



PISA UNIVERSITY

**Electrochemical deposition of mixed  
Self-assembled monolayers for biosensors  
-Bachelor Thesis proposal-**

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## Overview

The reproducibility of covalent surface functionalizations is a topic of utmost importance for biosensor development. Self-assembled monolayers are organic assemblies that spontaneously form on the (semi)-crystalline surfaces of metals, metal oxides and semiconductors and can readily be functionalized with recognition elements for analytes (Antibodies, Aptamers, Enzymes, ...).<sup>1</sup> The deposition of ethyleneglycol terminated thiols is herein a frequently used substrate as it does not only readily form SAMs, but is also resistant to the non-specific adsorption of proteins from solution. The formation of these monolayers is usually formed simply by dipping the electrodes in an ethanolic solution of alkylthiols.<sup>2</sup> Methods for electrochemically assisted deposition of SAMs have been reported,<sup>3</sup> but to our best knowledge no publication towards the optimization of the potential dependent deposition of these mixed monolayers has been published. This Bachelor thesis project is supposed to deal with the questions of optimization of a mixed monolayer deposition of ethyleneglycol terminated thiols as base for functionalization with recognition elements for biosensor formation.

**The two principal questions to solve in the thesis are:**

*In what time does a complete monolayer form?*

*And what electrode potential is optimal?*

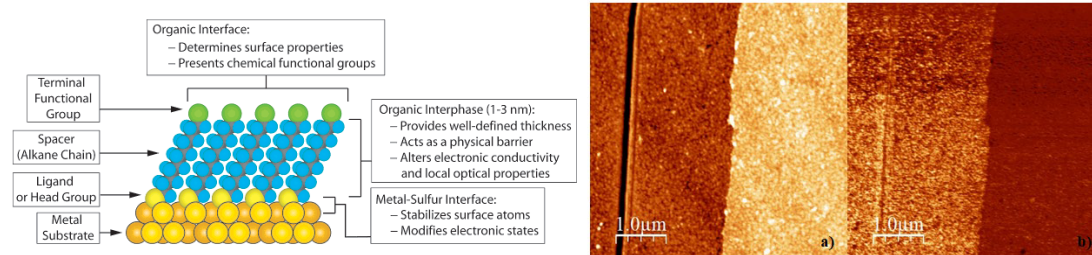


Figure 1: General build-up of alkylthiol SAMs on gold electrodes. Immagini ottenute all'AFM a) la superficie di un elettrodo a film sottile con SAM e parzialmente grattata b) l'immagine di suscettibilità elettrica, la zona a sinistra indica la superficie parzialmente grattata. (Bachelor thesis Stefano Pieroni)

Electrochemical techniques for the characterization of the surface layer formed are Voltammetric impedimetric and capacitive measurements. Further microscopical assessment of the formed monolayers we intend to perform STM and AFM studies. (QCM?)

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<sup>1</sup>Love et al. Self-Assembled Monolayers of Thiolates on Metals as a Form of Nanotechnology, Chem. Rev. 2005, 105, 1103-1169

<sup>2</sup>Carrara et al. Label-free cancer markers detection by capacitance biochip, Sensors and Actuators B 136 (2009) 163-172

<sup>3</sup>Riepl et al. Electrical Control of Alkanethiols Self-Assembly on a Gold Surface as an Approach for Preparation of Microelectrode Arrays, Mikrochim. Acta 131, 29-34 (1999)

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## Background

The thesis is embedded in our greater intent to develop a biosensor for proteinaceous analytes in solution as depicted in Figure 2. Herein a gold sensor surface was functionalized with an ethylenglycol terminated SAM surface in order to produce a SPR (Surface-Plasmon Resonance) based biosensor. A mixed monolayer, comprised of ethylenglycol terminated mercaptoundecane and mercaptoundecane terminated with a carboxylic acid were used therein. The carboxylic acid groups were further activated for amine coupling (EDC/NHS) and then an enzyme inhibitor analogue (Benzenesulfonamide) for the target analyte carbonic anhydrase deposited. Varying

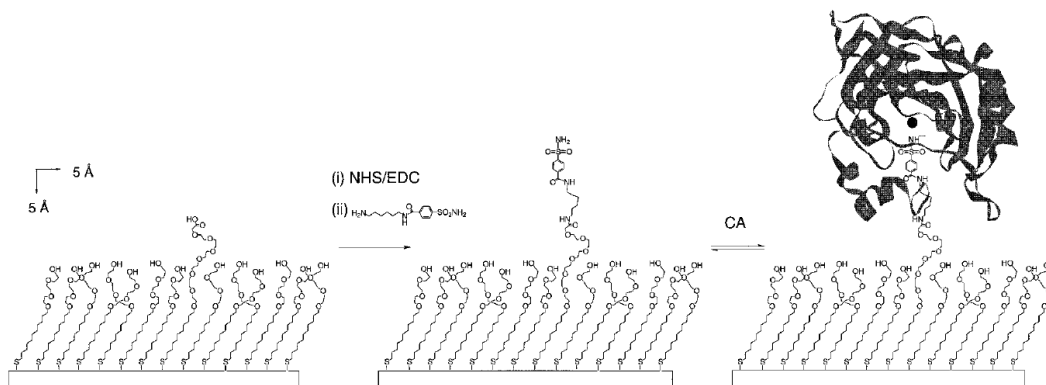


Figure 2: Benzenesulfonamide functionalized sensor surface for the detection of carbonic anhydrase.[Lahiri2002a]

the ration of carboxylic acid termnted alkylthiol with the EG terminated thiols allows to fixate the amount of surface active carboxylic acids and hence the amount of inhibitors deposited on the surface. A maximal amount of deposited carboxylic acids relative to EG-terminated alkylthiols of 10% was reported in order to prevent steric clashes of the surface bound analyte.

As has been reported in literature, the formation of the SAM can be performed at different potentials, typically without potential, but also with small positive values. So we want to perform a series of time dependent depositions of a mixed monolayer at three different potentials (0, +0.3V, +0.5V) at five different times. (0, 1h, 3h, 6h, 9h, 24h)

*This thesis is intended to optimize this first step of this biosensor formation, the deposition of the SAM*

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## Ethylenglycol terminated undecanethiol:

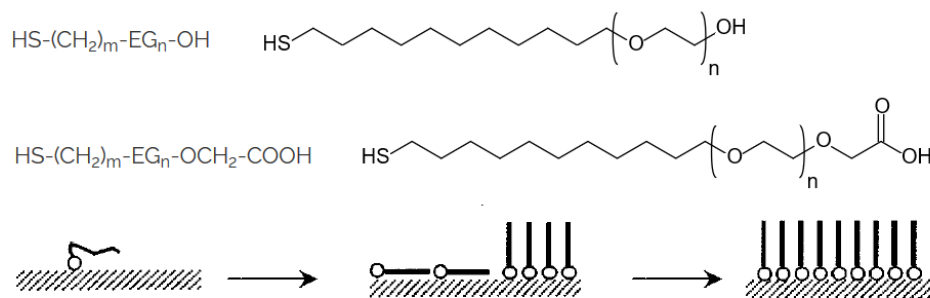


Figure 3: Structure of  $\omega$ -ethylenglycol terminated thiols for SAM formation and general scheme of SAM formation on a surface with time.

As proteins tend to adhere to hydrophobic surfaces, we intend to create monolayers that resist these. Herein, known resistant groups are ethylenglycol terminated SAMs. The more EG-units are in the chains the more resistant is the final surface for the attachment of non-specific recognition. Using 3 EG units is the standart in the literature, and represents a trade-off. The more EG units in the chain, the higher the capacity to resist non-specific adsorption, but less sensitive the sensor surface.

## Micrux sensor chip

The sensor surface we are going to functionalize is a vapor deposited gold surface of Micrux Inc. (Figure 4) This gold surface has a roughness of roughly 1nm and is very homogenous. It contains a thin gold layer on a titanium adlayer on a glass slide.

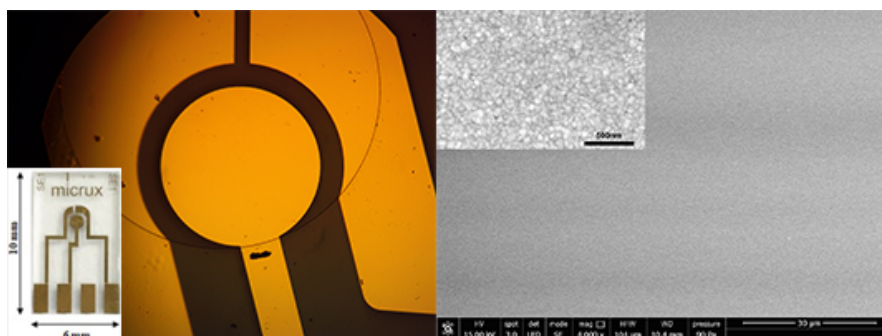


Figure 4: Sensor microchip in dimesion 6x10mm, an image of an optical microscope and an SEM image

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## Flowcell

The single tests will be performed in our little flow-cell.(Figure 5) We constructed this three

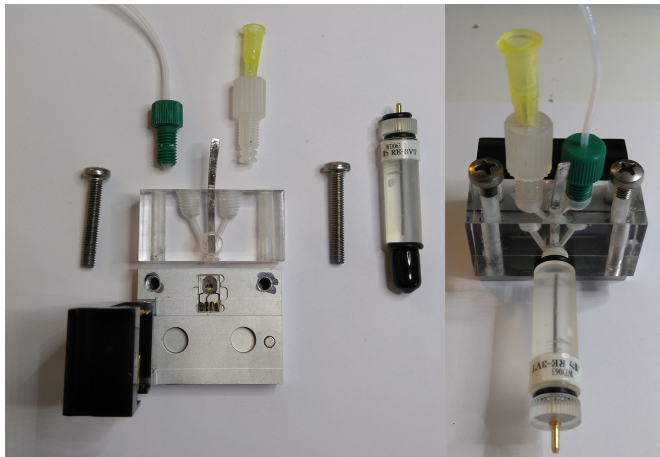


Figure 5: Flow-cell in parts/FlowCell assembled

electrode setup in order to work with smaller volumes and also to be quickly able to switch between solvents. It consists of a readout unit for the Micrux Inc. (which we extended with a custom flowcell system consisting of a block of PMMA in which a Pt-wire is implemented as counted electrode. The flow cell is designed to be either used with syringes (so in an injection mode) and analyse steady fluids, or otherwise can be run with pumps in a continuous mode.

## Palm-Sens potentiostat

A potentiostat is a self controlled device which is intended to keep the potential in the reference electrode constant at the intended input voltage. Herein, a control amplifier (CA) keeps the voltage and reference electrode as constant as possible as the input voltage  $E_i$ . It works by adjusting the current that applies between the exit of the CA and the working electrode. We use herein a potentiostat from PalmSensInc.[Grossi2017]

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## Methods

### Cyclic Voltammetry

Cyclic voltammetry is a powerful electrochemical method for the determination of redox potentials, reaction kinetics, diffusion properties and electrode geometry. In a three-electrode setup consisting of working, counter and reference electrode the current flowing between the working and counter electrode is measured as a function of the potential applied between working and reference electrode. Cyclic Voltammetry measurements are performed by cycling the potential between two values. Figure 6 shows results of a CV experiment in which potential is cycled between +0.8 V and -0.2 V: the redox behavior of a 6 mM  $\text{K}_3[\text{Fe}(\text{CN})_6]$  /  $\text{K}_4[\text{Fe}(\text{CN})_6]$  in water with  $\text{KNO}_3$  as auxiliary electrolyte can be observed with a platinum working electrode and a saturated calomel electrode as reference electrode.[Kissinger1983]

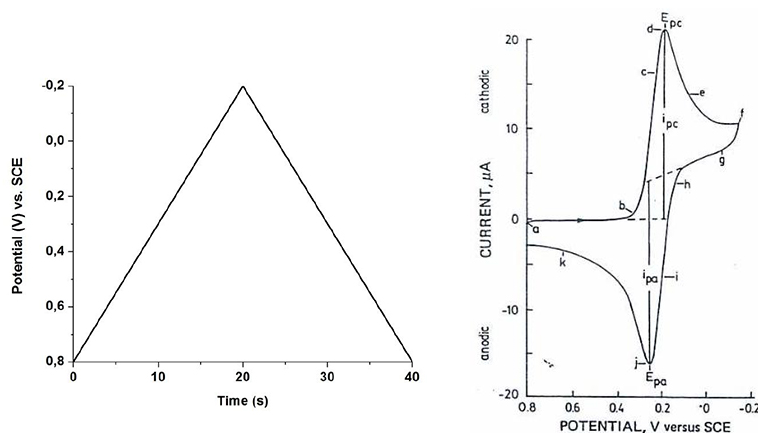


Figure 6: The triangular voltage applied in a cyclovoltammetry measurement and the current response of a solution 6.0 mM in  $\text{K}_3[\text{Fe}(\text{CN})_6]$  /  $\text{K}_4[\text{Fe}(\text{CN})_6]$  and 1.0 M in  $\text{KNO}_3$ . [Kissinger1983]

Once potential is set at about +0.4 V, a cathodic current appears at position b and the Fe (III) species is reduced to an Fe (II) species. As more and more Fe (III) is reduced, the concentration rises and the current reaches a maximum at point d. The continuous local increase of the Fe(II) concentration slows down the diffusion of Fe(III) to the electrode, so that once more negative potentials are reached it becomes impossible to reduce further Fe (III) and the current decays. At the switching point f, the potential reaches the minimum and is shifted back towards positive values, but it is still negative enough for the reduction to happen and to produce a positive current. As more positive potentials are reached, no reduction happens so that the current switches over to the negative side to reach a maximum oxidation rate at point j. When the Fe(II) species are mostly oxidized, the local concentration of Fe(III) now grows and again the current decays as no further Fe(II) ions can reach the electrode.

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## Electrochemical impedance spectroscopy (EIS)

Electrochemical impedance ( $Z$ ) can be defined as the opposition that an electrical circuit presents to the passage of an alternating current. The flow of direct current through a conductor faces a resistance according to Ohm's law ( $R = U/I$ ) as a relation of the applied potential to applied current. For alternating currents this definition has to be extended as circuit components (capacitors, inductors, etc.) may show a delayed response to the applied potential. In the same way the current response to an applied potential of a sensor surface can be modeled as a system of resistors and capacitors. (Figure 7)

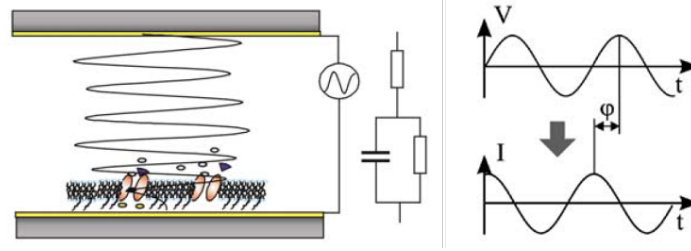


Figure 7: Description of applied potential, equivalent circuit and current response in EIS.[Kasemo2002a](Don't worry, we'll explain it in detail)

The recorded alternating current is shifted in time with respect to the applied potential. (Equation 1).[Banica2012]

$$Z = \frac{V_{AC}}{I_{AC}} = |Z| \exp(j\theta) \quad (1)$$

With Eulers relationship ( $\exp(j\phi) = \cos\phi + j\sin\phi$ ) the impedance can be separated in a real  $Z_{re}$  as well as an imaginary part  $Z_{im}$ . Accordingly, the impedance modulus can be displayed as a vector with its components  $Z_{re}$  and  $Z_{im}$  spanning a Cartesian coordinate system.

When an insulating layer is deposited on a sensor surface, no electrochemical reaction takes place and the system can be modeled by an equivalent circuit including only the ohmic resistance of the solution ( $R$ ) and the double layer capacitance at the electrodes surface, which are strictly related to the characteristics of the recognition layer. This is represented by a simple in line circuit of a Resistor  $R$  and a capacitor.

$$C = \epsilon_0 \epsilon \frac{A}{d} \quad (2)$$

The changes of magnitude in capacitance can herein be evaluated, as with the recognition of an analyte the medium between the electrodes changes from water to an organic compound. With capacitive measurements time dependent phenomena can be monitored, as for example the formation of a SAM in a solute [dilimon2013] or its stability with time.

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## Time Schedule

### **Fist month: Deposition tests and Electrochemistry**

EIS, CV, CV surface coverage and writing of the first version of the thesis ( which is rather a brainstorming than a complete text).

What we want to achieve herein is to write already the "skeleton" of the thesis. For this we want to write the most basic introductory part and the skeleton of the experimental section.

For results and discussion we would like to achieve the following: Three graphs of Surface coverage vs. time(h) dependent on three electrode potentials (0, +0.3V, +0.5V). Herein experiments at at least 5 different times (0,1,3,6,9,24h) in order to answer the question, how long does it take to form a monolayer and at what potential do they form best? Experiments will be performed at each potential, at each time at least with 2 replicate measurements. A determination of a kinetic constant of the formation of the monolayer would be nice. As well is to be depicted the three graphs as a barplot of Surface coverage vs. potential and the bars according to the immersion times.

### **Second month: Microscopic/spectroscopic analysis of the surface**

For the second part we intend to perform general microscopic analysis of the surface of the samples functionalized for 24h. Herein gaining insight into the morphology/structure of the surfaces is interesting as well as gaining insight into the electronic properties. Herein we would like to perform AFM-XXX studies in order to see the local electronic properties. of the monolayer, as well as the the monoatomic defectstructure with STM. With these measurements it is gonna to be more than enough for a bachelor thesis, so from that point on it will be mostly data analysis and writing.

### **Third month:**

Personal experience shows us that very few things in the lab go according to plan, so from the start we leave the third month reserved for eventualities and writing. (Further experiments, for the following steps in Figure 1 would maybe be possible, if there is time. Or an extension to the chosen potential by a pulse method as according to Jambrec et al. [[Jambrec2016](#)])

## People involved

Prof. Di Francesco, Arno, Andrea (maybe), Federico (maybe).