Advanced Surface-Chemical Gradients

A dissertation submitted to the
ETH ZURICH

for the degree of
Doctor of Sciences

presented by
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2011
FOR STEFAN, MY PARENTS AND MY SISTER
A scientist in his laboratory is not only a technician: he is also a child placed before natural phenomena which impress him like a fairy tale.

Marie Curie
Surface properties influence many processes, ranging from friction through protein adsorption to cell adhesion. Therefore it is important for many applications to optimize the properties of the surfaces involved. Self-assembled monolayers (SAMs) are frequently used to modify the properties of a surface. A broad variety of surface properties can be achieved by mixing components with different chemical functionalities in a SAM. Screening for the ideal surface properties can either be done by testing many individual samples with different compositions or surface-chemical gradients can be used as high-throughput screening devices to test many different surface-mixtures simultaneously. A broad variety of methods is available to create surface-chemical gradients. In a previous thesis performed in our group a simple and reproducible method to prepare unidirectional surface-chemical gradients was established. Alkanethiol density gradients were created by gradual immersion of a gold-coated substrate into a dilute alkanethiol solution, followed by a complete immersion step to backfill the empty binding sites. On a unidirectional surface-chemical gradient the concentration of two components can be varied continuously.

The mixing of three components is of interest whenever the interplay of effects is the subject of investigation. Orthogonal surface-chemical gradients provide the right platform to study ternary mixed SAMs. Only very few methods to prepare orthogonal surface-chemical gradients are known. The aim of this thesis was to extend the gradual immersion technique in order to develop a method to prepare orthogonal surface-chemical gradients and to test their applicability. Two very similar methods were developed to prepare orthogonal surface-chemical gradients differing only in the breadth of the dynamic range and the relative orientation of the component-density gradients. However, both are based on the gradual-immersion technique to prepare unidirectional surface-chemical gradients extended by a further gradual immersion
step in an orthogonal direction to the first immersion step before backfilling with a third component.

In the first approach, the density of the first two components is restricted to a maximum of 50% in order to have orthogonally oriented density gradients of both components. A wettability gradient with different hydrophobicity and oleophobicity profiles was created with this method. The gradient was characterized by its chemical composition, wettability and conformational ordering. In the second approach the dynamic range of the component mixture was extended. The immersion directions were kept orthogonally oriented to each other. But the density of the first two components was extended up to complete coverage. As a result of this, the density gradients are not oriented orthogonally to each other anymore because the available binding sites in the second adsorption step were restricted due to preadsorbed alkanethiols of the first component. A neutral, negatively chargeable and positively chargeable component were combined in this way resulting in an orthogonal charge-density versus net-charge gradient.

The applicability of the orthogonal surface-chemical gradients to interaction studies was tested on the example of the orthogonal charge-density versus net-charge gradient. Positively and negatively charged nanoparticles and proteins were adsorbed on the gradient surface. The adsorption was clearly driven by electrostatic interactions. Positively charged particles and proteins preferentially adsorbed on the negatively charged region and negatively charged particles and proteins in the positively charged region of the gradient. In a feasibility study the potential of surface-chemical gradients as a crystallization template for high-throughput polymorph screening was investigated. The wettability of the surface turned out to have a bigger impact on the crystallization outcome than the specific surface chemistry. By taking advantage of this effect, wettability gradients could be used as polymorph-screening tools.
Zusammenfassung


Für die Herstellung von unidirektionalen, chemischen Oberflächengradienten sind viele Methoden entwickelt worden. Unter anderem wurde in einer früheren Doktorarbeit, welche in unserem Labor durchgeführt wurde, eine auf graduellem Ein tauchen basierte Methode etabliert. Als selbstorganisierte, monomolekulare Schichten wurden die auf Gold bindenden Alkanthiole verwendet. Um orthogonale, chemische Oberflächengradienten herzustellen sind allerdings nur wenige Methoden bekannt. Das Ziel der vorliegenden Doktorarbeit war die Erweiterung der Methode zur Herstellung von unidirektionalen, chemischen Oberflächengradienten auf orthogonale, chemische Oberflächengradienten. Dies wurde durch einen zusätzlichen, graduellen...
Eintauchschritt in orthogonaler Richtung zum ersten Eintauchschritt erreicht. In einem dritten Schritt wurden die Proben vollständig in eine dritte Alkanthiollösung eingetaucht um die leeren Bindungsstellen zu füllen.

Durch dieses Prinzip konnten zwei verschiedene Arten von Gradienten hergestellt werden. Bei der ersten Methode zur Herstellung von orthogonalen Gradienten ist die Ausrichtung der Konzentrationsgradienten der ersten und zweiten Komponente orthogonal zueinander. Um dies zu ermöglichen muss die maximale Konzentration dieser beiden Komponenten auf 50% limitiert werden, was den dynamischen Bereich des Gradienten eingeschränkt. Bei der zweiten Methode ist der dynamische Bereich des Gradienten durch erhöhte Adsorption der beiden ersten Komponenten erweitert. Dafür ist jedoch die Ausrichtung der Konzentrationsgradienten nicht mehr orthogonal zu einander orientiert. Durch die erhöhte Konzentration der ersten Komponenten an der Oberfläche wird die Adsorption der zweiten Komponente teilweise verhindert, was zu einer radialen Form des zweiten Konzentrationsgradienten führt.

## Contents

1 Introduction ................................. 1
  1.1 Scope of the Thesis ....................... 2

2 Theoretical Background ..................... 7
  2.1 Self-Assembled Monolayers ............... 7
    2.1.1 Alkanethiol SAMs on Au ............. 8
    2.1.2 Mixed SAMs .......................... 12
  2.2 Surface-Chemical Gradients .............. 16
    2.2.1 Alkanethiol SAM based Gradient Preparation ............. 16
    2.2.2 Orthogonal Gradients .................. 19
    2.2.3 Studied Phenomena .................... 20

3 Materials and Methods ..................... 23
  3.1 Materials ............................... 23
    3.1.1 Chemicals ............................ 23
    3.1.2 Substrates ........................... 23
  3.2 Surface Characterization ................. 24
    3.2.1 Contact-Angle Measurements .......... 24
    3.2.2 X-Ray Photoelectron Spectroscopy .... 26
    3.2.3 PMIRRAS .............................. 29
    3.2.4 Atomic Force Microscopy ............. 31
    3.2.5 Ellipsometry .......................... 33
  3.3 Crystal and Particle Analysis .......... 34
    3.3.1 Light Microscopy ..................... 34
    3.3.2 Raman Spectroscopy ................... 35
    3.3.3 Infrared Spectroscopy ................. 36
3.3.4 XRD ................................................................. 37

4 Orthogonal Wettability Gradients .......................... 39
4.1 Abstract ......................................................... 39
4.2 Introduction .................................................. 40
4.3 Experimental .................................................. 41
4.4 Results and Discussion ..................................... 42
4.5 Conclusion .................................................... 55
4.6 Additional Material .......................................... 56

5 Orthogonal Charge-Density versus Net-Charge Gradients 59
5.1 Introduction .................................................. 59
5.2 Experimental .................................................. 62
5.3 Results and Discussion ..................................... 65
  5.3.1 Gradient Preparation ..................................... 65
  5.3.2 Surface-Chemical Composition ......................... 67
  5.3.3 Organization and Thickness ............................ 76
  5.3.4 Wetting Behavior ........................................ 77
  5.3.5 Effect of Hydrochloric Acid in the Backfilling Solution 80
  5.3.6 Particle Adsorption ................................... 83
  5.3.7 Protein Adsorption ..................................... 85
5.4 Conclusion .................................................... 88

6 Surface-chemical Gradients as Crystallization Templates 91
6.1 Introduction .................................................. 91
  6.1.1 Polymorphism ............................................ 91
  6.1.2 Thermodynamics and Kinetics ......................... 93
  6.1.3 Surfaces as Templates for Crystallization .......... 95
6.2 Experimental .................................................. 98
  6.2.1 Chemicals ............................................... 98
  6.2.2 Crystal-Analysis Methods .............................. 98
  6.2.3 Active Pharmaceutical Ingredients ................... 98
  6.2.4 Surfaces ................................................. 104
  6.2.5 Methods ................................................ 105
6.3 Results and Discussion ..................................... 108
  6.3.1 Surface-chemical Gradient on Polyimide Foil ....... 108
The fabrication of tools for various applications demand a big deal on the materials properties. Often it is impossible to meet all the demands with the selection of one material. The modification of a material by a chemical coating allows the adjustment of the surface properties separated from those of the bulk material. Since it is the surface of an object that is in contact with the surroundings, the requirements on its properties are especially high. Even the production of an apparent simple tool like a cooking pan requires the engineering of a non-stick coating. For biomedical applications such as implant materials, the demands on the surface properties are often twofold. On the one hand the surface has to be resistant against bacterial adhesion, which can cause inflammation, and on the other hand a fast adherence of the surrounding tissue material is desirable. Also for contact lenses, two requirements as to the surface properties have to be met. The surface of the lens has to be resistant to protein adsorption to avoid ophthalmitis and for a good wearing comfort the lens has to be hydrophilic so that it is well embeded in the tear liquid. To optimize the properties of a chemical coating of a material for a specific application it is often required to adjust the mixing ratio of different components. This can be done by testing many uniform samples with different mixing ratios in the chemical coating. Beside being slower, this approach has also the drawback that small variations might be overlooked due to sample-to-sample variation. Surface-chemical gradients offer the possibility for high-throughput screening with higher reproducibility.

In conventional, unidirectional surface-chemical gradients the mixing ratio of two components can be optimized. If a third component needs to be included or if the density of two components has to be optimized unidirectional gradients en-
counter their limits. Orthogonal surface chemical-gradients allow the study of three-component mixtures and the interplay of two effects. However, the number of methods available to prepare orthogonal surface-chemical gradients is very limited.

1.1 Scope of the Thesis

The scope of this thesis is to develop a simple method to prepare orthogonal surface-chemical gradients and to test their applicability. Morgenthaler et al.[1] from our laboratory developed a simple and reproducible way to fabricate unidirectional gradients. By gradual immersion of the sample into a diluted adsorbent solution a density gradient is created. The empty binding sites were occupied in a second adsorption step, in which the sample was immersed completely.

In order to prepare orthogonal surface-chemical gradients, the preparation method for unidirectional gradients was extended by applying an additional gradient preparation step in orthogonal direction to the first density gradient. A schematic sketch of the gradient preparation protocol is shown in the upper part of Figure 1.1. Since the unidirectional gradients are oriented in one direction and the orthogonal in two directions, they are often referred as 1D and 2D gradients, respectively. But since it is in the nature of a surface to be two dimensional this terminology was not used in this thesis.

While for the unidirectional gradients, the surface densities of two components are varied gradually, three components are present on a orthogonal surface-chemical gradients. With three components it is possible to create a surface-chemical gradient with all possible mixing ratios. The implementation of a fourth component would require the extension of the gradient dimensionality from a 2D gradient surface into a 3D volume.

The method presented in this thesis to prepare orthogonal surface-chemical gradients is therefore based on ternary, mixed-alkanethiol self-assembled monolayers (SAMs) with a gradually changing mixing ratio on the surface. In Chapter 2, alkanethiol SAMs are introduced with a strong emphasis on mixed alkanethiols SAMs. Additionally, an overview about alkanethiol-based gradient preparation techniques is given, orthogonal surface gradients studies are reviewed and the phenomena which were studied so far by means of surface-chemical gradients are discussed. In Chapter 3 the materials and methods used for the experiments of this thesis are presented.
Figure 1.1: Schematic sketch of the orthogonal surface-chemical preparation methods. In the upper part the sequential immersion steps were sketched and in the lower part the individual component concentrations on the surface after each immersion step are indicated by a color gradient.

In Chapter 4 the preparation of orthogonal surface-chemical gradients is shown by the example of an orthogonal wettability gradient. By using three components with different hydrophobic and oleophobic properties, a wettability gradient was created with the density gradients of two components perpendicular to each other. In the middle part of Figure 1.1 the distribution of the component is indicated by color gradients. The density gradient of the first two components (green and blue) are oriented orthogonally to each other and the third component (red) fills the remaining empty binding sites, resulting in a density gradient oriented diagonally to the two other components’ gradients. The hydrophobicity and oleophobicity profiles on these surfaces are differently oriented. In order to obtain a gradient with
the density gradients of the two components oriented orthogonal to each other, the maximum coverage of the first and second component is restricted to 50%. With this is a approach the dynamic range of two components is limited to 50% and not all possible mixing ratios are present on the surface.

In Chapter 5 a method to extend the dynamic range of three component surface-chemical gradients is presented. The adsorption time and concentration of the solutions are adjusted in a way that the densities of all components vary possibly between 0 and 100%. With this approach the density gradients are not oriented orthogonally to each other anymore, although the immersion directions of the gradient preparation steps are still orthogonal. The adsorption of the second gradient component is partially inhibited due to a lack of empty binding sites. In the lowest part of Figure 1.1, the density profiles of the gradient components are indicated by color gradients. The higher density of the components with respect to the unextended orthogonal gradient is indicated by the more intense color for the high density region. It is also visualized that the density profile of the second component is rather radial than linear in contrast to the first method. As components, a neutral, a positively and a negatively charged alkanethiol were selected, in order to create a charge-density gradient orthogonal to a net-charge gradient. The influence of the net-charge and the charge-density on the adsorption of charged particles or proteins was tested by means of the orthogonal charge-density versus net-charge gradient.

Surfaces can influence the polymorphs formed in a crystallization process. Therefore it was appealing to test the crystallization of active pharmaceutical ingredients on surface-chemical gradients. In Chapter 6 the applicability of surface-chemical gradients as crystallization templates for high-throughput polymorph screening was tested.

In the Appendix A, a collection of miscellaneous studies related to the previous chapters is presented. Most of these studies were performed on unidirectional gradients. The ripening process of an alkanethiol sub-monolayer assembly under different storage conditions was investigated. Gradual replacement of preadsorbed alkanethiols was tested as an alternative gradient preparation method. Unidirectional gradients with hydrophilic or hydrophobic alkanethiols versus oligo or poly(ethylene glycol) components were investigated, in order to test the influence of the ethylene glycol density and the wettability on the protein adsorption. An orthogonal surface-chemical gradient consisting of a hydrophilic and a hydrophobic alkanethiol gradient
perpendicular to each other, backfilled with poly(ethylene glycol) was prepared. In the last study it is shown that amine-terminated compounds in a unidirectional gradient can be coupled with a photocatalytic coupling reagent, which could be used to further modify the surface chemistry.
In the first part of this Chapter, SAMs are introduced with a strong emphasis on the preparation methods and properties of mixed SAMs. In the second part an overview about alkanethiol-based gradient-preparation techniques and orthogonal surface gradients studies is given. In the last part of this Chapter the phenomena which were studied so far by means of surface-chemical gradients are listed.

2.1 Self-Assembled Monolayers

A very frequently used technique to modify the chemistry of a surface is the adsorption of SAMs. SAMs are formed of molecules that are laterally ordered into monomolecular layers on a surface or interface. The generalized structure of molecules that can form SAMs is schematically sketched in Figure 2.1. They have an elongated structure with two functional end-groups. While the anchoring group interacts with the substrate, the functional group exposed at the surface or interface determines the surface chemistry. The spacer group enhances the stability of the SAM due to the intermolecular interactions between the spacer groups of the SAM molecules. Some SAMs are tilted ($\alpha$) with respect to the surface normal in order to maximize the intermolecular interactions.

Different SAMs systems with different substrate anchoring-group interactions are known. Often silanes SAMs are used to modify SiO$_2$[2], ITO[3] and TiO$_2$[4]. Phosphates are able to bind on different metal oxide surfaces (TiO$_2$, Ta$_2$O$_5$, Nb$_2$O$_5$, Al$_2$O$_3$).[5] Catechols bind on a wide variety of surfaces, such as Au, Ag, Ti, Cu,
2. Theoretical Background

2.1 Alkanethiol SAMs on Au

Alkanethiol SAMs on Au are a widely used and well investigated system. Also in this thesis alkanethiols on Au are used modify the surface chemistry. The main advantages of the alkanethiol system are its great reproducibility and flexibility. Besides that, the system is well investigated and a broad variety of functionalized alkanethiols is commercially available. As mentioned above, alkanethiols also adsorb on other metals than Au, but Au is used as substrate in this thesis because it is relatively inert, solid, the preparation is simple and the thiols bind with high affinity. Due to the reflective nature of the Au the samples can be readily analyzed with infra-red reflection-adsorption spectroscopy (IRRAS).

Bonds and Interactions in the SAM

The thiol group serves as an anchoring group and binds to the gold surface (see Figure 2.2). Different desorption studies measured a bond strength of the thiolate bond between the S anchoring-group and the Au substrate of 50 kcal/mol.[7] The sulfur is only slightly negatively charged (between -0.1e and -0.2e).[8, 9] This strongly suggests that the Au-S bond is mainly covalent. However, the degree of charge transfer and therefore the nature of the S-Au bond is a lively topic of discussion.[8] Additional stabilization of the SAM comes from the intermolecular interactions between Fe, Si and polystyrene.[6] Carboxylic acids functional groups interact with Al$_2$O$_3$, Fe$_x$O$_y$ and Ti/TiO$_2$, and alkanethiols SAMs can be formed on Au, Ag, Pt, Pd, Cu, Hg, GaAs, InP, ZnSe and Ni.[7]

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**Figure 2.1:** Schematic sketch of the structure of a SAM. The anchoring group binds to the substrate. The spacer groups stabilize the monolayer by intermolecular interactions. The outermost functional group determines the surface chemistry. The molecules are tilted by angle $\alpha$. Often the anchoring group bound to the surface is confusingly referred to as the head group.
2.1. Self-Assembled Monolayers

the spacer groups. The longer the chain length of the alkanethiols the higher the additional stabilization in the SAM layer. Each methylene group contributes with 1-2 kcal/mol to the van der Waals interactions between the chains.[10] Between perfluorinated thiols the intermolecular interaction is small. Therefore they do not experience a significant stabilization from being adsorbed as a SAM. Due to the reduced intermolecular interactions between the chains, perfluorinated[11] moieties in a SAM are not tilted. Normal alkanethiols are tilted with respect to the surface normal ($\alpha$) by about 30° in order to maximize the intermolecular interactions. Depending on the the functional group the tilt can deviate slightly.[12]

Functional Groups

As mentioned above, a broad variety of alkanethiols with different functional groups are commercially available. The main restriction is that the functional group does not interact with the gold surface. Otherwise it would be a competing adsorption and the quality of the SAM would be reduced. The structure of the alkanethiols used in this thesis are displayed in Figure 2.3. They are divided into different categories: hydrophobic (dodecanethiol ($C_{11}CH_3$), perfluorododecanethiol ($C_{11}CF_3$)), hydrophilic (mercaptoundecanethiol ($C_{11}OH$)), chargeable (mercapoundecanoic acid ($C_{10}COOH$), aminoundecanethiol ($C_{11}NH_3$)) and EG functionalized (methoxy-mercapto poly(ethylene glycol) (PEG-SH), methoxy-hexa(ethylene glycol)undecanethiol ($C_{11}EG_6$)) thiols. Only the nitrogen group of the amine-terminated SAM has a tendency to interact with the gold surface if the functional group is uncharged. This issue will be addressed in Chapter 5.
2. Theoretical Background

Figure 2.3: Chemical structures and abbreviations of all thiols used to modify the surface chemistry in this thesis.

Adsorption Kinetics

Depending on the chain length and the functional group of the thiol, the speed of the adsorption kinetics on the surface is different. But in general the thiols follow a slightly modified Langmuir-type adsorption kinetics.[13] In this kinetic model the adsorption rate is not only dependent on the available adsorption sites, as for a simple Langmuir-type adsorption model, but also takes different binding sites due to intermolecular interactions between the adsorbates into account. Different
2.1. Self-Assembled Monolayers

Approaches have been made to describe the adsorption process. Himmelhaus et al. studied the kinetics of the adsorption process by infra-red sum-frequency spectroscopy (SFG).[14] His focus was on the orientation and tilting of the alkanethiol molecules. He described the adsorption as a three-step process. Following the first step, during which the thiol-gold bonds are formed, the alkanethiols are still quite disordered and in the spacer chain gauche kinks can be observed. In a second step the spacer chains are straightened and in the last step the crystallinity of the SAM is increased by transforming gauche defects into an all-trans conformation. The different adsorption steps have very different time scales. While it needs hours to days in \(\mu\text{M-mM}\) alkanethiol solution to form a perfectly ordered crystalline SAM, even after seconds in nM solutions a low-coverage SAM is formed.[15] Xu et al. studied the SAM formation by atomic force microscopy (AFM).[16] His focus is on the relative orientation of the molecules to each other and to the surface. He observes the adsorption of alkanethiols in a lying-down orientation on the surface until a complete monolayer of lying-down thiols is formed. Only after that does a standing-up phase begin to be formed. During this adsorption step, alkanethiols adsorb preferentially on binding sites adjacent to already-bonded thiols due to intermolecular interactions with preadsorbed molecules. This leads to an island-like microstructure until the islands are merged to a complete monolayer.[17] The island structure can evolve over time in a ripening process if an incomplete SAM is stored.[18]

**Oxidation of SAMs**

Experimental results obtained on stored alkanethiol SAMs must be handled with care, because the susceptibility of alkanethiols to oxidation is often neglected. Well-ordered SAMs in a sealed container are reasonably stable.[19] But the stability is strongly dependent on the storage conditions. Ozone was identified to cause oxidation in most cases.[19, 20] SAMs can be stored under inert atmosphere such as argon or nitrogen or even under air or oxygen atmosphere if exposure to UV light is prevented, since oxygen can react into ozone under UV light irradiation. However, especially in ventilated buildings the ozone concentration is elevated and can cause oxidation if the SAM is exposed to open air for a significant time. The oxidation depends also on the quality of the SAM. In a dense SAM, the sulfur anchoring group, which is prone to oxidation, is well protected from oxidative attack by ozone present in the air. The lower the SAM density, the more susceptible they are to oxidation.
Especially sub monolayer coverage assemblies turned out to oxidize easily. Longer thiols are less prone to oxidation, since the sulfur group is better protected by the thicker SAM layer.[20] But also the substrate can influence the oxidation sensitivity. Alkanethiols SAMs formed on Ag surfaces are more stable than SAMs formed on Au due to the higher thiol density.[21] Also bigger grain size[22] or nanostructured Au[23] surfaces reduce the oxidation rate. Once an alkanethiol is oxidized it remains in the SAM layer due to van der Waals interaction with its neighboring thiols. Only upon rinsing or immersion into a solvent is the thiol removed.[24]

2.1.2 Mixed SAMs

Often thiols are used as single component, uniform and densely packed SAMs (see Figure 2.2 A). Due to the broad variety of available alkanethiols already many surface-properties can be selected e.g. hydrophilic, hydrophobic, protein-resistant, chargeable. But the properties of SAMs are not restricted to the properties of the individual building blocks. By mixing alkanethiols with different functional groups on the surface, the surface-properties can be finely tuned. In this section mixed alkanethiol SAMs are discussed. They can be prepared either by coadsorption of the components from one solution, by sequential adsorption steps of the different components separately in different solutions or by partial modification of the functional groups of the adsorbed component.

Coadsorption

The mixing of alkanethiols with different functional groups on the surface in a certain ratio is usually more complex than mixing the thiols in that ratio in solution and let them adsorb simultaneously. The adsorption kinetics of a alkanethiol is inversely correlated with the solubility of the compound in the adsorption solution and depends therefore on the spacer and the functional group of the compound. So, the surface chemistry of a SAM does not necessarily represent the mixing ratio of the two alkanethiols in the solution.[25, 26] The relative solubility of the components can also be different in different solvents. Even the concentration of the alkanethiol solution influences the ratio of the compounds on the surface.[27] To tune the surface chemistry by coadsorption, unbalanced adsorption rates have to be taken into account. If the surface affinity of the two compounds is very different it
can even be challenging to get a high surface density of one of the compounds by coadsorption. This difficulty can be circumvented by performing a preadsorption step of the less surface-affine thiol before immersion into a mixed solution.[28] The surface affinity of the alkanethiols can also be influenced by each other. Oppositely charged alkanethiols favor a charged-balanced ratio when adsorbing from a mixed solution.[29]

**Sequential Adsorption**

Another method to create mixed SAMs is sequential adsorption of the different components in separate adsorption steps. The simpler way to prepare mixed monolayers by sequential adsorption is by forming sub-monolayer assemblies in one or several adsorption steps, followed by a final adsorption step in which all unoccupied sites are occupied with the final component.[1, 30] The other way to create mixed SAMs by sequential adsorption is to remove preadsorbed alkanethiols from a uniform SAM in order to create empty binding sites for other components. Different methods can be used to remove bound thiols. Electrochemical desorption[31], displacement by exchange[32–34] and photooxidiation[24, 35] are frequently used methods.

Patterned SAMs are normally formed by sequential adsorption.[36] When µ-contact printing is used to create a pattern, the sequential adsorption steps involve a sub-monolayer adsorption step. Patterns created by photolithography are formed by removing thiols from a complete SAM.

An important prerequisite for mixed SAMs being created by sequential adsorption is that the backfilling step to fill the empty binding sites is significantly faster than the replacement of the preadsorbed thiols. Later in this section, the replacement of adsorbed thiols by other thiols will be discussed.

**Modification of the Functional Groups**

The third way to prepare mixed SAMs is to partially modify preadsorbed SAMs. Different ways are known to modify the functional groups of alkanethiol SAMs. Sullivan et al.[37] has published a nice overview on modification reactions on SAMs. Depending on the functional groups different reactions are possible. Carboxylic acid, hydroxyl and amine functionalized SAMs can undergo different reactions.
Replacement

Although alkanethiols are bound quite strongly to the gold they can be desorbed and replaced by another alkanethiol. Since the intermolecular interactions between the spacer groups also contributes to the stabilization of a SAM, the replacement kinetics depends on the chemical nature of the alkanethiol. If the SAM is densely packed and the components are long, the replacement is slower.[33] The more inhomogeneous or shorter the thiols the more easily the thiols can be replaced.[38] Thiols with perfluorinated or oligo(ethylene-glycol) (OEG) spacer chains are replaced more easily due to reduced intermolecular interactions between the thiols. Reduced intermolecular interactions are possibly also the reason why alkanethiols bound on gold grain boundaries are replaced faster.[39, 40] Additional stabilization due to hydrogen-bonding between the alkanethiols[41, 42] can lead to reduced replacement. Due to steric hinderance the replacement rate is drastically reduced when dithiols are used instead of thiols.[43]

The exchange kinetics are temperature dependent. The higher the temperature the faster the replacement.[34] Therefore also the fraction of replaced alkanethiols increases with increasing temperature.

Microstructure

Mixed SAMs can either be homogeneously mixed on the molecular level or they can exhibit a microstructure with separated domains for the different components. In Figure 2.4, homogeneously mixed and mixed monolayers with separated domains are sketched schematically.

**Figure 2.4:** Schematic sketch of (A) homogeneously mixed monolayer and (B) mixed monolayer with component islands.
This island structure can have different origins. On the one hand, the phase separation can occur during coadsorption of different components.[44, 45] The microstructure depends then on the chemical nature of the thiols. The more different the chemical head-group or the chain-length, the more likely are the thiols to separated into islands.[27, 46–49] On the other hand, sequential adsorption often leads to island microstructure. The SAM growth involves island formation due to intermolecular interactions between the spacer chains[50, 51] and the island structure remains upon backfilling with further components. As discussed above, sequential adsorption by replacement leads to higher concentration of the replacing component on defect sites and on gold grain boundaries.[39, 40] Also removal of bound alkanethiols by photooxidation starts at defect sites.[52]

The domain size in SAMs observed after sequential adsorption involving a sub-monolayer coverage step is only approximately 20 nm.[50] Therefore the microstructure will not have an impact on analysis techniques that are probing larger areas. Static contact-angle measurements, x-ray photoelectron spectroscopy (XPS) and PMIRRAS will not be influenced by the microstructure. But with an AFM the microstructure can be seen if there is either friction or height contrast between the components. The hysteresis of the dynamic contact-angle measurements will be increased on phase-separated SAMs.

The microstructure can become very important if the interaction of the SAM with features smaller than or with comparable size to the islands are probed. For example adhesion studies probed with a sharp AFM tip will display a bimodal distribution if the SAMs possess an island microstructure.[53] If the SAMs are probed with a soft colloidal tip, the bimodal distribution will vanish. Hobara et al. studied the adsorption of cytochrome c on phase-separated mixed monolayers.[54] He showed that protein adsorption on mixed SAMs can be influenced strongly by the microstructure.

**Ternary mixed system**

Binary mixed SAMs are frequently used to optimize the surface chemistry. But ternary mixed SAMs are barely studied.[55–57] The adsorption kinetics are most likely different for all three components. To achieve a certain mixture on the surface in a coadsorption step, the different adsorption kinetics have to be balanced by adjusting the concentration. Phong showed that the ratios between the components also depend on the overall concentration.[56] While for higher concentrations the
ratio between the different components is invariant for longer adsorption times, a shift of the surface composition is observed for lower concentrations.

By preparing ternary mixed SAMs by sequential adsorption, the different replacement rate of the pread sorbed components has to be taken into account. Selecting the sequence of the adsorption steps according to the stability of the components on the surface enables the preparation of SAMs with a wider variation in surface compositions.

2.2 Surface-Chemical Gradients

If the density of a chemical component on a surface has to be optimized, this can be done by testing many individual samples with varying chemical composition. Surface-chemical gradients allow the variation of the density of the chemical component on one sample with spatial variation. High-throughput testing can therefore be performed on surface-chemical gradients. Especially if phenomena with high sample-to-sample variation are studied, the use of surface-chemical gradients is advantageous because all tests were performed simultaneously on one sample. In the last decade a broad variety of gradient-preparation methods based on polymers or SAMs were developed.[58–60]

An overview of the alkanethiol-SAM based gradient preparation methods is given in Section 2.2.1. In Section 2.2.2, studies based on orthogonal gradients are reviewed. The phenomena investigated by means of surface-chemical gradients are listed in Section 2.2.3.

2.2.1 Alkanethiol SAM based Gradient Preparation

Adsorbing mixed alkanethiol SAMs on gold surfaces is a very flexible and reproducible method to vary the surface chemistry. Therefore many surface-chemical-gradient techniques are based on mixed alkanethiol SAMs. Since the gradients presented are based on mixed SAMs they can be prepared in the three principal ways that mixed SAMs can be prepared: coadsorption, sequential adsorption and modification of the functional groups. A schematic sketch of these ways can be seen in Figure 2.5.
2.2. Surface-Chemical Gradients

Figure 2.5: Schematic sketch of three principle ways to prepare alkanethiol-SAM-based surface-chemical gradients.

Preparation by Coadsorption

Only few gradient preparation methods are based on coadsorption of the gradient components. Liedberg et al. developed a method to prepare surface-chemical gradients by crossdiffusion of alkanethiols in a polysaccaride matrix above a gold-coated substrate.[61] Geissler et al. [62] created sub-micrometer sized gradients by taking advantage of different migration rates of two alkanethiols. Silica beads were adsorbed on a clean gold coated surface. A poly(dimethylsiloxane) (PDMS) stamp inked with a mixture of two alkanethiols was brought into contact with the silica
beads. The different alkanethiols migrated on the gold surface with different migration rates, resulting in radial, chemical-composition gradients. While in the first method the alkanethiols are diffusing across each other, the diffusion direction in the second method is the same for both components (see uppermost part of Figure 2.5).

**Preparation by Sequential Adsorption**

Most of the gradient preparation methods are based on sequential adsorption of the different components (see Figure 2.5 middle). The following methods include an adsorption step in which a sub-monolayer assembly is formed. Linear unidirectional gradients can be created by the gradual immersion technique developed by Morgenthaler et al.[1] A clean gold-coated substrate is immersed into a diluted alkanethiol solution by means of a linear-motion drive. The created coverage gradient is backfilled with a second component, in order to obtain a complete monolayer. Kraus et al. prepared an alkanethiol coverage gradient by bringing a gold-coated substrate into contact with a PDMS stamp with varying thickness.[63] The PDMS stamp was inked with a diluted alkanethiol solution and depending on the thickness of the stamp, different amounts of alkanethiol can diffuse and adsorb on the surface. The coverage gradient is also here backfilled with a second component in order to create a complete monolayer. Cai et al. presented a gradient preparation method based on ink-jet printing.[64] The first gradient component is ink-jet printed on a clean gold surface and the empty binding sites are subsequently backfilled with a second component.

Gradients can also be formed by removal and replacement of preadsorbed alkanethiols. Hayashi et al. used the gradual immersion technique to gradually replace preadsorbed short-chain alkanethiol by tri(ethylene glycol) terminated alkanethiol and vice versa.[65] In this method the removal and replacement step are happening simultaneously. The same is true for the gradient-preparation technique involving replacement lithography by means of a scanning tunneling microscope (STM) developed by Fuierer et al.[66] After desorption of the preadsorbed alkanethiols by the STM tip the empty binding site is immediately occupied by the second component, since the removal is performed in a solution containing the second component.

In other techniques the removal and replacement take place in separated steps. Blondiaux et al. partially oxidized a preadsorbed SAM by oxygen radicals origi-
nating from a TiO$_2$ layer irradiated by UV light through a gray-tone mask. [24] The oxidized alkanethiol molecules remain in the SAM until they are washed away by ethanol. The empty binding sites were subsequently backfilled with a second component. A very similar procedure was applied by Burgos et al. [67] They oxidized the thiolate bond by UV irradiation in presence of oxygen before backfilling with a second component. Depending on the UV-light intensity the degree of oxidation can be varied. Ballav et al. used variable doses of X-ray irradiation to locally destabilize the thiolate bond. [68] Subsequently the destabilized thiols were replaced by a second component. Applying a electrochemical potential is another method to remove alkanethiol in order to create a density gradient, which can be backfilled with second component. [69–72]

**Preparation by Modification**

Few methods have been based on modification of an alkanethiol SAM. Herbert et al. used a photoimmobilization reaction to implement oligopeptides in a OEG SAM. [73] By varying the light exposure the amount of immobilized species is changing. Mougin et al. [74] created a PEG-density gradient by modifying a cysteine SAM to different degrees by a gel-diffusion method. By applying a temperature gradient which influences the kinetics of a surface reaction, a surface-chemical gradient was created by Shovsky et al. [75]

**2.2.2 Orthogonal Gradients**

All gradient-preparation methods presented in the section above are based on two-component mixtures with variable mixing ratio. But often it would be interesting to study the interplay of two different interactions by varying the concentration of two components on the surface independently.

Orthogonal gradients allow the optimization of two surface properties simultaneously. Only few studies report the preparation of orthogonal gradients. Most of these studies are based on a roughness gradient perpendicular to a chemistry gradient. Clements et al. prepared polymer thickness gradients perpendicular to a porosity gradient on silicon wafers. [76] Yang et al. created a roughness gradient by varying the distance of grooves. Perpendicular to it a polymer thickness gradient
2. Theoretical Background

was applied.[77] Zhang et al. created a polystyrene micropatterned based roughness gradient perpendicular to a sulfonation degree gradient.[78]

In order to vary the surface chemistry in an orthogonal manner two different approaches were found in literature beside the work presented in this thesis. Khire et al. gradually modified a thiol-terminated, silane-based SAM on a glass-slide by thiol-acrylate conjugate addition with PEG acrylate and hexyl acrylate in orthogonal directions.[79] Genzers and coworkers developed an orthogonal surface-chemical gradient preparation technique based on block-copolymerization.[80–82] Either the grafting density of the polymers is varied perpendicular to the molecular weight of the polymer or the molecular weights of the two polymer-components are varied independently in orthogonal directions.

To our knowledge, with the exception of the work presented in this thesis, no alkanethiol based orthogonal gradient preparation method has been published so far. Only Riepl et al. hint at preliminary results on four-component orthogonal gradients based on alkanethiols with an OEG conformational gradient in one direction and a \( \text{COO}^{-}\text{NH}_{3}^{+} \) density gradient in the other direction.[83]

2.2.3 Studied Phenomena

The use of surface-chemical gradients can be divided into three categories. Either gradients can be created in order to study the properties of the gradient material itself, to study the interaction with other objects or to drive a phenomenon. In this section some examples are given for each category.

Materials Property

The chemical composition of a surface-chemical gradient is obviously an important materials property. Most studies of surface-chemical gradients involve the characterization of the chemical composition. The most frequently used technique to determine the chemical composition is X-ray photoelectron spectroscopy.[24, 63, 75] The height or thickness of a gradient film is often of interest especially for grafted polymer gradients[82] or gradients formed from components differing significantly in height.[74] The most frequently used techniques to measure the height are ellipsometry and atomic force microscopy (AFM). Conformational changes and the ordering
of the gradient layer are studied by infra-red spectroscopy-based techniques or by dynamic contact-angle measurements.\cite{84–86} Besides the properties of the organic adlayer also changes of the underlying substrate can occur, such as a change in the metal work function.\cite{87}

### Interactions

Most frequently, surface-chemical gradients are used to study the interaction between particles, proteins, cells, liquids and the gradient surface. Especially the wettability of a gradient surface is frequently used as a way to illustrate the gradient.\cite{1, 30, 68, 75}

Different studies have investigated the adsorption of nano-particles on surface-chemical gradients. Depending on the chemical functionalities present on the surface, the adhesion of the particles is different. Since most nano-particles are charged in order to prevent agglomeration, they interact by electrostatic interactions with charged surfaces. Iqbal et al. showed that the amount of adsorbed positively charged ternary-amine-functionalized polystyrene nano-particles increases with increasing density of negatively-charged carboxylic-acid functionalities.\cite{88} Bhat et al. studied the opposite charge pairing.\cite{89} Negatively charged gold nano-particles adsorb due to positive charges immobilized on the surface either from amine-terminated organosilanes or on poly(acryl-amine) polymers. The study was extended to an orthogonal gradient with polymer-brush-density gradient perpendicular to a polymer brush molecular weight gradient.\cite{90} With increasing molecular weight and increasing density of the polymer brushes, the amount of adsorbed nano-particles increases.

A large number of publications report protein-surface interaction studies based on surface-chemical gradients. Early work in this area was mainly based on wettability gradients. Most studies reported increasing protein adsorption with increasing hydrophobicity.\cite{91–94} The fact that also the opposite behavior has been observed illustrates that the protein-surface interactions are more complex than just being based on the hydrophobic effect.\cite{95} Poly(ethylene glycol)-based surfaces have attracted a lot of attention over recent years due to their excellent resistance toward nonspecific protein adsorption. Recent studies investigated the effect of the PEG-chain density on the protein adsorption by means of surface-chemical gradients.\cite{96, 97} They report decreasing protein adsorption with increasing PEG
density. Also OEG-terminated SAMs are reported to be protein resistant. An extensive study about influence of the OEG chain density, conformation, length and end functional group was published by Riepl et al.[85] A newer approach to render surfaces protein-resistant is the adsorption of zwitterionic mixtures.[98] The effect of the charge balance on the protein adsorption was studied on a charged polymer gradient.[99] Indeed a region with very low protein affinity was found. But the protein-adsorption behavior was mainly found to depend on the charge of the protein. That protein adsorption on charged surfaces is driven to a great extent by electrostatic interactions was already demonstrated on an alkanethiol-based charge gradient.[85]

Since the initial step of cell adhesion on a surfaces is often the adsorption of cell adhesive proteins such as fibrinogen or fibronectin, the adsorption of these proteins and the cell adhesion on surface-chemical gradients are often studied together.[96, 100, 101] To investigate the correlation between fibronectin adsorption and cell adhesion or migration, fibronectin-density gradients were created by immobilizing the proteins on a surface with gradually changing protein density.[36, 71]

Driving

Gradients can also be used to drive a phenomenon. Chaudhury and Whitesides showed that a water droplet can run uphill on a wettability gradient.[102] The water contact angle on the surface-chemical gradient ranged from 25° on the hydrophilic side to 97° on the hydrophobic side. Kraus et al. demonstrated the collection of heptane droplets in the center of a radial, lipophobicity gradient.[63, 102] Ionov et al. sorted proteins according to their size by a density gradient of immobilized kinesin.[103] Smith et al. measured the cell migration of bovine aortic endothelial cells on fibronectin-density gradients.[36]
Materials and Methods

3.1 Materials

3.1.1 Chemicals

Dodecanethiol (98%, Sigma-Aldrich, USA, C_{11}CH_{3}), 11-mercaptoundecanol (97%, Sigma-Aldrich, USA, C_{11}OH), mercaptoundecanoic acid (95%, Sigma-Aldrich, USA, C_{10}COOH), aminoundecanethiol (99%, Sigma-Aldrich, USA, C_{11}NH_{3}Cl) and 1H,1H,2H,2H-perfluorododecanethiol (available from previous studies[63], C_{11}CF_{3}) were used for experiments presented in different sections of this thesis. For all self-assembled monolayer preparation, analytical-grade ethanol (Scharlau Chemie, Spain) was used as solvent. Sulfuric acid (95-97%, Sigma-Aldrich, USA), hydrogen peroxide (30%, p.a., Merck, Germany), chromium (99.6%, Balzers Materials, Liechtenstein) and gold (99.99%, Umicore Materials AG, Liechtenstein) were used as received. For contact-angle measurements, ultrapure water (TKA GenPure, Germany) and hexadecane (≥98%, Fluka, Germany) were used. Bovine serum albumin (96%, Sigma-Aldrich) was dissolved in phosphate-buffered saline (PBS, Sigma-Aldrich, Switzerland, pH=7) for the protein-adsorption studies.

3.1.2 Substrates

Glass slides (4 x 4 cm, Menzel GmbH, Germany) or precut silicon wafers (POWATEC, Switzerland) were cleaned for 10 min in hot piranha solution (7:3 sulfuric acid/
hydrogen peroxide) and rinsed with copious amount of water. **Caution**: Piranha solution reacts violently when placed in contact with organic material and should be handled with care! The substrates were coated with a 10 nm chromium adhesion layer and 100 nm of gold by physical vapor deposition (MED 020 coating system, Bal-Tec, Liechtenstein). Alternatively, the silicon wafers were sonicated for 10 min in toluene, twice in ethanol and air-plasma treated (high power, Harrick Plasma Cleaner/Sterilizer PDC-32G instrument, USA) for 2 min prior to coating.

### 3.2 Surface Characterization

There is a broad variety of surface-analytical techniques that can be used to characterize a surface. The simplest among them to assess the chemistry of a flat surface is contact-angle measurement. However, to fully characterize the surface chemistry X-ray photoelectron spectroscopy (XPS) measurements are necessary. Polarization-modulation infrared reflection-adsorption spectroscopy (PMIRRAS) can be used to investigate the ordering and crystallinity of an organic layer on the surface. The topography of a surface can be determined by atomic force microscopy (AFM). The layer thickness of a species (e.g. SAM or protein adlayer) on the surface can be determined by variable-angle spectroscopic ellipsometry (VASE).

#### 3.2.1 Contact-Angle Measurements

Contact-angle measurement is a quick method to probe the outermost surface chemistry. The way in which a surface is wetted by the contacting liquid depends on the interfacial tension ($\gamma$) between surface (S), liquid (L) and gas-phase (G). The correlation between the interfacial tension and the contact angle on an ideally flat surface was described by Young in the early 19th century (Equation 3.1).[104]

$$\cos \theta^Y = \frac{\gamma_{SG} - \gamma_{SL}}{\gamma_{LG}}$$

(3.1)

Figure 3.1 schematically sketches the interfacial tension at the three-phase contact line. If the interfacial tensions cancel each other out, the droplet is in an equilibrium state and the contact angle is constant. By keeping the liquid and the gas-phase fixed, changes on the surface can be probed.
3.2. Surface Characterization

Figure 3.1: Schematic sketch of a droplet on a surface. A constant contact angle is observed if the interfacial tensions cancel each other out.

The standard liquid used to measure contact angle is water. Roughly speaking, hydrophilic surfaces have contact angles $\leq 90^\circ$ and hydrophobic $\geq 90^\circ$. Contact angles of other liquids can provide additional information. Hexadecane is often used to probe the oleophobicity of a surface. By measuring contact angles with series of liquids with different liquid tensions the surface tension $\gamma_{SL}$ can be determined.

From the Young’s equation, only one contact angle can be calculated. But real surfaces exhibit a range of possible contact angles due to inhomogeneities and roughness. By constantly increasing and decreasing the volume of the droplet during the measurement the advancing and the receding contact angle can be recorded, respectively. The so-called hysteresis is the difference between the advancing contact angle and the receding contact angle. If dynamic contact-angle measurements on SAMs are performed on flat surfaces, so that roughness effects can be ruled out, information about the homogeneity of the SAM can also be obtained. For static contact-angle measurements, the droplets were created in the gas phase and placed on the surface. Static contact angles only provide information about the wettability but can be performed much faster than dynamic contact-angle measurements.

Quantitative analysis is difficult with contact angle measurements, especially for surfaces with different surface chemistries but comparable wettabilities, since they cannot be distinguished by contact-angle measurements. The roughness of a sample should be low and constant in order to avoid roughness effects on the contact angle. But for many surfaces, contact-angle measurements allow quick and crude characterization of the surface chemistry without the requirement of ultra-high vacuum (UHV) conditions. By performing dynamic contact-angle measurements even information concerning the homogeneity of the surface layer can be obtained.

Contact-angle measurements were carried out with a contact-angle goniometer (DSA 100, Krüss GmbH, Hamburg, Germany or Ramé Hart model100, Ramé Hart Inc., Mountain Lakes, USA). The contact angles were evaluated using the instrumental software (DSA 3, Krüss GmbH, Hamburg, Germany) in the case of the DSA 100.
3.2.2 X-Ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) can be used to characterize the surface chemistry in detail. If a surface is irradiated by X-rays of sufficient energy, electrons are removed from the surface atoms due to the photoelectric effect. In Figure 3.2 the removal of an electron from the carbon 1s orbital is schematically displayed. The X-ray interacts with an electron, which is then removed from the atom. The measured kinetic energy (KE) of the removed electron depends on the binding energy (BE) of the electron, the energy of the X-ray irradiation beam (hν) and the work function (Φ) of the instrument. The binding energy of the electron can therefore be calculated using equation 3.2, if the work function of the instrument and the energy of the X-ray are known.

\[ \text{BE} = h\nu - KE - \Phi \]  

(3.2)

The binding energies of the electrons are characteristic for the orbitals and for the elements. Therefore it is possible to determine the different chemical components on the surface from XPS spectra by comparing the measured binding energies with reference values. The intensity of the signals is proportional to the amount of atoms present on the surface. The proportionality (see Equation 3.3) depends on the ionization cross-section (sensitivity factor \( \sigma_A \)), the transmission function (\( T_A \)) of the instrument, the inelastic mean free path (\( \lambda_A \)) and the sine of the take-off angle (\( \theta \)).

\[ N_A = \frac{I_A}{(\sigma_A T_A \lambda_A \sin \theta)} \]  

(3.3)
The sensitivity factor and the inelastic mean free path are specific for each element. Therefore it is necessary to correct for these values, in order determine the atomic ratio of the different elements.

Depending on the chemical environment of an atom, the binding energy of the electrons varies slightly. This results in a characteristic shift of that XPS signal. Therefore more detailed information on the surface chemistry can be obtained by acquiring high-resolution spectra.

After emission of a photon by a photoelectron process from the inner shells, the ion is in an exited state. To relax, an electron from the outer shells can be transfered into the vacancy and another electron can be emitted simultaneously by an Auger process. The kinetic energy of the emitted electron is characteristic for an Auger process. Since in an XPS spectrum the binding energy of the electrons is displayed, the the Auger signals change depending on the source used, although the actual process does not depend on the source.

XPS has to be carried out under ultra-high vacuum in order to avoid collision of the emitted electrons with the gas atoms and to reduce contamination of the surface.

\[ \text{Figure 3.3: Schematic sketch of an XP spectrometer equipped with a tiltable sample stage and a hemispherical analyzer.} \]
from the atmosphere. An XPS spectrometer consists of a X-ray source, an analyzer where the electrons are separated according to their kinetic energy, and a detector. In Figure 3.3 a possible measuring setup is shown. The X-ray source is often a Al Kα or a Mg Kα source because they have a narrow natural bandwidth, leading to well-resolved XPS signals. Often a quartz crystal is used as a monochromator before the X-ray beam hits the sample surface, in order to narrow the X-ray line. If the sample stage is tiltable, angle-resolved XPS measurements are possible. In the lens system the emitted electrons are focussed before they enter the hemispherical analyzer, where the electrons are separated according to their kinetic energy. With the detector, the electrons with the selected energy are recorded.[105]

By varying the angle between the sample and analyzer (changing the take-off angle), the sampling depth of the XPS measurements can be varied. The smaller the angle, the higher the surface sensitivity. The information depth can be varied between 1–10 nm.

Samples can degrade during XPS measurements due to damage induced by the X-ray flux. To minimize sample degradation during the measurement, some precautions needs to be taken. A compromise between signal-to-noise ratio and measuring time has to be found. The use of a monochromatic source reduces the sample damage during the measurement due to the narrow X-ray line.

The disadvantages of XPS are the requirement for ultra-high vacuum, the long acquisition times and the possibility of sample degradation. The advantage is the amount of information that can be extracted from the measurements. Almost all elements can be detected and it is possible to carry out quantitative analysis. From the peak-shift, information about chemical bonds can be obtained and from angle-resolved measurements information about the depth profile.

X-ray photoelectron spectra were acquired with a VG Thetaprobe spectrophotometer (Thermo Electron Corporation, West Sussex, UK) equipped with a radian lens, a concentric hemi-spherical analyzer, a two-dimensional channel-plate detector with 112 energy and 96 angle channels. A monochromatic Al Kα source with a spot size of 400 or 450 µm was used. Electrons are emitted at 53° to the surface normal and the acceptance angle was ±30°. The instrument was operated in the standard lens mode and the analyzer in the constant-analyzer-energy mode. To analyze the data, the CasaXPS program [Version 2.3.5, www.casaxps.com] was used. The signals were fitted using Gaussian-Lorentzian functions and least-squares-fit rou-
3.2. Surface Characterization

times following Shirley iterative or linear-background subtraction. For calculation of the sensitivity factors, published photoionization cross sections were used,[106] corrected for the attenuation length dependence with kinetic energy and for the angular asymmetry.[107]

3.2.3 PMIRRAS

Polarization-modulation infrared reflection-absorption spectroscopy (PMIRRAS) provides information about the various chemical functional groups present in the surface layer, as well as the orientation and the conformational ordering of the surface layer.

Infrared light (10-4000 cm$^{-1}$) excites non-centrosymmetric vibration of bonds or groups in molecules. Non-centrosymmetric vibrations are vibrations where the dipole changes during the movement. Bonds can vibrate in different modes: symmetric and antisymmetric stretching, scissoring, rocking, wagging and twisting. The resonance frequencies of these vibrations are characteristic for the excited functional groups and the vibrational modes. Therefore it is possible to obtain information about the chemical bonds present in the molecules from their infrared absorption spectra.

Normally infrared absorption is recorded by transmission measurements of the compounds as a KBr pellet. These kind of measurements will be discussed later in section 3.3.3 as a method to identify polymorphic forms in bulk material. To measure the absorption spectrum of a thin organic layer on a metallic surface, such as a self-assembled monolayer, infrared measurements can be carried out in reflection mode. The gold-coated silicon wafers used in this work as substrate are ideal surfaces to measure infrared reflection-adsorption spectroscopy (IRRAS). The sample surface is irradiated with an infrared beam under a grazing angle of 80° and the intensity of the reflected beam is recorded. By taking the ratio of it with respect to the adsorption spectrum of a uncoated surface, the infrared spectrum of the adsorbed film can be obtained.

Background measurements of a reference sample become redundant, if the absorption spectrum is recorded simultaneously with s- and p-polarized light. While p-polarized light interacts with the adsorbed organic film on the surface, the s-polarized light does not. Therefore the absorption spectrum of the s-polarized light
3. Materials and Methods

**Figure 3.4:** Schematic sketch of a PMIRRA spectrometer.

A photoelastic frequency-modulator (PM) is used to change the state of polarization of the light during the measurement and therefore enables polarization-modulation infrared reflection-absorption spectroscopy measurements (PMIRRAS). The setup of a PMIRRA spectrometer is schematically sketched in Figure 3.4.

In IRRAS measurements on metallic surfaces, only vibrational modes whose dipole moments are oriented normal to the surface are observable, due to the surface selection rules. From the intensity of the different IR modes, information about the orientation and the ordering of the layer can be obtained. Especially in the case of monolayers composed of alkyl chains, the bandwidth and exact position of the peaks provide additional information about the conformational ordering.

This technique is highly surface sensitive. In contrast to XPS, no vacuum is required. Signals arising from gas molecules in the air are canceled out by the s-polarized background measurement. Besides that, the technique is non-destructive. The disadvantages of the technique are the low lateral resolution due to the high-incident grazing angle and the difficulty to carry out quantitative analysis of contributions from different compounds.

Polarization-modulation infrared reflection-absorption spectra were recorded on a Bruker IFS 66v IR equipped with a PMA 37 polarization-modulation accessory. The incoming beam from the external beam port of the spectrometer, polarized by a KRS-5 wire-grid polarizer and modulated by a ZnSe photoelastic modulator, was reflected off the sample surface at an angle of 80° and detected with a liquid-nitrogen-cooled MCT detector. The maximum of polarization retardation was set at 3000 cm\(^{-1}\) and the polarization was modulated with a frequency of 50 kHz. The sample compartment was continuously purged with dry air during the measurements. The data was processed with OPUS software (Bruker Optics, Germany) and background-corrected with a polynomial.
3.2.4 Atomic Force Microscopy

Atomic force microscopy (AFM) provides information about the surface topography and the chemical contrast between species on a surface with a lateral resolution down to the nanometer level.

A cantilever with a sharp tip is brought into close proximity to the sample surface. First the tip experiences increasing attractive forces up to a certain limit. If repulsive forces start to be the dominating interaction, the tip is in contact with sample surface. This behavior can be described with a Lennard-Jones potential (see Figure 3.5). AFM images can either be acquired in contact mode when the tip is in contact with the surface or in tapping mode when the tip is oscillating above the sample surface. By measuring in tapping mode, less force is applied between the tip and the sample surface as illustrated by the areas in Figure 3.5. Therefore the tapping mode is applied to measure sensitive samples, such as biological samples or samples with mobile species, which could be moved away. During the measurement, either the force or height between tip and sample surface is kept constant.

The tip is scanned over the sample surface. A piezoelectric scanner in the sample stage (see Figure 3.6) is used to precisely move the sample below the tip over the small scanning range. In some AFMs the piezoelectric scanner is attached to the cantilever and instead of the sample, the tip is moved. The cantilever is deflected by forces acting between the sample tip and the surface. Information about the vertical
and for the contact mode also lateral movements of the cantilever is transferred by the reflection of a laser beam on the backside of the cantilever to a photodiode detector. By recording the vertical and the lateral deflection of the cantilever, friction and height images can be acquired simultaneously. The height image represents the surface topography. If the tip was calibrated before the measurements, the height of features can be extracted from these images. From the friction image, information about the chemical contrast can be obtained.

The resolution of the AFM depends to a great extent on the tip. Clean, sharp tips with a single asperity are needed to obtain high-quality, high-resolution images.

To study alkanethiol SAMs on a gold surface with AFM, ultraflat-gold surfaces with roughness below the dimensions of the alkanethiol monolayer are required. AFM provides topographical and chemical contrast images with very high lateral resolution. It is not restricted to conductive samples as it is the case for scanning tunneling microscopy (STM). In contrast to scanning electron microscopy (SEM) AFM can be performed under ambient conditions.

The AFM measurements shown this thesis were performed with a AFM (Dimension 300, Veeco Instruments, Plainview NY) equipped with a standard scanner. The used cantilever (Si$_3$N$_4$, Veeco Instruments Inc., Plainview, NY) was V-shaped and had a spring constant of 0.12 N/m.
3.2.5 Ellipsometry

The thickness of a thin film can be determined by variable-angle spectroscopic ellipsometry (VASE). For films thicker than 50 nm also optical constants of the material can be established. Figure 3.7 shows the experimental alignment of an ellipsometer. The excitation light becomes polarized, passes through a compensator, is reflected from the sample surface and then detected by an analyzer and a detector. The reflection spectrum is recorded at three different angles (θ). The amplitude ratio (tanΨ) and the phase of the reflected light (∆) are the measured properties. By fitting the experimental curves to a theoretical model, the layer thickness can be obtained. For films thinner than 50 nm, the film thickness and the optical constants are correlated, necessitating the use of an approximation for the optical constants. For an insulating film, the Cauchy model is used to approximate optical constants. It is used in this thesis to model the optical constants of a SAM layer and adsorbed protein layers.

![Figure 3.7: Schematic sketch of a the experimental alignment of an ellipsometer.](image)

The precondition of the sample for ellipsometric measurements are a reflective surface, an optically homogeneous film and for film thicknesses lower than 50 nm approximations for the optical constants. It is also necessary to have information about the layer system. If background measurements of the samples can be done prior to film adsorption, the accuracy of the result is improved.

The lateral resolution of this technique is 200 µm. Film thicknesses from Ångströms to µm can be determined. Because the technique is non-destructive and contactless, samples can still be used to perform further experiments after ellipsometric measurements.

In this thesis, organic adlayer thicknesses were determined by variable-angle spectroscopic ellipsometry (VASE, M-2000F, L.O.T. Oriel GmbH, Germany). The instrument was equipped with focussing probes (spot size ~ 200 µM). Measurements were
conducted in a dry state under ambient conditions at an angle of incidence of 70° in the spectral range of 370-1000 nm. Measurements were fitted with the WVASE32 analysis software using a bilayer model for an organic adlayer on gold. The n and k values for the gold were fitted, and the adlayer thickness was determined using a Cauchy model (A=1.45, B=0.01, C=0).

3.3 Crystal and Particle Analysis

Different methods are used to analyze compounds in their crystalline state and to determine their polymorphic forms.\[108\] The most frequently used techniques are differential scanning calorimetry (DSC), X-ray diffraction (XRD), Raman spectroscopy, infrared spectroscopy (IR) and light microscopy. In this thesis, mainly light microscopy and Raman and IR spectroscopy were used. Few crystal analysis are made by XRD in transmission mode on X-ray transparent SAMs (see section 6.3.1). Light microscopy was also used to analyze the particle distribution on surfaces after particle adsorption.

3.3.1 Light Microscopy

Often the differences in the crystal structure between polymorphic forms lead to different crystal morphologies. In many cases the morphologies are so different that one can distinguish between polymorphs from their crystal shape, without the need for any additional analysis. Optical light microscopes were used to obtain images of the crystals present on the surface.

The central part of a optical microscope is an array of optical lenses. The species of interest is illuminated by a light source. The reflected or transmitted light is enlarged by the optical-lens system and the images were recorded by a CCD camera.

Optical microscopy is a non-destructive technique, which allows fast acquisition with excellent spatial resolution. In the case of polymorph characterization, unfortunately not all polymorphs can be distinguished by their crystal habit. Optical light microscopy is therefore not suitable as a unique characterization method for polymorph screening, since unknown polymorphs with crystal shapes identical to known polymorphic forms would be overlooked.
3.3. Crystal and Particle Analysis

Crystals and nanoparticles were analyzed with a optical microscope (Axo Scope, Carl Zeiss, Germany).

3.3.2 Raman Spectroscopy

When light is scattering by matter, three different processes are possible. The most frequent process is elastic scattering, in which no energy is transferred between the molecule or atom and the photon. The process is called Rayleigh scattering. Since no energy is transferred, the wavelength of the light is unaffected. About 1 out of $10^6$ of the photons are scattered inelastically and energy is transferred between the matter and the photon. This so called Raman effect was discovered by Sir Chandrasekhara Venkata Raman (Nobel Prize in Physics, 1930). During the scattering process the molecule or atom is excited into a virtual higher energy level. If it relaxes back to the same vibrational level of the ground state as before excitation, no energy is transferred (Rayleigh). If the final vibrational level is higher in energy, the photon lost energy (Stokes) and if it was lower the photon gained energy (Anti-Stokes). In Figure 3.8, the scattering processes are sketched schematically. The transition between different vibrational levels is possible, if the polarizability of the two states is different. In a Raman spectrum the intensity of the scattered photons is displayed against the Stokes shift.

The Raman spectrometer can be coupled to a microscope, in order to focus on small features. The higher the magnification of the microscope, the better is the collection of the scattered light into the detector, which improves the signal-to-noise ratio and therefore the quality of the spectrum. Small amounts of material can be analyzed due to the high intensity of the focused laser of the Raman microscope.
Depending on the bonding pattern in a crystal, the vibrational energy states have slightly different energies.[109] Therefore Raman spectra can be used to distinguish between different polymorphic forms of a compound since their bonding network is different. If a compound is present in its amorphous form, the Raman spectrum will be similar to that of the liquid, but with broader bands. If water is present in a compound, a broad and intense signal around 3500 cm\(^{-1}\) and a weak signal around 1560 cm\(^{-1}\) can be observed.

Many organic compounds are emitting fluorescence, if they are irradiated with a visible (VIS) light. Therefore often near-infrared (NIR) lasers are often used to avoid fluorescence, but they normally provide less intense spectra than VIS lasers.

Raman microscopy is the most frequently used technique in this thesis to identify polymorphic forms. The advantages of the technique are manifold. It requires only a small amount of crystals (micrometer in diameter, picogram in weight) to measure a Raman spectra, orders of magnitude less than what is required for XRD. The lateral resolution is very good due to the microscope. Besides that, no sample preparation step is needed.

Raman spectra presented in this thesis were recorded either with a Raman imaging microscope (Renishaw plc Transducer Systems Division, Gloucestershire, England) or a confocal Raman microscope (CRM 200, WITec GmbH, Germany).

3.3.3 Infrared Spectroscopy

Infrared spectroscopy can also be used to identify the polymorphic form of crystals. The principle of infrared spectroscopy is described in section 3.2.3.

In contrast to the infrared-spectroscopy technique used to analyze SAMs, crystals are analyzed in the transmission mode and not in the reflection mode. The crystals are embeeed in a matrix that is optically transparent in the IR region (KBr). They have to be ground together with the KBr, which might cause polymorph transformation in some cases. Therefore the results obtained by this technique have to be interpreted with care and the stability of the polymorphic form during the grinding process has to be confirmed.
3.3.4 XRD

X-ray diffraction is the most direct method available to characterize the crystal structure of a material. Diffraction of X-rays on the electron density of the regularly ordered molecules in a crystal show a characteristic diffraction pattern. The wavelength of the irradiation has to have the same order of magnitude as the distances in the grid in order to observe diffraction. The wavelength of the X-ray irradiation is comparable to the distances of atoms in a crystal. Constructive interference of the diffracted X-ray beams leads to a diffraction pattern, from which the crystal structure of the compound can be calculated.

X-ray diffraction (XRD) measurements can be performed either on single crystals (SCXRD) or on crystal powder (PXRD). The former method was developed by Laue and the later by Debye and Scherrer.

With XRD, the crystal structure is determined directly and not via a hydrogen-bonding network as it is the case for Raman and IR spectroscopy. But the quality and quantity of material needed for this analysis technique is some order of magnitudes higher than for Raman microscopy.
Orthogonal Wettability Gradients

This work was published in 2010 in Langmuir.[30] The supplementary materials have been added at the end of this chapter. The major part of this work was done by myself, including all experiments and the first version of the paper. Nevertheless, the contribution of the co-authors has been important: Antonella Rossi helped me to measure XPS on the orthogonal gradients and answered my questions concerning XPS data analysis. Roman Engeli and Florian Bachmann, two undergraduate students, assisted me by performing the first set experiments of the project during a lab course. Nagaiyanallur V. Venkataraman and Nicholas D. Spencer contributed in discussions about the project steps and by correcting the manuscript.

4.1 Abstract

An orthogonal surface-chemical gradient composed of self-assembled monolayers on gold has been prepared by successive, controlled immersions in orthogonal directions into dilute solutions of dodecanethiol and perfluorododecanethiol. The resulting two-component orthogonal gradient in surface coverage was backfilled with 11-mercaptoundecanol, leading to a two-directional, three-component surface-chemical gradient. Water and hexadecane show distinctly different wetting behaviors on the gradient surface because of the differences in the hydrophobic and oleophobic natures of the three different constituents. These results are correlated with the chemical composition maps of the surface obtained by X-ray photoelectron spectroscopy. The homogeneity and the ordering of the self-assembled monolayer were investigated by
dynamic water contact-angle measurements and polarization-modulation infrared reflection-absorption spectroscopy.

4.2 Introduction

The physicochemical characteristics of surfaces are central to a wide variety of applications ranging from tribology to cell biology. Self-assembled monolayers (SAMs) offer the possibility to precisely tailor the surface chemistry by exposing high concentrations of a certain chemical functional group (or groups) at the interface. In this regard, alkanethiol SAMs on gold have been heavily scrutinized as model systems due to their ease of preparation and high degree of monolayer ordering. A host of thiols with different chemical or bio-active functional groups at their chain termini, and their applications, have been extensively studied and reviewed. Mixed monolayers comprising two or more components are as important as uniform SAMs, since they allow the systematic tuning of surface characteristics such as wettability, surface charge, work function or exposed ligand density. Screening many individual mixed SAMs for optimal surface characteristics for a certain application by testing is very time consuming. However, surface gradients offer the possibility of optimizing a desired property by testing a range of surface compositions on a single sample, in a single, high-throughput experiment. The use of gradients also minimizes sample-to-sample variation and therefore enhances reliability. This is particularly important when two surface properties are to be simultaneously optimized, which can be achieved by combining two geometrically orthogonal gradients on the same sample. Such orthogonal gradients could be composed of either two different chemical functionalities or a combination of a morphological and a chemical gradient, for example. Clements et al., Zhang et al. and Yang et al. have reported orthogonal gradients composed of surface roughness and surface chemistry, and orthogonal gradients combining two different grafted polymers were reported by Khire et al. and Bhat et al.

In this chapter, alkanethiol SAMs-based orthogonal surface-chemical gradients are presented. The methodology used to generate such gradients is similar to the generation of a one-directional gradient. The method is characterized by the gradual immersion of a gold-coated substrate into a dilute thiol solution, followed by back-filling with a complementary thiol, to produce a continuous change in surface com-
4.3. Experimental

position of the two components. This simple methodology has since been utilized by our group and others[115] to generate a variety of thiol-based gradients on gold with different chemical functionalities. In this study, the methodology has been extended to create orthogonal surface-chemical gradients using a further immersion step in the orthogonal direction, before backfilling with a third component. Experimental conditions have been optimized to minimize the replacement of pre-adsorbed thiols. The three different thiols have been chosen in order to result in very different wetting behavior by water and oil at different positions on the sample. In these orthogonal gradients, each location on the sample represents a unique combination in surface composition of three different components. These gradients are also relevant for fundamental studies on ternary SAMs, since very few systematic studies have been carried out on such systems.[55] This is partly due to the fact that the number of samples required to study multi-component SAMs increases exponentially with the number of components. Also, the surface composition of ternary SAMs is likely to be severely influenced by the different adsorption and replacement kinetics of each component, making it extremely difficult to precisely control the exact surface composition. Orthogonal gradients offer the possibility to study systematically the effects of varying the concentration of three different surface species independently.

4.3  Experimental

Substrates. Prior to the immersion steps, the substrates (4x4 cm glass slides coated with 10 nm Cr and 100 nm gold as described in Section 3.1.2) were sonicated in ethanol for 10 min, plasma-cleaned in air plasma (PDC-001, Harrick Scientific Corporation, NY) for 30 s and subsequently immersed in ethanol for 10 min.

Gradient preparation. The stock solutions were prepared by dissolving dodecanethiol, 11-mercaptoundecanol or perfluorododecanethiol in ethanol at a concentration of 1 mM for dodecanethiol and mercaptoundecanol and 100 μM for perfluorododecanethiol. All other solutions were prepared by further dilution of the corresponding stock solution. In a first step the sample was immersed into 5 μM dodecanethiol solution by means of a linear-motion drive (OWIS GmbH, Germany) with a speed of 150 μm/s. It was removed immediately from the solution, rinsed with ethanol and blown dry with nitrogen, followed by a second immersion in 5 μM perfluorododecanethiol solution at the same immersion speed but with the immer-
sion axis being perpendicular to the first. Subsequently the sample was immersed for 30 min in 1 mM 11-mercaptoundecanethiol solution at 5 °C. Different immersion times and temperatures were tested but these conditions resulted in a reasonably well-ordered SAM without substantial replacement of the previously adsorbed thiol molecules, as judged from static contact-angle measurements.

**Contact-Angle Measurements.** Static contact-angle measurements were performed with 6 µl droplets for water and 3 µl for hexadecane. Dynamic water contact-angle measurements were carried out with drop volumes from 3 µl to 11 µl and with a dosing speed of 15 µl/min. Contact angles were measured every 5 mm on the orthogonal gradient resulting in 49 measurements per sample. The presented contact-angle values are an average over 7 samples for water contact angles and over 4 samples for hexadecane contact angles. For the non-backfilled samples, an average value over 3 samples is reported for both the water and the hexadecane contact angles.

**Infrared Spectroscopy.** PMIRRA spectra were acquired at every 5 mm along the dodecanethiol gradient direction with a 2-mm aperture and a resolution of 4 cm$^{-1}$ using 1024 scans of multiplexed interferograms.

**XPS Measurements.** A monochromatic Al Kα source with a spot size of 400 µm was used. Pass energies used for survey scans and detailed scans were 200 and 100 eV, respectively, for Au 4f, C 1s, F 1s, O 1s and S 2p. The energy resolution (full width at half-maximum height, fwhm) under these conditions measured on gold Au 4f$_{7/2}$ is 1.55 and 0.95 eV, respectively. In order to obtain an adequate signal-to-noise ratio in a minimum time and to limit beam-induced damage, acquisition times of approximately 7 min for survey scans and 35 min (total) for high-energy-resolution elemental scans were chosen. These conditions provided reproducible XPS spectra and sample damage was negligible. The signals were fitted using Shirley iterative background subtraction. The total analysis time for one orthogonal gradient sample with these chosen conditions was about 5 days (see below).

### 4.4 Results and Discussion

**Gradient Preparation.** The experimental conditions, such as the solvent, concentration of the thiols, temperature and substrate cleaning protocols, are similar to
4.4. Results and Discussion

those for 1-directional gradients.[1] However, the preparation of orthogonal gradients requires some additional considerations due to the additional controlled-immersion step into a second thiol. First, the immersion speed during the first step was increased by a factor of 2 compared to the protocol for a linear, 2-component gradient, in order to result in only half the surface coverage. This was clearly necessary, in order to leave a sufficient number of adsorption sites to allow the generation of an additional gradient along the orthogonal axis. The second consideration was the order in which the thiol immersions were carried out. From our earlier studies on alkyl-perfluoroalkyl gradients,[87] it is known that in ethanol, perfluoroalkanethiols are replaced more rapidly by alkyl thiols than the other way around; therefore, we chose the order of immersion to be alkyl followed by perfluoroalkyl. The third and most important set of parameters that needed to be reoptimized for the orthogonal gradients included the concentration, immersion time, and temperature during the backfilling with the third component. During this final step, the replacement of the previously adsorbed thiols needs to be minimized. However, such replacement-kinetics data is sparse in the literature for a single preadsorbed component[32, 33, 39, 40, 116–119] and nonexistent for two preadsorbed components. Therefore, several trials were carried out under different sets of conditions, and the static water contact angles were assessed every 5 mm along the $4 \times 4 \text{ cm}^2$ surface (49 measurements per sample). In the first step, the backfilling process was performed at three different temperatures (5, 22, and 50 °C) because the replacement kinetics are significantly slowed by decreasing the temperature.[34] Because the highest difference in the contact angle was found on samples backfilled at 5 °C, the immersion time was varied for this backfilling temperature in a second step. After a backfilling time of 10 min, the difference in the contact angle between the two extremes started to drop. PMIRRAS measurements on samples backfilled for 10 min showed a high degree of disorder in the SAM. A backfilling time of 30 min was chosen as a trade off between the contact angle slope and degree of order in PMIRRAS. Thus, the conditions for the backfilling step have been chosen to be the following: 1 mM 11-mercaptoundecanol and an immersion time of 30 min at 5 °C. It is emphasized that this set of conditions is not necessarily unique and represents one of the several possible combinations of these parameters that lead to the desired wettability changes along the orthogonal axes.

Wetting behavior. The hydrophobicity and oleophobicity of a surface can be determined by measuring the water and hexadecane contact angles, respectively. The
water contact angle of a SAM-covered surface depends on the nature of the chemical termini and can be adjusted over a wide range of values by changing the ratio between different components. A full monolayer of hydrophilic mercaptoundecanol had a contact angle of 20°, and those of the two hydrophobic components, dodecanethiol and perfluorododecanethiol, had high water contact angles of 108° and 118°, respectively.

Figure 4.1 shows a 3D surface plot of the static water contact angles on the orthogonal gradient before (top panel) and after (bottom panel) backfilling with mercaptoundecanol. The water contact angles on orthogonal gradients before backfilling show only a small increase along both immersion axes with increasing coverage of the hydrophobic components. This is expected because static water contact-angle measurements do not sensitively distinguish between exposed methyl and methylene groups or between -CF₂ and -CF₃ groups. Moreover, the presence of adventitiously deposited material on the nonbackfilled regions cannot be ruled out. However, the static water contact angles measured on the backfilled gradients (Figure 4.1b) show a clear increase along the two immersion axes. The presence of a gradient in water wettability is clearly visible. These values show the effect of backfilling with mercaptoundecanol along both alkyl and fluoroalkyl axes. The water contact angles measured on backfilled gradients reflect the extent of exposed -OH and hydrophobic groups. A change in the contact angle to >30° can be seen between the two extremes. Every position on the orthogonal surface-chemical gradient shows a different surface molar ratio of dodecanethiol, perfluorododecanethiol, and mercaptoundecanol. The ratio between hydrophilic and hydrophobic functional groups of the thiols is reflected in the static water contact-angle measurements. This is more clearly shown in Figure 4.2, wherein contact angles measured along the two diagonals of the sample are plotted. A wettability gradient with a linear increase of almost 40° can be observed along one of the sample diagonals (Figure 4.2, ■). This line corresponds to the increasing total immersion time into the two hydrophobic components. Along the complementary diagonal (Figure 4.2, ●), the contact angle does not show any significant variation. These data illustrate well the symmetric increase in the contact angle arising from dodecanethiol and perfluorododecanethiol. A small increase in the contact angle on moving from the dodecanethiol-rich region toward the perfluorododecanethiol-rich region, as might be expected from the pure-monolayer values, is not visible. Two effects could explain the absence of this increase. Either the adsorption of the perfluorododecanethiol component is slightly slower than the
Figure 4.1: Surface plots of static water contact angle measured on the orthogonal gradient, plotted against the immersion time into the two hydrophobic components (a) before and (b) after backfilling with mercaptoundecanol. Each intersection of the black gridlines marks a measured contact-angle value. The more yellow the color, the more hydrophobic is the location on the gradient sample. Color scales are displayed beside the plots. Additionally, for clarity, here and in the subsequent figures, the immersion times into dodecanethiol and perfluorododecanethiol solutions are indicated with a green and a blue color intensity gradient, respectively.

dodecanethiol adsorption or the replacement of perfluorododecanethiol with mercaptoundecanol is faster than the replacement of dodecanethiol. From our previous adsorption[87] and replacement kinetics measurements, we conclude that explanation two is more likely. Moreover, it is to be noted that during the second immersion
4. Orthogonal Wettability Gradients

Figure 4.2: The contact angles measured along the diagonals of a backfilled orthogonal wettability gradient are displayed; the two diagonals correspond to the total immersion time in the hydrophobic solutions increasing (■) or remaining constant (●).

The number of available adsorption sites for perfluorothiol adsorption is reduced (almost linearly) along the dodecanethiol immersion axis.

Although the water contact-angle data do not sensitively distinguish between the two hydrophobic components, the hexadecane contact angle is a measure of the oleophobicity of a surface.[120] Because the alkanethiols are oleophilic and fluoroalkanethiols oleophobic, this method is expected to reflect the compositional differences between the two hydrophobic components more sensitively. The single-component monolayers have very different hexadecane contact angles. The hexadecane contact angles for dodecanethiol SAM (45°) and mercaptoundecanol SAM (17°) are considerably lower than for the perfluorododecanethiol SAM (83°). Figure 4.3 shows the static hexadecane contact-angle maps on nonbackfilled and backfilled orthogonal gradients. Unlike the water contact angles, the static hexadecane contact angles show an entirely different behavior on nonbackfilled gradients. The contact-angle values increase rapidly along the perfluorothiol axis whereas along the dodecanethiol axis the contact angle is almost invariant. This is due to the different wetting behavior of hexadecane toward oleophilic or fluorinated surfaces. Along the perfluoro axis at low surface coverage a -CF$_2$-terminated surface is formed since the molecules are likely to adopt a lying-down conformation. The wetting behavior of hexadecane is similar on surfaces exposing -CF$_2$ or -CF$_3$ groups. Therefore, the wetting behavior is mainly determined by the coverage and not by the ordering of the thiols. Along the dodecanethiol axis, at the low coverage of a nonbackfilled gradient, mainly the methylene groups of the alkyl chains are exposed, leading to much lower contact angles.[26] The surface coverage of both molecules is far below that of full SAMs, as
4.4. Results and Discussion

Figure 4.3: Surface plots of static hexadecane contact angle measured on the orthogonal gradient plotted against the immersion time into the two hydrophobic components (a) before and (b) after backfilling with mercaptoundecanol. Each intersection of the black gridlines marks a measured contact-angle value. The more yellow the color on the surface plot the higher the hexadecane contact angle on the surface of the orthogonal gradient. Color scales are displayed beside the plots.

...evident from the measured values at the two extremes of the gradient. The hexadecane contact angles after backfilling (Figure 4.3b) show a gradient mainly along the perfluoro axis and only a small increase along the dodecanethiol axis. After the backfilling process, the surface density of thiols increases, thereby reducing the number
of exposed methylene groups; however, the resulting surface exposes an increasing
number of methyl groups together with the hydroxyl groups of mercaptoundecanol.
This is one of the reasons that the static hexadecane contact angles, after backfilling,
do not show an appreciable change in the dodecanethiol-rich regions of the sample
compared to that for the perfluoroalkyl-rich regions. However, because of the short
immersion time employed for the backfilling process the surface coverage of thiols
is not maximal, leading to an increase in the hexadecane contact angle with the
alkyl-rich end being smaller than expected. The slightly higher replacement of the
perfluorothiol compared to alkylthiols by mercaptoundecanol is also reflected as a
small increase toward the alkyl-rich end. The apparent lack of distinction between
the mercaptoundecanol-rich and dodecanethiol-rich regions in the hexadecane con-
tact angle may be understood by considering the disordered conformation of the
alkyl chains leading to a considerable fraction of methylene groups still exposed at
the interface. This is further evident from the dynamic contact angle and infrared
spectral data discussed below.

Dynamic water contact-angle measurements on backfilled gradients were performed
to assess the homogeneity of the gradients. The difference between the advancing
and receding contact angles (hysteresis) is a measure of the homogeneity of a surface.
The larger the hysteresis, the less ordered and less homogeneous the surface. On
the orthogonal gradient, the hysteresis of the dynamic water contact angle varies
between 25° and 30° with no significant trend (see Section 4.6). This indicates that
the homogeneity of the SAMs is comparable over the whole orthogonal gradient.
However, this value is higher than that of the dodecanethiol-mercaptoundecanol
two-component gradient system (around 14°) or those measured on full SAMs (10°
for dodecanethiol SAMs and 15° for mercaptoundecanol SAMs). This could be due
to the backfilling process not being complete, leading to less-well-organized SAMs.
Increasing the backfilling time or the concentration of mercaptoundecanol did not
lead to a significant improvement in the ordering without substantially increasing
the fraction of replaced dodecanethiol and perfluorododecanethiol. The fact that the
gradient components are not entirely homogeneously distributed but rather form
nanoscopic islands rich in individual components during the two immersion steps
could further contribute to this observed contact angle behavior.\cite{50}

Chemical composition. Although contact-angle measurements are only an in-
direct measure of the surface chemistry, X-ray photoelectron spectroscopy (XPS)
allows the determination of the exact chemical composition of a surface. Unfor-
4.4. Results and Discussion

tunately, this analysis is time-consuming for such orthogonal gradient samples. Whereas an orthogonal gradient is analyzed with static water contact-angle measurements in about 20 min, the careful characterization of one orthogonal gradient demands an XPS acquisition time of more than 5 days. Therefore, only two sets of identically prepared samples were subjected to XPS analysis. Concerns about sample stability over such long acquisitions times and possible X-ray-induced sample degradation necessitated some precautions and led to severe restrictions on the number of repetitions carried out on such samples. To minimize the danger of sample degradation, a monochromatic X-ray source was used and the acquisition time for a single measuring spot was restricted to approximately 7 min for survey scans and 35 min (total) for high energy-resolution elemental scans. After acquiring the spectra for all components (Au 4f, C 1s, F 1s, O 1s, S 2p, survey, and valence band) on one position, we re-recorded the C 1s peak to confirm that the sample was not degrading significantly during the measurements. For the quantification of the components distribution, only the C1s high-energy-resolution elemental scan has been analyzed. The high-energy-resolution elemental scans of F1s and O 1s at selected positions close to the edges of the orthogonal gradient are shown in Section 4.6. In the C 1s signal of the XPS spectra, different components of the carbon signal characteristic of the three components are observable because of their different chemical shifts. A typical C 1s XPS spectrum along with the fitted components is depicted in Figure 4.4. The aliphatic C 1s peak appears in the range between 284.5 and 284.7 eV binding energy, and this signal encompasses components arising from aliphatic dodecanethiol and the alkyl parts of mercaptoundecanol and the fluorinated thiol, together with any possible adventitiously deposited material. The C 1s (C-O) component arising from mercaptoundecanol gives rise to a carbon signal with peak maxima ranging from 286.1 to 286.6 eV. Characteristic of the fluorinated thiol are the C 1s, C-F2, and the C-F3 components, with peak maxima ranging from 291.5 to 291.9 and 294.0-294.5 eV, respectively. The signal of the C 1s (C-S) component is buried in the C 1s (C-O) component. In Figure 4.5 and Figure 4.6, the contributions of the individual components to the total intensity are shown. To extract the true intensity of the C 1s (C-O) component, the contribution from the C 1s (C-S) signal has been subtracted in the following way: Assuming that no sulfur-containing contamination is present on the surface, the C 1s (C-S) contribution to this intensity can be calculated from the S 2p signal, correcting for the different sensitivity factors of carbon and sulfur signals. The contribution of the C 1s (C-S)
Figure 4.4: Typical C1s XPS spectrum along with the fitted C aliphatic, C-OH and C-S, CF\textsubscript{2} and CF\textsubscript{3} components.

signal is then simply subtracted from the total C 1s (C-O) signal. For clarity, in Figure 4.5, the axes of the surface plots are turned by 180 with respect to the plots of the contact-angle measurements. In the first plot, the C 1s (C aliphatic) intensity is shown. It is decreasing mainly with increasing immersion time into the perfluorododecanethiol solution. Also, a minor drop is observable with decreasing immersion time into the dodecanethiol solution. The decrease in this intensity with increasing immersion time in perfluorododecanethiol arises because the perfluorododecanethiol component contains only 1 carbon atom bonded to hydrogens while dodecanethiol and mercaptoundecanol have 11 and 10 such carbon atoms, respectively. The minor decrease in carbon intensity along the axis corresponding to decreasing immersion time into dodecanethiol arises from two sources: the reduction in chain length from 12 carbons in dodecanethiol to 11 carbons atoms in mercaptoundecanol, and the decrease in the perfluorododecanethiol concentration on the surface for longer immersion times into dodecanethiol solution due to saturation effects (i.e., nonlinear behavior in the adsorption kinetics at high coverage). In the lower part of Figure 4.5, the contribution of C 1s (C-O) to the total C 1s peak is displayed. The maximum observed value of this signal normalized to the total C 1s signal is 0.103, which is slightly higher than the expected value of 0.091 for a full monolayer of mercaptoundecanol. The deviation from the expected value is observed even though the
4.4. Results and Discussion

The components of the XPS C 1s signal are normalized to the total C 1s intensity. In the upper surface plot, the C 1s (C aliphatic) is displayed and the bottom surface plot shows the C 1s (C-O) component. Each intersection of the black gridlines marks a measured value. Note that, for ease of display, the axes of the surface plots are turned by 180° in comparison to the plot of the contact-angle measurements shown earlier. Color scales are displayed beside the plots.

Figure 4.5: The components of the XPS C 1s signal are normalized to the total C 1s intensity. In the upper surface plot, the C 1s (C aliphatic) is displayed and the bottom surface plot shows the C 1s (C-O) component. Each intersection of the black gridlines marks a measured value. Note that, for ease of display, the axes of the surface plots are turned by 180° in comparison to the plot of the contact-angle measurements shown earlier. Color scales are displayed beside the plots.

plotted C 1s (C-O) has been corrected for the C 1s (C-S) signal, as described above. This is due to the fact that these two signals (C-S and C-O) originate from different positions within the monolayer, leading to different amounts of attenuation: The C 1s (C-S) signal is attenuated by the entire monolayer above it, whereas the C 1s
Figure 4.6: The surface plot represents the XPS C 1s (C-F) component normalized to the total C 1s intensity. Each intersection of the black gridlines marks a measured value. A color scale is displayed beside the plot.

(C-O) signal is not attenuated at all because it arises from the outermost carbon, thus leading to an underestimation of the C 1s (C-S) signal and a consequent overestimation of the C 1s (C-O). Nevertheless, the C 1s (C-O) intensity, as expected, shows an increase with increasing concentration of the mercaptoundecanol. In Figure 4.6, the contribution of the C 1s (C-F) components to the total C 1s signal is shown. The C-F signals appear to be well separated from the other signals and therefore do not suffer from any of the above difficulties and clearly reflect the increasing concentration of perfluorododecanethiol with increasing immersion time. Almost no variation in this intensity is observed for any given immersion time along the dodecanethiol immersion axis, indicating the homogeneity of the gradients except for the longest immersion times into dodecanethiol, where the concentration of perfluorododecanethiol drops off slightly. This is entirely consistent with the contact-angle data presented earlier. This behavior can be explained by considering slightly modified Langmuir-type isotherm\[13\] absorption behavior and a progressively lower concentration of free binding sites for the perfluorothiols as a result of the previously adsorbed dodecanethiols. In the hexadecane contact-angle measurement on backfilled gradients, this drop was not observable because the increase in the hexadecane contact angle due to increasing dodecanethiol concentration on the
4.4. Results and Discussion

**Figure 4.7:** Static water contact angles are displayed against the normalized peak intensity of the XPS C 1s (C-O) signal.

Surface somewhat balanced out this effect. The mercaptoundecanol concentration, as evident from C1s (C-O) on the surface, reflects well the water-wetting behavior shown in Figure 4.1. The higher its concentration, the lower the water contact angle. (Note that the axes of the surface plots are turned 180° with respect to each other.) This is more clearly seen in Figure 4.7, in which the intensity of the C-O

**Figure 4.8:** Correlation of the perfluorododecanethiol concentration on the surface (C1s C-F) with the hexadecane contact angle.
signal is plotted as a function of the static water contact angle. A good correlation between the water contact angle and the C 1s (C-O) signal can be seen over the entire gradient. In Figure 4.8, the correlation between the perfluorododecanethiol concentration on the surface and the hexadecane contact angle is shown. Here again, excellent correlation between the two quantities is evident. The increased scattering about the regression line for high hexadecane contact angles is due to the variation of the dodecanethiol concentration for constant perfluorododecanethiol concentration.

**Organization.** The crystallinity and organization of the alkyl chains of the SAM can be investigated by polarization-modulation infrared reflection-absorption spectroscopy (PMIRRAS).[86] Figure 4.9 shows the PMIRRA spectra of the orthogonal gradient every 5 mm along the dodecanethiol gradient axis. On the upper and lower ends of the gradient, the spectra of the uniform dodecanethiol and mercaptoundecanol SAM, respectively, are shown. The position of the peak maxima for the symmetric and antisymmetric CH$_2$ stretching mode with respect to the corresponding bands in the full SAMs shows that the alkyl chains are not well organized. On full monolayers, the symmetric and antisymmetric stretching modes are seen at 2850 and 2920 cm$^{-1}$, respectively. Although the positions of the symmetric and antisymmetric CH$_2$ stretching modes measured along the gradient decrease slightly with increasing immersion time into dodecanethiol, they do not reach the full-monolayer values. Nevertheless, the presence of a gradient in dodecanethiol concentration can be seen from the increase in the asymmetric CH$_3$ stretching mode. Increasing the backfilling time or concentration of mercaptoundecanol did not lead to a significant improvement in the crystallinity and ordering of the SAM without substantially increasing the fraction of replaced dodecanethiol and perfluorododecanethiol. This is in contrast to the situation seen in the two-component gradients composed of dodecanethiol and mercaptoundecanol,[86], where the ordering and the crystallinity of the SAM after the backfilling step are not much different from those of the full monolayer, reflecting the complex nature of the three-component SAM systems. The spectra in the C-F stretching mode are not shown because the intensities of the peaks are quite low and the difference along the gradient was not observable. Unlike the two-component alkyl-perfluoroalkyl gradients, the typical helical modes of the CF$_2$ groups are not observable, even at the highest perfluorothiol coverage.[87] This is due to the lower concentration and poor organization of the perfluoromoieties on the orthogonal gradient rather than on the two-component gradients.
4.5 Conclusion

A method to fabricate orthogonal surface-chemical gradients composed of three different thiols on gold is presented. Small deviations from orthogonal behavior occur in the most hydrophobic region because of saturation effects. The experimental conditions and the specific thiols have been chosen in such a way as to result in an orthogonal gradient that exhibits very different wetting behavior toward water and...
oil. The water contact angle shows a gradient along both immersion axes whereas the hexadecane contact angle varies only along one of the axes. This difference in wetting behavior can be switched by simply changing the order in which the immersions are carried out. For example, controlled immersions into dodecanethiol and mercaptoundecanol followed by backfilling with perfluorododecanethiol would lead to a complementary gradient in wetting behavior by the two liquids. The method can readily be extended to thiols with different functional groups (e.g., biologically active functional groups), providing a tool to investigate three-component mixed SAMs systematically, which can be utilized as a platform to optimize the effects of two surface properties simultaneously.

4.6 Additional Material

Figure 4.10 shows the hysteresis of the dynamic contact-angle measurements. As discussed above the hysteresis shows no significant variation along the gradient surface what indicates comparable homogeneity over the whole gradient surface.

![Figure 4.10: Dynamic water contact-angle hysteresis measured on the orthogonal gradient. The brighter the color on the surface plot, the higher the contact-angle hysteresis on the surface. The immersion times into dodecanethiol and perfluorododecanethiol solutions are indicated with the green and the blue gradient, respectively. Each intersection of the black gridlines marks a measured contact-angle value. The color scale is displayed beside the plot.](image-url)
In Figure 4.11 the F 1s and O 1s spectra measured close to the edges of the orthogonal gradient samples are shown. The intensity of the F 1s signal reflects very well the immersion time of the orthogonal gradient sample in the perfluorododecanethiol solution. The O 1s signal increases with decreasing immersion time into the dodecanethiol and perfluorododecanethiol solution.

**Figure 4.11:** F 1s (top) and O 1s (bottom) XPS signals measured at four different locations (A, B, C and D) close to the edges of the orthogonal gradient sample. A schematic representation of these positions on the orthogonal gradient sample is also shown.

In Figure 4.12 the XPS S 2p (upper) and F 1s (lower) signal on the orthogonal gradient is displayed. Little variation in the S 2p signal is observed, which illustrates the uniform coverage density over the whole sample. The variation of the F 1s signal reflects the variation observed for the C 1s (C-F) signal displayed in Figure 4.6.
Figure 4.12: Surface plot of the XPS S 2p (upper) and F 1s (lower) signal on the orthogonal gradient. The more yellow the color on the surface plot the more intense the XPS signal from the surface of the orthogonal gradient. Each intersection of the black gridlines marks a measured contact-angle value. The color scale is displayed beside the plot.
5.1 Introduction

Charged surfaces can interact with charged objects by electrostatic interactions. The adsorption of nanoparticles on charged surfaces is relevant for different applications.[121–123] While charged nanoparticles are simple examples of charged objects, proteins are more complex examples, since their net charge is not just a sum of either positive or negative charges, but corresponds to the excess of amino acids, which are either negatively (aspartic acid, glutamic acid) or positively (lysine, arginine, histidine) charged at physiological pH. The charge is also not necessarily equally distributed over the protein surface. Different protein regions may have different net charges. The interaction of proteins with surfaces consisting of a mixture of charged components is especially interesting since zwitterionic surfaces have been found to be protein resistant.[98, 99, 124–126]

Functionalized alkanethiols can be used to modified the surface chemistry with chargeable functional groups. For example carboxylic acid and amine-terminated alkanethiol SAMs are partially negatively and positively charged, respectively, at neutral or physiological pH. By mixing both components the net charge of the surface can be varied and when surface-chemical gradients of these components are prepared, the surface-net charge can be varied gradually. Riepl et al.[85] reported protein adsorption experiments on a unidirectional surface-net-charge gradient. The positively charged lysozyme is adsorbing preferentially on the negatively charged carboxylic acid side and the negatively charged pepsin on the positively charged amine
side. Chuang et al. [127] observed that platelet adhesion is minimized for mixed amine and carboxylic acid terminated SAMs if the charge is balanced. If only positive or negative charges are present on the surface, the protein adsorption is higher than on neutral hydrophilic surfaces. [128] Lestelius et al. [129] showed that protein adhesion on OH terminated SAMs was reduced in comparison with carboxylic acid terminated SAMs.

Not only is the charge balance of importance, but also the charge density plays a role in particle and protein adsorption. Gessner et al. [130] studied protein adsorption on nanoparticles with different surface-charge densities. They observed higher protein adsorption for higher charge densities. With the binary mixtures of positively and negatively chargeable components it is not possible to independently change the surface net charge and the charge density. Ternary mixed systems of a positively charged, negatively charged and a neutral component are needed to address this interplay. Three-component surface-chemical gradients allow the systematic study of ternary mixed systems. By varying the density of positively and negatively charged functional groups on the surface independently, it is possible to study the influence of the charge balance and the charge density simultaneously. Therefore it would be interesting to study the nanoparticle and protein adsorption on surfaces with variation of surface charge density and perpendicular variation of surface-net-charge.

The method for the preparation of orthogonal, three-component, alkanethiol-based surface-chemical gradients [30], presented in Chapter 4, allows the combination of three different components with variable ratio on one surface. Two density gradients of two components were applied on a surface independently, in such a way that the two gradients are in orthogonal directions. However, with this method the dynamic range of the two components is limited to half the density of the full monolayer. In this chapter a modification of this method is presented, which allows the preparation of three-component surface chemical gradients with an extended dynamic range. The immersion time into the first two component was increased in order to increase the maximum density of these components. The dynamic range of the gradient is extended and instead of the two-components density gradients, the charge-density gradient and the net-charge gradient are oriented orthogonal to each other. The neutral mercaptoundecanol (C_{11}OH), the negative chargeable mercaptoundecanoic acid (C_{10}COOH) and the positive chargeable aminoundecanethiol (C_{11}NH_{3}) were used as gradient components.
The formation of high-quality SAMs of chargeable alkanethiols is more demanding than for neutral alkanethiols, such as dodecanethiol or mercaptoundecanol. If amine-terminated alkanethiols are adsorbed from pure ethanol, a significant fraction of the sulfur is oxidized.[131, 132] This partial oxidation of the amine-terminated thiol in the SAM has often been neglected.[113] Oxidation of exposed sulfur groups due to double-layer formation by hydrogen bonding between the chargeable functional groups is one possible explanation. For this reason Wang et al.[131] suggested the use of small molecules that can form hydrogen bonds with the SAMs and reduce the dimer formation of the acid- and amine-terminated thiols. They showed that the oxidized sulfur species observed by XPS on the amine-terminated SAM was reduced. However, on the carboxylic acid terminated SAM no oxidized sulfur species were observed although it is known to form double layer.[133] This indicates that the double-layer formation is not the only reason for the oxidized sulfur species. Another explanation for the presence of oxidized sulfur species in the amine-terminated SAM is that in pure ethanol a significant fraction of the amine-terminated thiols are uncharged and that the lone pair of the nitrogen can therefore interact with the gold during the adsorption process. This leads to adsorption of thiols in upside-down orientation in the SAM film. The sulfur groups of these thiols are exposed to the air and are therefore more susceptible to oxidation. Lee et al.[132] suggest the use of hydrochloric acid to maintain the amine group in a charged state during the adsorption and therefore to prevent the adsorption of thiols oriented with the amine functional group towards the gold. Performing the adsorption in the charged state has the drawback that the density of adsorbed thiols is reduced due to electrostatic repulsion, but the implementation of miss-oriented amine-terminated thiols can be suppressed.

Unfortunately the use of strong inorganic acids can lead to ester formation between the carboxylic-acid-terminated thiol and the alcohol group of the ethanol.[134, 135] Therefore the conditions needed to adsorb amine-terminated thiols without oxidized sulfur groups conflict with the conditions under which the carboxylic-acid-terminated thiol is stable. This conflict had to be considered during the three-component gradient preparation.

Arnold et al. suggest the use of an acid in the carboxylic acid solution, in order to obtain denser SAMs when the carboxylic acid groups are uncharged.[134] For our purpose the adsorption of C\textsubscript{10}COOH from pure ethanol in its charged state[136] is favorable. As mentioned in Chapter 2 the microstructure of SAM gradients prepared
by the dip-coating method adsorption exhibit island structure with islands of about 20 nm in diameter for the dodecanethiol. The islanding can influence the adsorption of nanoparticles and of proteins. Although the nanoparticles used in this study are, with 500 nm and 900 nm diameter, much bigger than possible thiol islands, the contact area of the nanoparticles is much smaller and therefore their adsorption could still be influenced by the microstructure. We believe that by adsorbing the C\textsubscript{10}COOH in its charged state the island formation can be significantly reduced.

Although using sequential adsorption steps to adsorb the different components has the disadvantage of possible island formation, it has a clear advantage over coadsorption of charged components. When charged thiols are mixed in various ratios in solution the species on the surface are charged balanced over a broad range of solution compositions.\cite{29} Chuang et al. \cite{127} observes preferential adsorption of the amine-terminated thiol over the adorption of the carboxylic-acid-terminated thiol during coadsorption. By applying sequential adsorption steps this difficulty can be circumvented.

In Sections 5.3.1-5.3.4 of this chapter, the preparation and characterization of a charge-density gradient with a perpendicular net-charge gradient are presented. The measures, that were necessary to create a well-defined SAM gradient are discussed in Section 5.3.5. The results of the nanoparticle and protein adsorption tests on these gradients are shown in Sections 5.3.6 and 5.3.7 of this chapter, respectively.

### 5.2 Experimental

**Chemicals.** Amino-modified silica (462 nm diameter, 5 wt.-%, microparticles, Germany) and unmodified silica (900 nm diameter, 10 wt.-%, Polyscience Inc., USA) particle suspensions were diluted in HEPES 0 (aqueous 1 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, Bio-Chemika Ultra, Fluka, Switzerland)) for the particle-adsorption experiments. Bovine serum albumin (96%, Sigma-Aldrich, USA), fibrinogen from human plasma (Hexa Fluor 546 conjugate, Invitrogen USA) and lysozyme from hen egg white (106'509 U/mg, Sigma-Aldrich, USA) were dissolved in phosphate-buffered saline (PBS, Sigma-Aldrich, USA) for the protein-adsorption experiments.

**Substrates.** Prior to the sample preparation, gold-coated glass slides (4 x 4 cm) or silicon wafers (1 x 1 cm or 2 x 2 cm) were sonicated in ethanol for 10 min, UV/O\textsubscript{3}
cleaned (UV Clean Model 135500, Boekel Scientific) for 30 min and subsequently immersed in ethanol for at least 10 min.

**Gradient Preparation.** The stock solutions were prepared by dissolving mercaptoundecanol, mercaptoundecanoic acid or aminoundecanethiol in ethanol at a concentration of 5 mM for mercaptoundecanethiol and mercaptoundecanoic acid and 1 mM for aminoundecanethiol. To 30 ml of the aminoundecanethiol stock solution, 5 ml of 1 M HCl were added and it was stored at 5 °C. All solutions were prepared by further dilution of the corresponding stock solution. In a first step the sample was immersed over 32 mm into 10 µM mercaptoundecanol solution by means of a motion drive (OWIS GmbH, Germany) with a variable speed. It was removed immediately from the solution, rinsed with ethanol and blown dry with nitrogen, followed by a second immersion in 20 µM mercaptoundecanoic acid solution with the same immersion protocol but with the immersion axis being perpendicular to the first. Subsequently the sample was immersed for 4 h in 17 µM aminoundecanethiol solution containing 14% 1 M HCl. Finally the samples were sonicated in ethanol and in water for 3 min to remove any possibly formed double layer, rinsed with ethanol and blown dry with nitrogen.

**Uniform SAMs.** Samples of uniform single-component SAMs were prepared by immersing clean, gold-coated silicon wafers into 1 mM mercaptoundecanol, mercaptoundecanoic acid solution or 220 µM aminoundecanethiol solution containing 14 % 1 mM HCl for at least 14 h.

**Contact-Angle Measurements.** Static contact-angle measurements were performed with 3 µl droplets. Prior to the contact-angle measurements the samples were rinsed for 15 s with ethanol containing 10 mM HCl and blown dry with nitrogen, in order to make sure that the aminoundecanethiol is charged. Contact angles were measured every 5 mm on the gradient region, resulting in 25 measurements per sample. The presented contact-angle values are an average over 3 samples.

**Infrared Spectroscopy.** The polarization was modulated with a frequency of 50 kHz and the maximum of polarization retardation was set at 3000 cm\(^{-1}\). Spectra were acquired at every 3 mm along the mercaptoundecanol gradient direction with a 3-mm aperture and a resolution of 4 cm\(^{-1}\) using 1024 scans of multiplexed interferograms.

**XPS Measurements.** 100 eV was used as pass energy for the detailed scans of Au 4f, C 1s, N 1s, O 1s, Cl 2p and S 2p. The energy resolution (full width at
half-maximum height, fwhm) under this condition measured on gold Au 4f7/2 is 0.95 eV. Acquisition time of approximately 50 min for all spectra was chosen in order to obtain an adequate signal-to-noise ratio in a minimum time and to limit beam-induced damage. In the inner 2x2 cm gradient area a grid pattern of 7x7 points was measured. The total analysis time for one orthogonal gradient sample with these chosen conditions was about 40 h.

**Particle Adsorption.** 2 µl of the amino-modified silica or of the unmodified silica particle suspensions were diluted in 6.25 ml HEPES 0. On top of the gradient sample a UV/O3-cleaned 4x4 cm glass slide, separated with 800 µm thick teflon spacer, was fixed with clamps as schematically displayed in Figure 5.1. The gap between the the gradient and the glass slide was filled with the particle solution. After 30 min of adsorption, the gap was first rinsed with EtOH:HEPES 0 mixture (1:1), then with pure EtOH. After complete evaporation of the ethanol the gradient and the glass slide were separated. Microscope images of the adsorbed particles were taken by an optical microscope and analyzed by the software Image J. The presented particle adsorption densities are an average over 3 samples.

**Protein Adsorption.** BSA and lysozyme were dissolved in PBS to a concentration of 1 mg/ml. Fibrinogen was diluted in PBS from a 1.5 mg/ml solution to a 0.1 mg/ml solution. The protein solutions were filtered immediately prior to use with a 0.2 µm filter, to remove undissolved material. The gradient samples were covered with the protein solutions for 30 min, subsequently rinsed with PBS, ultrapure water and finally blown dry with nitrogen. SAM and protein adlayers thicknesses were determined by variable-angle spectroscopic ellipsometry. Spectroscopic scans were taken after cleaning of the sample, after gradient preparation and after protein adsorption in a 5x5 grid pattern within the gradient area. The presented protein adsorption thicknesses are an average over 3 samples.
5.3 Results and Discussion

5.3.1 Gradient Preparation

The gradient preparation conditions have been chosen, in order to obtain a broad variation in composition of the three components over the gradient area. In contrast to the method used to fabricate orthogonal wettability surface-chemical gradients[30] presented in Chapter 4, the coverage of the alkanethiols used in the first and second adsorption step was extended to almost full coverage for the highest immersion end. For the orthogonal wettability surface-chemical gradients, the coverage of the two components used in the first and second immersion step was restricted to a maximum concentration of 50% in order to obtain two orthogonal density gradients of the components. Using the method presented in this Chapter to prepare three component surface-chemical gradients the component-density gradients are not orientated orthogonal to each other. While the amount of adsorbed C\textsubscript{11}OH is varying along the immersion axis and stays constant perpendicular to the adsorption axis, the amount of adsorbed C\textsubscript{10}COOH is varying along the immersion axis but also perpendicular to the immersion axis. This is due to reduction of free binding sites along the coverage gradient of the preadsorbed C\textsubscript{11}OH perpendicular to the immersion axis of C\textsubscript{10}COOH. Therefore the coverage gradient of the second component is radially shaped. By backfilling the remaining empty binding sites with C\textsubscript{11}NH\textsubscript{3}Cl a radial shaped coverage gradient of C\textsubscript{11}NH\textsubscript{3}Cl as third component is created. The distribution of the components are displayed in Figure 5.2.

![Distribution of the components](image)

Figure 5.2: Distribution of the three components indicated with a color scale gradient.

As already stressed for the orthogonal wettability surface-chemical gradients, the sequence of immersion has to be selected carefully. C\textsubscript{11}OH was selected as first component, in order to create the charge-density gradient perpendicular to the net-charge gradient. Besides that it was expected that the C\textsubscript{11}OH thiol would be less susceptible to replacement than the charged thiols, since it more easily forms dense
SAMs than the thiols with bulky and charged functional groups. C$_{11}$NH$_3$Cl is adsorbed as last component in the backfilling step due to its susceptibility to oxidation. Therefore C$_{10}$COOH was adsorbed as second component.

C$_{11}$OH and C$_{10}$COOH adsorb more slowly on the gold surface than dodecanethiol used to create full-range unidirectional gradients[1] by approximately a factor of two and four, respectively. The static water contact angle after immersion of clean samples into 5 µM solution of C$_{11}$OH and C$_{10}$COOH is shown in Figure 5.3. While for dodecanethiol full coverage is reached after about eight minutes, it takes 16 and 32 minutes for C$_{11}$OH and C$_{10}$COOH, respectively, to reach full coverage.

![Figure 5.3: Adsorption kinetics of C$_{11}$OH (○) and of C$_{10}$COOH (●) individually, onto clean substrates. Static water contact angle displayed against the immersion time into 5 µM alkanethiol solution.[137]](image)

Instead of increasing the immersion time with respect to the unidirectional dodecanethiol gradient, the concentration was raised in order to obtain comparable immersion times. By doubling (10 µM) and quadrupling (20 µM) the concentration for C$_{11}$OH and C$_{10}$COOH, respectively, with respect to the concentrations used in former studies[1, 30], the maximum immersion time could be restricted to 9 min for the highest-coverage end of the gradient. This reduces both oxidation of the preadsorbed thiols and hydrocarbon contamination adsorbed from the air during the immersion in the non-immersed region of the sample.

The immersion profile is selected in a way that only the inner 2x2 cm form the gradient area. Having the area of interest located in the sample centre has the
advantage that observed effects are less sensitive to be caused by artefacts due to close proximity to the sample edge.

In order to maximize the dynamic range of the gradient the immersion speed is increased towards the short-immersion end of the gradient. In Figure 5.4 the immersion time is displayed against the position on the sample surface.

![Figure 5.4: Immersion time of sample in the alkanethiol solution along the gradient axis during the gradient preparation steps.](image)

To the $\text{C}_{11}\text{NH}_3\text{Cl}$ backfilling solution HCl was added to a final concentration of 140 mM. This measure is discussed in detail in section 5.3.5.

### 5.3.2 Surface-Chemical Composition

X-ray photoelectron spectroscopy (XPS) was carried out, to determine the chemical composition of the gradient. In the first part of this section it will be discussed how the fraction of the components on the gradient area can be derived from the XPS signals and the fractional distribution will be displayed. In the second part the distributions of the components will be discussed in the sequence they were applied on the surface. In the third part the charge state of the $\text{C}_{11}\text{NH}_3\text{Cl}$ component will be discussed. In the last part of this section the ratio between the ionizable functional groups will be displayed.
Derivation of the Fractions of the Three Components from XPS measurements

XPS was measured on the gradient area in order to analyze the chemical composition on the charge gradient. The Au 4f, C 1s, N 1s, O 1s, Cl 2p and S 2p signals were recorded. The distribution of the $\text{C}_{11}\text{NH}_3\text{Cl}$ component can be illustrated by the N 1s signal, since no other components contribute to the N 1s signal. In Figure 5.5 the atomic percentage of nitrogen divided by the atomic percentage of nitrogen for the homogeneous $\text{C}_{11}\text{NH}_3\text{Cl}$ SAM is displayed.

![Graph showing the distribution of $\text{C}_{11}\text{NH}_3\text{Cl}$](image)

**Figure 5.5:** Fraction of $\text{C}_{11}\text{NH}_3\text{Cl}$ on the gradient surface calculated from the atomic percentage of the N 1s XPS signal with respect to the full monolayer. Each intersection of black gridlines corresponds to a measured value. The color scale is displayed beside the plot. Additionally, for clarity, here and in subsequent Figures, the immersion directions into $\text{C}_{11}\text{OH}$ and $\text{C}_{10}\text{COOH}$ solutions are indicated by green and a blue color intensity gradients, respectively.

The distribution of $\text{C}_{10}\text{COOH}$ was determined from the C 1s signal. In Figure 5.6 the C 1s signal from the region with high $\text{C}_{10}\text{COOH}$ density is shown. The most intense signal at 284.8 eV arises from the aliphatic carbon. The signal from the carbon of the carboxylic acid group, observed at 289.2 eV, is shifted by 4.4 eV with respect to the aliphatic carbon. The signal of the carbon bound to the sulfur group (C-S) can be seen at 287.3 eV. In the curve-fitting process the shift has been derived from the shift of the C 1s (C-S) signal of the dodecanethiol SAM where only the C 1s (C-S) and the aliphatic C 1s peaks are observed. The contribution of the C 1s (C-S) has also been restricted to the fraction of the C 1s (C-S) on the
5.3. Results and Discussion

The C 1s signal determined from a dodecanethiol SAM. Although the stoichiometry of the sulfur-bound carbon is 1:1 with carbons bound to functional groups of the thiols, the intensity of its XPS signal is significantly lower than their signal due to attenuation. While the functional groups are present at the outermost part of the SAM, the carbon bound to the sulfur is covered by the alkyl chains of the thiols and the signal is therefore attenuated by the SAM layer. The shift of the C 1s (C-O) signal with respect to the C 1s (C aliphatic) was determined from the C_{11}OH SAM and kept fixed during the fitting process. The same procedure was applied for the shift of the C 1s (COOH) from the C_{10}COOH SAM and the C 1s (C-NH_{3}) from the C_{11}NH_{3}Cl SAM. The shift of the C 1s (C-NH_{3}) is 1.6 eV, resulting in a signal position of 286.4 eV and for the C 1s (C-OH) signal the shift is 1.7 eV, resulting in a signal position of 286.5 eV.

Figure 5.6: Typical C 1s XPS spectrum along with the fitted C aliphatic, C-S, COOH and C-NH_{3}. The C-OH signal is not shown because the C_{11}OH concentration on that spot is zero.

The C 1s (COOH) signal is the only signal arising from the functional end groups that is clearly separated from the others. Therefore the signal can be used to calculate the fraction of C_{10}COOH from ratio of the atomic fraction of the C 1s (COOH) signal over the atomic fraction in the single component monolayer. Figure 5.7 shows the distribution of C_{10}COOH fraction over the gradient area.

The shift of the C 1s signal from the carbon bound to the hydroxyl group is only 1.7 eV and therefore overlapping with the C 1s signal from the carbon bound to
the amine group, which is shifted 1.6 eV with respect to the aliphatic carbon. The distinction of the two signals is hardly possible and the determination of the C_{11}OH component distribution has to be derived in another way. Since the O 1s signal has contributions of the C_{11}OH, C_{10}COOH components and from water bound to the C_{11}NH_3Cl component[138], this signal is also not very suitable to determine the C_{11}OH distribution on the gradient surface. Therefore the distribution of the C_{11}OH component was calculated from the difference of the sum of the fractions of the other two components to one, assuming that no contamination is present on the surface. Figure 5.8 displays the fraction of the C_{11}OH component on the gradient surface, calculated in this way.

**Discussion of the Fractional Distribution of the Three Components**

The fraction of the C_{11}OH component increases with increasing immersion time into the C_{11}OH solution and remains constant perpendicular to the immersion direction. It varies from 0 for the region immersed only for a second up to 0.71 for the region immersed for nine minutes. Towards the long-immersion end the steepness of the gradient levels off. This reflects the slightly modified Langmuir-type adsorption kinetics[13]. By applying a nonlinear-immersion speed profile with lower immersion speeds for the high-coverage end, this saturation effect has already been reduced.
5.3. Results and Discussion

Figure 5.8: Fraction of C$_{11}$OH on the gradient surface calculated from by the difference of the fraction of the two other components to one. Each intersection of black gridlines makes a measured value. The color scale is displayed beside the plot.

If linear adsorption profiles would have been applied, this effect would have been more pronounced. Besides the just mentioned Langmuir-type adsorption kinetics, it is mainly replacement effects during the backfilling process that are responsible for the C$_{11}$OH fraction not reaching higher values than 0.71. From the uniform concentration distribution perpendicular to the C$_{11}$OH immersion axis, it can be seen that replacement effects during the second immersion step can be neglected. If replacement effects were to play an important role during the second immersion step, the C$_{11}$OH concentration would decrease with increasing immersion time into the C$_{10}$COOH solution.

The C$_{10}$COOH concentration (see Figure 5.7) is increasing with increasing immersion time into the C$_{10}$COOH solution and with decreasing immersion time into the C$_{11}$OH solutions leading to a radial-shaped type of gradient. This shape is apparently different to the shape of the second component for the orthogonal wettability gradient, where both gradients have a unidirectional shape oriented perpendicular to each other. In order to create a surface-chemical charge gradient with the components’ density gradients oriented orthogonal to each other, it would be necessary to restrict the immersion time into the C$_{11}$OH solution to a maximum of about 40 s. For higher immersion times, the adsorption kinetics of the C$_{10}$COOH component is
already influenced by the preadsorbed species, as is observable in Figure 5.7. This illustrates that the dynamic range of orthogonal component-density gradients is limited. The fraction of the C\textsubscript{10}COOH component ranges from 0.13 to 0.71 and at 0.27 for the C\textsubscript{11}NH\textsubscript{3}Cl-rich region is quite high. Further decrease of the immersion time into the C\textsubscript{10}COOH component for the briefly immersed end of the gradient becomes difficult, since it is already as low as 1 second. It would be necessary to decrease the concentration of the adsorption solution, in order to obtain lower fractions for the short immersion end. However, this would lead to increased immersion times required for the long immersion end and therefore to increased hydrocarbon contamination from the air during the adsorption process. One alternative would be to dilute the adsorption solution during the adsorption process with pure ethanol in a controlled way, although this would inevitably involve some challenges relating to homogeneous mixing.

The C\textsubscript{11}NH\textsubscript{3}Cl component distribution is also radially shaped (see Figure 5.5). The lower the immersion time into the two first gradient components, the higher the C\textsubscript{11}NH\textsubscript{3}Cl concentration due to the increase in available binding sites. The fraction ranges from 0.18 to 0.71. The concentration drops quite linearly in all directions (although the immersion time into the two other components has been deliberately chosen to be nonlinear) and it levels off at a fraction of about 0.2. Only for the region where the C\textsubscript{10}COOH component is highest in concentration does the fraction remain slightly higher (0.25). On the one hand, the C\textsubscript{11}NH\textsubscript{3}Cl fraction does not exceed 0.71 because of the substantial fraction of C\textsubscript{10}COOH at the briefly immersed end as discussed above. On the other hand, the C\textsubscript{11}NH\textsubscript{3}Cl fraction does not drop below 0.18 due to replacement effects. The immersion time into the C\textsubscript{11}NH\textsubscript{3}Cl solution is, at 4 h, much longer than the maximal immersion time into the other two alkanethiol solutions. During this backfilling step, not only are the empty binding sites filled but also previously adsorbed species can desorb and be replaced by the C\textsubscript{11}NH\textsubscript{3}Cl component. The longer the sample remains in the backfilling solution, the higher the fraction of replaced species. Therefore the backfilling time was restricted to 4 h even though the SAM layer ordering could be improved by longer immersion into the backfilling solution. This issue will be further discussed in Section 5.3.3.

Two effects could be responsible for the higher C\textsubscript{11}NH\textsubscript{3}Cl fraction in the C\textsubscript{10}COOH-rich region, in comparison to the C\textsubscript{11}OH-rich region. On the one hand, the C\textsubscript{11}NH\textsubscript{3}Cl and C\textsubscript{10}COOH thiols are oppositely charged and electrostatic interactions between the charged groups could lead to increased backfilling and replacement rate. On the
other hand, it takes much longer for the C\textsubscript{10}COOH component than for the C\textsubscript{11}OH to form a dense SAM, due to the bulky and charged functional group and the less dense a SAM is, the higher is the backfilling and replacement rate.

**Charge State of C\textsubscript{11}NH\textsubscript{3}Cl on the Surface**

The N 1s signal of the amine is to be found at 399.6 eV. If the nitrogen is charged due to protonation, the nitrogen signal shifts to a higher binding energy (401.9 eV). Depending on the rinsing procedure of the SAM prior to the XPS measurements, the ratio between the two signals can be very different. If a uniform C\textsubscript{11}NH\textsubscript{3}Cl SAM is rinsed with ethanol only, the fraction of charged nitrogen groups remains 0.63, but after the rinsing procedure used for the gradients, which includes a sonication step in water, only a fraction of 0.21 remains protonated. In Figure 5.9 N 1s, Cl 2p and O 1s XPS signals of a sample sonicated solely in ethanol (lower) and of a sample sonicated in both ethanol and water (upper) are shown. While the chlorine signal can clearly be observed on the former sample, it is not detectable in the latter. Oxygen shows the opposite behavior: while on the ethanol-sonicated sample no oxygen is observed, a clear signal on the sample sonicated also in water is detectable. In the sulfur signal no oxidized species can be observed for either samples. Recently Baio et al. [138] et al. pointed out that the oxygen found on amine-terminated SAMs arises from tightly bound water or other oxygen-containing coadsorbates. The appearance of the oxygen signal after sonication in water supports this finding. Hydrogen bonding between the lone pair of nitrogen in the uncharged amine functional group and the

![Figure 5.9: N 1s, Cl 2p and O 1s XPS spectra on uniform C\textsubscript{11}NH\textsubscript{3}Cl SAMs sonicated in ethanol only (lower) or sonicated in ethanol and in water (upper).](image-url)
hydrogen of a water molecule is possible. From the N 1s signal it can be observed that in the absence of chlorine a significant lower fraction of nitrogen groups is charged. The degree of protonation therefore depends on the amount of available counter-ion.

On the three-component surface-chemical gradient, no chlorine Cl 2p signal is detectable. However, the C_{10}COOH thiol can serve as counter ion. In Figure 5.10 the fraction of the total amine groups present on the surface that are charged is displayed. The fraction changes from a predominant charged fraction of 0.62 in the C_{10}COOH-rich region to a sparsely charged fraction of 0.25 in the hydroxyl- and amine-rich region. In the C_{11}OH-rich region, the fraction of the charged C_{11}NH_3Cl is about 0.3 — slightly higher than in the amine-rich region, but considerably lower than in the C_{10}COOH-rich region. The shape of the surface plot displaying the charged fraction of the amine functional groups on the gradient surface resembles very much the surface plot in Figure 5.7 displaying the C_{10}COOH fraction on the gradient surface. This illustrates that the presence of the C_{10}COOH thiol, acting as counter ion in its deprotonated state C_{10}COO^-, is necessary in order to keep the majority of the amine functional groups charged in absence of chlorine as counter ion. This illustrates that the pK_d (dissociation constant) of the amine functional group can be very different depending on the local environment.

**Figure 5.10:** Surface plot of the fraction of protonated amine signal N 1s (NH_3^+) on the total amine signal, plotted against the immersion time into the C_{11}OH and C_{10}COOH solutions. Each intersection of black gridlines correspond to a measured value. The color scale is displayed beside the plot.
5.3. Results and Discussion

Ratio between Ionizable Functional Components

The ratio between the ionizable functional groups varies over the gradient area. In Figure 5.11(a) the ratio of the C\textsubscript{10}COOH to the C\textsubscript{11}NH\textsubscript{3}Cl and in (b) the ratio of the C\textsubscript{11}NH\textsubscript{3}Cl to the C\textsubscript{10}COOH fraction are displayed. In the C\textsubscript{11}OH-rich region, the ratio is over a broad range around 1. Thus an equal amount of functional groups that can be positively charged (–NH\textsubscript{3}\textsuperscript{+}) and negatively charged (–COO\textsuperscript{−}) are present on the surface. Towards shorter immersion time into C\textsubscript{11}OH and longer immersion time into the C\textsubscript{10}COOH solution, negatively chargeable functional groups are present.
in excess. For shorter immersion times into both $C_{11}OH$ and $C_{10}COOH$ solution, positively chargeable functional groups are adsorbed in excess. The plots do not represent the surface net charge, since that depends on the pH of the surroundings. However, the shapes of the plots give an idea of the charge distribution over the gradient area.

5.3.3 Organization and Thickness

Figure 5.12 shows the PMIRRA spectra along the $C_{11}OH$ immersion axis for 1, 15, 40, 70, 180, 340, 540 s immersion time into the $C_{11}OH$ solution. The reference spectra of the uniform single-component $C_{10}COOH$ and $C_{11}OH$ monolayers are shown at the top and at the bottom of the graph, respectively. The position of the symmetric and asymmetric $CH_2$ stretching modes are shifted with respect to the uniform single-component monolayers, which are observed at 2850 and 2920 cm$^{-1}$, respectively. This shift shows that the alkyl chains on the gradient surface are not well

![Figure 5.12: PMIRRA spectra for 1, 15, 40, 70, 180, 340, 540 s immersion (from top) along the $C_{11}OH$ immersion axis on the three-component gradient. The reference spectra of the uniform single-component $C_{10}COOH$ and $C_{11}OH$ monolayers are shown at the top and at the bottom of the graph, respectively.](image)
organized. The same effect was observed for the orthogonal wettablity gradient presented in Chapter 4. The backfilling time could be increased to improve the ordering and crystallinity of the SAM. However, as discussed in Section 5.3.2, even after 4 h immersion time substantial replacement is observed. Increase of the backfilling time could not only lead to increased replacement but also to an increased fraction of transformed carboxylic acid groups into carboxylic acid ester. This issue will be discussed further in Section 5.3.5.

In Figure 5.13 the thickness of the three-component charge gradient SAM measured by ellipsometry is shown. The thickness is between 9 and 11 Å and does not vary significantly along the gradient surface. The thicknesses for the uniform single-component SAMs are higher than the SAM on the gradient surface at 14 Å for the C\textsubscript{11}COOH, 11 Å for the C\textsubscript{11}OH and 14 Å for the C\textsubscript{11}NH\textsubscript{3}Cl SAMs. Nevertheless, as already mentioned above, the ordering and crystallinity of the SAM is reduced with respect to the uniform single-component SAMs, which also can be observed by the reduced SAM thickness.

5.3.4 Wetting Behavior

Many very different contact angles are reported in the literature for acid- and amine-terminated alkanethiol SAMs. For a carboxylic-acid-terminated SAM, Bain
et al. [139] reported a static contact angle of <10°, Fears et al. [140] an advancing contact angle of 18° and Min Sze Wang et al. [141] an advancing contact angle of 72°. Wang et al. [131] showed that the advancing contact angle could be reduced from 35° to 10° if CF₃COOH was added to the solution, which inhibited double-layer formation. Double-layer formation is probably an important reason why such a broad range of water contact angles are reported for carboxylic acid terminated SAMs. Another issue is the dependence of the contact angle on the pH of the water. Lee et al. [142] showed that the advancing water contact angle of a carboxylic-acid-terminated SAM measured under cyclooctane can vary between <10° and 60° if the pH changes from 8 to 5. The pH of ultrapure water can vary from 7 to 4.5 due to the uptake of CO₂ from the air. Therefore the contact angle observed can change depending on the preparation, storage and freshness of the water.

Also for the amine-terminated SAM, different contact angles are reported in the literature. Wang et al. [131] reported that the advancing contact angle was reduced from 43° to 28° if N(CH₂CH₃)₃ was added to the solution, which inhibited double-layer formation and reduced the fraction of oxidized sulfur groups. Fears et al. [140] observed a contact angle of 38° and Lee et al. a contact angle of 87°!

The decrease of the static contact angle with increasing immersion time in the alkanethiol solution for the carboxylic-acid-terminated SAM displayed in Figure 5.3 shows that the contact angle is decreasing with increasing density and ordering of the SAM. The same was observed for the amine-terminated thiol.

The difficulty in preparing dense, unoxidized SAMs with chargeable functional groups without an adsorbed double layer and the influence of the pH of the water on the contact angle makes it difficult to compare contact angles reported in the literature with contact angles measured on the three-component gradient. Therefore only the contact angles measured on uniform, single-component SAMs prepared with our method and measured with our measuring setup are used as reference values. These reference values are listed in Table 5.1.

In Figure 5.14, static water contact angles measured on the gradient surface before (upper) and after (lower) the backfilling step in the C₁₁NH₃Cl solution are plotted against the immersion time into the C₁₁OH and C₁₀COOH solution. Please note that the axes of the two surface plots are inverted because of the better presentability. Before the backfilling process the contact angle is highest where the immersion time in the C₁₁OH and C₁₀COOH solution is lowest. This was expected since hydrocarbon
Table 5.1: Static and dynamic contact angles on uniform single-component C\textsubscript{11}OH, C\textsubscript{10}COOH and C\textsubscript{11}NH\textsubscript{3}Cl SAMs. The same rinsing protocol as for the three-component gradient is applied prior to the measurements.

<table>
<thead>
<tr>
<th>SAM</th>
<th>CA\textsubscript{static} [°]</th>
<th>CA\textsubscript{advancing} [°]</th>
<th>CA\textsubscript{receding} [°]</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{11}OH</td>
<td>13</td>
<td>13</td>
<td>&lt;10</td>
</tr>
<tr>
<td>C\textsubscript{10}COOH</td>
<td>10</td>
<td>11</td>
<td>&lt;10</td>
</tr>
<tr>
<td>C\textsubscript{11}NH\textsubscript{3}Cl</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

Figure 5.14: Surface plots of the static water contact angle measured on the three-component gradient area, plotted against the immersion time into the C\textsubscript{11}OH and C\textsubscript{10}COOH (a) before and (b) after backfilling with C\textsubscript{11}NH\textsubscript{3}Cl. Each intersection of black gridlines marks a contact angle measured value. The color scale is displayed beside the plot.
contamination on bare gold has a contact angle of about 80°. The contact angle drops faster towards the C\textsubscript{11}OH-rich region than towards the C\textsubscript{10}COOH-rich region, but a saturation value is reached in both directions. The saturation values are surprisingly high. For the C\textsubscript{10}COOH-rich region the contact angle does not drop below 46°, although on a uniform single-component C\textsubscript{10}COOH SAM, contact angles of 10° are measured. Also, the contact angle on the C\textsubscript{11}OH-rich region (35°) is significantly higher than the value for the uniform single-component C\textsubscript{11}OH SAM (13°). After backfilling the contact angle decreases to various extent. The most drastic change is, as expected, observed in the regions which has not been covered before. The contact angle drops from 71° to 14°, which is close to the wetted state observed for a uniform single-component SAM (<10°). In the C\textsubscript{10}COOH-rich region, the contact angle changes only from 46° to 42°, the contact angle in the C\textsubscript{11}OH rich region dropping from 35° to 26°. In the region immersed longest into both the C\textsubscript{11}OH and C\textsubscript{10}COOH solutions, the change is the smallest (41° to 39°).

As shown in Section 5.3.3 the density and ordering of the alkanethiols on the three-component gradient is significantly reduced in comparison to the uniform single-component SAMs. This can also explain the higher contact angles observed for the gradient in contrast to the uniform single-component SAMs. What also has to be taken into account, is that it takes significantly longer to measure the contact angle on all different positions on a three-component gradient than on a uniform single-component SAM. During this time it is also possible that some hydrocarbon contamination from the air is adsorbing onto the surface. The measurements were always started within seconds after the rinsing process, but while for the single-component SAMs only seconds were needed to perform the contact-angle measurements, it took about 10 min until the gradient was measured.

5.3.5 Effect of Hydrochloric Acid in the Backfilling Solution

Lee et al. showed [132] that the oxidation of the amine-terminated SAMs can be prevented by adding hydrochloric acid to the adsorption solution. The interaction of the amine with the gold by the nitrogen lone-pair can be inhibited by protonating the amine group and therefore the adsorption of aminoundecanethiol in top-down orientation in the SAM is not probable. If no thiols are embedded upside-down in the SAM, the thiol groups are not exposed to the air and therefore not as susceptible to oxidation. The lowest spectra in Figure 5.15 show the S 2p and O 1s signal of a
5.3. Results and Discussion

Figure 5.15: High-resolution XPS S 2p and O 1s spectra of aminoundecanethiol SAMs prepared from different solution. Top: no HCl in stock solution and no HCl in adsorption solution. Middle: no HCl in stock solution, but HCl in adsorption solution. Bottom: HCl in stock solution and HCl in adsorption solution.

C\textsubscript{11}NH\textsubscript{3}Cl SAM prepared from a solution containing hydrochloric acid. If a fraction of the sulfur groups were to be oxidized, a S 2p signal of at a binding energy of 169 eV would be observed. Since no such signal can be detected it can be concluded that our SAM preparation protocol led to unoxidized C\textsubscript{11}NH\textsubscript{3}Cl SAM.

Alkanethiols are often stored as stock solutions with higher concentrations before further diluting to the required concentration. The most frequently used alkanethiols are stable in ethanolic solution. However, aminoundecanethiol is very sensitive to oxidation. In the upper XPS spectra of Figure 5.15 the S 2p and O 1s spectra SAMs prepared from ethanolic alkanethiol solution from a stock solution, which was stored over weeks in the shelf, were displayed. It can be observed that the majority of the thiol groups were oxidized. If hydrochloric acid was added to the adsorption solution, although the stock solution was the same, the fraction of the oxidized species was drastically reduced. This can be observed from the middle XPS spectra of Figure 5.15. If hydrochloric acid was already added to the stock solution the effect could be reduced even further (see lowest spectra in Figure 5.15). These measurements show that the hydrochloric acid on the one hand supresses the oxidation of the C\textsubscript{11}NH\textsubscript{3}Cl in ethanol, and on the other hand inhibits the implementation of miss-oriented thiols. It is likely that the oxidized sulfur species is negatively charged at neutral pH.
As mentioned in the introduction, the coadsorption of oppositely charged components can lead to a more balanced concentration on the surface than in the solution. Therefore it is possible that traces of oxidized thiols present in the solution are implemented preferentially in the SAM in upside-down orientation. The change in pH of the adsorption solution renders the sulfonate group uncharged, which would eliminate the electrostatic attraction between the amine and the oxidized sulfur group. No preferential adsorption of the thiol with the oxidized sulfur group should then be observed.

Unfortunately the hydrochloric acid added to the C$_{11}$NH$_3$Cl solution in the backfilling step can interact with the preadsorbed thiols. Carboxylic-acid-terminated SAMs can undergo an esterification reaction with ethanol in presence of hydrochloric acid.[134] In order to test how fast the reaction is and if the backfilling step in ethanol containing hydrochloric acid is short enough to prevent the transformation of a significant fraction of the preadsorbed C$_{10}$COOH thiols, the stability of a homogeneous C$_{10}$COOH monolayer in an ethanol containing 140 mM HCl was tested. PM-IRRAS and static water contact-angle measurements were performed after 0 s, 4 h and 6 days of immersion into ethanol containing 140 mM HCl. The static water contact angle was unchanged low (11 °) after 4 h of immersion. After 6 days the contact angle was increased to 66 °. Also the PM-IRRA spectra of the fresh sample and the sample, which was immersed for 4 h in acidified ethanol, look the same (see Figure 5.16). After 6 days, ester-bands were observed. Therefore it can be concluded that the esterification of the carboxylic group of the C$_{10}$COOH SAM is a slow process. The immersion time in the backfilling solution was not long enough that the transformation could be observed by IR or by the contact-angle measurements. Longer immersion times in the backfilling solution could lead to substantial ester-formation between the carboxylic acid functional group of the SAM and the ethanol in solution. Because of that and because of the above mentioned replacement process, the backfilling time was restricted to 4 h.

Ester formation on the surface is slowed down with respect to ester formation in solution, since the backside attack of the alcohol on the carboxylic acid group is sterically hindered if the thiol is embedded in the SAM.[37] Besides inhibiting the upside-down adsorption of the C$_{11}$NH$_3$Cl component in the SAM, the presence of the hydrochloric acid has another advantage. By keeping the carboxylic acid groups protonated, the interchain anhydride formation can be suppressed and no coupling with the amine-functional group of the C$_{11}$NH$_3$Cl component should occur.[143]
5.3. Results and Discussion

Figure 5.16: PM-IRRA spectra of C\textsubscript{10}COOH freshly prepared (bottom), immersed into ethanol containing 140 mM HCl for 4 h (middle) and for 6 days (top).

5.3.6 Particle Adsorption

Silicon with a natural oxide layer is negatively charged. Therefore unmodified SiO\textsubscript{2}-nanoparticles are used to study the adsorption of negatively charged particles on the gradient. If the surface is functionalized with amine groups, the particles become positively charged. Zeta-potential measurements on the amine-modified silicon particles were performed in ultrapure water showed that the particles are clearly positively charged (56 mV). Ultra-pure water has an acidic pH due to the uptake of CO\textsubscript{2} from the air. In order to perform particle-adsorption measurements in an almost neutral environment, the buffer HEPES 0 (pH=7.4) was used as solvent. In HEPES 0 the charge of the amine-modified SiO\textsubscript{2}-particles is slightly reduced (47 mV), but still positive with a good stability. In Figure 5.17 the surface plots of particle density of adsorbed particles on the gradient area are shown. The adsorption of positively charged amine modified SiO\textsubscript{2}-nanoparticles (Figure 5.17a) is strongly correlated with the C\textsubscript{10}COOH density on the surface. The higher the C\textsubscript{10}COOH density the higher the amount of adsorbed particles. But while the C\textsubscript{10}COOH fraction (5.7) is almost linearly decreasing towards the C\textsubscript{11}NH\textsubscript{3}Cl rich region, the particle density is decreasing faster towards the C\textsubscript{11}NH\textsubscript{3}Cl rich edge. The C\textsubscript{10}COOH fraction is still changing clearly between 1 and 15 s of immersion time into the C\textsubscript{10}COOH solution, while the particle density decreased to a zero-level at an immersion time of
5. Orthogonal Charge-Density versus Net-Charge Gradients

Figure 5.17: Surface plots of the density of adsorbed (a) SiO$_2$-NH$_2$ and (b) unmodified SiO$_2$ nanoparticles measured on the three-component gradient area, plotted against the immersion time into the C$_{11}$OH and C$_{10}$COOH. Each intersection of black gridlines corresponds to a measured value. The color scale is displayed beside the plot.

15 s. This illustrates that not only the fraction of C$_{10}$COOH but also the C$_{11}$NH$_3$Cl fraction has an influence on the density of adsorbed particles. Towards the C$_{11}$OH rich region, the particle adsorption density is dropping slower than the C$_{10}$COOH fraction. The C$_{10}$COOH fraction is already low at an immersion time of 70 s into the C$_{11}$OH solution, but the particle density is only substantially reduced at an immersion time of 180 s. This, in contrast, shows the expected effect that the adsorption is less influenced by diluting the surface charge with a neutral species than by adding oppositely charged species.
5.3. Results and Discussion

The negatively charged particle density is highest in the C\textsubscript{11}NH\textsubscript{3}Cl-rich region and lowest in the C\textsubscript{10}COOH-rich region. In the C\textsubscript{11}OH-rich region the particle density is intermediate. The shape of the particle density plot resembles the shape of the C\textsubscript{11}NH\textsubscript{3}Cl over C\textsubscript{10}COOH ratio plot (see Figure 5.11b). If the ratio between the two chargeable functional groups is 1 the adsorption of the negative particles is intermediate. While little excess of the C\textsubscript{11}NH\textsubscript{3}Cl functional group leads to increased particle density, a high C\textsubscript{10}COOH excess is needed to completely suppress the particle adsorption. The particle density reaches a saturation value although the C\textsubscript{11}NH\textsubscript{3}Cl excess would still be increasing. This is probably due to electrostatic repulsive forces between the particles, what inhibits higher particle density.

In contrast to the negatively charged particles, the positively charged particles do not adsorb in the area where the ratio between the two chargeable groups is 1. This discrepancy indicates that the surface net charge is not balanced when the molecular ratio of the chargeable groups is 1. A higher fraction of the amine groups are charged compared with the carboxylic acid groups.

5.3.7 Protein Adsorption

The adsorption of three different proteins was tested on the three-component charge gradients. The first protein was lysozyme. It is a small protein (4.5 x 3.0 x 3.0 nm) and with a pI of 10.5-11 it is positively charged at physiological pH (+9 e).\cite{144, 145}

Then the adsorption of bovine serum albumin (BSA), which is the most abundant serum protein, was tested. BSA has with dimensions of 11.6 x 2.7 x 2.7 nm double the size of lysozyme. With a pI is 4.7-4.8\cite{144} it is negatively charged at physiological pH (-17 e)\cite{146}. Albumin is a biological passsivator.\cite{147, 148} Platelet adhesion is reduced on albumin-coated surface. Fibrinogen is the third protein for which the adsorption was tested on the gradient surface. It is the biggest protein among these three (45 x 6 x 6 nm) and is with a pI of 5.1-6.3\cite{144} also negatively charged at physiological pH (-21 e)\cite{149}.

Table 5.2 lists the thicknesses of the adsorbed protein layers measured on uniform single component SAMs. As expected, the positively charged lysozyme adsorbs mainly on the negatively charged C\textsubscript{10}COOH SAM and barely on the neutral C\textsubscript{11}OH and positively charged C\textsubscript{11}NH\textsubscript{3}Cl. This trend is also observable on the gradient, as shown in Figure 5.18. The higher the C\textsubscript{10}COOH/C\textsubscript{11}NH\textsubscript{3}Cl ratio on the surface (see
Table 5.2: Upper part: Protein adsorption on uniform single component C\textsubscript{11}OH, C\textsubscript{10}COOH and C\textsubscript{11}NH\textsubscript{3}Cl SAMs. Lower part: charge at physiological pH and size of the three proteins.

<table>
<thead>
<tr>
<th>SAM</th>
<th>Lysozyme [Å]</th>
<th>BSA [Å]</th>
<th>Fibrinogen [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{11}OH</td>
<td>4 (±2)</td>
<td>2 (±2)</td>
<td>8 (±3)</td>
</tr>
<tr>
<td>C\textsubscript{10}COOH</td>
<td>11 (±4)</td>
<td>8 (±4)</td>
<td>7 (±3)</td>
</tr>
<tr>
<td>C\textsubscript{11}NH\textsubscript{3}Cl</td>
<td>2 (±3)</td>
<td>26 (±2)</td>
<td>41 (±7)</td>
</tr>
<tr>
<td>Charge [e]</td>
<td>+9</td>
<td>-17</td>
<td>-21</td>
</tr>
<tr>
<td>Size [nm]</td>
<td>4.5 x 3.0 x 3.0</td>
<td>11.6 x 2.7 x 2.7</td>
<td>45 x 6 x 6</td>
</tr>
</tbody>
</table>

As listed in Table 5.2 the adsorption of the negatively charged proteins BSA and fibrinogen is highest on the positively charged uniform C\textsubscript{11}NH\textsubscript{3}Cl SAM. For both...
proteins, the adsorption is significantly reduced on the C$_{10}$COOH and C$_{11}$OH SAMs. In Figure 5.19 the thickness of the adsorbed BSA (upper) and fibrinogen (lower) layer are shown. The shapes of the surface plots for the adsorbed protein layer thickness resembles the C$_{11}$NH$_3$Cl/C$_{10}$COOH fraction ratio (see lower part of Figure 5.11). The thickness of the adsorbed BSA layer varies between 7 and 20 Å. The maximum thickness observed on the gradient is lower as for the uniform single component C$_{11}$NH$_3$Cl SAM. For the adsorbed fibrinogen the thickness varies between 14 and 31 Å, which is also lower than the adsorption on the C$_{11}$NH$_3$Cl SAM. However, as observed for the lysozyme no protein resistance due to a charge-balanced zwitterionic
orthogonal Charge-Density versus Net-Charge Gradients

mixture is observed, although the protein adsorption stays low down to a immersion time of 70 s in the C\textsubscript{10}COOH solution. In contrast to the positively charged lysozyme fibrinogen and BSA adsorption is intermediate in the C\textsubscript{11}OH rich region, although the protein adsorption on the uniform C\textsubscript{11}OH is low. The same behavior was observed for the negative particle adsorption. This is probably due to a higher fraction of the C\textsubscript{11}NH\textsubscript{3}Cl being charged compared with the C\textsubscript{10}COOH compound, which probably leads to a rather positively charged C\textsubscript{11}OH-rich region. Different reasons could be responsible for the absence of a protein-resistant region. First of all, the microstructure of the SAM created by sequential adsorption could be different to that of the SAM prepared by coadsorption, although we believe that the adsorption of the chargeable thiols in the charged state reduces the island formation drastically in contrast to the situation for dodecanethiol adsorption.[50] AFM or Kelvin-probe force microscopy (KPFM) measurements could possibly confirm this assumption. The neutral C\textsubscript{11}OH component probably still shows some island microstructure, but since a purely zwitterionic region for the lowest immersion time into the C\textsubscript{11}OH component exists, this cannot be the reason why no protein-resistant region is observed. Secondly the protein-adsorption minima for charged balanced SAMs might be a combination of low protein adsorption for both positively and negatively charged SAMs. This could be tested by adsorbing a mixture of differently charged proteins (e.g. blood serum). Thirdly, the certain degree of disorder observed for the gradient could lead to increased protein adsorption due to partial exposure of hydrophobic -CH\textsubscript{2}- groups from the alkane-chain of the thiol. It is known that protein adsorption is increased on hydrophobic surfaces.[128, 150–152]

5.4 Conclusion

A three-component surface-chemical gradient exposing a net-charge gradient perpendicular to a charge density gradient was prepared from the neutral C\textsubscript{11}OH, the negatively chargeable C\textsubscript{10}COOH and the positively chargeable C\textsubscript{11}NH\textsubscript{3}Cl. The dynamic range of the three-component on the gradient presented in this chapter is extended compared to that of the three-component gradient presented in Chapter 4. The adsorption of positively and negatively charged objects (both nanoparticles and protein molecules) could be correlated very well with the excess of negatively and positively chargeable groups on the surface, respectively. No protein-resistant
region has been observed on the gradient surface, although charged balanced zwitterionic surfaces have been claimed in literature to be protein resistant. However, the protein adsorption in the region with the same charge as the protein shows reduced protein adsorption compared to the full monolayer. The comparison of the protein adsorption on zwitterionic SAMs prepared by coadsorption and an investigation of the microstructure of the gradient could allow better understanding of the protein adsorption behavior on the gradient.

It would be interesting to study the protein coadsorption of two fluorescently labeled proteins of opposite charged proteins to evaluate the potential of these gradients to sort proteins. The influence of the wettability on the protein adsorption could be studied if dodecanethiol instead of mercaptoundecanol were to be used as a neutral component. The modification of the amine-terminated component by amide formation (see appendix) would allow the immobilization of different, biologically active functional groups. Recent studies have shown that also the surface potential[113, 153] and the work function[154] of a surface can be tuned by mixing charged SAMs. The three-component charge gradients would allow the study of the charge balance and the charge density on the surface potential and the work function.
6.1 Introduction

6.1.1 Polymorphism

Crystallization is one of the most important process steps in the production of organic chemicals. Controlling the crystallization process of an organic molecule during their production has further implications, especially in the pharmaceutical industry, where it is important to control not only the chemical purity but also the crystal structure. Different crystal structures of the same material are called polymorphs. Depending on the interplay of kinetic and thermodynamic effects, one or another polymorphic form is preferentially formed during the crystallization process. Sometimes even more than one polymorph is formed simultaneously – a process known as concomitant crystallization.[155] Polymorphs have different physical properties such as solubility, melting point, density, dissolution rate. If the crystal structures include solvent molecules, the polymorphic forms are called pseudopolymorphs. The most important class of pseudopolymorphs are hydrates, because they can be formed during storage due to uptake of water from the environment.

Polymorphism in the Pharmaceutical Industry

Most active pharmaceutical ingredients (API) have polymorphic forms (although there is a very famous exception, in aspirin). Hence, it is clear why polymorphism
is so important for the pharmaceutical industry: if the solubility of a drug depends on the crystal form, the biological effect depends on the polymorph present. On the one hand it is essential to control the polymorph formed during the crystallization process and on the other hand it is as important to avoid polymorph transformation during tablet production and storage. In 1998 it was necessary to adjourn the production of a drug called Novir because suddenly a much less soluble polymorph of the API Ritonavir was formed during production.\[156\] To avoid such expensive recall and reformulation of a drug, it is necessary to carefully screen for all possible polymorphs in the early stages of a drug-formulation process. Besides that patent claims to protect drugs from being copied include information about the polymorphic form of API used in a drug formulation. It is possible to circumvent the patenting protection by formulating the pharmaceutical with another polymorphic form if not all the polymorphs are protected in the patent claims. Because of these compelling reasons the pharmaceutical industry spends a lot of time, effort and money on polymorph screening of APIs.

Polymorph Screening Methods

Screening for polymorphs of a compound is typically done by performing many different crystallizations under broad variety of crystallization conditions. Parameters which are changed routinely are temperature, solvent, pressure, humidity, degree of supersaturation and the crystallization method. The crystallization solution can reach the supersaturated state by evaporation of the solvent, by cooling of the solution or by antisolvent precipitation.\[108, 157\] In section 6.1.2 the interplay of thermodynamic and kinetic effects during polymorph formation is discussed. Besides these conventional means of controlling crystallization process, a newer approach to influence the crystal structure is to use chemically modified surfaces as crystallization templates. In section 6.1.3 a brief overview on the results in this area reported in literature is given. The aim the work presented in this chapter is to evaluate the aptitude of surface-chemical gradients as crystallization templates, and thereby to create high-throughput screening platforms for surface-templated nucleation and crystal growth.
6.1.2 Thermodynamics and Kinetics

Thermodynamic and kinetic effects determine the outcome of a crystallization process. The outcome can be influenced by varying the above mentioned conditions during the crystallization process. The thermodynamic stability can be influenced by changing the temperature, solvent, pressure or humidity. By influencing the degree of supersaturation and the crystallization method the kinetics of the crystallization process can be varied.

Homogeneous and Heterogeneous Nucleation

During a crystallization process, molecules in solution form agglomerates and dissolve again until an agglomerate exceeds a critical size. Once a nucleus reaches a critical size the crystal starts to grow. In a clean, unperturbed solution the nucleus is solely formed from crystal components and the nucleation process is therefore referred to as homogeneous. However, when impurities, rough edges or perturbations are present, they induce the formation of a crystallization nucleus, leading to heterogeneous nucleation. The idea behind the use of physicochemically modified surfaces as crystallization templates is to control heterogeneous nucleation with the surface acting as the nucleation site. Heterogeneous nucleation is normally faster than homogeneous nucleation. The faster nucleation and crystal growth take place the more likely the kinetically favored polymorph is formed. In contrast the likelihood of the thermodynamically favored polymorph being formed can be enhanced by suppressing heterogeneous nucleation.[158]

Enantiotropically and Monotropically Related Polymorphs

It is also important to note that the thermodynamically favored polymorph is not necessarily the same at all temperatures. If the relative thermodynamic stability of two polymorphs is inverted below their melting points they are called enantiotropically related. Figure 6.1 shows schematically the relative energies of such a system. At the transition point \( t_{II/I} \) both polymorphs have the same energy. If there is only a virtual transition point above the melting point the polymorphs are monotropically related. But the energy difference between the polymorphs can be very small over a broad energy range, even when there is no transition point. Therefore kinetic effects
6. Surface-chemical Gradients as Crystallization Templates

Figure 6.1: Energy versus temperature diagram of an enantiotropically related dimorphic system. \( H \) is the enthalpy and \( G \) the Gibbs free energy. \( t \) indicates the transition point between the polymorphic form I and II. Scheme is adapted from reference [155].

Figure 6.2: The activation barrier for a dimorphic system is displayed schematically. The Gibbs free energy is plotted against the reaction coordinate. Scheme is adapted from reference [155].

can overcome this energetic difference and even monotropically related polymorphs can crystallize concomitantly. Figure 6.2 schematically displays the relative Gibbs free energies of two polymorphs during the crystallization process. Polymorph II is thermodynamically favored but kinetically hindered. The faster the nucleation
6.1. Introduction

and crystallization process occurs, the more likely it is that the kinetically favored product dominates the outcome.

Ostwald Ripening

Small crystal nuclei are not very stable. Although crystal growth in a supersaturated solution is thermodynamically favored once the nuclei reached a critical size, the energetic gain with respect to crystal shrinking is minor for small crystals. The larger the crystal gets the bigger is this energetic gain. To maximize the energetic gain of the system bigger crystals grow at the expense of the smaller crystals. This process is called Ostwald ripening.[159] Around bigger crystals on the surface often a depletion zone[160] is observed, in which no crystals grew.

Solution-Mediated Polymorph Transformation

Crystals of one polymorphic form grown in solution can also undergo transformation to another polymorphic form in a solution-mediated polymorph transformation process by dissolving and recrystallization in solution. The more stable a polymorph, the lower is its solubility.[155] However, due to kinetic effects polymorphs with lower stability can also be formed in solution. These crystals grow until the solution is not supersaturated anymore with respect to less stable polymorph. Crystals of the more stable polymorph continue growing due to their lower solubility. If the concentration of the compound goes beyond the solubility limit of the kinetic polymorph, the polymorph begins to dissolve while the more stable polymorph is still growing until all of the kinetic polymorph is transformed into the more stable polymorph.[161]

6.1.3 Surfaces as Templates for Crystallization

Surfaces can influence crystallization processes in two principal ways. Either the kinetics of the crystallization process are altered by changing nucleation or crystal growth speed, or the relative thermodynamic stabilities of the polymorphic forms are influenced by epitaxial match of the periodic structure on the surface with crystal structure. A brief overview on different methods used in the past to influence the crystallization outcome through surfaces is presented in this section.
Nucleation and Crystallization Speed

The nucleation process can be influenced by either providing nucleation sites or by suppressing nucleation. Aizenberg et al.[162] created artificial defect sites in alkanethiol SAMs on gold on the edges of topographical features. These defects sites act as nucleation sites during CaCO$_3$ crystallization, creating a crystal pattern. In contrast to that, Cox et al.[158] used nonsticky crystallization containers to suppress heterogeneous nucleation on the crystallization container walls. While on glass vials modified with perfluorosilane monolayers, indomethacin was solely crystallizing in the thermodynamically favored but kinetically hindered form I, in all other vials also form II was found. This work will be further discussed in section 6.3.3. The ability to prevent crystal nucleation is also important in order to grow defect-free multilayer thin films of organic molecules. Jeong et al.[163] studied the growth of pentacene layers on binaphthyl monolayers. Crystal nucleation is inhibited by exposing an amorphous surface to the nucleation solution. The missing epitaxy between the surface and the crystal form is attributed as the main reason for the low nucleation rate. Pham et al.[164] used hydrophobic SAMs to increase the size of protein crystals. High-quality protein crystals are needed for single-crystal X-ray diffraction to obtain information about their 3D structure. With the hydrophobic surfaces, nucleation was inhibited and therefore fewer, bigger crystals were grown. Lee et al.[165] used lyophilic islands with different dimensions in a lyophobic grid as a crystallization platform. The substrate was dipped into the crystallization solution and the solution was trapped on the surface in the region of the lyophilic islands. Depending on the size of the island, droplets of a certain volume remain on the surface. The bigger the droplet the longer it takes until the solvent is evaporated, thus lowering the nucleation and crystal growth rate leading to the formation of the thermodynamically favored but kinetically hindered product.

Epitaxy and Orientation

Epitaxy between building-block units of the surface and of the formed crystal is often reported to be the reason for a certain crystallization outcome. Several studies report that the polymorph formed depends on the SAM surfaces, which serves as a crystallization template. In 1994 Carter et al.[166] showed that crystallizing anthranilic acid by sublimation leads to form III on trimethoxysilane SAMs
but to form II on chlorotriisobutylsilane. Dressler et al. [167] found that the metastable \( \alpha \) polymorphic form of glutamic acid can be stabilized by crystallizing it on L-2-amino-N-{[2-(2-amino-3-phenyl-propionylamino)-ethyl]disulfanyl-ethyl}-3-phenyl-propionamide SAMs. Hiremath et al. [168, 169] reported that 2-iodo-4-nitroaniline is crystallizing on 3'-nitro-4-mercaptobiphenyl and on 3'-iodo-mercaptobiphenyl only in the orthorhombic phase. Küther et al. [170] used thiol-based SAMs as crystallization templates for CaCO\(_3\) crystal modifications (calcite, vaterite, aragonite). They studied the effect of the functional group, chain length and crystallization temperature. The high-temperature crystal modification aragonite was shown to be crystallizing on polar, rough and disordered surfaces. Quist et al. [171] found that carbamazepine is crystallized exclusively as form II on biphenyl SAMs. Cox et al. [172] studied the crystallization of theophylline on hydrophobic and hydrophilic surfaces. On hydrophilic surfaces the anhydrous form II was formed and on hydrophobic the monohydrate form was obtained. This work will be discussed further in section 6.3.2.

SAMs can also serve as templates for entantioselective crystallization. Nakanishi et al. [173] used chiral leucin-modified surfaces to crystallize leucin enantioselectively.

Epitaxial matching between SAM and crystal structure has an influence also on the nucleation density. Briseno et al. [174] studied the nucleation density of anthracene on different SAMs. While methyl-terminated SAMs show low nucleation density, terphenylthiol SAMs exhibit the highest nucleation density.

The orientation or the crystal morphology can also be influenced by using SAMs as crystallization templates. Dabros et al. [175] selectively crystallized different phases of carbamazepine on thiol SAMs of different chain lengths having the same end functional group. While on a 11-mercaptoundecanoic acid SAM the 012 phase of form III polymorph was dominant, on a 16-mercaptohexadecanoic acid SAM mainly the 101 phase was shown to be growing due to different spacial orientation of the functional groups. Kang et al. [176] reported that mixed SAMs with methyl and hydroxyl end-functional groups leads to different orientations of glycine crystals formed on the surface. For 25%, 50% and 75% the crystals were in contact with the surface with their \{010\}, \{121\} and \{1105\} planes, respectively. Hsu et al. [177] found that the morphology of ZnO crystals can be influence by surfaces. While most of the above studies and several others, have shown a definite influence of the type of chemical functional group present on the surface on the heterogeneous crystal
nucleation, unequivocal evidence for the necessity of an epitaxial match between
the surface and the resulting polymorphic phase of an organic crystal has not been
shown.

6.2 Experimental

6.2.1 Chemicals

The crystallization experiment were performed with theophylline (Fluka, \( \geq 99\% \)), in-
domethacin (Fluka, \( \geq 99\% \)) and carbamazepine (TCI, \( \geq 97\% \)). As solvents, ethanol,
toluene (Fluka, \( \geq 99.7\% \)), acetonitrile (Fluka, \( \geq 99.5\% \)) and ultra-pure water were used.
Substrates were silicon wafers, glass slides or polyimide foil (DuPont, USA) all
coated with 10 nm chromium and 100 nm (or 80 nm for polyimide) gold. To
modify the surface chemistry of glass crystallization containers (1H,1H,2H,2H-
perfluoroocetyl)trichlorosilane (ABCR GmbH, 97\%) was used.

6.2.2 Crystal-Analysis Methods

The different methods used in this thesis to characterize compounds in their crys-
talline state and to determine their polymorphic forms are described in Chapter
3. Mainly Micro-Raman spectroscopy was used to characterize the formed crystals,
since crystals on the surface can be analyzed without further sample preparation.
Infrared spectroscopy was used to analyze the crystals formed in solution and to
confirm the polymorphic forms of indomethacin determined by light microscopy.
Crystal analysis in the multiwell plate experiments were made by XRD in transmis-
sion mode on X-ray transparent SAMs (see section 6.3.1) by the company Roche.

6.2.3 Active Pharmaceutical Ingredients

In the following sections the API used to perform the crystallization experiment
are described in detail. The relative stability of the different polymorphic forms is
discussed, the protocols to obtain the pure form are listed and the IR and Raman
intensities reported in literature are summarized.
Theophylline

Figure 6.3: Structure of theophylline

Theophylline (3,7-dihydro-1,3-dimethyl-1H-purine-2,6-dione) is a bronchodilator used in asthma therapy. It has two stable anhydrous polymorphic forms (form I and form II) and a monohydrate form[178]. Besides that the dehydration of the monohydrate form at room temperature under reduced pressure leads to a metastable anhydrate form (form III) which transforms into form II over time. Depending on the conditions under which the compound is stored, the conversion to the stable form takes between 1 h and up to several months. Form II is stable at ambient atmosphere and a relative humidity of 0-79%.[179] It only transforms into the monohydrate above 79%. The monohydrate does not transform into the anhydrate between 30-100% relative humidity. Therefore both forms are stable between 30-79% humidity. The nomenclature to describe the different polymorphic forms of theophylline has not been used in a consistent way throughout the literature.

Table 6.1: Crystal structure and preparation methods of the polymorphic forms of theophylline

<table>
<thead>
<tr>
<th>Form</th>
<th>Crystal structure</th>
<th>Pure Form Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>not solved</td>
<td>heat II for 1 h at 260-268 °C in hermetically sealed glass test tube[180]</td>
</tr>
<tr>
<td>II (anhydrate)</td>
<td>orthorhobic (Pmnb)[178]</td>
<td>use as received[181]</td>
</tr>
<tr>
<td>III (I*, metastable)</td>
<td>not solved</td>
<td>monohydrate at 15 °C in vacuum[181]</td>
</tr>
<tr>
<td>hydrate</td>
<td>monoclinic (P2_1)[178]</td>
<td>dissolve anhydrate in water at 85 °C, cool down to RT, filtration, dry at ambient atmosphere[181]</td>
</tr>
</tbody>
</table>
The fact that no publication includes information about all possible polymorphic forms makes it hard to compare the information. In this thesis the nomenclature used by Suzuki et al. in 1989[180] and further extended by Matsuo et al. in 2007[181] is used. Table 6.2 shows the most intense IR and Raman signals and the melting point for the different polymorphic forms.

**Table 6.2:** Raman and infrared signals of theophylline published in the literature

<table>
<thead>
<tr>
<th>Form</th>
<th>Raman [cm(^{-1})]</th>
<th>IR [cm(^{-1})]</th>
<th>Melting Point [°C]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>not published</td>
<td>not published</td>
<td>275</td>
</tr>
<tr>
<td>II (anhydrate)</td>
<td>668, 928, 3123[179]</td>
<td>1717, 1667[182]</td>
<td>271</td>
</tr>
<tr>
<td>III (I*, metastable)</td>
<td>not published</td>
<td>not published</td>
<td>272</td>
</tr>
<tr>
<td>hydrate</td>
<td>673, 919, 3109[179]</td>
<td>broad 3450[172]</td>
<td>270</td>
</tr>
</tbody>
</table>

Theophylline forms needle-shaped crystals regardless of the polymorphic form present. Therefore the polymorphs cannot be identified simply by imaging techniques.

**Indomethacin**

![Structure of indomethacin](image)

Indomethacin (2-{1-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methyl-1H-indol-3-yl}acetic acid) is a non-steroidal anti-inflammatory drug. It has four confirmed different polymorphic forms (form I-IV). While form I (γ) is the most stable form, form II (α) is also often found. Form III and form IV (δ) [183] are less stable. The
Disagreements exist about a possible fifth polymorphic form ($\beta$), which was first reported by Yamamoto et al. in 1968. Spychala et al. reported again in 1977 a $\beta$ form, although Borka et al. pointed out in 1974 that the $\beta$ form of Yamamoto is a benzene solvate and not a different polymorphic form. The IR spectrum published by Spychala looks very much like the IR spectrum of the solvent-containing form reported by Borka. Even the higher melting point of the beta form in contrast to the reported melting point of the solvate (95 °C) can be explained by a melting process of the solvate, a subsequent loss of the solvent and crystal formation of a higher polymorph with a melting point at the higher temperature. Solvates of indomethacin with many different solvents are known: methanol, tert-butanol, acetone, benzene, dichloromethane, tetrahydrofuran, propanol, propan-1-ol, propan-2-ol, chloroform, diethyl oxide, carbon tetrachloride, cyclohexanone, ethanol, isoamyl alcohol, octan-2-ol and cyclohexanol. In Table 6.3 the crystal cell and the preparation methods for the pure polymorphic forms of indomethacin are reported.

Table 6.3: Crystal structure and preparation methods of the polymorphic forms of indomethacin

<table>
<thead>
<tr>
<th>Form</th>
<th>Crystal Structure</th>
<th>Pure Form Preparation Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>I ($\gamma$)</td>
<td>triclinic (P-1)</td>
<td>used as received[188]</td>
</tr>
<tr>
<td>II ($\alpha$)</td>
<td>monoclinic (P2$_1$)</td>
<td>dissolving in hot EtOH, precipitation by addition of water[189]</td>
</tr>
<tr>
<td>III</td>
<td>not solved</td>
<td>putting a crystal film on the Kofler hot bench at 110-115 °C[184]</td>
</tr>
<tr>
<td>IV ($\delta$)</td>
<td>not solved</td>
<td>crystallization from warm methanol[184]</td>
</tr>
<tr>
<td>$\beta$</td>
<td>not solved</td>
<td>crystallization from benzene[185]</td>
</tr>
</tbody>
</table>

Table 6.4 shows the most intense IR and Raman signals and the melting point for the different polymorphic forms. The morphologies of the polymorphic forms I and II are very different. Form I forms plates and prisms and form II forms needles. In Figure 6.5 light microscope images of the different forms are displayed.
Figure 6.5: Microscope images of the polymorphic form I and II of indomethacin. Form I forms prisms and plates and form II needles.

Table 6.4: Raman and infrared signals and melting points of the polymorphic forms of indomethacin published in literature

<table>
<thead>
<tr>
<th>Form</th>
<th>Raman [cm$^{-1}$]</th>
<th>IR [cm$^{-1}$]</th>
<th>Melting Point [°C][187]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I ($\gamma$)</td>
<td>1699, 1620, 1591, 1467[190]</td>
<td>1717, 1692[188]</td>
<td>159-161</td>
</tr>
<tr>
<td>II ($\alpha$)</td>
<td>1688, 1650, 1579, 1458, 1155[190]</td>
<td>1735, 1692, 1680[188]</td>
<td>153-154</td>
</tr>
<tr>
<td>III</td>
<td>not published</td>
<td>not published</td>
<td>148-149</td>
</tr>
<tr>
<td>IV ($\delta$)</td>
<td>not published</td>
<td>not published</td>
<td>133-134</td>
</tr>
<tr>
<td>solvate or $\beta$</td>
<td>not published</td>
<td>1690, 1675[186]</td>
<td>158-161</td>
</tr>
<tr>
<td>amorphous</td>
<td>broad signal 1680[190]</td>
<td>1666[190]</td>
<td></td>
</tr>
</tbody>
</table>

Carbamazepine

Figure 6.6: Structure of Carbamazepine
Carbamazepine (5H-dibenzo[b,f]azepine-5-carboxamide) is an anticonvulsant drug, which is used to treat epilepsy and trigeminal neuralgia. It is often used as a model system for polymorphic studies since it has four different anhydrous polymorphs[191] and a dihydrate pseudopolymorph[192]. Many different nomenclatures for the different polymorphs are found in literature. Grzesiak et al. summarize the different nomenclatures[191] and in this thesis their nomenclature is used. The anhydrous polymorphic forms are labeled as form I-IV. Their stability decreases in the following order: III, I, IV, II.[191] Protocols to produce the pure forms and the crystal structure of the polymorphs are listed in table 6.5.

**Table 6.5:** Crystal structure and preparation methods of the polymorphic forms of carbamazepine

<table>
<thead>
<tr>
<th>Form</th>
<th>Crystal Structure</th>
<th>Pure Form Preparation [191, 192]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>triclinic (P-1)[191]</td>
<td>preparation from melt in vacuum sealed capillary heated up to 192 °C or heating the pure compound for 3 h at 150 °C</td>
</tr>
<tr>
<td>II</td>
<td>trigonal (R3)[193]</td>
<td>slow cooling from 80 °C to 5 °C</td>
</tr>
<tr>
<td>III</td>
<td>primitiv monoclinic</td>
<td>slow evaporation from ethanol</td>
</tr>
<tr>
<td>IV</td>
<td>c-centered monoclinic (C2/c)[194]</td>
<td>slow evaporation from methanol in presence of hydroxypropyl cellulose</td>
</tr>
<tr>
<td>dihydrate</td>
<td>monoclinic (P2₁/C)[195]</td>
<td>stirring a suspension of I or III in water for 25h at RT, filtration an drying at RT for 30 min</td>
</tr>
</tbody>
</table>

**Figure 6.7:** Concomitant crystallization of polymorphic form I (needles) and III (prisms) of carbamazepine.

The different crystal forms of carbamazepine have partly different morphologies. While form I and II both form needles and are therefore hard to distinguish, form
### Table 6.6: Raman and infrared signals and melting points of the polymorphic forms of carba-mazepine published in literature

<table>
<thead>
<tr>
<th>Form</th>
<th>Raman [cm$^{-1}$]</th>
<th>IR [cm$^{-1}$]</th>
<th>Melting Point [°C] [191]</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>not published</td>
<td>3485, 1690, 1395 [191]</td>
<td>135-170 (transformation)</td>
</tr>
<tr>
<td>III</td>
<td>1624, 1565, 1042, 723 [196]</td>
<td>3466, 1677, 1386 [191]</td>
<td>162-175 (melt + transformation)</td>
</tr>
<tr>
<td>IV</td>
<td>not published</td>
<td>3474, 1674, 1394 with a small shoulder 1418 [191]</td>
<td>178-187 (melt + partial transformation)</td>
</tr>
<tr>
<td>dihydrate</td>
<td>not published</td>
<td>not published</td>
<td>50-90 (dehydration) [192]</td>
</tr>
</tbody>
</table>

IV form plate-like crystals and form III prisms. The resemblance of forms I and II have often caused confusion.

### 6.2.4 Surfaces

#### Samples with uniform surface chemistry

Most of the experiments were performed on uniform, single-component alkanethiol SAMs on gold-coated silicon wafers or gold-coated glass slides. Glass slides or silicon wafers were coated with 10 nm Cr and 100 nm Au. The substrates were sonicated in ethanol for 10 min and plasma cleaned for 2 min before they were immersed into 1 mM alkanethiol solution for at least 14 h. As alkanethiols, dodecanethiol, perfluorododecanethiol, mercaptoundecanoic acid or mercaptoundecanol were used.

To artificially increase the roughness of the samples, silicon wafers coated on the unpolished side were used as substrates.

#### Gradients

Surface-chemical gradients were prepared according to the method of Morgenthaler et al. [1]. Glass slides or silicon wafers coated with 10 nm Cr and 100 nm Au were sonicated in ethanol for 10 min, plasma cleaned for 30 s and immersed in ethanol for
10 min. To create a coverage gradient, the substrates were immersed into diluted thiol solution (5 µM) by means of a linear-motion drive. Subsequently the samples were backfilled in a 10 µM solution of the second component for 14-16 h. Dodecanethiol, perfluorododecanethiol, mercaptoundecanoic acid or mercaptoundecanol were used as components to create the gradients.

In section 6.2.5 a crystallization method is described which uses gradients on polyimide foil. To prepare such gradients the foil was coated with 80 nm Au by magnetron sputtering (PSI Villingen, Switzerland). Prior to the coating step the foil was rinsed with ethanol and plasma treated for 2 min. Directly before gradient preparation the gold-coated foil was ultrasonicated in ethanol for 10 min, plasma cleaned for 30 s and immersed in ethanol for additional 10 min. To create a surface-chemical gradient the substrate was immersed into a slightly stirred 5 µM ethanolic dodecanethiol solution during 8 min 10 s. To obtain a uniform dense coverage, the sample was backfilled in 10 µM mercaptoundecanoic acid for 18 h.

### 6.2.5 Methods

**Glass Vial**

Most of the crystallization experiments were performed in glass vials. SAMs used as crystallization templates were immersed into the crystallization solution in four different ways. In the first method, samples were simply placed at the bottom of the glass vial with the SAM facing upward. The advantage of this method is that the sample is still completely immersed in the crystallization solution even if most of the solvent is evaporated, this is especially preferable if the solubility of a compound in a solvent is very high. However, with this method besides the crystals formed on the SAM surface all the crystals formed in the solution or at the liquid-air interface will also be found on the SAM. To avoid this sedimentation the samples were leaned against the glass vial wall either with the SAM facing up or down. Immersing the samples with the SAM facing down often led to detachment of the crystal formed on the SAM surface due to gravity. To vary the immersion time into the crystallization solution the SAMs were immersed and gradually withdrawn by means of the linear motion drive.

The wetting of the glass vial by the crystallization solution was varied by treating the glass walls before the experiment (see Figure 6.8B). The glass vials were cleaned for
Figure 6.8: A) SAMs immersed into the crystallization in four different ways: lying on the bottom, standing upright with the SAM either facing up or down, immersed by means of the linear motion drive. B) Perfluorinated and piranha cleaned glass vials show different wetting behavior with the crystallization solution.

10 min in hot piranha solution (7:3 sulfuric acid/hydrogen peroxide) and rinsed with copious amounts of water. Such freshly piranha cleaned glass vials have hydrophilic and oleophilic surfaces and are wetted by the crystallization solution.

Wetting of the glass vial can be avoided by modifying the glass surface with perfluorosilane coating[158]. Plasma cleaned glass vials were filled with 1 mM (1H,1H,2H,2H-perfluorooctyl)trichlorosilane toluene solution for 3 h. After the solution was removed from the vial it was rinsed with toluene, sonication for 20 min in acetone, washed three times with ethanol and dried with nitrogen.

Teflon Container

Home-made teflon containers exhibiting fluorinated surfaces to the crystallization solution were used to perform some of the crystallization experiments. The containers were constructed in such a way that only the SAM surface of the crystallization template and the fluorinated teflon surfaces were in contact with the crystallization solution, as depicted in Figure 6.9. To achieve that a 3 cm thick teflon block was fixed with screws on a aluminum plate. The teflon block had a hole with 0.9 cm inner diameter. Below the hole a depression for a 1x1 cm SiO$_2$-wafer sample was made in the aluminum plate. The system was sealed simply by fixing the teflon block on the aluminum plate with four screws. The teflon became sealed on to the aluminum block by applying pressure on it. Prior to the crystallization experiments the teflon block was piranha cleaned to make sure that the surfaces were clean.
6.2. Experimental

Figure 6.9: Teflon container: teflon block with hole fixed on aluminum plate with a depression for a 1x1 cm SiO$_2$-wafer.

Droplet Container

Placing droplets of crystallization solution directly on the SAM is a way to completely avoid contact of the crystallization solution with any other surface than the SAM chosen as crystallization template. Volumes of crystallization solution deposited on the surface range from 6 to 24 $\mu$l. Depending on the volume, the solvent, the contact angle and the storage conditions (desiccator or open air) the evaporation time varies from seconds to hours.

Figure 6.10: Droplets of crystallization solution placed on surfaces with different wettabilities.

Roche Multiwell plate

All the methods described above to perform crystallization experiments are low-throughput methods. To perform several experiments simultaneously a tool with several crystallization chambers in parallel is needed. A multiwell plate for crystallization experiments was developed by the company Roche. The multiwell plate is built up as follows: a teflon-coated metal block was fixed with screws on another metal block. Both metal blocks had holes on the same positions. A polyimide foil was clamped between the metal blocks.
6. Surface-chemical Gradients as Crystallization Templates

Figure 6.11: Multiwell plate for high-throughput crystallization experiments consisting of two metal blocks with holes and a polyimide foil fixed in between.

The polyimide foil allows XRD measurements of the formed crystal to be performed in transmission mode due to the high transmittance of the polyimide foil to X-rays. To expose chemically modified surfaces to the crystallization solution, the polyimide foil was coated with a thin layer of gold, which was modified with thiols to vary the surface chemistry. In section 6.3.1 it is shown that the surface wettability can be varied on such a polyimide foil and how the gold coating affects XRD transmission and Raman reflection measurements.

6.3 Results and Discussion

6.3.1 Surface-chemical Gradient on Polyimide Foil

In order to establish that it is possible to create a surface-chemical gradient on a gold coated polyimide film using similar procedures used for silicon wafers static contact angle measurements were performed on two-component gradients composed of methyl and carboxyl terminated thiols. Figure 6.12 shows that preparation of a wettability gradient on the gold coated polyimide foil is possible. The contact angle is plotted against the immersion time in the hydrophobic dodecanethiol solution.

XRD transmission measurements (see Figure 6.13) showed that the gold coating doesn’t affect the diffraction measurements significantly. Only the (111) diffraction peak at 38 ° and the (222) diffraction peak at 65 ° arising from the Au (111) gold coating are observable. Therefore it is still possible to distinguish between polymorphs grown on these surfaces by transmission XRD measurements.
6.3. Results and Discussion

Figure 6.12: Static contact angle measurements along the wettability gradient on the gold-coated polyimide foil. The gold-coated polyimide foil was immersed by means of a linear-motion drive into a 5 \( \mu \text{M} \) \( \text{C}_{11}\text{CH}_3 \) solution up to 8 min 10 s and backfilled in a 10 \( \mu \text{M} \) \( \text{C}_{10}\text{COOH} \) solution for 18 h.

Figure 6.13: XRD transmission measurement on a gold-coated polyimide foil.

Besides that the thin gold coating made Raman measurements possible. The polyimide foil itself shows several intense Raman signals (see Figure 6.14), but the gold layer is not transparent to infrared light. Therefore no signal of the polyimide foil
is detectable after the gold-coating step and only the signals of the crystals were observable.

A thin layer of gold on a polyimide foil allows chemical modification of the surface as well as Raman measurements on crystals on top of the foil, while ability to measure XRD in transmission mode is not disturbed.

### 6.3.2 Theophylline

As mentioned in Chapter 6.1.3, Cox et al. [172] published results in 2007 on the pseudopolymorph-selective crystallization of theophylline on hydrophilic (mercaptoundecanol, mercaptoundecanoic acid) and hydrophobic (dodecanethiol) SAMs. The anhydrous form II and the monohydrate form preferentially crystallized on the hydrophilic and on the hydrophobic SAMs, respectively. Based on this study, theophylline seemed to be a good model system for polymorph-selective crystallization on SAMs and possibly on gradients.

### Pure Forms

The infrared absorption spectra of the two pseudopolymorphs hydrate and anhydrous form I of theophylline are shown in Figure 6.15. The corresponding Raman
spectra are displayed in Figure 6.16. The peaks labeled with an arrow were used to identify the pseudopolymorphs formed during the crystallization experiments according to the reference values from literature reported in Table 6.2.

**Figure 6.15:** Infrared absorption spectra of the hydrate pseudopolymorph (upper) and the anhydrate pseudopolymorphic form I (lower) of theophylline.

**Figure 6.16:** Raman spectra of the hydrate pseudopolymorph (upper) and the anhydrate pseudopolymorphic form I (lower) of theophylline.
Crystallization from Pure Ethanol

Initial crystallization experiments were carried out in pure ethanol since the experiments of Cox et al. were performed in pure ethanol. But in our crystallization studies from ethanol the anhydrous pseudopolymorph was crystallizing independent of the crystallization container, the temperature, the degree of supersaturation, the roughness of the sample, the chemistry and the orientation of the template, the crystallization speed, the beaker walls and the humidity in the atmosphere (30-50%, as well as under controlled humidity of 66% over saturated NaNO₂ solution). Even if the monohydrate pseudopolymorph was used as starting material to prepare the crystallization solution only the anhydrate pseudopolymorph was formed. This, though in contrast to the results of Cox et. al, is actually not very surprising since the amount of water present in the system due to humidity in the air or water coming from the dissolved monohydrate is very small. For the water-methanol mixture as solvent, Zhu et al.[198] published in 1996 that solution-mediated pseudopolymporphic transformation of theophylline occurs between the anhydrous and the monohydrate pseudopolymorphic form at a water activity of 0.25 in methanol. Below this activity, exclusively the anhydrate form is formed and above exclusively the monohydrate is formed. It is very likely that theophylline in a water-ethanol mixture shows a very similar behavior. Therefore the water content of the crystallization solution was artificially increased in order to obtain concomitant crystallization of both pseudopolymorphs.

Crystallization from Ethanol-Water Mixture

Crystallization of theophylline from ethanol-water mixture led exclusively to the anhydrate form up to a water fraction of 20%. Above 30% of water in the crystallization solution the monohydrate is formed exclusively. In the range from 20-30% either the anhydrous, the monohydrate or concomitant crystallization was observed. In above 30% and below 20% of water content regimes the energy difference between the two different pseudopolymorphic form is so high that the kinetics of the crystal formation doesn’t influence the outcome of the crystallization process. Only the thermodynamically favored pseudopolymorph was formed. Between 20% and 30% water content in the crystallization solution kinetics plays a role. The energy difference between the two pseudopolymorphic forms is so small that kinetic effects
can overcome this energy difference. Therefore all parameters influencing the nucleation and crystal growth kinetic were dominating. All further experiments were performed in ethanol-water mixtures containing 20-30% water. There are two principal ways to reach supersaturation: either by reducing the amount of solvent in the system by evaporation or by decreasing the solubility of the compound by lowering the temperature of the solution. The experiments of Cox et al. [172] were performed by evaporation-controlled crystallization.

**Evaporation-Controlled Crystallization**

For evaporation-controlled crystallization experiments, two difficulties arose with the theophylline in ethanol-water mixture. One difficulty was crystal growth on the beaker walls. On the three-phase contact line of the crystallization solution on hydrophilic glass beaker walls, nucleation and crystal growth occurred first. The concentration was highest where evaporation was happening and therefore the crystals began to grow there first. On the beaker on the right side of Figure 6.17 this behavior can be seen. Due to this crystal growth at the three-phase contact line, supersaturation in solution did not reach a level where crystal growth in the solution could occur. This could be avoided by chemically modifying the beaker walls as described in Section 6.2.5. Perfluorosilanized glass beaker walls showed no crystal growth (see left side of Figure 6.17).

![Figure 6.17: Crystal formed on perfluorinated (left) and plasma-cleaned (right) beaker walls.](image)

Another difficulty was the variation of the water content. When evaporation-controlled crystallization was performed, the water content of the crystallization
solution changed during the experiment. It was increasing due to the higher vapor pressure of ethanol with respect to water (if the ethanol concentration was lower than 95.63%, what correspond to the azeotropic mixture). Therefore, the exact water content in the crystallization solution at the nucleation point and during the crystal growth phase was not known and might have varied significantly from one experiment to the other. This might also be the reason why Cox et al. observed monohydrate crystals in the crystallization solution from pure ethanol. An initially small water content might have been increased during the evaporation due to the above described effect. Since the solubility of the theophylline in water is by a factor of three higher than the solubility in ethanol the increasing water fraction causes an increasing solubility of the compound in the crystallization solution. This slowed down the transition into the supersaturation state. In order to avoid increasing water content, further experiments were performed by control cooling of the crystallization solution.

**Cooling Controlled Crystallization**

When the supersaturation of the crystallization solution was so high that the crystallization already started at elevated temperature, the anhydrous polymorphic form was formed even at higher water concentration, than if the crystallization was performed at RT. This behavior can have two reasons. On the one hand, faster crystallization leads to the anhydrous form and slower crystallization leads rather to the monohydrate form (see Figure 6.18) due to kinetic effects. The time-scale of crystallization process ranged from minutes for a fast, cooling-controlled crystallization to days for an evaporation-controlled crystallization. The fact that if the nucleation and crystallization speed was increased due to the presence of nucleation sites the same tendency was observed at lower temperature supports this interpretation.

On the other hand, the temperature is of course strongly influencing which polymorphic form is kinetically and thermodynamically favored. The temperature at which the nucleation and crystal growth occurs should consequently not deviate too much from room temperature, in order to have comparable parameters as Cox et al. in their experiments. Therefore the concentration of the crystallization solution was adjusted such that crystallization occurred only after cooling to room temperature. Adjusting the solution concentration in that way turned out to be demanding. Depending on the amount of nucleation sites, the degree of supersatu-
6.3. Results and Discussion

The water content reached before crystallization occurs varied considerably. Possible nucleation sites were impurities in the solution or vibrations of the crystallization container. Even the presence or absence of a SAM in the crystallization solution changed the moment at which crystallization started. Additionally, it was necessary to adjust the theophylline concentration for the different water concentrations, since the solubility was varying substantially. But in few cases it was possible to achieve conditions where concomitant crystallization started at RT without substantial loss of solvent due to evaporation. The findings of these experiments are described in the following section.

Concomitant Crystallization

In three different cases, concomitant crystallization of both forms was observed. The first two cases are results obtained with the above-described method of carrying out cooling-controlled crystallization at room temperature. Once a mixture of both pseudopolymorphs was found in an ethanol solution with 20% of water added without SAM-modified surfaces in the crystallization solution. A second time crystals of different forms are found on the surface of the SAMs in a crystallization solution containing 30% of water. But the results did not match the findings of Cox et al. (see Table 6.7) Attempts to repeat these results failed.
Table 6.7: Comparison of the obtained results of the crystallization experiments with the results of Cox et al.

<table>
<thead>
<tr>
<th>SAM</th>
<th>Cox et al.</th>
<th>Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{11}CH_3</td>
<td>hydrate</td>
<td>anhydrate</td>
</tr>
<tr>
<td>C_{11}OH</td>
<td>anhydrate</td>
<td>monohydrate</td>
</tr>
<tr>
<td>C_{10}COOH</td>
<td>anhydrate</td>
<td>anhydrate</td>
</tr>
</tbody>
</table>

The fact that concomitant crystallization of the pure polymorphs can occur at very different water contents in the crystallization solution (20% without SAM and 30% with SAM) demonstrate the importance of the kinetics for the crystallization process. The presence of a foreign material such as a SAM substrate allowed faster crystallization due to heterogeneous nucleation sites. Therefore the kinetic product rather than the thermodynamic product formed in the presence of a SAM.

The third time, concomitant crystallization was observed when droplets of the crystallization solution were deposited on C_{11}CH_3 and C_{11}OH SAMs. The hydrophilic C_{11}OH SAM was completely wetted by the crystallization solution, which leads to very fast evaporation of the solvent and therefore to fast crystallization. The kinetically favored pseudopolymorph (anhydrate) formed exclusively. On the hydrophobic C_{11}CH_3 SAM the contact angle was higher and due to surface-volume ratio effects, the evaporation rate significantly decreased. Therefore also the thermodynamically favored pseudopolymorphic form (monohydrate) was crystallizing. Table 6.3.2 summarize all experiment series in which the crystallization of theophylline was performed with the droplet method. On the C_{11}OH SAM the anhydrate form was found exclusively. However, on the C_{11}CH_3 SAM both pseudopolymorphs are found in experiment series 1-3. In the first experiment series 35% of crystals were hydrates, in the second 20% and it the third 30%. In the fourth experiment series solely the anhydrate was formed even when the water content was increased to 22% and 24%.

Over all this led to same trends in selectivity as observed by Cox et al. Besides that the crystals formed on the C_{11}OH SAM were smaller than those on the C_{11}CH_3 SAM. This is due to the different crystal growth velocities. The faster a crystallization process proceeds, the smaller are the formed crystals. The reason for that is that the diffusion driving the Ostwald ripening, which is responsible for the formation of larger crystals, is slower than the crystal growth on the hydrophilic SAMs.
Table 6.8: Pseudopolymorphic forms found in four different experiment series of droplets crystallization experiments on SAMs with different wettability properties. The water and theophylline concentration is listed for each experiment.

<table>
<thead>
<tr>
<th>Nr</th>
<th>H₂O content [%]</th>
<th>TP conc. [mg/ml]</th>
<th>Hydrate on C₁₁CH₃ [%]</th>
<th>Anhydrate on C₁₁OH [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>21.6</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>20</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>20, 22, 24</td>
<td>20</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

From the microscope crystal images displayed in Figure 6.19 the crystal size on the dodecanethiol SAM could be estimated as 1 mm long and 100 \(\mu\)m broad crystal needles. On the mercaptoundecanol SAM the needles are about 200 \(\mu\)m long and about 20 \(\mu\)m broad. These crystals have approximately the same size as the crystals in the microscope images in the publication of Cox et al. [172]. It is likely that the crystals formed on the hydrophilic surfaces in the experiments of Cox et al. are formed after withdrawing the samples from solution. It cannot be avoided that crystallization solution remains on the surface if a hydrophilic surface is withdrawn from the solution. The crystals are small enough to be formed in a wetting film. In contrast the hydrophobic SAM will not be wetted after removal from the crystallization solution. Therefore only crystals that were formed slowly in solution remain on the hydrophobic SAMs. If these assumptions are true, the effect observed by Cox et
al. was not caused by a serendipitous coincident epitaxy as the authors claim, but by a kinetic effect driven by different wettabilities of the substrates.

These results clearly show how many different factors can have an influence on the outcome of a crystallization experiment. Depending on the temperature, the water concentration in the crystallization solution and the crystallization speed one or the other pseudopolymorph is formed. The higher the temperature of the crystallization solution, the higher is the solubility of the compound and the slower is the crystallization speed. Also the solubility is very much influenced by the water concentration in the solution. For evaporation-controlled crystallization, the water concentration is increasing during the evaporation process and therefore difficult to control. The variation of the temperature, water concentration, theophylline concentration and the crystallization speed are closely related and influence one another. Additionally, the presence of an external SAM surface in the crystallization medium, requires further optimization of these parameters, making the crystallization of theophylline on SAMs a complicated system. One positive outcome of these set of experimental results is that the tendentiously polymorph-selective crystallization obtained in droplet containers demonstrate that it is possible to take advantage of the different wetting behavior of surfaces in order to tune the evaporation rate and thereby influence the crystallization outcome using chemically modified surfaces.

6.3.3 Indomethacin

Cox et al.[158] also published in 2007 a method to selectively grow one or the other polymorphic form of indomethacin as described in chapter 6.1.3. While in untreated glass beakers, form II was formed, crystallization in perfluorosilanized glass beakers led to form I. In the following section, investigations made in order to evaluate if different surfaces can affect the predominantly formed polymorphic form of indomethacin are shown.

Pure Forms

The infrared absorption spectra of polymorphic forms I and II are shown in Figure 6.20. The peaks labeled with an arrow were used to identify the pseudopolymorphs formed during the crystallization experiments.
Figure 6.20: Infrared absorption spectra of the pure polymorphic form I (lower) and II (upper) of indomethacin.

**Pure Beakers**

In clean, oleophobic, perfluoro-silanized glass crystallization containers, the kinetically hindered but thermodynamically favored form I was preferentially formed. The container was not wetted by the crystallization solution, avoiding local supersaturation at the three-phase contact line. But in piranha-cleaned glass beakers the form I was often formed also. The absence of nucleation sites in these clean, unscratched glass beakers allowed the growth of form I. Sharp edges and irregularities of the SAM samples immersed in the crystallization solution provided nucleation sites, and, independent of the SAM surface chemistry the form II was formed. It could clearly be observed that nucleation was starting on the sample edges. The contact of the crystallization solution with the sharp SAM sample edges had to be avoided, in order to make epitaxial crystal growth possible.

**Teflon Container**

Teflon container, as described in section 6.2.5, were used to avoid the contact of the SAM sample edges with the crystallization solution. But in these experiments most of the crystals were formed on the teflon container walls (see Figure 6.21) themselves, although the surface chemistry was not very different from the perfluorosilanized
container. The surface roughness of the mechanically polished teflon crystallization container is estimated to be much higher than the roughness of the glass beaker, and this had a significant influence on the wetting behavior.[199]

Figure 6.21: Crystals formed on teflon walls. The hole in the teflon block is shown here. The slightly brighter round part of the image is due to the crystals formed on the teflon wall. The very bright part in the middle is air and the darker part is the teflon block.

**Multiwell plate**

In the multiwell plate described in section 6.2.5 the metallic container walls were coated with a teflon layer. The surface roughness of the teflon coating is lower than that of the mechanically formed teflon block. Evaporation-controlled and cooling-controlled crystallization experiments of indomethacin were performed in these multiwell plates. Ethanol and acetonitrile were used as solvents. Two different surfaces were used to form the bottom of the crystallization container: pure polyimide foil and polyimide foil coated with gold modified with an alkanethiol wettability gradient (as described in section 6.2.4). To create the wettability gradient, the gold coated polyimide foil was immersed by means of a linear motion drive into a 5 µM \( \text{C}_{11}\text{CH}_3 \) solution up to 8 min 10 s and backfilled in a 10 µM \( \text{C}_{10}\text{COOH} \) solution for 18 h.
Figure 6.22: Outcome of the crystallization experiment with indomethacin performed on a pure polyimide foil or a wettability gradient in the multiwell plate. In the upper part crystallization experiments were performed by letting the solvent evaporate completely. In the lower part the temperature of the wellplate was slowly decreased from 80 °C to RT in order to reach supersaturation and start crystal growth. Crystal analysis was performed by XRD.

Evaporation-controlled crystallization led uniquely to form I, independently of which solvent and which surface were presented to the crystallization solution. Cooling-controlled crystallization led to form I in acetonitrile and to form II in ethanol. No trend along the gradient was observable. Apparently the amorphous form is more likely if a SAM is present in the crystallization solution. The formation of amorphous material can occur if crystallization is hindered. The presence of a SAM might even hinder crystallization instead of promote a certain crystal structure. But the solvent, the absence or presence of crystallization nuclei and the method by which the crystallization is achieved influence the crystallization outcome more than the nature of the SAM present in the system.
6.3.4 Carbamazepine

Pure Forms

The infrared absorption spectra of the two polymorphs I and III of carbamazepine are shown in Figure 6.23. The corresponding Raman spectra are displayed in Figure 6.24. The peaks labeled with an arrow were used to identify the pseudopolymorphs formed during the crystallization experiments.

![Infrared absorption spectra](image)

**Figure 6.23:** Infrared absorption spectra of form I (lower) and form III (upper) of carbamazepine.

Crystallization in Ethanol

Crystallization experiments of carbamazepine in ethanol showed that carbamazepine is first crystallizing on rough features. Crystals were formed on the sample edges if the crystallization was performed in perfluorosilanized beakers and on the teflon walls if the teflon containers described in section 6.2.5 were used. The same effect has already been observed for indomethacin an has been described in section 6.3.3. Artificial increase of the roughness of the SAM sample led to higher crystal density on the surface because more nucleation points were present at the SAM surface than on the teflon beaker walls. But on C\(_{11}\)CH\(_3\) as well as on C\(_{11}\)OH\(_3\) surfaces form I is formed. It is important to note that the SAM present on these nucleation...
6.3. Results and Discussion

Figure 6.24: Raman spectra of form I (lower) and form III (upper) of carbamazepine.

points is less well ordered than on a flat surface. If rough features or poorly ordered SAMs cause nucleation on the SAM surfaces the chance of polymorph selective crystallization due to epitaxial crystal growth is lowered since the crystal growth is not starting on well ordered SAM regions. Therefore it is not surprising that the kinetic product is formed independent from the surface chemistry. The rough edges and poorly ordered regions acts as nucleation points and therefore the nucleation and crystal growth can occur fast. Since our aim is to test the influence of the surface chemistry on the crystallization process, rough surfaces are not the ideal substrates. But these experiments demonstrate that is possible to direct the nucleation and crystal growth spatially by artificially introducing rough features.

Crystallization in Toluene

Quist et al. used toluene as a solvent for carbamazepine. The solubility of carbamazepine in toluene is a factor of ten lower than in ethanol. The effect of the roughness on the crystallization process of carbamazepine in toluene was reduced significantly with respect to the effect in ethanol. Crystallization experiments in teflon containers mainly showed crystals formed on the SAM surface. The crystal density was much higher on hydrophilic substrates (C\textsubscript{11}OH) than on hydrophobic substrates (C\textsubscript{11}CH\textsubscript{3}) but the crystal forms varied more from batch to batch than
from surface to surface. Using the teflon box as a crystallization container had the additional drawback that it was not possible to distinguish between sedimented crystals formed in the solution and crystals actually formed on the surface. When the crystallization solution was removed, the C\textsubscript{11}OH SAM was still wetted and some precipitated crystals might not have been washed away. To confirm that the higher crystal density was not arising from this effect, crystallization experiments with SAMs standing upright and facing down (as described in section 6.2.5) were performed. For the same immersion time no crystal could be found on either surface. This finding indicates that the major fraction of the crystals found on the surface in the teflon crystallization box were formed in solution. In this case a wetting phenomena is also causing differences in the crystallization behavior on different surfaces, as already found for theophylline.

**Crystallization at the Three-phase Contact-line**

Withdrawal of a uniform SAM or a gradient by means of a linear-motion drive turned out to be an alternative to the droplet-container crystallization shown in section 6.3.2. The crystallization process is influenced by the wetting behavior in both methods. Three types of unidirectional surface-chemical gradients were withdrawn simultaneously from the same saturated crystallization solution. Microscope images of the crystals formed on the SAM surface are displayed in Figure 6.26, 6.27 and 6.28. The crystallization was mainly happening at the three-phase contact line. Depending on the contact angle of the crystallization solution on the surface, the evaporation speed and therefore the crystallization rate was different. For high contact angles the crystallization rate was lower than for lower contact angles, which makes the crystallization of the kinetically hindered but thermodynamically favored product more likely. Thus the outcome of the crystallization experiment was more influenced by the contact angle of the crystallization solution on the surface than by the specific surface chemistry. With this method, crystal-density gradients could be achieved. Along these gradients no variation in the polymorphic form was observable, although small changes in the evaporation rate could lead to different polymorphic forms, as demonstrated in section 6.3.2. Probably the difference in the evaporation rate was too small to have a major influence on the polymorph formed. The biggest wettability difference of the crystallization solution is observed for gradients of perfluorododecanethiol versus 11-mercaptopoundecanoic acid (see Fig.6.26, 6
min 30 s immersion in 5 \( \mu \text{M} \) C\textsubscript{11}CF\textsubscript{3} solution, backfilling 16 h in 10 \( \mu \text{M} \) C\textsubscript{10}COOH solution). It can be clearly seen that on perfluorododecanethiol rich side of the gradient no crystals were formed. In the middle of the gradient the transition between wetting and not wetting of the substrate can be observed. The reason for this is that perfluorododecanethiol SAMs are oleophobic (\( \text{CA}_\text{Toluene}=78 ^\circ \)), while nonfluorinated surfaces are oleophilic (dodecanethiol SAM (\( \text{CA}_\text{Toluene}=43 ^\circ \)), mercaptoundecanol (\( \text{CA}_\text{Toluene}=7 ^\circ \)) and mercaptoundecanoic acid (\( \text{CA}_\text{Toluene}=8 ^\circ \))).

Nevertheless, the contact angle of toluene on a dodecanethiol SAM is 43 \(^\circ\) – still significantly higher than on hydrophilic SAMs. This difference can be observed when the crystallization with the linear-motion drive technique is performed on a C\textsubscript{11}CH\textsubscript{3} versus C\textsubscript{11}OH gradient (8 min 17 s immersion in 5 \( \mu \text{M} \) C\textsubscript{11}CH\textsubscript{3} solution, backfilling 16 h in 10 \( \mu \text{M} \) C\textsubscript{11}OH solution). We observe a much higher crystal density on the C\textsubscript{11}OH rich side (see Figure 6.27), but the difference is smaller than for the gradient in Figure 6.26.
C\textsubscript{11}OH and C\textsubscript{10}COOH SAMs were both wetted by toluene. On the C\textsubscript{11}OH versus C\textsubscript{10}COOH gradient (8 min 10 s immersion in 5 µM C\textsubscript{11}OH solution, backfilling 16 h in 10 µM C\textsubscript{11}OH solution) many crystals were observed all over the gradient. But on the C\textsubscript{10}COOH rich side more crystals were observable. Two different properties of the C\textsubscript{10}COOH SAMs could cause this effect. On the one hand it takes longer to form a perfectly well-ordered SAM due to the bigger size of the end-functional group.[86] As was shown in section 6.3.3, disordered features can serve as nucleation points. On the other hand the ionizable functional groups could interact with the ionizable functional group of the carbamazepine molecules (see structure in Figure 6.6). This enhanced nucleation density does not lead to polymorph-selective crystal growth. While a method has been found to create a crystal density gradient of carbamazepine, no polymorph-selective crystal growth was observed.
6.4 Conclusion

The results presented in this chapter illustrate that crystallization is a very complex process. Many different parameters influence the outcome of a crystallization experiment. Often the variation of one parameter also influences other conditions. For example, an increase in water concentration in the ethanolic theophylline crystallization solution led to a lowered degree of supersaturation. This led to the formation of the thermodynamically stable anhydrous form despite the presence of more water. Another important connection between variable parameters, which is often ignored in literature, is the connection between wetting behavior of the crystallization solution and the evaporation speed at the three phase contact line. If the solution wets the surface well, the surface to volume ratio is very high. The same amount of liquid is evaporated in a shorter time, which increases the nucleation and crystal growth speed. It is therefore more likely that the kinetically favored polymorph is formed. During the course of several crystallization experiments described in this thesis, often the clearest difference in the crystallization outcome was observed between surfaces with very different wetting behaviors. Although this suggestion puts in doubt the role of the surface as an epitaxial crystal growth template, it supports the idea that surfaces can be used to influence the crystallization outcome. The driving force might not always be epitaxial match between the surface and the polymorph but the difference in crystal growth rate due to different wetting behavior can lead to different polymorphs formed during the crystallization process. This conclusion is further supported by the crystallization experiments performed with the linear-motion drive, where more wettable regions nucleated more crystals than the non-wetted regions. But none led to a clear polymorphic selection on surfaces despite presenting different chemical functional groups. The use of a linear-motion drive to grow crystals on wettability gradients by gradual withdrawal of the sample from crystallization solution or the droplet container method to tune the evaporation rate of the crystallization solution are both promising approaches to exploit surfaces with differences in wettability as crystallization template.

The results found in this chapter also illustrates the importance of roughness of a surface during the crystallization process. Therefore roughness gradients could potentially be used to serve as crystallization templates.
The high nucleation density of carbamazepine on the C\textsubscript{10}COOH surface in comparison to the hydrophilic but not ionizable C\textsubscript{11}OH surface suggests the possible use of surface-charge gradients as crystallization template.
Conclusions and Outlook

7.1 Conclusions

A broad variety of surface-chemical gradient preparation methods are available, but the number of methods which have been extended in order to create orthogonal surface-chemical gradients is limited. In a previous thesis accomplished in our research group, a gradual-immersion technique to prepare unidirectional surface-chemical gradients was established. The aim of the present thesis was to advance this method in order to prepare orthogonal surface-chemical gradients. To do so, a further gradual immersion step was included in the gradient-preparation protocol to create an alkanethiol density gradient of a second component perpendicular to the first density gradient before backfilling with a third component. An orthogonal wettability gradient with different hydrophobicity and oleophobicity profiles has been prepared with this method and the wettability, chemical composition and the ordering of the gradient surface have been characterized.

The dynamic range of the orthogonal surface-chemical gradients prepared by this method is limited to maximum concentration of 50% for two of the three components. For many studies this dynamic range is too limited. Therefore the method has been refined in order to extend the dynamic range of the three component gradient. The adsorption time and concentration of the alkanethiol solutions used for the gradient preparation were adjusted that complete coverage was reached in the region where the sample was immersed longest during the gradual immersion steps. The density gradient of the second component was not longer oriented orthogonal to
the density gradient of the first component due to the restricted amount of available binding sites towards the high density region of the first adsorbed component. The density profiles of the second and third component are therefore radially shaped. This extended method was applied by combining a neutral, negatively charged and positively charged species on the surface, leading to a charge-density gradient perpendicular to a net-charge gradient.

The applicability of the orthogonal surface-chemical gradients to investigate surface-object interactions was tested by the adsorption of positively and negatively charged nanoparticles and proteins. The adsorption of both, proteins and nanoparticles, is clearly influenced by electrostatic interactions with the orthogonal charge-density versus net-charge gradient. Besides that the impact of the surface-chemistry of a gradient on the polymorphic crystal growth was investigated in order to evaluate the potential of surface-chemical gradients as high-throughput crystallization templates. Wetting effects were found to have a bigger influence on the formed polymorph than the specific surface-chemistry. Therefore surface-chemical gradients with changing wettability show the potential for the development of high-throughput screening-devices for polymorphic crystal formation.

7.2 Outlook

The exploration of the preparation and implementation of orthogonal surface-chemical gradients can be advanced in different directions.

As seen in Chapter 4 and 5 the ordering and crystallinity of the SAM from the orthogonal gradients is not as good as for the uniform single-component SAMs. Due to substantial replacement and — in the case of the orthogonal charge-density versus net-charge gradient — ester formation, the backfilling time was not increased further, although it would have improved the SAM ordering and crystallinity. Different attempts could be made to improve the ordering without reducing the gradient range. First of all, longer alkanethiol species could be used, since the replacement rate of longer thiols is reduced due to the enhanced intermolecular interaction.[33] The backfilling step could be split into two parts: first a third gradual immersion along the gradient axis of the backfilling component could be performed followed by complete immersion. Besides that, for the backfilling step a solvent in which
the solubility of the alkanethiols is low could be used, since the desorption rate is reduced in a bad solvent.[200]

In Chapter 5 it was shown that positively charged proteins have the tendency to adsorb in the negatively charged region of the charge-density versus net-charge gradient and vice versa. By performing coadsorption of two different fluorescently labeled, oppositely charged proteins the protein sorting potential of the gradients could be evaluated. As a further extension, the net-charge gradient could be combined with the kinesin density gradient published by Ionov et al.[103], which was used to sort proteins according to their size. A successful combination of these two approaches would lead to an on-surface alternative to 2D gel electrophoresis.

From the supposition that the chargeable alkanethiols are adsorbed in their charged state during the orthogonal charge-density versus net-charge gradient preparation, it was assumed that the gradient exhibit almost no island structure. Because the phase separation of mixed alkanethiol SAMs has an impact on the protein adsorption[54], it would be interesting to investigate the effect of island structure of the charge-density versus net-charge gradient by AFM and to compare the microstructure and protein adsorption on the gradient with mixed zwitterionic SAMs prepared by coadsorption.

With the presented methods to prepare orthogonal surface-chemical gradients, a broad range of surface-chemistry mixtures can be obtained. The chemistry is by far not restricted to the presented combinations. The chemical functional groups of the gradient surfaces can be changed in two ways. Either an alkanethiol with a different functional group is adsorbed on the surface during the gradient-preparation steps or the chemical functionality is changed after the adsorption in a modification step. For the former approach, a broad variety of alkanethiols is commercially available with functional groups ranging from bioresistant (PEG, OEG), through biospecific (biotin, growth factors such as RGD), chelating agents (nitrilotriacetic acid), electroactive (ferrocene, hydroquinone), fluorescent (fluorosceine, triphenylimidazole, coumarin) to derivatizable (thiol, azide, alkene) functional groups. In Sections A.3 and A.4 of the appendix, two preliminary studies are presented in which PEG and OEG terminated thiols are used to prepare gradient surfaces. In order to implement different compounds in the gradient, their adsorption kinetics need to be determined and it needs to be tested whether any measures have to be taken to stabilize the compound during the adsorption.
In the latter approach, the modification takes place after the gradient was formed. In Section A.5 of the appendix it is shown that the amine functional group, used in the charge-density versus net-charge gradient study, can be modified with a photocatalytic coupling reagent after gradient formation.\cite{201} The coupling reagent becomes activated with UV light into a nitrene, which can be insert into NH and CH bonds. Therefore this coupling scheme can be used to modify the surface chemistry with a broad variety of functionalities. Also for the two other functional groups present on the charge-density versus net-charge gradient, specific chemical reactions are known to modify them.\cite{37} Therefore this gradient promises to be a very versatile platform that can be used to modify the surface chemistry with specific reactions.

The applicability of the orthogonal gradient preparation method presented in this thesis is not restricted to alkanethiols SAMs. Also other anchoring-group substrate interactions could be used to create density gradients by gradual immersion, such as PLL-\textit{g}-PEG or catechols on TiO\textsubscript{2}.\cite{6, 96}

The mixing of different components in all possible mixing ratios is limited to three components on one surface. Including a fourth component requires an additional dimension. Since the surface is by definition a two-dimensional construct the extension in the third dimension can only be achieved by applying a trick. A stacked assembly of flat surfaces could be used to create kind of a volume and would lead to a step-wise gradient in the third dimension. The only requirement for the gradient preparation with the serial alignment is that the surfaces have to be separated enough to avoid an advancing liquid surface due to capillary forces. The stacked assembly of flat surfaces can be disassembled for analysis. Such quaternary SAM gradients would raise the number of possible mixtures to an even higher power.
In this appendix five different preliminary studies that came out of the projects described in Chapter 4-6 are presented. All of these smaller projects are somewhat incomplete in nature, but nevertheless, the information contained here might be of use for further investigations. All studies were performed on unidirectional gradients. Only in one case was the study extended to an orthogonal surface-chemical gradient. The first section describes a study of the island structure of sub-monolayer alkanethiol layers and the growth of these island structures under different storage conditions. In the second section, an alternative method is explored to prepare surface-chemical gradients. In the third and fourth section the influence of ethylene-glycol chain density and wettability on the protein adsorption was studied on C\textsubscript{11}CH\textsubscript{3} and C\textsubscript{11}OH versus PEG- or OEG-terminated thiol gradients. In the last study, amine-terminated SAMs were coupled with a photocatalytic coupling reagent, which could be used to further modify the surface chemistry.

A.1 Islanding

As mentioned in Chapter 2, alkanethiol SAMs at sub-monolayer coverage have the tendency to form island domains due to van-der-Waals interaction between the alkyl chains. This effect is observable only if the coverage is lower than a full monolayer. On uniform, single-component SAMs at complete coverage, these islands merge to a continuous monolayer. Since the procedure employed in this thesis for the preparation of gradients interrupts the adsorption of one or more gradient components
before complete coverage is reached, the microstructure of the presented gradients will exhibit islands.[50] Barrena et al. have studied alkanethiol island growth upon storage of sub-monomonolayer covered SAMs.[202] In the present study the change of the microstructure of single-component density gradients as well as uniform sub-monomonolayer alkanethiol assemblies during storage under dry and humid atmosphere was recorded.

Ultraflat gold (mean roughness $R_a$ below 0.3 nm) is required, in order to observe the microstructure of an alkanethiol layer. The ultraflat-gold preparation protocol presented by Blackstock et al.[203] was slightly modified and adapted to our purpose. Gold-coated mica (either prepared by the protocol presented by Wagner et al.[204] or delivered from Georg Albert PVD) was cold welded on top of a gold-coated silicon wafer, prepared as perviously described (see Chapter 3). The cleanliness of the gold surfaces is crucial for the cold-welding process. Simultaneous coating to obtain two clean gold surfaces as suggested by Blackstock was not possible. The gold surfaces were cleaned by air plasma prior to the welding step and immersed in pure ethanol to reduce the oxidized gold. The welded samples were cleaved directly before the thiol adsorption and used without cleaning. Two types of samples were prepared: unidirectional dodecanethiol ($C_{11}CH_3$) density gradients, prepared by gradual immersion by means of a linear-motion drive into 5 $\mu$M $C_{11}CH_3$ thiol solution up to 4 min and uniform single-component sub-monomonolayers prepared by complete immersion into 5 $\mu$M $C_{11}CH_3$ thiol solution for 30 s. The samples were analyzed directly after preparation, and after 7 and 14 days of storage under humid or dry conditions.

Figure A.1: Friction images (1x1 $\mu m$ size) from contact-mode AFM on a freshly prepared sample of a $C_{11}CH_3$ thiol layer at sub-monomonolayer coverage and after storage under dry and humid atmosphere for 1 week.
In Figure A.1 lateral force microscopy (LFM) images representing the island structures on a freshly prepared sample of dodecanethiol on gold at sub-monolayer coverage and after 1 week storage under dry and humid condition are shown. The island size on the sample, which was stored under dry conditions is not very different to the island size observed on the fresh sample. In contrast the islands on the sample was stored under a humid atmosphere are clearly bigger. Figure A.2 summarizes the average island size measured on single-component dodecane thiol gradients as a function of time. Similar to the above presented friction images on uniform samples

![Average island size plotted against the immersion time into the dodecanethiol solution on unidirectional single component density gradients of a fresh sample (○) and of samples stored for 1 week under dry (□) and humid (▼) atmosphere.](image)

the average island size on the gradient samples are also bigger when the sample was stored under humid conditions. The size of the islands also varies along the gradient. For intermediate immersion times the island size is biggest. For longer and shorter immersion times the size is smaller. The growth process of the islands becomes even more pronounced when the sample is stored in humid atmosphere for longer. Figure A.3 shows the average island size on the uniform single-component samples stored in dry and humid atmospheres for 0, 7 and 14 days. While for the sample stored under dry conditions the island size remained constant, the island size increased drastically after 14 days storage in humid atmosphere. A possible explanation for the observed phenomena is the diffusion of desorbed thiols through a liquid film on the sample surface and readsorption on the island edge. The mobility of the thiols in the liquid film is restricted to two dimensions. Therefore desorbed thiols can not
leave the surface. The attachment on island edges is energetically favorable due to van-der-Waals interaction between the alkyl-chains. Another explanation would be that the presence of water facilitates the desorption of the bound thiols.

The longer the samples were stored, the more frequently high-friction features were observed, as seen on the humid sample displayed in Figure A.1. They vanished when the samples were rinsed with ethanol before the AFM measurements. However, simultaneously a decrease of the island size was observed after rinsing. XPS measurements performed on the single-component SAMs directly after the AFM measurements showed that the majority of the thiol groups were oxidized during the AFM measurements on a fresh sub-monolayer C\textsubscript{11}CH\textsubscript{3} layer. Figure A.4 shows the S 2p spectrum measured on a single-component C\textsubscript{11}CH\textsubscript{3} sub-monolayer after AFM measurements. The S 2p 3/2 signal is observed at a binding energy of 168.9 eV. The S 2p signal of unoxidized alkanethiols bound to gold is observed in traces at a binding energy of 162.0 eV.

This finding is consistent with the observation of Willey et al.[19] They showed that alkanethiol SAMs oxidize in air due to ozone. Ozone is present in the air, especially in ventilated buildings. In sealed containers the oxidation was very limited. But the AFM images were performed in open air and therefore oxidation could take place during the measurement. Willey et al. performed measurements on complete monolayers. The sub-monolayer samples used in this study are probably even more

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**Figure A.3:** Average island size measured on two single component sub-monolayer samples plotted against the storage time in dry (●) and humid (○) atmosphere.
susceptible to oxidation since the sulfur groups, which are normally protected by the alkanethiol overlayer, are partly exposed to the air. To avoid oxidation during the AFM measurements it would be necessary to perform the measurements under nitrogen or argon gas. It is also possible that the oxidized alkanethiols are the mobile species causing the island growth effect, since the oxidized sulfur species is not bound to the surface anymore. Blondiaux et al.[24] showed that oxidized alkanethiol could be rinsed away simply by ethanol. The structure of the sub-monolayer has often been investigated in air.[18, 50] It is unclear to what extent the island formation and growth observed in these studies were influenced by the presence of an ongoing oxidation process.

A.2 Gradient Preparation by Replacement

As just discussed in Section A.1, gradients formed by sequential adsorption tend to have a island microstructure. One way to reduce the island formation is to avoid partial coverage during the gradient preparation. This could be done by gradual immersion of a complete, uniform single-component SAM of one alkanethiol in a concentrated solution of a second alkanethiol. By replacement of the first component by the second component, a density gradient could be formed.[65] Although it is still
possible that the mixture is not perfectly homogeneous due to increased replacement rates on defect sites and grain boundaries,[39, 40, 205] the blending is expected to be improved. The aim of the work presented in this section was to test the feasibility of applying this method. Besides the improved homogeneity of the SAM, the ordering and crystallinity could also possibly be improved. If the gradients were prepared by the sequential-adsorption method, including partial coverage steps, they are often not perfectly ordered because the backfilling time needs to be restricted, in order to avoid substantial replacement of the other components. Starting from an already perfectly ordered SAM and taking advantage of this replacement step could lead to reduced island formation and improved ordering of the SAM.

Gold-coated silicon wafers were sonicated in ethanol for 10 min, air-plasma cleaned for 2 min and immersed into 1 mM C_{11}OH solution over night (about 15 h). Subsequently the samples were immersed into 10 mM C_{11}CH_{3} solution for different times. The sequence of immersion has been selected in this way since we know from previous studies[206] that C_{11}OH gets replaced more easily by C_{11}CH_{3} than the other way around. In order to test the degree of replacement the static water contact angle was measured. In Figure A.5 the resulting contact angles are plotted against the immersion time in the C_{11}CH_{3} solution. The higher the replacement of the hydrophilic

\[ \text{Contact Angle [°]} \]

\[ \text{Cosine Contact Angle []} \]

\[ \text{Immersion Time 2nd Step [h]} \]

Figure A.5: Static water contact angle of uniform C_{11}OH SAMs immersed as a second step into 10 mM C_{11}CH_{3} solution for different times.

C_{11}OH with the hydrophobic C_{11}CH_{3} the higher the contact angle. As expected the contact angle increases with increasing immersion time into C_{11}CH_{3} solution. This confirms that the C_{11}OH thiols are replaced by C_{11}CH_{3} thiols over time. In
the beginning the replacement is faster and then it slows down. This is consistent with the findings of former studies.\[34\] The initial faster replacement is due to the exchange at defective regions. An almost linear increase of the cosine of the water contact angle with the immersion is observed after the faster initial replacement. By applying Cassie’s equation\[207\], it can be estimated that about 40% of the C\(_{11}\)OH thiols are replaced by C\(_{11}\)CH\(_3\) after 67 h. Compared to the time needed to prepare unidirectional surface-chemical gradients by the di-coating method, the time scale is very long. The longest immersion times that can be obtained with our linear motion drive for a for 4 cm long sample is about 5 h. After 5 h, only an increase in contact angle by 20° could be observed. For such long immersion times the evaporation of the solvent becomes relevant and makes the control of the immersion process more complicated.

Different measures could be taken to enhance the replacement. By elevating the temperature of the solution the replacement kinetics would be accelerated. But the evaporation rate of the alkanethiol solution would be enhanced and vapor-phase exchange in the non-immersed region of the sample could be possible.\[34\] Other compounds, such as perfluorinated thiols or shorter alkanethiols\[38\] are more susceptible to replacement than the C\(_{11}\)OH. Therefore other replacement pairings (such as short against long or perfluorinated against alkyl spacer) could increase the replacement rate. Hayashi et al. prepared surface-chemical gradients of short chain alkanethiol (C\(_6\)) versus oligo(ethyleneglycol)-thiols with this method.\[65\] Also starting from less-ordered SAMs would increase the replacement rate,\[33\] but all these measures would lead to reduced ordering and crystallinity that is at odds with the initial objective. Using another solvent, such as chloroform or THF, for the backfilling step in which the thiols are more soluble could increase the fraction of replaced thiols over time.\[38, 200\] Since these solvents have a lower boiling point than ethanol, the gain in replacement kinetics needs to be substantial in order to compensate for the more unstable liquid line.

The gradual-immersion gradient-preparation method cannot simply be substituted by the replacement-gradient preparation method due to the very limited dynamic range achievable within a reasonable preparation time. However, if only a very narrow gradient range is required the method probably produces gradients with a higher crystallinity and degree of ordering.
A.3 PEG Gradient

One way to render surfaces protein resistant is to modify the surface chemistry in a way that poly(ethyleneglycol) (PEG) chains are exposed to the surroundings. PEG is a non-toxic, biocompatible, uncharged, water soluble polyether. The most frequently used strategy in our group is to immobilize PEG chains on the surface by adsorbing poly-L-lysine-grafted-PEG (PLL-g-PEG) on metal oxide surfaces.[208] Another approach is to assemble PEG-based thiols on gold-coated surfaces.[209–211] Pasche et al.[212] showed that the PEG chain density influences the protein adsorption. On the other hand, several studies demonstrate that the wettability has a clear influence on the protein adsorption.[128, 151] The combination of a PEG-chain density gradient with a wettability gradient would allow us to address the interplay of both effects. The aim of the project presented in this section is the preparation of orthogonally oriented C$_{11}$CH$_3$ and C$_{11}$OH alkanethiol density gradients backfilled with PEG-thiol in order to change the wettability and the PEG-chain density independently.

First C$_{11}$CH$_3$ and C$_{11}$OH gradients, backfilled with a PEG-thiol, were studied and the backfilling time optimized before the gradients were combined into an orthogonal gradient. The samples were prepared as follows: Gold-coated silicon wafers were sonicated in ethanol for 10 min, air-plasma cleaned for 30 s and again immersed in ethanol for 10 min before gradient preparation. The samples were immersed into C$_{11}$CH$_3$, C$_{11}$OH or α-methoxy-ω-mercapto poly(ethylene glycol) (PEG-SH, m.w. 5000 Da, Iris Biotech GmbH, Marktredwitz, Germany) for various times, either by complete immersion or gradual immersion by mean of a linear-motion drive. In Figure A.6, the thickness of C$_{11}$CH$_3$ and C$_{11}$OH density gradients, both backfilled by PEG-SH are shown. The PEG-SH SAM with a thickness of 70 Å is much thicker than the alkanethiol SAMs, which are about 15 Å thick. Thickness is an indication of PEG-chain density. A thickness gradient can be observed for both alkanethiols and the shorter the immersion time in the alkanethiol during the first step, the thicker the layer. The thickness of the gradient containing C$_{11}$OH is higher, indicating a lower alkanethiol concentration on the surface than on the C$_{11}$CH$_3$ gradient. On the one hand, the C$_{11}$OH adsorption kinetics are slower than those for C$_{11}$CH$_3$ adsorption and on the other hand the C$_{11}$OH is more susceptible to replacement. At the high-density end of the C$_{11}$CH$_3$ component, the thickness of the layer is reduced close to the thickness of the alkanethiol, indicating that C$_{11}$CH$_3$ is mainly...
A.3. PEG Gradient

Figure A.6: SAM-layer thickness on a $C_{11}OH$ and $C_{11}CH_3$ versus PEG-thiol gradient measured by ellipsometry.

present on the surface. For the PEG-SH rich end, the thickness is lower than that of a pure PEG-SH SAM. But for the $C_{11}OH$ gradient the thickness in the PEG-SH rich region is close to the thickness observed for a pure PEG-SH SAM.

In Figure A.7 the static water contact angles of the $C_{11}CH_3$ and the $C_{11}OH$ density gradient both backfilled with PEG-SH are shown. While the contact angle is invariant on the $C_{11}OH$ gradient, it is increasing with reduced thickness for the $C_{11}CH_3$ gradient. This illustrates the wettability difference between the PEG-SH
(CA\textsubscript{water}=31°) and the C\textsubscript{11}OH (CA\textsubscript{water}=20°) compared to C\textsubscript{11}CH\textsubscript{3} (CA\textsubscript{water}=107°). By combining these gradients in an orthogonal manner, it is therefore possible to change the PEG-chain density and the wettability independently.

The backfilling process was optimized, in order to create gradients with the highest difference in thickness. The layer thickness of blank samples and samples first immersed in the alkanethiol solutions was recorded as a function of immersion time into backfilling solution. In Figure A.8 the resulting thickness difference between the two measuring series is displayed. The thickness difference is first increasing with increasing immersion time into the backfilling solution, but after 4 h the difference is leveling off.

Orthogonal surface-chemical gradients were created to study the interplay of the PEG-chain density and the wettability on the protein adsorption. To do so, the following conditions have been chosen: a 4x4 cm gold coated glass slide was gradually immersed into 5 \( \mu \)M C\textsubscript{11}CH\textsubscript{3} solution over 4 min 10 s, in a second step the sample was immersed in orthogonal direction into 5 \( \mu \)M C\textsubscript{11}OH solution over 8 min 20 s and finally the samples was backfilled for 4 h in 50 \( \mu \)M PEG-SH solution. The
immersion into $\text{C}_{11}\text{CH}_3$ was restricted to half of the immersion time as used for the preparation of the unidirectional gradients by Morgenthaler et al.[1] in order to allow the formation of gradients of $\text{C}_{11}\text{CH}_3$ and $\text{C}_{11}\text{OH}$ orthogonal to each with the maximum coverage of 50%. Otherwise not enough free binding sites will be available for the second component. The immersion time into the $\text{C}_{11}\text{OH}$ component was double with respect to the first immersion step since, as can be seen in Figure A.6, the $\text{C}_{11}\text{OH}$ adsorption and subsequent back-filling by PEG thiol is slower than that of $\text{C}_{11}\text{CH}_3$ by a factor of two. The backfilling of 4 h as chosen in order to create a thickness gradient with the highest possible thickness difference as can be seen from Figure A.8. The resulting thickness gradient is displayed in Figure A.9. The shorter

![Figure A.9: SAM thickness of an orthogonal gradient of dodecanethiol, mercaptoundecanethiol backfilled with PEG-5K-thiol measured with ellipsometry. Each intersection of black gridlines marks a measured value. The color scale is displayed beside the plot.](image-url)

the immersion time into both alkanethiol solutions the higher the SAM thickness. The thickness over the gradient is symmetric with respect to the sample diagonal. The thickness difference from one extreme to the other is 35 Å almost similar to what was observed for the backfilling kinetics (see Figure A.8). In the $\text{C}_{11}\text{CH}_3$ and $\text{C}_{11}\text{OH}$ rich region, the thickness is 30 Å high compared to the pure $\text{C}_{11}\text{CH}_3$ and $\text{C}_{11}\text{OH}$ SAM thickness, what is due to the PEG-SH present on the surface. From that thickness we can estimate that PEG-SH its fraction is roughly about 30% in this region. In the PEG-SH rich region its fraction is about 90%. A PEG-SH density gradient with a fraction varying between 30 and 90% was achieved with this gradient preparation method.
In Figure A.10 the static water contact angles measured on the gradient are shown. The longer the immersion time into the C\textsubscript{11}CH\textsubscript{3} solution, the higher the contact angle. Only little variation is observed perpendicular to the immersion axis. Along the sample diagonal from the the C\textsubscript{11}OH to the C\textsubscript{11}CH\textsubscript{3} rich region the angle varies clearly but no variation in the SAM thickness was observed along this diagonal (see A.9). This shows that we were able to change the wettablity and the PEG-chain density separately. But the variation of the contact angle by only 9° is small. This is probably due to the fact that the flexible PEG-chains, which are much longer than the alkanethiols, mask the alkanethiol functional groups and therefore the water contact angle is dominated by the wettability behavior of the PEG-chain.

When protein adsorption tests were performed with 1 mg/ml BSA in PBS for 1 h and analyzed with ellipsometry, negative protein-layer thicknesses were obtained. Different effects can be responsible for this decrease in thickness. Some PEG-SH chains might have been entangled during the adsorption process and if they were released during the protein-adsorption test the layer thickness would have been reduced. Adsorbed PEG-SH might have desorbed from the surface. One way to reduce this effect is to stabilized PEG-thiols by lateral interaction in a similar way as it was done for OEG-thiols.\cite{213} Then oxidation of the PEG chains either by autooxidation\cite{214} or by oxygen present in the protein solution has been observed. To increase the stability the use of deoxygenated protein solution was suggested.\cite{215}
But for many applications the oxygen content of the surrounding solution cannot be controlled.

Besides that the PEG-chain density was too high on all positions of the gradient for differences in protein adsorption to be observed. As observed from the contact-angle measurements, the outermost surface chemistry was dominated by the PEG chains. Therefore no variation in the protein adsorption was observable. Higher alkanethiol densities would be necessary for the proteins to also be able to sense the alkanethiol functional groups. To make this possible, the immersion times into the alkanethiol solutions would have to be increased. The density gradients would not be oriented orthogonal to each other anymore, but as shown in Chapter 5 it is possible to increase the dynamic range of three-component gradients above 50% of the individual components. Another possibility would be the use of oligo ethylene glycol (OEG) terminated thiols instead of PEG-SH, since they are much shorter and the masking effect could be reduced. A completely different strategy to render surfaces protein-resistant is the immobilization of oppositely charged components (see Chapter 5).

A.4 EG Gradients

Oligo-ethylene-glycol SAMs (OEG) are also reported to be protein resistant.[152, 216, 217] Due to the reduced EG chain length, the SAM thickness is much lower than for PEG thiol SAMs. The masking effect reported in Section A.3 due to the long PEG chains is therefore expected to be reduced significantly. In contrast to the PEG thiols, the OEG thiols lose their conformational freedom for high densities of the OEG chains, affecting the protein resistance.[218, 219] The protein resistance of OEG-terminated SAMs depends on the EG-density and on the wettability.[85, 220, 221] Therefore surface-chemical gradients of OEG thiols and C_{11}CH_{3} or C_{11}OH were prepared to study the influence of the OEG-chain density and wettability on the protein adsorption.

Gold-coated silicon wafers were sonicated in ethanol for 10 min, air-plasma cleaned for 30 s and again immersed into ethanol for 10 min. Subsequently the samples were immersed gradually over 8 min and 20 s into 5 \mu M C_{11}CH_{3} solution or over 14 min 20 s into 5 \mu M C_{11}OH solution, before backfilling in 10 \mu M \alpha-methoxy-hexa(ethylene glycol)undecanethiol (C_{11}EG_{6}, Asemblon, Inc., Redmond, USA) solution for 16 h.
Besides that a gradient with longer maximum immersion time (16 min 40 s) into the C\textsubscript{11}CH\textsubscript{3} solution and mixed C\textsubscript{11}CH\textsubscript{3} and C\textsubscript{11}EG\textsubscript{6} SAMs were prepared for the protein-adsorption tests. The mixed SAMs were prepared by coadsorption from 10 µM C\textsubscript{11}CH\textsubscript{3} and C\textsubscript{11}EG\textsubscript{6} solution containing 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% C\textsubscript{11}CH\textsubscript{3} over 16 h.

In order to characterize the gradients the SAM layer thickness and the static water contact angle were measured every 5 mm along the sample surface. In Figure A.11 the SAM layer thickness and the static contact angle of the C\textsubscript{11}CH\textsubscript{3} versus C\textsubscript{11}EG\textsubscript{6} gradient are displayed. A SAM-layer thickness gradient is observable with only a small variation from 20 to 24 Å. As expected, the maximum thickness is much lower than for the C\textsubscript{11}CH\textsubscript{3} versus PEG-SH gradient, since the thickness of a dense C\textsubscript{11}EG\textsubscript{6} SAM is only 30 Å in contrast to 70 Å for the PEG-SH SAM. The thicker the SAM-layer, the higher the C\textsubscript{11}EG\textsubscript{6} density. The C\textsubscript{11}EG\textsubscript{6} density is decreasing with increasing immersion time into the C\textsubscript{11}CH\textsubscript{3} solution. In contrast to that, the contact angle is increasing with increasing immersion time into the C\textsubscript{11}CH\textsubscript{3} solution, because the C\textsubscript{11}CH\textsubscript{3} (107°) component is more hydrophobic than the C\textsubscript{11}EG\textsubscript{6} (70°) component. It is remarkable that the contact angles measured on the gradient are below the contact angles of the single components. The reason for that is that the contact angle measured on a dense C\textsubscript{11}EG\textsubscript{6} SAM is significantly higher than for a SAM with slightly reduced density. For the densely packed C\textsubscript{11}EG\textsubscript{6} SAM
the water senses mainly the methyl group of the methoxy end-functional group, but with reduced density the OEG chains are exposed to the water and a contact angle of about 50° can be observed. The same behavior was observed for mixed SAMs prepared by coadsorption. In Figure A.12 the SAM layer thicknesses and the static water contact angles measured on the C_{11}OH versus C_{11}EG_{6} gradient are shown. The thickness of the SAM is decreasing with increasing immersion time into the C_{11}OH solution due to the reduced C_{11}EG_{6} density. But the thickness is in general higher than on the C_{11}CH_{3} versus C_{11}EG_{6} gradient, although the immersion time into the C_{11}OH was increased with respect to the immersion into the C_{11}CH_{3} solution. On the one hand it could be that the increase in immersion time was not sufficient to balance the slower adsorption kinetics and on the other hand the replacement of the C_{11}OH component by the C_{11}EG_{6} might be more substantial. The contact angle is increasing with increasing SAM layer thickness in contrast to the situation for C_{11}CH_{3} versus C_{11}EG_{6} gradient since the C_{11}OH component is hydrophilic and not hydrophobic as C_{11}CH_{3}. Also here a contact angle of about 50° is observed for the C_{11}EG_{6} rich region, which indicates that the EG chains are not perfectly dense packed. In Figure A.13 the C-H vibrational spectra measured every 5 mm along a C_{11}OH versus C_{11}EG_{6} gradient are displayed. The spectra are changing gradually with decreasing immersion time into C_{11}OH solution. For longest immersion the spectra clearly resembles the C_{11}OH spectrum. For shorter immersion times, the intensity at 2893 cm\(^{-1}\) is increasing with respect to the band
Figure A.13: PMIRRA spectra of a unidirectional two component gradient of C$_{11}$OH and C$_{11}$EG$_6$ measured every 5 mm along the gradient axis. Each spectrum is labeled with the time the sample was immersed into C$_{11}$OH solution at this position. The spectra of the pure C$_{11}$OH and C$_{11}$EG$_6$ SAMs are shown at the top and bottom of the graph, respectively.

at 2850 cm$^{-1}$. This is arising from the most prominent vibration band in the C-H region of the C$_{11}$EG$_6$ SAM, which is the CH$_2$ symmetric stretching.[222] Besides that it can be observed that the intensity of the CH$_3$ antisymmetric and symmetric stretching at 2980 and 2816 cm$^{-1}$ of the C$_{11}$EG$_6$, respectively, are increasing with decreasing immersion time into the of the C$_{11}$OH solution.

Protein-adsorption tests were performed with 1 mg/ml BSA in PBS for 1 h and analyzed with ellipsometry. In Figure A.14 the protein adsorption on all these surfaces is plotted against the SAM-layer thickness. An almost linear correlation between the SAM-layer thickness and the protein adsorption can be observed. The higher the SAM-layer thickness, the lower the protein adsorption.

No difference in protein adsorption can be observed between the mixed SAMs prepared by co-adsorption and the gradients prepared by sequential adsorption. Only the SAM layer thickness range is broader for the mixed SAMs prepared by co-adsorption. Therefore we can conclude that islanding is not affecting the protein adsorption.
A.5. Amide formation

Amine-terminated SAMs are not only important as a means to create charge surfaces (as shown in Chapter 5) but they can also serve as a tool to attach a variety of other functional moieties through amide linkage. N-hydroxysuccinimide (NHS) functionalized molecules are often used to form amide bonds with amines.[223] In this study, NHS-bound perfluorophenyl azides (PFPA) groups are used for modification of the amine groups. PFPA groups can be further modified by photocatalytic reactions.[201] The azide functional group is converted into a nitrene by UV activation. The nitrene functional group is highly reactive and can insert into CH and NH bonds. This coupling mechanism is interesting for e.g. biological applications since many different biological active moieties can be coupled to the surface, such as carbohydrates,[224], proteins or PEG chains.[225]

This modification protocol was tested on unidirectional C_{11}CH_{3} density gradients backfilled with C_{11}NH_{3}. Gold-coated silicon wafers were sonicated in ethanol for 10 min, air plasma cleaned for 30 s and again immersed into ethanol for 10 min. Subsequently the samples were immersed gradually over 8 min and 10 s into 5 µM C_{11}CH_{3} solution, before backfilling in 10 µM C_{11}NH_{3}Cl solution for 16 h. In order

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**Figure A.14:** Thickness of the adsorbed protein layer plotted against the SAM layer thickness for single component C_{11}CH_{3} (+) and C_{11}EG_{6} (×) SAMs, mixed C_{11}CH_{3} and C_{11}EG_{6} SAMs (●), short (▽) and long (△) unidirectional two component gradients of C_{11}CH_{3} and C_{11}EG_{6} and a unidirectional two component gradient of C_{11}OH and C_{11}EG_{6} (◁)
to characterize the gradient static water contact angles were measured every 5 mm along the sample surface.

![Figure A.15: Static water contact angle on linear, unidirectional C$_{11}$CH$_3$ versus C$_{11}$NH$_3$ gradient plotted against the immersion time in the C$_{11}$CH$_3$ solution. The measurements were averaged over two measurement series.](image)

Prior to the amide bond formation, the gradient was immersed in 10 mM K$_2$CO$_3$ for 15 min. Then the sample was stirred in 1 mM N-hydroxy-succinimidyl-4-azidotetrafluorobenzoat (NHS-PFPA, [226]) for 1 h. After rinsing with ethanol the PMIRRA spectra were recorded every 5 mm along the gradient surface. In Figure A.16 a section of the PMIRRA spectra measured every 5 mm along the gradient are displayed. At 2131 cm$^{-1}$ the characteristic asymmetrical stretch vibration of the azide functional group[227] can be observed. With decreasing immersion time into the C$_{11}$CH$_3$ solution the azide signal increases. Only the functional group of the C$_{11}$NH$_3$ functional group reacts with the NHS-PFPA and therefore the lower the C$_{11}$NH$_3$ density on the surface due to preadsorbed C$_{11}$CH$_3$ the lower the azide signal. This shows that the density gradient created by gradual immersion can be transported into another gradient via coupling reaction.

Note: The C$_{11}$NH$_3$ adsorption has been carried out in pure ethanol. Therefore part of the C$_{11}$NH$_3$ thiols might have been adsorbed in a upside-down configuration in the SAM layer and expose oxidized sulfur groups on the surface.[132] This issue is discussed more intensively in Chapter 5. To make sure that no oxidized sulfur
A.5. Amide formation

Figure A.16: PMIRRA spectra of a unidirectional tow component gradient of $C_{11}CH_3$ and $C_{11}NH_3Cl$ modified by amide bond formation with PFPA.

groups are interfering with the coupling reaction, the experiment should be carried out with HCl in the $C_{11}NH_3$ adsorption solution.
References


I was fortunate to be accompanied by many wonderful people during my PhD and this thesis wouldn’t have been possible without their help and encouragement. Therefore I take the opportunity to acknowledge all the people who supported me during the last years.

First of all, thanks to Nicholas D. Spencer for accepting me as a PhD student and for always being optimistic, when had I had lost faith in my results. It is a real pleasure to work with him and in his group. He leads the group with a excellent balance between letting people take their own decisions and supporting them with suggestions, when they are needed. The overview he has of the different projects going on in his group and his scientific knowledge and experience are impressive. Many thanks also for the correction of this thesis (including all the missing hyphens and commas).

Thanks to Venkataraman Nagayianallur for being a great supervisor. His calm and critical way to address scientific problems, his fundamental understanding of science in combination with his creative ideas make him an outstanding researcher. I was really fortunate to be able to work with him. I’m also very thankful for the correction of this thesis and for his interest in my projects.

Thanks to Bo Liedberg for examin my thesis and for coming to Zürich to attend my defense. Thanks to Ludwig J. Gauckler for attending my defense as the representative of the Department of Materials.

Thanks to all the former and current team members of the LSST for the nice working atmosphere. It was always possible to get a helping hand or an open ear whenever it was needed. It was wonderful and interesting to work in such a international group. Thanks for all the off-site group meetings, BBQs, movie nights
Acknowledgements

and coffee-breaks. Some of the group members I would like to thank especially. Many thanks to:

**Sara Morgenthaler** for starting the gradient project and for supervising the first part of my PhD. She was always there to answer questions patiently concerning scientific problems, organizational matters or wedding preparation. Thanks a lot for the memorable time we spent together in New York and for all the discussions during coffee-breaks.

**Doris Spori** for being a wonderful friend and co-worker. I always enjoy the time we spend together and our scientific and private discussions. I learned a lot from the way in which she starts talking to strangers with an open ear and from her spontaneous way of organizing things.

**Whitney Hartung** for sharing my interest in food, movies, books and for being a dear friend. Spending time with her always cheers me up.

My students, **Roman Engeli, Florian Bachmann, Florian Furrer, Marianne Sommer**, who performed their laboratory course and their master projects within the framework of my PhD project, for their work, questions and inputs. My research assistants, **Maike Quandt and Payam Payamyar**, for testing different particle adsorption setups and alternative API for the crystallization project.

**Antonella Rossi** for teaching me how to measure XPS and for her critical mind. **Jia Pei** for the help with the fluorescence microscope. **Clément Cremmel** for the help with the nanoparticle adsorption, for the particle-counting macro and for his open ears to my difficulties to prepare sugar flowers for my wedding cake. **Olof Sterner** for the discussions about the ellipsometry measurements and for collaborating on the plasma-cleaner issue. **Stefan Zürcher** for his inputs concerning the crystallization project. **Lucio Isa** for the ideas about the particle adsorption. **Seunghwan Lee** for his supporting and motivating words on his good-bye party and for all his inputs during the gradient meetings. **Josephine Baer** for the administrative work she has done and for always being in a good mood. **Giovanni Cossu** for the support of the XPS. **Martin Elsener** for the fabrication of the crystallization boxes. **Tomas Bartos** for the computer support.

**Maura Crobu** for being so generous with her table and her sofa, for her pleasant company and for all the gorgeous food she cooked. **Christoph Mayer** for all the phone calls he answered for me pretending to being my secretary, for always
drinking a coffee with me when I needed a break and for his nice company. Torben Gillich for all the scientific and political discussions we had and for being a excellent entertainer. Sina Saxer for her motivating words at a defence party.

Prathima Nalam, Bidhari Pidhatika, Chiara Perrino, Marcella Roba, Akshata Rao, Tomoko Hirayama, Saiko Aioki, Karthik Kumar, Raphael Heeb, Tobias Balmer, Mathias Rodenstein, Filippo Mangolini, Robert Bielecki, Stefan Kaufmann, Firat Durmaz, Roman Heuenberger, Shivaprakash Ramakrishna, Ang Li, Fabian Anderegg and Ke Zeng for being pleasant office-mates.

Erich Schurtenberger for all the silly discussions we had about chili and wasabi.

Rowena Crockett and Paul Hug for the help with the Raman at the Empa Dübendorf. Thomas Wermelinger for the help with the Raman at ETH. Urs Schwitter and Pirmin Hidber from Roche for performing part of the crystallization experiments together and for their inputs.

Thanks to the SBB for the save and punctual transportation between Zürich and Bern. I traveled roughly 170’000 km with the SBB during my PhD and many pages of this thesis were written in the train. The Swiss National Science Foundation is acknowledged for generous financial support.

Last but not least, I’m very grateful to have a wonderful family and dear friends who gave me the self-esteem to start this PhD and who always helped me with words and deeds. Especially many thanks to:

My parents, Julia and Bruno Beurer, for giving me life, for the opportunity to go to the university and for believing in me. It is priceless to have such a warm home.

My sister, Andrea Beurer, for teaching me many lessons you have to learn in life and for being a great sister. I wouldn’t swap her for any money in this world.

My cats for their company while writing at home and for their unconditioned affection.

My in-laws, Vreni, Nicole, Anouk, Kurt, Dominic, Lars Amacher, for embracing me as part of the family.

My love and husband, Stefan Amacher, for everything he is and he has done for me. He always knew how to cheer me up whenever it was difficult and how to share
my happiness when things went well. He served me coffee in bed when I had troubles to get up in the morning and prepared dinner when I returned late from work. I'm thankful and proud to be your wife and I'm looking forward to our joint future and to all our projects.
Curriculum Vitae

Personal

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Education

2006 – 2011 Dissertation at the ETH Zürich in the Department of Materials in the Laboratory for Surface Science and Technology under the supervision of Prof. N. D. Spencer (Advanced Surface-Chemical Gradients)

2005 Diploma thesis in the research group of Prof. H. U. Güdel (Synthesis and Optical Light Absorption and Emission Properties of Tm\textsuperscript{2+} Doped CsCaI\textsubscript{3} and RbCaI\textsubscript{3} Crystals)

2004 Research project during an Erasmus exchange study at the University of Leuven in Belgium in the research group of Prof. K. Binnemans (Synthesis and spectroscopy of organic lanthanide complexes)

2001 – 2005 Diploma study of Chemistry at the University of Bern, Switzerland

1996 – 2001 Gymnasium in natural science (Typus C) in Bern Neufeld, Switzerland
Working Experience and Personal Commitment

2007 – 2009  **Assistant** in the "Scientific Working" lecture for Materials Science students

2006 – 2007  **Assistant** in a chemistry lab course for Material Science students

2006  **Research assistant** in the research group of Prof. H. U. Güdel at the Department of Chemistry and Biochemistry (optical spectroscopy)

2003 – 2005  **Vice president** of the students’ council for chemistry and biochemistry at the University of Bern, responsible for the organization of visiting days for high-school students

2003  **Junior research assistant** at the the Department of Chemistry and Biochemistry at the University of Bern in the research group of Prof. S. Decurtins (inorganic synthesis)

2002  **Junior research assistant** at the the Department of Chemistry and Biochemistry at the University of Bern in the research group of Prof. R. Häner (organic synthesis)

Publications Related to this Thesis

Presentations

2010  Two Types of Orthogonal Surface-Chemical Gradients: Variation in Wettability and in Surface Charge Density *(poster presentation)*, **E. Beurer**, N.V. Venkataraman, A. Rossi, F. Bachmann, R. Engeli, and N.D. Spencer, MRC Graduate Symposium, May 10, Zürich, Switzerland

Orthogonal, Three-Component, Alkanethiol-Based Surface-Chemical Gradients *(poster presentation)*, **E. Beurer**, N.V. Venkataraman, A. Rossi, F. Bachmann, R. Engeli, and N.D. Spencer, SAOG meeting, January 22, Fribourg, Switzerland
2009  Alkanethiol-Based Orthogonal Surface-Chemical Gradients (*poster presentation*), **E. Beurer**, N.V. Venkataraman, A. Rossi, F. Bachmann, R. Engeli, and N.D. Spencer, MRC Graduate Symposium, June 10, Zürich, Switzerland

Surface Chemical Gradients: Analysis and Preparation by DSA 100 (*oral presentation*), **E. Beurer**, Krüss, February 20, Hamburg, Germany

2008  Surface Chemical Gradients as Templates for Heterogeneous Crystallization (*poster presentation*), **E. Beurer**, S. Morgenthaler, and N.D. Spencer, Gordon Research Conference on Chemistry At Interfaces, July 13-18, Waterville Vally, New Hampshire, USA

Heterogeneous Crystallization Testing Tool- Self-assembled Monolayers on Gold-Coated Polyimide Foil (*poster presentation*), **E. Beurer**, S. Morgenthaler, and N.D. Spencer, MRC Graduate Symposium, May 14, Zürich, Switzerland

2007  Is the Ripening of Thiol Islands influenced by Humidity? (*poster presentation*), **E. Beurer**, S. Morgenthaler, S. Lee, and N.D. Spencer, Gordon Research Conference on Chemistry of Supramolecules and Assemblies, May 06-11, Il Ciocco, Italy

Is the Ripening of Thiol Islands influenced by Humidity? (*poster presentation*), **E. Beurer**, S. Morgenthaler, S. Lee, N.V. Venkataraman, and N.D. Spencer, MRC Graduate Symposium, June 27, Zürich, Switzerland

**Publications**