New methods for the preparation of (bio)sensor surfaces: Molecular gradients and mixed monolayers containing oligo(ethylene glycols)

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Alkanethiols form very close-packed and well ordered self-assembled monolayers on gold surfaces. The simple preparation of the organo-metal interface and the possibility to tailor the ω-functional group of the thiol individually for each target makes it attractive for a variety of applications. In the recent years many biosensors, for example affinity sensors [1, 2], DNA chips [3] and array systems, have been developed, which include a thiol sublayer for the covalent binding of receptor molecules. One important problem that must be avoided in biosensor design is non-specific adsorption of (bio)molecules on the sensing surface. Therefore, creating an optimal surface or basis layer is a major goal in biosensor applications.

Two main directions for the preparation of thiol basis layers are described in this paper: 1) mixed monolayers and 2) molecular gradients. Oligo(ethylene glycol) terminated thiols (Eg4, Eg6 = HS-$(CH_2)_{15}$-CONH-$(CH_2)_2$-O$)_{4,6}$-H), mercaptooctadecane (MOD), 16-mercaptohexadecan-1-ol (MHD), 16-mercaptohexadecanoic acid (MHA) and 16-mercaptohexadecan-1-amine (MDA) were chosen as model thiols for the investigations. All gold surfaces were cleaned using the TL1 procedure (NH_3:H_2O_2:H_2O 1:1:5 at 80°C). FT-IR spectroscopy, ellipsometry, impedance and contact angle measurements were used to characterize the monolayers. The combination of optical and electrochemical methods allows detailed statements about quality, structure and stability of the organic layer. The infrared reflection-absorption spectra were recorded at room temperature on a Bruker IFS 66, system equipped with a grazing angle (85°) infrared reflection accessory and a liquid-nitrogen-cooled MCT detector. The measurement chamber was continuously purged with nitrogen gas during the measurements. The acquisition time was around 10 min at 2 cm^{-1} resolution. A spectrum of a deuterated hexadecanthiolate (HS-$(CD_2)_{15}$-CD_3) was used as reference.

First, co-adsorption of two thiols at different molar ratios was performed, and the influence of the organic solvent was studied. It was investigated, whether the composition of the monolayers followed simple rules of statistical mixing or whether phase-segregation occurred. Oligo(ethylene glycol) modified thiols as Eg6 are well known to hinder non-specific adsorption of enzymes and proteins on metal surfaces [4,5] and is therefore an attractive candidate for biosensing applications. Contrary to the shorter analogues, with less ethylene glycol groups, which form monolayers with the OEG chain in the all-trans structure, the Eg6 portion adopts a helical structure, which can be easily detected by significant FT-IR bands near 1350, 1115 and 965 cm^{-1} [6]. Eg6 was mixed with MOD, MHD and MHA at different molar ratios. The composition range started with the pure compound, over 10:1, 3:1 and ended with equimolar mixtures. The total concentration of thiols was about 30-50 µmol/L and the incubation time 36 h or longer. Ethanol, hexane, water, ethanol/hexane and ethanol/water solutions were used as solvents. It was observed that the molar ratio of the thiols in the solution did not correlate with the composition on the gold surface. In ethanol MOD seemed to stick much quicker to the gold surface than Eg6. In the mixed monolayer of MOD/Eg6 prepared from a solution containing 20% MOD the helical peaks of Eg6 were drastically reduced. After increasing the amount of MOD to 50% no Eg6 peaks appeared in the FT-IR spectra. These results correlate with the impedance (capacitance) and ellipsometric data. The capacitance values increased from 0.95 (pure Eg6) to 1.15 $µF/cm^2$ (MOD), while the layer thickness decreased from 38 to 23 Å. When hexane/ethanol solutions were used as solvent the helical peaks characteristic for ordered Eg6 appeared at significantly lower molar ratios of Eg6 in the solution mixture. For example, at a mixing ratio of 6/1 (hexane/ethanol) 20% of Eg6 in the MOD/Eg6 mixture was enough to create a monolayer of Eg6 with predominantly helical conformation, and in pure hexane Eg6 was the dominant molecule on the gold surface [Fig. 1]. Thus, our data clearly display that the solvent has a great influence on the layer composition. Increasing fractions of hexane shifts the surface composition to the Eg6 side. One plausible reason for this could be the different solubility behavior of the molecules in solvent mixtures. MOD is a more hydrophobic molecule than Eg6 and therefore better dissolved in hexane. This behavior will definitely favor adsorption of Eg6 in mixtures containing large fractions of hexane.
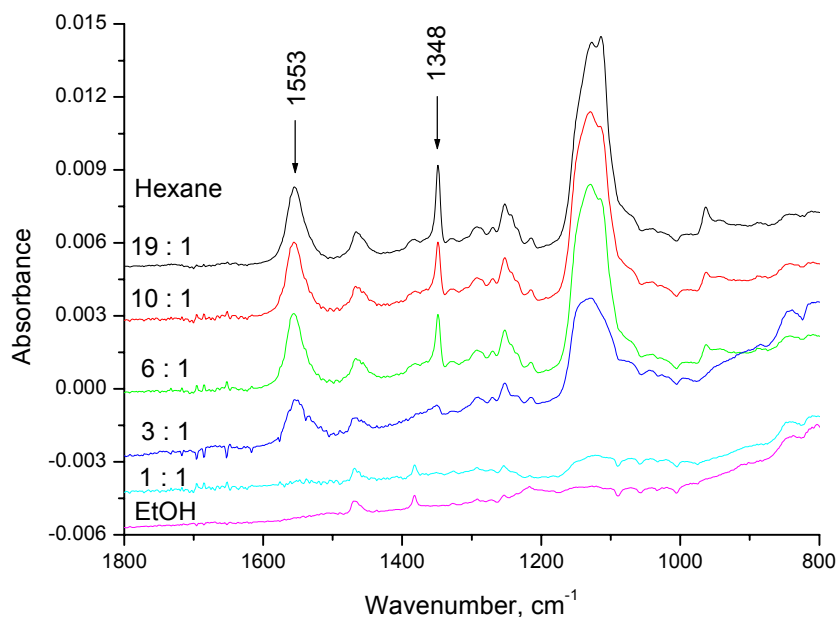
Fig. 1. *FT-IR spectra of mixed monolayers of Eg6 and MOD on gold. The molar ratio of Eg6 in solution was 0.2 (20 %). The numbers on the left side indicate the mixing ratio of hexane to ethanol. A ratio of 19:1 was displayed as pure hexane. The helical peak at 1348 cm^{-1} and the peak for the amide II bond at 1553 are labeled.*

The phase and aggregation behavior of the mixed monolayers can be addressed qualitatively by studying the lateral hydrogen bonding between the amide groups in MOD/Eg6 mixtures. The amide II mode (around 1553 cm^{-1}), which is sensitive to changes in the local hydrogen bonding pattern between neighboring CO and NH groups remains almost constant over a broad range of solvent mixing ratios and monolayer compositions (≤ 3 cm^{-1}). Thus, only small changes in the hydrogen bonding pattern appear to occur with monolayer composition, suggesting that the lateral hydrogen bonding in the mixtures is similar to that observed in the pure (100%) Eg6 monolayers. It should be pointed out that a complete screening of lateral hydrogen bonding is expected to red-shift the amide II peak to 1545 cm^{-1} (≈ 8 cm^{-1}) [7], i.e. to substantially lower frequencies than observed in our work. The most plausible explanation to our observations is therefore that the two molecules phase-segregate at the surface. However, we are at present unable to provide information about the size of these segregated domains. Detailed AFM studies are required to address this issue.

When MHD and MHA, that are more hydrophilic than ODT, were used in the mixed monolayers it was much more difficult to create a monolayer with the OEG portion in helical conformation. We believe that the -functional groups (OH and COOH, which both have hydrogen bond donor and acceptor sites) will intercalate and act as hydrogen bonding bridges (NH···OH···CO) between the

amide groups of the Eg6 molecules. This will increase the average distance between the Eg6 tails preventing them from interacting and thereby adopting the helical conformation. We suggest therefore that statistical mixing of the two compounds is more likely when the -functional groups are of the type OH or COOH. Thus, our data shows that the choice of the organic solvent has a great influence on the composition of mixed monolayers, and that the -functional group of the thiols can be used to control the phase behavior.

Second, 1D- and 2D-gradients were prepared using a "cross-diffusion" methodology. In detail, a clean and bare gold surface, size 2 x 2 cm^2, was inserted in the reaction cell. The setup is schematically outlined in Fig. 2. The diffusion matrix was formed by mixing 1 g of Sephadex LH-20, 5 g of ethanol (99.5%) and a few droplets of Millipore water. After 15 min of swelling it was spread and homogenized on top of the gold slide. Glass filters with a pore size of about 100 μm were inserted and used as reservoirs and starting points for the thiol solutions.
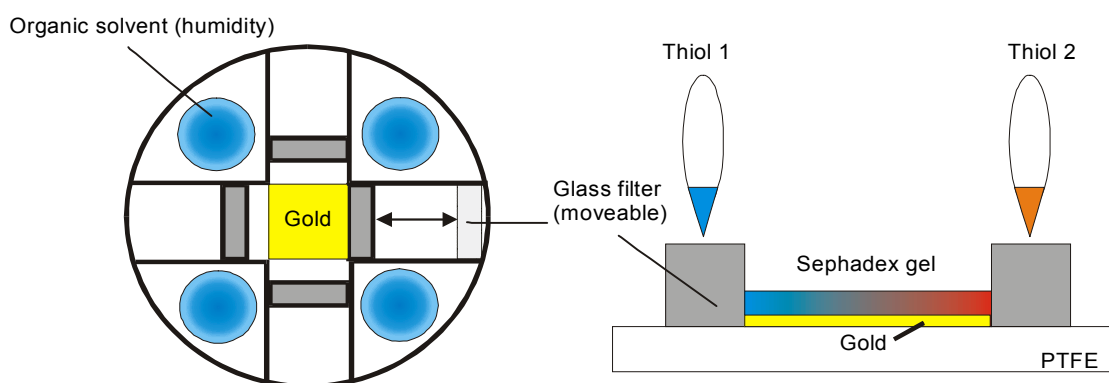

Fig. 2. Schematic drawing of the top (left) and the side (right) views of the cross-diffusion geometry used for the preparation of the molecular gradients.

One-dimensional gradients with different hydrophilic and hydrophobic head groups were formed according the above description [8]. In this case, two of the glass filters, displayed in Fig. 2, were replaced by PTFE barriers. In a first step molecular gradients of Eg6 and MOD or MHA were formed and characterized. Fig. 3 shows the ellipsometric thickness before and after the adsorption of fibrinogen. While nearly no fibrinogen adsorption takes place on the Eg6 dominated side it seems to adsorb strongly on the MHA side. A change in thickness of about 35 Å was observed over a distance of about 6 mm. We believe that this type of gradients can assist in the development of new protein resistant surfaces for biosensing applications.
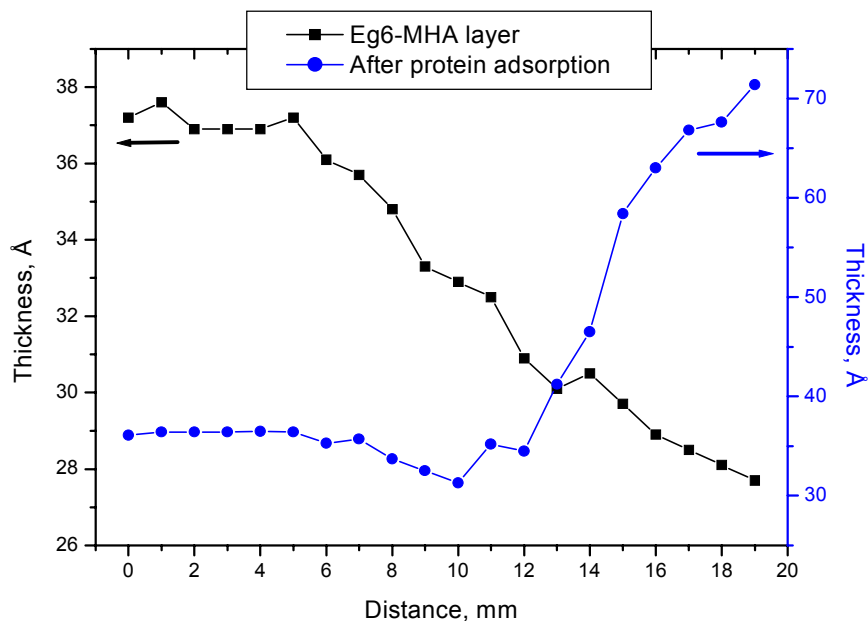
Fig. 3. *Ellipsometric thickness of an one-dimensional gradient of Eg6 and MHA before (squares) and after (diamonds) adsorption of fibrinogen.*

Two-dimensional gradients were formed by diffusing two pairs of thiols in perpendicular directions with respect to each other. This set-up allows the introduction of molecules with specific   - terminal binding groups for covalent coupling of (bio)molecules into a non-adsorptive matrix. As a model system Eg4 and Eg6 was used as one thiol pair, MHA and MDA as the other pair. Preliminary results indicate the high potential of these type of molecular gradients in (bio)sensor applications.

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