# Letters

## A Simple, Reproducible Approach to the Preparation of Surface-Chemical Gradients

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We demonstrate a very simple and reproducible preparative approach for the fabrication of surface-chemical gradients. A surface concentration gradient of adsorbed methyl- or hydroxyl-terminated thiolates was achieved upon gradually immersing a gold-coated substrate into a very dilute thiol solution (0.0033 mM) by means of a linear-motion drive. Subsequent immersion of the substrate into the complementary thiol solution provided a hydrophobicity gradient with a large range (50° of the water-contact angle) and over a significant distance (35 mm). The self-assembled monolayer gradient produced in this way also displayed a high packing density, as demonstrated by dynamic contact-angle and X-ray photoelectron spectroscopy measurements.

### 1. Introduction

The self-assembly of alkanethiols on gold is a well-known process that has been the subject of considerable research.[1-10] The mechanisms leading to the formation of single-component and mixed self-assembled monolayers (SAMs) have been studied extensively. The mixed systems investigated have often consisted of methyl- and hydroxyl-terminated thiols because the results of adsorption can be readily monitored by water contact-angle measurements. Such mixed monolayers were found to be stable and readily produced.

Chemical gradients are of great interest for numerous practical applications, such as investigating biomolecular interactions, cell-motility studies, diagnostics, nanotribology, or microfluidics, and naturally lend themselves to combinatorial studies because an entire spectrum of chemical properties can be covered in a single experiment. A number of gradient preparation techniques for various substrates have been described,[11-26] and such gradients have been used for further experiments and applications.[11,23-29] Several methods have been reported for the generation of thiol-based chemical gradients: the cross-diffusion of two thiol solutions through a polysac-

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charide matrix,[12–14] applying an electrochemical potential to a substrate during adsorption,[15,16] the use of microfluidic devices,[17,18] and scanning-tunneling-microscopy-based replacement lithography.[19] With the exception of the electrochemical potential approach, the gradients formed have been limited in physical size. The aim of our research was to develop a very simple, generally applicable method for fabricating chemical gradients of SAMs on gold on the millimeter–centimeter scale.

## 2. Materials and Methods

**Materials.** The two alkanethiols employed in this study were dodecanethiol [$CH_3(CH_2)_{11}SH$] and 11-mercapto-1-undecanol [$HO(CH_2)_{11}SH$], both purchased from Aldrich Chemicals (Milwaukee, WI). Ethanol (purity >99.8%, Merck, Darmstadt, Germany) was used as a solvent. The substrates for SAM films were prepared by evaporating gold (purity >99.99%, Unaxis, Balzers, Liechtenstein) onto silicon wafers (POWATEC, Cham, Switzerland), according to a standard method.[1] The silicon wafers were coated with a 6-nm-thick chromium adhesion layer, followed by an 80-nm gold film in an evaporation chamber (MED 020 coating system, BALTEC, Balzers, Liechtenstein) at a pressure of about $2 \times 10^{-5}$ mbar. All glassware was cleaned with *piranha solution* (7:3 concentrated $H_2SO_4$/30% $H_2O_2$) for 20 min and rinsed copiously with deionized water and ethanol.

**Preparation of Gradient SAM Films.** The stock solutions were prepared by dissolving $CH_3(CH_2)_{11}SH$ or $HO(CH_2)_{11}SH$ in ethanol at a concentration of 1 mM. All other solutions were prepared by further dilution of the corresponding stock solution. The gradient SAM films were generated by varying the immersion time in alkanethiol-containing solutions along the longitudinal axis of the gold-coated silicon substrate (length 4 cm, width 1 cm). The immersion of the substrates was controlled by a computer-driven linear-motion drive (OWIS, Staufen, Germany). For comparison with static experiments, small pieces ($1 \times 1$ cm) of the substrate were also immersed in the solutions for different lengths of time. All substrates were rinsed with ethanol, dried with nitrogen, and plasma-cleaned (30 s $N_2$, high power, Harrick Plasma Cleaner/Sterilizer PDC-32G instrument, Ossining, NY) before immersion. The speed of the linear-motion drive can be varied between 2.5 $\mu$m/s and 2.5 mm/s. Before characterization, the substrates were again rinsed with ethanol and dried with nitrogen.

**Characterization of Gradient SAM Films.** The hydrophobicity variation of the gradient SAM films was characterized by water contact-angle measurements as a function of position along the longitudinal axis of the sample. Both static and dynamic contact angles were measured employing a contact-angle goniometer (Ramé Hart model 100, Ramé Hart, Inc., Mountain Lakes, NJ, U.S.A., and G2/G40 2.05-D, Krüss GmBH, Hamburg, Germany, respectively). The results of dynamic contact-angle measurements were evaluated using digital image analysis. X-ray photoelectron spectroscopy (XPS) spectra were obtained using a PHI 5700 spectrometer with an Al K$\alpha$ source (350 W, 15 kV) at a takeoff angle of 45°. We have chosen a pass energy of 46.95 and 0.1 eV per step to limit the exposure time
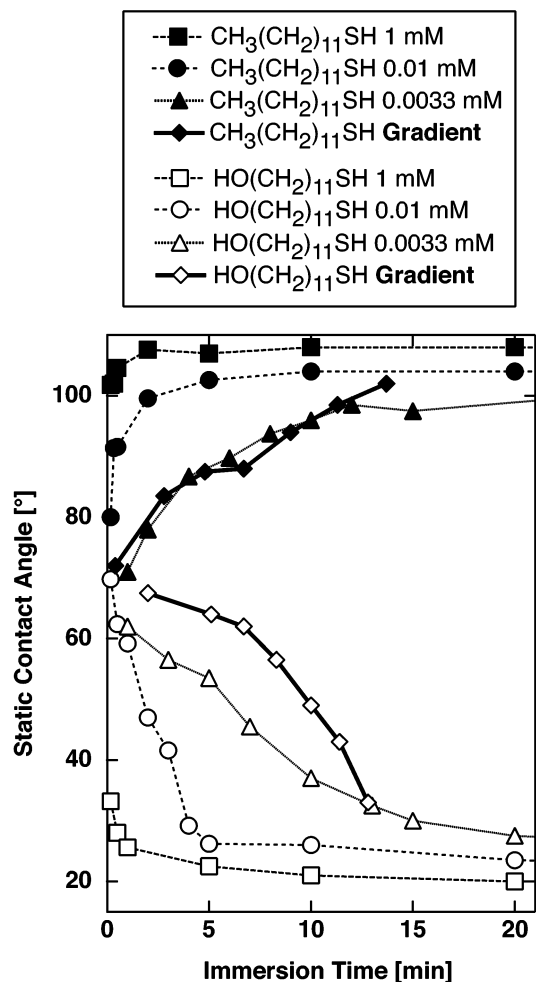


**Figure 1.** Gold substrates immersed in either $CH_3(CH_2)_{11}SH$- or $HO(CH_2)_{11}SH$-containing ethanol solutions. Static contact angles were measured after different immersion times. The lower the concentration of the solutions, the longer it takes to reach the saturation values. For comparison, two gradient samples are also shown: the gradient sample was prepared by immersing a gold-coated substrate (4 cm in length) in 0.003 mM $CH_3(CH_2)_{11}SH$ solution at a speed of 40 $\mu$m/s by means of a linear-motion drive.

and, therefore, keep X-ray damage to a minimum while having reasonable signal-to-noise ratios. The exposure time for each measurement of the four regions [C(1s), O(1s), S(2p), and Au(4f)] was 700 s.[30,31]

## 3. Results and Discussion

In previous studies, the kinetics of alkanethiol adsorption on gold substrates has been investigated by varying the exposure time of a series of samples in solutions containing alkanethiol moieties.[1–7] In this work, this approach was reproduced for both $CH_3(CH_2)_{11}SH$ and $HO(CH_2)_{11}SH$ at different concentrations: 1, 0.01, and 0.0033 mM. In all cases, water contact angles (static) after 24 h of immersion reached the expected saturated values [110° for $CH_3(CH_2)_{11}SH$ and 26° for $HO(CH_2)_{11}SH$]. As shown in Figure 1, however, the adsorption behavior in the initial stages (<30 min) showed a strong dependence on the solution concentration and the type of alkanethiol.

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For the highly concentrated solution (1 mM), both thiols [$CH_3(CH_2)_{11}SH$ and $HO(CH_2)_{11}SH$] reached water contact angles with less than 5% deviation from the saturated monolayer values immediately after immersion (<1 min), while systematically longer times were needed to reach these values for the dilute solutions (0.01 and 0.0033 mM). It was also noted that $HO(CH_2)_{11}SH$ displayed a slower adsorption behavior than $CH_3(CH_2)_{11}SH$, presumably as a result of its greater affinity for ethanol.

The different hydrophobicity values on separate samples shown in Figure 1 can be produced on a single substrate if the immersion time is spatially controlled, and, thus, a *gradient* can be generated. This was accomplished through a controlled immersion of a substrate, such that the position along the sample corresponds directly to a particular immersion time. Thus, the immersion speed has to be carefully selected according to the adsorption kinetics. In this study, a concentration of 0.0033 mM and a speed of 40 $\mu$m/s were chosen. The water contact angles (static) measured at several discrete points along the gradient films are also plotted in Figure 1, after the conversion of the spatial position to the corresponding immersion time. As expected, the contact-angle variation achieved along the gradient is in good agreement with that obtained from a series of homogeneously function-alized samples (70–100°) for a $CH_3(CH_2)_{11}SH$ gradient. For the $HO(CH_2)_{11}SH$ gradient, agreement was less good, presumably as a result of the higher intrinsic surface energy of the layer and, thus, a greater susceptibility to the adsorption of airborne contamination: A Au substrate that was rinsed with ethanol and briefly exposed to laboratory air showed a water contact angle of 65°.

A hydrophobicity gradient film composed of a single component, however, consists of coverage gradients of alkanethiols along the immersion axis of the substrate. Because partial monolayers are less ordered than full monolayers, this initial surface also displays a gradient in order. To remove this inhomogeneity in order and to promote the formation of a complete monolayer, while maintaining the hydrophobicity gradient, the sample was immersed in the complimentary thiol solution in a second step. This is also expected to provide an extended hydrophobicity gradient range.

Two approaches have been employed: (a) the sample was immersed in the same way as in the first step, allow-ing the end that was least exposed to the first component to be initially immersed in the complementary solution (*head-to-tail* method); (b) following the initial step, the sample was fully immersed in the complementary solution for a given time (*full-immersion* method). In addition, the issue of sequence, that is, whether a $CH_3(CH_2)_{11}SH$ or $HO(CH_2)_{11}SH$ gradient is generated first for each ap-proach, provides four alternatives in total, denoted as *head-to-tail (CH₃-first)*, *head-to-tail (OH-first)*, *full-immersion (CH₃-first)*, and *full-immersion (OH-first)*. To facilitate the filling of vacant binding sites, a higher concentration (0.01 mM) was selected for the second solution. The samples were rinsed with ethanol and blown dry with a stream of nitrogen prior to their immersion into the second solution.

All four alternatives showed that the hydrophobicity gradient range is extended after immersion into the second solution: For both the *head-to-tail* and the *full-immersion* methods, after the second immersion step, *CH₃-first* showed a range of 40–100° and *OH-first* showed a range of 30–90° (static water contact angles). However, in terms of monolayer completion and reproducibility/stability, *full-immersion (CH₃-first)* provided the best results. The advancing and receding contact-angle measurements
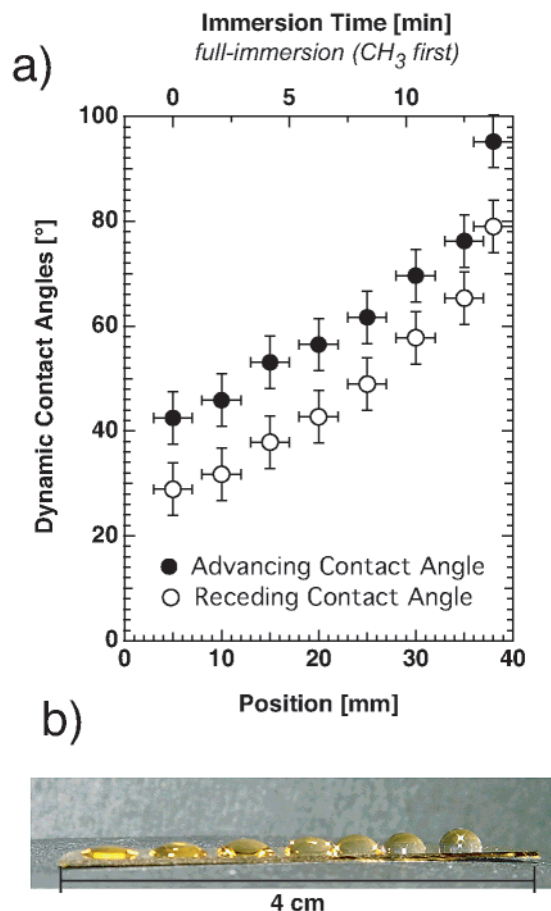


**Figure 2.** (a) Dynamic contact angles along a hydrophobicity gradient using *full-immersion (CH₃-first)* as the preparation method. The small hysteresis of less than 15° between the advancing and the receding contact angles is an indication for the formation of a full monolayer. (b) Water droplets along a hydrophobicity gradient using *full-immersion (CH₃-first)* as the preparation method.

obtained from *full-immersion (CH₃-first)* overnight are shown in Figure 2a. In this plot, the results obtained from five different gradient films are plotted to show their reproducibility (±5°). A fairly linear hydrophobicity gradient with an average water contact-angle slope of 1.5°/mm over 35 mm is obtained. The average hysteresis of 14° between advancing and receding contact angles indicates that the monolayer formation is nearly complete along the gradient.[1] The photograph in Figure 2b provides a more direct illustration of the hydrophobicity gradient generated by this method.

All the other approaches, including the *head-to-tail* methods and *full-immersion (OH-first)*, have the common drawback that the immersion time in the second solution is practically limited (<15 min under the experimental parameters selected in this work). For the *head-to-tail* method, this is because the immersion speed can only be varied between 2.5 $\mu$m/s to 2.5 mm/s. The *full-immersion (OH-first)* method has a limitation because an elongated immersion (>1 h) of the $HO(CH_2)_{11}SH$ precoated film in the second solution (0.01 mM $CH_3(CH_2)_{11}SH$) results in a loss of the hydrophobicity gradient as a result of the displacement of adsorbed $HO(CH_2)_{11}SH$ by $CH_3(CH_2)_{11}$-SH. Thus, for the *full-immersion (OH-first)* method the immersion time in the second solution needs to be a compromise between the full saturation of sites and the maintenance of a hydrophobicity gradient (10 min was selected in this work). In contrast, *full-immersion (CH₃-first)* suffered virtually no corresponding displacement in
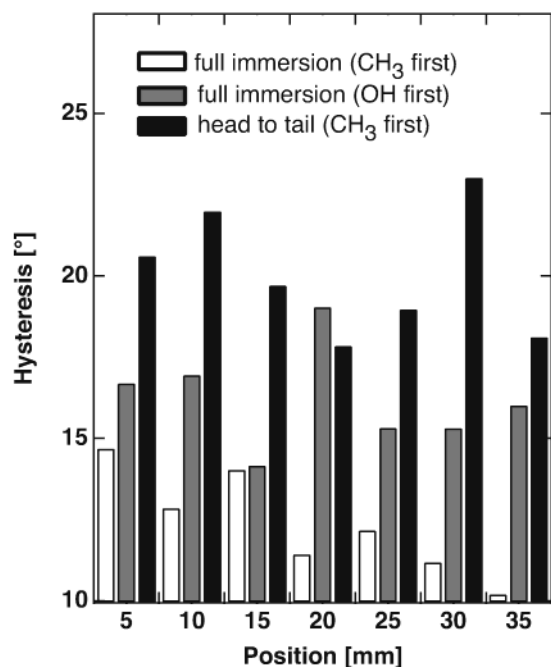
**Figure 3.** Comparison of the hysteresis of the advancing and the receding contact angles for three different preparation methods (see text). *Full-immersion (CH₃-first)* shows the smallest hysteresis values indicating the most complete monolayer formation.



**Figure 4.** Normalized detected atomic concentrations along a hydrophobicity gradient [*full-immersion (CH₃-first)*] by XPS. Both ends are in good agreement with samples immersed in either $CH_3(CH_2)_{11}SH$ or $HO(CH_2)_{11}SH$. Theoretical values for a full CH₃-terminated or a full OH-terminated thiol film were calculated using a 15-Å-thick model [value from ellipsometry and modeling; electron takeoff angles of 45°: attenuation length of $0.085 \times$ (kinetic energy)$^{0.5}$]. The discrepancy between the calculated and the experimental values in the case of the OH-terminated film can be explained by additional carbon contamination of the hydrophilic sample.

the second step, even after 24 h of immersion, thus leading to an improved packing of the film. This behavior was confirmed by comparison with the behavior of single-component monolayers generated from either $HO(CH_2)_{11}SH$ or $CH_3(CH_2)_{11}SH$ moieties, immersed in the complementary solution. *Full-immersion (CH₃-first)* displayed the lowest contact-angle hysteresis of all the approaches tested, as shown in Figure 3.

The chemical composition of such a gradient [*full-immersion (CH₃-first)*] was also characterized by XPS immediately after preparation. For a full monolayer, a constant sulfur concentration (about 6 atom %) is expected across the whole gradient. At the same time, the normalized detected atomic concentration of oxygen is expected to increase from the hydrophobic to the hydrophilic side, while the amount of carbon should decrease somewhat because the terminal methyl groups are increasingly replaced by hydroxyl groups. An almost linear increase for the O(1s), with a concomitant decrease in the C(1s) signals, was found in the experiment, in agreement with the contact-angle results (see Figure 4). The comparison of the two extreme ends of the gradient with two control samples immersed for 24 h in either 0.003 mM $HO(CH_2)_{11}SH$ or 0.003 mM $CH_3(CH_2)_{11}SH$ demonstrates that the chemical composition is changing from an almost complete monolayer of $CH_3(CH_2)_{11}SH$ to an almost complete monolayer of $HO(CH_2)_{11}SH$ in a very smooth and almost linear way. The composition of the pure monolayers was compared with a theoretical model, where we corrected for instrumental functions and the attenuation effects of the monolayer at a takeoff angle of 45°.[32] In the case of the pure $CH_3(CH_2)_{11}SH$ sample, a perfect agreement was observed, whereas in the case of the pure $HO(CH_2)_{11}SH$ sample, an excess of carbon was found. This can be explained by a higher affinity to carbon contamination by the higher-surface-energy samples, compared
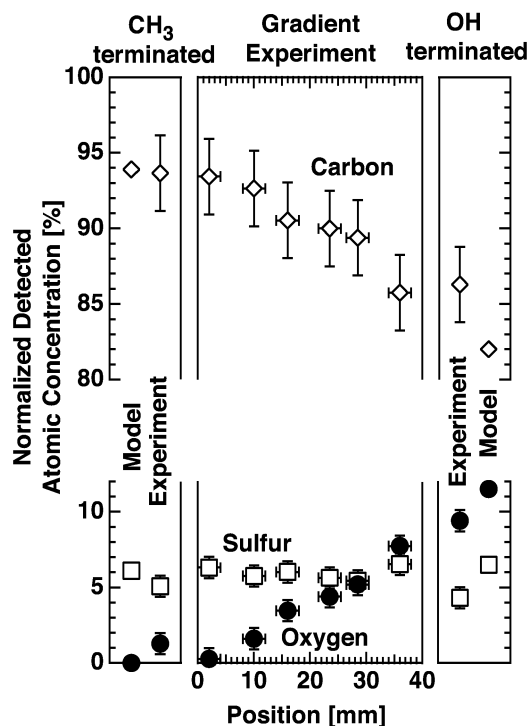
to the low-surface-energy, hydrophobic methyl-terminated surfaces. This explanation is in good agreement with results from ellipsometry, where films fabricated from $HO(CH_2)_{11}SH$ are always found to be a few angstroms thicker than $CH_3(CH_2)_{11}SH$ films.[12,13] If a monolayer of carbonaceous contamination is assumed to be present on the OH-terminated surface, the calculated normalized atomic concentrations match the experimental values within the error bars.

Gradients were stored in different media, such as air, water, ethanol, a vacuum, and nitrogen. The vacuum and nitrogen proved to be effective storage conditions for gradients for up to 5 days. In all other cases, considerable changes in the contact angle were detected after 5 days.

This very simple and reproducible method for the preparation of hydrophobicity gradients could be easily extended to the preparation of other gradients of various (bio)chemical functionalities. It can also be used to prepare gradients of tailored slopes and lengths, as determined by the concentrations of the adsorbing solutions and the velocity program of the linear-motion drive. While there is no general upper limit to the length of gradients that may be prepared in this way, a lower limit is presumably imposed by the finite size of the meniscus at the air–gold–thiol solution interface.

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