of photoactive biohybrid electrodes incorporating stable natural photosynthetic complexes has attracted increasing attention over the past few years. A proper combination of the photosynthetic core protein and key materials attracts great attention to build hybrid devices for solar energy conversion. These biohybrid electrodes can also be used for different applications such as biosensors, biophotovoltaic devices, circuits, nanotransducers etc [1-3]. The effective electrical wiring of Photosystem I (PSI) to various materials is of fundamental importance for functional photobiohybrid devices generating higher photocurrent densities. Up to now, several strategies has been published applying different surface design and

chemistry. These studies reveal that it is essential to use electrical signal chains with electron shuttling systems or conducting junctions for the strong improvement in electrode performance [4-6]. Graphene with its intriguing properties: unique electron transport properties and flatness of the surface, high elasticity, large thermal conductivity and elevated transparency over the entire visible spectrum, is close to an ideal electrode to promote energy transfer from ultrastable and highly active natural photosynthetic systems. Most of the reported graphene-based surface plasmon resonance biosensors utilize graphene oxide, reduced graphene oxide, or graphene decorated metal nanoparticles as a sensing platform. However, the main limitation of this approach is the lack of homogeneous and defect-free monolayers on the surface plasmon resonance sensing platform, hindering the exploitation of the benefits of graphene. Here we report design and preparation of biohybrid electrodes bearing extremely stable natural photosynthetic complexes.

**Acknowledgements:** This research has been financially supported by The Scientific and Technological Research Council of Turkey, TUBITAK (Grant number: 215M389).

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## Hybrid photosynthetic assemblies with plasmonically enhanced function

Sebastian Maćkowski

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In the presentation we describe several results that demonstrate the potential of hybrid photosynthetic assemblies for enhancing the function of the natural photosynthetic complexes in the context of light harvesting, as well as energy and charge transfer. While the actual architectures are different, the tools used to study them revolve around fluorescence microscopy and spectroscopy techniques, both in steady-state and time-resolved variations.

After describing the basic concept of plasmonic interaction using the simplest cases of a molecule and a single metallic nanoparticle, more complex structures will be discussed.

Particular emphasis will be put on devising proper design of the hybrid nanostructure, both in regard to the choice of metallic nanoparticles as well as coupling architecture. In addition, variety of photosynthetic complexes will be described, starting from the simplest light-harvesting systems, with two chlorophylls, up to reaction centers where hundreds of pigments are present. Complexity of an applied system substantially affects the degree of complication in analyzing and understanding the experimental results, Nevertheless, by proper design, enhancement factors reaching 100s can be achieved.

Overall, the results of optical spectroscopy of hybrid nanostructures that involve natural photosynthetic complexes, indicate that by proper choice and control of the morphology of plasmonic nanostructures offer an attractive pathway for manipulating and presumably improving the function of photosynthetic complexes.

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## Nanoscale molecular junctions incorporating peptides and peptidomimetics

Sławomir Sęk<sup>a,b</sup>

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Peptides are involved in numerous biologically relevant processes and their broad range of functions results from ability to adopt variety of structural motifs. Therefore when suitably designed, peptides can provide desired properties that are useful in biosensing devices as well as in nanoscale electronic circuits. However, there are still numerous problems which need to be solved in order to design and build functional molecular devices such as molecular wires, logic gates, switches, or molecular-scale transistors. One of the crucial issues is to understand the fundamental mechanisms determining efficiency of charge transport through these molecules. Numerous experimental approaches can be utilized to probe electron transport efficiency through

organic molecules. Among them, scanning probe microscopy (SPM) offers unique capability to investigate electric properties of individual molecules or molecular films at nanoscale.<sup>1</sup> In general this method involves entrapment of the molecules between two metallic contacts established by metal support and SPM probe. The electric properties of the resulting metal–molecule–metal junction can be probed while the bias voltage is applied between the electrodes. The additional advantage of SPM-based method comes from the fact that it enables modulation of the efficiency of peptide mediated ET by controlling mechanical strain or stress of the molecules entrapped within the junction.<sup>2</sup> Using such experimental approach, it is possible to probe electric properties of peptides as well as peptide-mimicking compounds.

#### References:

<sup>1</sup> Sęk, S. “Review - Peptides and proteins wired into the electrical circuits: an SPM-based approach” *Biopolymers Peptide Science* **2013**, *100*, 71-81

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## **New hybrid materials and biomaterials – design and modelling of properties**

Bartosz Trzaskowski

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The aim of our research project is to computationally study the interactions between light harvesting proteins and graphene, in order to gain better understanding on the direct electron transfer mechanism and binding interactions (improved stability) of the hybrid system. This work will contribute to shed light on the nature of the hybrid photoactive proteins and graphene materials and the possible use of this new hybrid system as biosensors, bio-organic photovoltaic cells (bio-OPV) and bio-organic transistors (bio-OFET).

## **Practical application of biophysical and bioinformatics methods for protein modeling**

Dominik Gront

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A few past decades witnessed a tremendous advance both in computational biophysics and bioinformatics. Although these two fields rely on different principles, both can be very helpful for experimental biologists, providing insights into biomolecular structure, dynamics and sometimes, its function. In this talk I briefly summarise the most important areas of applications from both fields.

# Preparation of perfect biological material for constructing truly ‘green’ viable biohybrid solar-to-fuel nanodevices.

Patrycja Haniewicz<sup>1</sup>, Mateusz Abram<sup>1,2</sup>, Joanna Kargul<sup>1</sup>

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Nature has created amazing process of photosynthesis that allows to convert the solar energy, water, and carbon dioxide into carbohydrates and oxygen. Nowadays it is very important to construct truly ‘green’ viable biohybrid solar-to-fuel nanodevices that mimic the most crucial steps of the early events of photosynthesis to produce fuels, such as molecular hydrogen and renewable carbon-based fuels.

The photosynthetic apparatus of extremophilic microalgae has gained considerable interest due to the exceptionally high enzymatic stability and activity of its photoactive components, making them attractive for biophotovoltaics and solar-to-fuel artificial leaf technology. The extremophilic red alga *Cyanidioschyzon merolae* contain the monomeric PSI-LHCI supercomplex that represents an intermediate evolutionary link between the prokaryotic cyanobacterial PSI reaction centre and green algal/higher plant counterpart.

In our work we developed a procedure to obtain highly homogenous PSI-LHCI samples by detergent-based solubilisation of thylakoids. We applied a 3-step anion exchange and a size exclusion chromatography (AEC and SEC) to obtain ultrapure supercomplex preparations. Our work shows that obtain *C. merolae* PSI-LHCI supercomplex is characterized by exceptional robustness in various extreme conditions, such as high temperatures, low/high pH and high light that makes it an ideal biologic material for construct truly ‘green’ viable biohybrid solar-to-fuel nanodevices.

**Acknowledgements:** PH, MA and JK are funded by the Polish National Science Center (OPUS8 grant no. UMO-2014/15/B/NZ1/00975).

## Towards well defined, highly organized nanoarchitectures for molecular engineering of bioelectrodes based on carbon materials

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The acceleration rate of our civilization development as well as dwindling stocks of fossil energy sources clearly show that the increase of energy production and management efficiency is a *condicio sine qua non* for the further growth. Widely understood artificial photosynthesis can cope with this great challenge supplying both new generation of eco-friendly solar fuels and high added-value chemicals. To make it real, it is required to find new approaches for molecular designing of photosynthetic devices utilizing the possibilities of recently developed materials and architectures.

Nowadays, the field of application of carbon-based materials is spreading fast due to some unique properties – either high electric conductivity or versatility and relative modification simplicity. Moreover, the production of these materials can be scaled up making real their industrial application. We are going to investigate graphene (or reduced graphene oxide) as well as glassy carbon as a base material for photoelectrodes construction.

Natural biomolecular photosystem as an “engine” for artificial photosynthetic devices is considered to be one of the most promising solutions. In this research we will use the robust photosystem I protein complex, obtained from an extremophilic red microalga *Cyanidioschyzon merolae*, as the biological module of the biohybrid graphene photoelectrode. Exposed to visible light this highly specialized natural photoactive protein-pigment complex is able to generate the charge separation state with an internal quantum yield reaching unity. Due to the high anisotropy of PSI complex the appropriate orientation with respect to the electrode surface is required for the efficient electron transfer.

Here we are going to report two approaches to obtain highly-organized, defined nanoarchitectures of PSI-graphene electrodes. Specifically, we will dissect radical grafting of substituted aryl moieties, able to react in Huisgen 1+3 dipolar cycloaddition. This modification enables designing of several architectures based on i.e. Ni-NTA immobilization strategy as well as a conception of self-assembly coordination molecular wires. We assume that our specific design of the surface interfaces will be suitable for (photo)electroactive proteins immobilization providing a new generation of efficient carbon-based (bio)photoelectrodes. In addition to this, we propose a highly universal chemical platform for designing various molecular constructs requiring high molecular organization as well as efficient charge transfer.

**Acknowledgements:** Research is supported by the grant no. DZP/POLTUR-1/50/2016 funded by the Polish National Centre for Research and Development.

# Fabrication and characterization of graphene hydrogel and flexible graphene oxide films

Ersan Harputlu

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Increasing attention of graphene hydrogel has been attracting in interdisciplinary research areas due to its marvelous physicochemical properties. Graphene, which is composed of single-layer  $sp^2$  hybridized carbon atoms, can be cost-effectively produced from natural graphite by using Hummers' method. By this method, Graphene Oxide (GO) is prepared by treating graphite with a mixture of sulphuric acid, sodium nitrate and potassium permanganate which are strong oxidizers. These oxidizers work by reacting with the graphite and removing an electron in the chemical reaction. GO has greater aqueous solubility than graphene due to its oxygen-functionalities. Various chemical methods can be applied to GO, which has encouraged a new research area called "wet chemistry of graphene" [1-4]. Moreover, graphene hydrogels prepared by hydrothermal reduction of GO dispersions. Graphene hydrogel and flexible reduced GO films demonstrate high performance for electronic and biological applications. For example, graphene hydrogel exhibits exceptional electrical conductivity and mechanical robustness to make it an excellent material for flexible energy storage devices and solid-state supercapacitors [5, 6]. Here we report fabrication and characterization of graphene hydrogel and flexible GO films, and also integration of semiconductor materials on graphene surface.

**Acknowledgements:** This research has been financially supported by The Scientific and Technological Research Council of Turkey, TUBITAK (Grant: 215M389).

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# Biohybrid electrodes based on positively and negatively charged reduced graphene oxide

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The photosynthesis is one of nature's most fundamental light-driven processes in which carbon dioxide and water are converted into oxygen, reducing equivalents and a cellular 'fuel', ATP. Recently, solar conversion technologies based on natural solar energy converters, such as photosystem I (PSI) have attracted extensive attention. PSI is a pigment-protein complex that captures light in its specialized reaction centre (P700) to power up the electron transfer chain, ultimately leading to reduction of NADP to NADPH. As such it constitutes one of the components of the photosynthetic apparatus that is present in cyanobacteria, plants and algae. Extremophilic algae, such as an acido-thermophilic red microalga *Cyanidioschyzon merolae*, provide a particularly good source of highly robust and highly active photosynthetic complexes that can be successfully incorporated by our laboratory into biophotocatalysts.

Here, we propose the design of biohybrid electrodes comprising positively and negatively charged reduced graphene oxide (rGO) and PSI modules. The variation in the surface charge of the rGO and PSI is proposed to allow for achieving the preferable orientation of the modules through their specific electrostatic interactions. Even though, reduced graphene oxide is not free of defects it is relatively easy and cheap to produce. In addition, electrode fabrication can be scaled up without any laborious steps using techniques such as dip-coating, spray-coating and spin-coating.

Moreover, we will discuss the use of cytochrome *c<sub>553</sub>* and ferredoxin from *Cyanidioschyzon merolae* as the native electron donor or acceptor of PSI, allowing for a better control of PSI orientation and electron flow within the biophotocatalyst.

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# Plasmon-induced absorption of blind chlorophylls in photosynthetic proteins assembled on silver nanowires

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The natural converters of solar energy, such as photosystem I (PS I), are more and more often applied as building blocks for photosensors, optoelectronic components, and photovoltaic devices. On the other hand, it is well known that interaction between metallic nanoparticles and fluorescent molecules can lead to strong modifications of their optical properties. Particularly attractive in this regard are systems with controlled morphology. In this work we assemble a hybrid nanostructure composed of an oriented monolayer of PSI-LHCI from *C. merolae* on functionalized silver nanowires (AgNWs). The results were compared to a system, where PSI-LHCI was simply physically adsorbed on AgNWs.

Silver nanowires were synthesized from silver nitrate in ethylene glycol, using polyvinylpyrrolidone as a capping agent. Controlled assembly of PSI-LHCI on AgNWs was achieved by immobilization of a monolayer of PSI-LHCI - using the cytochrome c553 - on silver nanowires modified with Ni(II) nitrilotriacetic acid (NTA) alkanethiol complex. This conjugation scheme can facilitate efficient electron tunneling between the protein-embedded electron-transfer cofactors and the metal surface due to very small distance between photosynthetic complexes and AgNWs.

The results of fluorescence imaging reveal two important effects: (1) the emission of the PSI-LHCI complex is not quenched upon coupling to the AgNWs, and (2) oriented deposition of protein complexes on silver nanowires triggers, via plasmon interaction, qualitative modifications in the absorption of the PSI-LHCI complex. Namely we find that due to proximity between certain cofactors to the AgNWs, the absorption in the green region is an order of magnitude stronger than for a structure where only physical adsorption was used.

We demonstrate, by using fluorescence imaging, that control over the morphology of plasmonically-active hybrid nanostructures offers a new pathway for manipulating and refining the optical properties of photosynthetic complexes, in particular inducing strong absorption of light in normally weakly active spectral regions. Therefore, such nanostructures can be considered as building blocks for future biohybrid functional solar energy conversion devices.

## References:

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## Unusual energy transfer from photosynthetic antennae to graphene

Marcin Szalkowski<sup>a</sup>, Alessandro Surrente<sup>b</sup>, Dorota Kowalska<sup>a</sup>, Izabela Kamińska<sup>a</sup>, Paulina Płochocka-Maude<sup>b</sup>, Sebastian Maćkowski<sup>a</sup>

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In last decade graphene and its derivatives have been the subject of intensive studies. Some of the research concerns exploiting the acceptor properties of graphene, as its absorption is constant and spans over the whole visible range <sup>[1]</sup>. Moreover, high electrical conductivity and broad possibilities of surface functionalization render graphene as an attractive material for electrodes in solar cell architectures.

We present optical studies of a hybrid system consisting of a graphene layer and photosynthetic complexes – antennae Peridinin-Chlorophyll-Protein complexes (PCP) from the algae *Amphidinium carterae*. This water-soluble complex has well-known structure and energetic relations between its components, therefore it is widely used as a model system for examination of nanoscale interactions.

We focus on the energy transfer from PCP to graphene. Fluorescence steady-state and time-resolved microscopy studies, have shown strong quenching of the fluorescence intensity of photosynthetic complexes when deposited on graphene. Surprisingly we found that fluorescence quenching in such hybrid system is nonlinear – despite the uniform absorption of graphene - and efficiency of this process strongly depends on the excitation wavelength <sup>[2]</sup>. Detailed studies, where fluorescence excitation spectra were measured, reveal that the efficiency of the energy transfer is significantly enhanced in the blue spectral region, whereas it is highly suppressed for longer wavelengths. This result points towards rather unique acceptor properties of graphene, that can be exploited in energy transfer assemblies.

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# Electrografting as an attractive way for covalent modification of graphene based materials.

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Electrografting is a method that uses the electrochemical reaction for modification of the surface of conducting materials. The highly reactive intermediates (such as aryl radicals) are generated in the close proximity to the surface in the course of the redox process and subsequently react with the surface functional groups (covalent bond is formed). The material functionalized with the anchor groups can be used for immobilization of more complex molecules (e.g. enzymes).

The popularity of this technique in the field of electrode modifications results from the high stability of the connection between conductor and organic layer. Electrografting can be applied to a variety of materials including metals and their oxides, different forms of carbon as well as semiconductors. As a precursors for organic layer can be used inter alia aromatic diazonium salts and other onium salts as well as aliphatic amines.

However, it should be noted that the radical nature of the process entails free radical polymerization and formation of thick and generally poorly conductive film which in turn reduces the electron transfer rate through the electrografted layer. This disadvantage for a long time hampered the usage of such chemically modified electrodes. Only recently procedures enabling the selective monolayer formation have been developed. Among the applied strategies, the most effective proved to be a method in which as the modifying agent is used aryl diazonium salt containing large substituent which can be easily removed after electrografting by using chemical or electrochemical reaction. The undoubted advantage of this approach is that even if a polymer film is formed, it may be readily removed in a subsequent treatment.

Electrode modification protocols using electrografting will be presented as well as advantages and disadvantages will be discussed. Furthermore, preliminary results of attempts to modify of the electrochemically reduced graphene oxide on glassy carbon electrode will be shown.

# In-situ Raman studies of solution gated graphene

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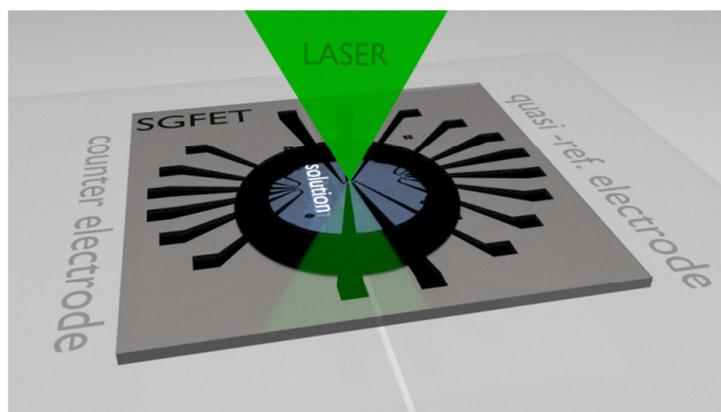
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Solution gated field-effect transistors (SGFETs) are promising candidates for future graphene based sensing devices. However, to obtain a selective detection, the graphene surface has to be functionalized. Having said that, one of the drawbacks of such functionalized transistors is that one has to rely only on the changes in channel resistance, which is a measure that can be influenced by many side effects.

We address this issue by reporting on an approach that combines a SGFET with *in-situ* Raman spectroscopy [1]. Epitaxial graphene on SiC is used as sensing material, which allows to measure Raman spectra through the transparent substrate (see Fig. 1) enabling to disentangle the behavior of the SGFET as a whole from chemical modifications of the graphene layer.

The use of a water / NaCl solution allowed us to steer the carrier concentration for bilayer graphene in a range of about  $\sim 2 \times 10^{13} \text{ cm}^{-2}$ , thanks to an electrical double layer at the surface. The changes in concentration enabled the observation of the *resonant electron-phonon coupling* as well as differences in *inversion symmetry breaking* in the Raman spectra of bilayer samples with and without a buffer layer.

Regarding more application oriented results, we were able to follow the reversible chemisorption of hydrogen on an SGFET *in-situ* with Raman spectroscopy, as evidenced by the emergence of C-H and Si-H bonds in the Raman spectra. Our setup hence constitutes a powerful tool for *spectroelectrochemical* measurements of graphene on the micrometer scale.



**Fig. 1.** 3D visualization of the SGFET combined with *in-situ* Raman spectroscopy.

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## **Biosensing applications of electrochemically reduced graphene oxide**

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The electrochemical reduction of graphene oxide (GO) enables the direct deposition of the graphene – like layer on various conductive surfaces. Another advantage of the electrochemical reduction is a possibility to carry out the partial reduction of GO, enabling easy modification of the surface with oxygen containing groups. The number of surface groups can be tuned by the parameters of the electrochemical process, for example by the number of the reduction cycles. In this talk the electrochemical reduction of GO on the glassy carbon and on the nanostructured gold surfaces will be discussed. The infrared and Raman spectra of the resulting reduced GO layers will be reviewed. Further the activity of model enzymes: laccase and horseradish peroxidase immobilized on the electrochemically reduced graphene oxide (ERGO) layers will be discussed.

The results show that the number of the reduction cycles influences the enzyme activity. The reduction of GO in first cycles improves the electric conductivity of the ERGO layer, what improves in turn the electrocatalytic signals of the immobilized enzyme. Although, removing all the oxygen containing surface groups causes the deterioration of the signal due to the worse communication between the enzyme active site and the electrode surface<sup>1</sup>. The gold nanostructures influences properties the ERGO and the electrocatalytic signals of the enzyme on the ERGO-nanoAu surface<sup>2</sup>.

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## **Lipidic cubic phase for hosting enzymes and improving their catalytic activity**

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Lipid cubic phase systems are an excellent immobilizing carriers for enzymes due to their biocompatibility and well-defined pore nanostructure.<sup>1</sup> They have been proposed as a convenient matrix for incorporating enzymes and holding them on the electrode surface in a fully active form. 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.<sup>2</sup>

We used cubic phase for immobilizing *Corynascus thermophilus* CDH (*Ct*CDH). CDH is an extracellular oxidoreductase secreted by wood degrading fungi. It oxidizes sugars to their respective lactones. The two electrons gained during the process are transferred to a monooxygenase which reacts with oxygen to form radicals and carry out the wood decomposition. It consists of two separate domains joined together with a short polypeptide linker region. The larger domain is the flavodehydrogenase domain ( $DH_{CDH}$ ), and it is the catalytically active. Electrons can be transferred to a one or two-electrons acceptor (in mediated electron transfer conditions, MET) or alternatively can be shuttle through an internal electron transfer (IET) to the cytochrome domain ( $CYT_{CDH}$ ). Electrons are then transferred in direct electron transfer (DET) conditions to the natural acceptor or to the electrode surface.

Here we report the improvement gained by the *Ct*CDH when trapped in a monoolein cubic phase in mediated and direct electron transfer conditions. Ruthenium ammonium chloride,  $Ru(NH_3)_2Cl_2$ , midpoint potential at pH 7.4 equal to -136 mV, was successfully used as mediator.

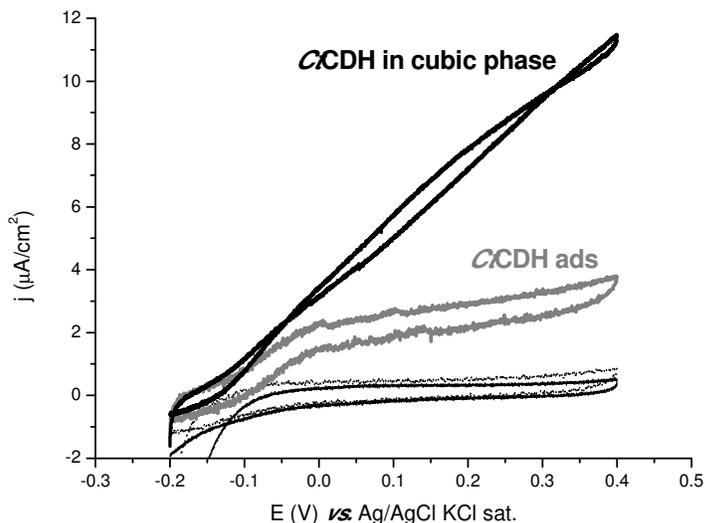


Figure 1 Comparison of CDH behavior in cubic phase or adsorbed on the electrode surface. Lactose 100mM. PBS pH 7

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## **Application of monomethyl ether methacrylate and ethylene glycol methacrylate phosphate copolymer as hydrogel electrolyte in enzymatic fuel cell**

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Research in the field of material engineering has led to advances in hydrogel science and technology supporting a wide spectrum of applications in biomedicine, agriculture, tissue engineering, aquaculture, infant care and nanotechnology [1].

In the last decade, utilization of a solid-state electrolyte containing an hydrogel has been considered as a substitute of a liquid electrolyte for different electrochemical devices. However liquid electrolytes have high ionic conductivity, thus allowing quick ion migration between opposite side electrode which enables obtaining large amount of electricity, they have also numerous disadvantages. Using liquid electrolyte can be dangerous both for its direct user, as well as for the environment. Hazard of electrolyte spill in form of concentrated inorganic compounds (acids, bases), sometimes even spill of organic solvents that can be used as constituents, low electrochemical stability, inability to miniaturize the battery. Therefore, application of hydrogel electrolytes were considered for various different electrochemical devices, such as supercapacitors, lithium-ion or zinc-air batteries [2].

Our idea was to utilize the same approach in enzymatic fuel cell field [3]. In our study, we describe application of solid state electrolyte in enzymatic fuel cell based on derivatized carbon nanotubes, fructose dehydrogenase for fructose oxidation and laccase for oxygen reduction. The hydrogel sheet ensures ion conduction between electrodes, serves as the fuel reservoir that could eliminate the necessity of packaging and prevent of quick desorption of enzymes, therefore enhance mechanical durability of modified electrodes. Also, such approach provides new architecture application, inaccessible for EFC with liquid electrolyte. Hydrogel composed of monomethyl ether methacrylate and ethylene glycol methacrylate phosphate with triethylene glycol dimethacrylate as a linking agent was thoroughly described and used in electrochemical characteristic of each electrode activity test and full EFC as well.

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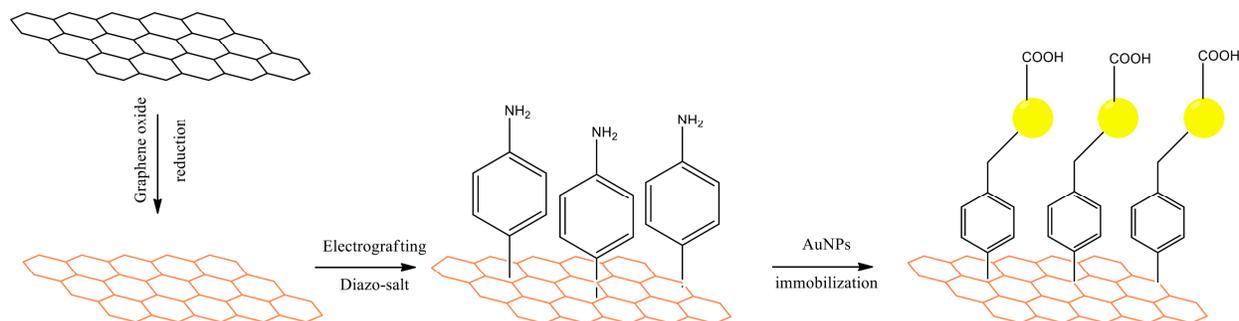
# Graphene – gold clusters hybrid matrix for enzyme immobilization

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The properties of metal nanoparticles depend on size and shape. Gold nanoparticles of 2 nm diameter differ from larger nanoparticles in terms of optical, electronic and chemical properties. Small particles can behave more like molecules than as particles and exhibit enhanced catalytic activities and interesting charging properties [1].

Here we present the synthesis of a hybrid matrix for enzyme immobilization using graphene layers and small gold nanoparticles for improvement of conducting properties of graphene and for binding protein molecules. We synthesized graphene oxide using modified Hummers-Offeman method [2], which was further electrochemically reduced to graphene electrodes. On the carbon substrate we introduced functional groups by electrochemical reduction of appropriate diazonium salts and which allow to bind the gold clusters [3].



Our procedure will enable to immobilize enzyme molecule effectively on the hybrid matrix and retain its catalytic activity.

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# Functional graphene oxide coatings on metallic surfaces

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In the presented project we propose a coating of graphene oxide (GO) onto stainless steel and gold surfaces. Such coatings can isolate the surface of metal from direct contact with tissues and will preserve beneficial characteristics of metals such as current conductivity. Moreover, the functionalisation of graphene oxide by attachment of peptides or antibodies can lead to novel properties of the material such as active prevention from blood clotting. Also the introduction of functional groups attached to gold via graphene layer can be useful in development of gold based biosensors.

## Materials and Methods

In the study a method of coating gold surfaces with GO which comprises binding GO to gold with the use of gold-sulphur bonds has been applied. For that purpose on a thoroughly cleaned surface of gold sputtered onto a glass slide, or a gold SPR sensing chip, a self assembled monolayer (SAM) of compound containing thiol and amine groups (such as cysteine or cystamine) has been created. Subsequently, a well known technique of amine coupling has been used for binding carboxyl groups of GO with amine groups in a two-step amine coupling process.

In order to prepare GO coatings on stainless steel we used two coating methods: electrodeposition and dip-coating. In both cases we focused on the selection of process conditions including the composition of the electrolyte solution, its pH, concentration of graphite oxide or the addition of the substance to ensure better adhesion of GO to the steel. We also tested a quality of obtained materials using techniques such as infrared spectroscopy or scanning electron microscopy.

Graphite oxide foils as well as foils made of graphite oxide copolymers with aldehydes and amines have been demonstrated and are currently under investigation as alternative coating techniques

## Results

FTIR spectra confirmed the presence of hydroxyl and carbohydrate moieties on a surface of the modified material. The modified gold sensing chip has been used in refractive index measurement experiment and exhibited sensitivity to glucose concentration. The results indicate a positive effect of coatings on biocompatibility of steel surface. In all cases the viability of cells incubated with extracts prepared from coated materials was higher than after incubation with extracts derived from uncoated steel. There is a possibility that obtained coatings increase biocompatibility of steel. We also found the best conditions for coating processes such as time, concentration or pH of solution.

## **Conclusions**

Presented methods for coating steel and gold surfaces are innovative, simple and can be used in large scale for coating different workpieces. Moreover, it is not required to use toxic, organic solvents. There is thus a possibility to create a new biomaterial, which could find an application in medicine, however further investigation on biocompatibility and sensing properties is strongly required and will be performed.

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