Abstract:

Title of Project: Combined electrochemical and vibrational spectro-electrochemical studies of enzymatic processes on MIP covered model electrodes

Co-supervisor 1: Ingo Zebger, TUB (SPP 1927, UniSysCat)

Co-supervisor 2: Frieder Scheller (Ulla Wollenberger), UP (UniSysCat)

The project is dedicated to establish methodical approaches for studying molecular mechanisms of binding and electro-enzymatic catalysis of hydrogenases and Moco-enzymes on MIP-covered electrodes. These enzymes are key components of biofuel cells and electro-enzymatic substrate conversion. The approaches will be based on vibrational spectro-electrochemical studies of electro-synthesized nm-thin cover layers of Molecularly Imprinted Polymers (MIP) on conducting surfaces. MIPs allow the regeneration of the protein target and uniform “productive” orientation of enzymes for bioelectrocatalysis via direct electron transfer. MIPs for hydrogenases and Moco-enzymes on three structural levels of the target protein will be analyzed by surface sensitive IR spectroscopy: (i) the holoprotein, (ii) a subunit or a domain of the protein and (iii) an exposed peptide sequence or peptide tags. We will focus on the oxygen tolerant bidirectional, NAD^+ reducing soluble hydrogenases, the isolated large subunit of regulatory hydrogenase and Moco-containing enzymes, such as TMAO reductase.

Extended version of the project:

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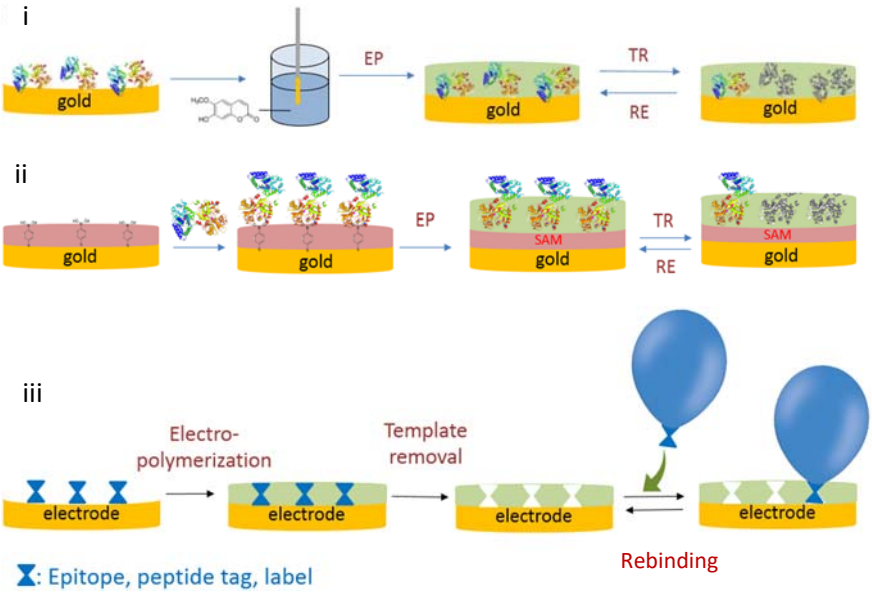
1. Overall goal of the project

The main objective of the project is the realization of an effective electron transfer between the Molecularly Imprinted Polymer (MIP) - covered (transparent) conductive surface and the active site of hydrogenases and Moco-enzymes on the basis of electrochemical and spectroscopic analyses. For this reason, conductive electropolymers containing metallic nanoparticles, carbon nanotubes and/or graphene-will be synthesized on the electrode surface. Applying a short peptide tag (e.g. Strep-Tag; His_6-tag) or the holoprotein as the template during electropolymerization will open pathways to molecularly imprinted layers with metallic conductivity. The molecularly Imprinted Polymer will provide a stabilizing scaffold for the enzymes will also allow spectro-electrochemical investigation of the enzyme during immobilization and catalysis.

2. State of the art

Whilst enzymes and antibodies are made up of 20 natural amino acids, Molecularly Imprinted Polymers (MIPs) can be synthesized from only ONE monomer. This is a remarkable reduction of complexity. In order to achieve structuring on the molecular level, conductive surfaces have been covered with ultrathin layers of MIPs. They allow the regeneration of the protein target, uniform "productive" orientation of enzymes for bioelectrocatalysis via direct electron transfer (DET) and potentially the stabilization. MIPs for proteins have been developed on three structural levels of the target protein: (i) the protein is entrapped in the polymer, (ii) a subunit or a domain of the protein is the target and (iii) an exposed peptide sequence of the target protein - the epitope- or artificial peptide tags of engineered proteins, sugars of glycoproteins and even chemical label of macromolecules are the template during polymerization.



Workflow of MIP preparation i) in two-steps that involves the pre-adsorption of the target protein followed by electropolymerization of the monomer around the surface-confined targets, ii) affinity binding of the target to a self-assembled anchor layer for oriented immobilization of the protein followed by electropolymerization. iii) only protein fragments were used as templates.

With this approach we established i.a. the controlled immobilization of the hexameric heme protein with direct electron transfer as well as peroxide electrocatalysis and elucidated the shape-speci-ficity for subunit-imprinted polymers (Cyt P450 BM3).[1, 2] Further, Surface Enhanced Infrared Absorption (SEIRA) was not only established to monitor the different steps of the MIP-film preparation (*in-situ*) in combination with electrochemical methods[3, 4], but also allowed to follow the immobilization and catalytic reaction of hydrogenase enzymes on the (gold) electrode surface.[5, 6]

3. Specific aims and how they may be reached

A. Combination of EC and SEIRAS to characterize (conductive) enzyme MIPs

Since electrochemical MIP-synthesis and DET represent the core of the project, the selection of appropriate electrode materials is essential. We will immobilize the target prior to electropolymerization either by affinity binding to a self-assembled mono layer for oriented immobilization a gold surface or by a hexahistidine-tag to the surface of transparent conducting electrodes. Around the target either non-conducting or conducting polymers will be deposited by electropolymerization. All the steps of MIP synthesis will be characterized in parallel by electrochemical methods and SEIRA spectroscopy. Subsequently, characteristic reactions of the protein will be monitored to prove the immobilized protein integrity. To ensure a sufficient electronic conductivity, metallic nanoparticles, carbon nanotubes and/or graphene will be entrapped in the polymer or alternatively the electron transfer might be realized by a conductive cover layer.

B. Target proteins: [NiFe] hydrogenase (subunit), MoCo-enzymes

Whilst membrane bound [NiFe]-hydrogenase [MBH] from *Ralstonia eutropha* is the subject in the UniSysCat supported MIP-project here we will focus on the cluster relevant oxygen tolerant bidirectional, NAD^+ reducing soluble hydrogenases (SH), the isolated large subunit of regulatory hydrogenase (HoxC) and the Moco-containing trimethylamine N-oxide (TMAO) reductase (TorA) from *E.coli.* In the two latter “minimal” enzymes, which carry only active sites, other or even “artificial” catalytic centers can be potentially introduced into the respective apoproteins to modify their pro-duct selectivities. The immobilization of the different target protein will be guided by attaching specific affinity tags, implementing a genetically engineered cysteine for the binding onto a gold electrode or complementary charged self-assembled monolayer for the interaction with specific surface regions of the protein. The latter will be guided by the use of molecular dynamic (MD) simulations.

C. Mechanistic Studies

Surface sensitive vibrational spectroscopic techniques like Infrared and Resonance Raman spectroscopy under potential in combination with electrochemical studies will be not only used to monitor the MIP formation but also to follow the different catalytic reactions. While redox states of the hydrogenase enzyme molecules can be followed in “isolated” spectral windows, the interaction of the other enzymes with their substrates and related structural change will be followed via IR-difference spectroscopy. Changes at the Mo-active site might be detected by (surface-enhanced) resonance Raman spectroscopy.

4. General information

The project is a combined project involving molecular imprinting, electrochemistry, carried out in the group of F. Scheller supported by A. Yarman, U .Wollenberger, and spectro-electrochemistry, which will be done in the Zebger group. Here all required set-ups are readily available, as well as the know-how for the assembly of MIP’s, which will transferred to Zebger group, with respect to the longterm perspective of this important protein immobilization technique in UniSysCat. A. Yarman, who applied for an own DFG project on her topic, will be the key person in supervising the development of protein-MIPs and the know-how transfer. F. Scheller will concomitantly act as a senior advisor. The project relies upon the established collaboration with the research groups of S. Leimkühler, UP (Moco enzymes) and O. Lenz, TU Berlin (hydrogenases). The work will be further supported by several groups which provide functional electrode materials (M. Driess, A. Thomas, TUB; A. Fischer, ext.), molecular catalysts (C. Limberg, HU) and computational support (M.A. Mroginski, TUB).

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