Surface dynamics of organic layers explored by scanning probe microscopy techniques

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SURFACE DYNAMICS OF ORGANIC LAYERS EXPLORED BY SCANNING PROBE MICROSCOPY TECHNIQUES

DISSERTATION

to obtain
the degree of doctor at the University of Twente,
on the authority of the rector magnificus,
Prof. dr. H. Brinksma,
on account of the decision of the graduation committee,
to be publicly defended
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General introduction

1.1 Introduction

Organic thin layers, especially self-assembled monolayers (SAMs), on well-defined solid surfaces have attracted tremendous attention owing to their interesting physical and chemical behavior. Apart from the interest in basic knowledge of the SAMs themselves, the attention also arises from their potential applications in a wide range of application fields, for instance, corrosion inhibition, surface patterning, wetting inhibition, molecular electronics and bio-sensing.¹⁻³ The structural and dynamical properties of organic thin layers are of interest from both a fundamental and an application point of view.⁴⁻⁸ The ultimate utility of SAMs for the potential applications, with respect to the reproducibility and the stability of devices as well as the possibility to control and tune the properties of devices, is critically dependent on their structural and dynamical properties, for instance, conformational changes upon external stimuli (temperature and electrochemical potential etc.).^{8, 9} Therefore, it is of importance to study the structural properties and dynamical process of SAMs. In this sense, high spatially resolved and time resolved techniques are needed.

Scanning probe microscopy (SPM) techniques, like atomic force microscopy (AFM) and scanning tunneling microscopy (STM), are among the most frequently employed characterization tools to examine the structural and electronic properties of SAMs. One advantage of these techniques is that they can be applied in ultra-high vacuum (UHV), liquids as well as under ambient conditions. In addition, high spatial resolution can be obtained utilizing SPM tips with an atomically sharp apex. Using STM, dynamics of SAMs have been observed down to the molecular scale.^{6, 7, 10} However, the time resolution for standard STM is limited, and is usually in the range from seconds to minutes.¹¹ Thus, processes that occur on a much faster timescale will not be accessible. In order to visualize and probe the dynamic phenomena on the surfaces, it is necessary to significantly enhance the temporal resolution. Thus specialized approaches have been developed. For example, current-voltage (I-V) converters can be employed in STM with a large bandwidth and without using a feedback loop, which allows one to monitor current-time spectroscopy (hereafter referred as I-t traces). In this way, a high temporal resolution can be achieved.¹¹

This, and similar options, in combination with the capability to retain molecular resolution, make STM a versatile tool for the characterization of the structure and dynamics of SAMs.¹² Despite the high promise and great potential, relatively few dynamical STM studies on molecular level have been performed on SAMs. Hence, we decided to complement this area and tackle hitherto unaddressed problems in this Thesis.

Stimuli responsive thin layers including biological layers, which switch their physical and chemical properties upon changes in the external environment (temperature, pH, solvent, radiation and electrochemical potential etc.), offer great possibilities in many technological areas.^{13, 14} Redox processes provide an effective way to control the interfacial properties (such as morphological and mechanical properties) for biological thin films.^{15, 16} One well-established technique for studying interfaces, which are electrochemically responsive, is the combination of AFM with electrochemistry (EC-AFM). Peak force tapping, which was developed recently, enables one to measure the soft biological samples with high spatial resolution.¹⁷ With the introduction of peak force tapping mode in EC-AFM, the capability of AFM in studying the responsiveness (*e.g.* morphological and mechanical properties) of biological SAMs will be improved. This method will also receive attention in this research.

1.2 Concept of this Thesis

In this Thesis, surface dynamics of organic thin layers, especially SAMs, are explored by scanning probe microscopy techniques, including time-resolved scanning tunneling microscopy (TR STM) and electrochemical AFM (EC-AFM).

In **Chapter 2**, fundamentals of SAMs and their dynamics as studied by TR STM are discussed. Firstly, fundamental aspects, potential applications, growth mechanisms and structure of SAMs, especially SAMs on Au(111) are introduced. Secondly, the basics and spectroscopic methods of STM are elucidated. In addition, strategies that have been applied to enhance the temporal resolution of STM are also briefly reviewed. Finally, dynamics of SAM surfaces investigated with STM are surveyed.

In **Chapter 3**, energetics and dynamics of decanethiol SAMs on Au(111) surfaces are described and discussed using TR STM at room temperature. It is revealed in the first part of this chapter that the massive dynamics of the decanethiol SAMs is due to diffusion of decanethiol-Au complexes, rather than the diffusion of individual decanethiolate molecules. In the second part of this chapter, the boundary free energies between the disordered and ordered phases are studied using a statistical analysis of the thermally induced meandering of the domain boundaries. On the basis of these results, it is possible to accurately predict the two-dimensional phase diagram of the decanethiolate/Au(111) system.

In **Chapter 4**, utilizing open loop current-time spectroscopy which has a time resolution down to a few μs , the ordering and dynamics (including phase transition and

conformational changes) of monothiol oligo(phenylene ethynylene) (termed hereafter as OPE) SAMs and dithiol OPE SAMs are studied. In the first part of this Chapter, it is revealed that the monothiol OPE molecules at the edges of the vacancy lines exhibit dynamic behavior and frequently jump back and forth between neighboring stripes. Subsequently, I-t traces recorded on the dithiol OPE SAMs are presented. The data suggest that the dithiol OPE molecules continuously switch back and forth between two nearly degenerate configurations.

In **Chapter 5**, STM was used to measure the transport through a single octanethiol molecule in the temperature range from 77 K to room temperature. The conductance of octanethiol is temperature independent, demonstrating that either quantum mechanical tunneling or ballistic transport is the main transport mechanism.

In **Chapter 6**, stimuli responsiveness (via changing electrochemical potential) of the redox-active metalloprotein Cu-azurin on Au(111) surface was investigated by *in-situ* EC-AFM. Height changes of the Cu-azurin were observed upon electrochemical redox switching. The origin of the height change of the Cu-azurin could be attributed to the conformational changes and redox-driven molecular orientation effects. Consistently, no large height changes have been found for Zn-azurin, which is non-redox active. In conclusion, it was found the height of the Cu-azurin could be modulated by the applied potential.

The research described in this Thesis significantly contributed to a better understanding of dynamics of organic thin layers. In the **Outlook**, directions for future research are provided to show the way from the authors' perspective. For instance, TR STM could be employed to study dynamics of mixed SAMs, which is under debate to date. Another future direction related to the dynamics of organic layers on surfaces encompasses the use of AFM. As a force sensitive technique, the time resolution of AFM could be significantly improved by performing measurements with the feedback loop disabled. All these developments are expected to contribute to an enhanced understanding of the dynamical processes of organic thin layers on surfaces.

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Dynamics of organic thin layers explored by scanning tunneling microscopy

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2.1 Self-assembled monolayers

2.1.1 Introduction

The preparation and characterization of ordered organic thin layers (especially self-assembled monolayers) on well-defined solid surfaces have attracted tremendous interest due to their interesting physical and chemical behavior. Apart from the interest in basic knowledge of the SAMs themselves, the interest is also attributed to their potential applications in a wide range of fields, for instance, corrosion inhibition,¹ surface patterning,²⁻⁴ wetting inhibition, molecular electronics⁵⁻⁵⁸ and bio-sensing.^{59, 60} Self-assembled monolayers (denoted as SAMs hereafter) are organic assemblies formed by adsorption of molecular constituents onto the surface of solids or in regular arrays on the surface of liquids, for instance, mercury.⁶¹ SAMs on solid substrates can be prepared by various methods. These include deposition from solution,⁶² deposition in vacuum⁶³ and micro-contact printing.⁶⁴ In all cases, the SAMs are formed spontaneously, that is to say, the adsorbates organize themselves spontaneously (and sometimes epitaxial) into crystalline (or semi-crystalline) structures.^{5, 65, 66}

Each of the molecules or ligands that constitute the building blocks of the SAMs consists in general of three different parts: the head group (linking group), the backbone (main chain) and the specific terminal (active) group (see Figure 2.1).^{5, 66} The chemical functionality of the head group arises from its specific affinity for a certain substrate; in many cases, the head group* also displays a high affinity for the surface and replaces previously adsorbed adventitious organic materials from the surface. There are a number of head groups that bind to specific metals, metal oxides, or semiconductors. Among different kinds of SAMs, those formed by thiol-derived molecules, alkanethiol for instance, on noble

---

* Here, head group refers to the linking group or anchoring group which is binding to the substrate. For example, the head group is thiol (SH) for alkanethiol.

metal substrates (like gold, silver, copper, palladium, platinum and mercury) have been extensively studied due to their stability and ease of fabrication.^{62, 63, 67-74} The strong bonding between thiols and the aforementioned surfaces of noble metals makes it possible to fabricate well-defined organic surfaces with highly tunable chemical functionalities displayed at the exposed interface. The presence of a S-metal bonding also results in the formation of a stable monolayer that remains intact even after the substrate being removed from the solution. Additionally, the adsorption can be carried out in a variety of solvents, polar and non-polar, enabling versatile possibilities in molecular design. The interactions among hydrocarbon chain backbones (including van der Waals and hydrophobic forces) ensure an efficient packing of the monolayer and contribute to stabilize the structures with increasing chain length. The terminal group endows the surface with specific properties (for instance, hydrophilic and hydrophobic), and can be readily used to anchor various molecules by weak interactions or covalent bonds.

Figure 2.1 Schematic diagram of an ideal, single-crystalline SAM of alkanethiolates on a Au(111) surface. The anatomy and characteristics of the SAM are highlighted. Reprinted with permission from Ref. 5. Copyright (2005) American Chemical Society.

As mentioned before, SAMs can form on various substrates. However, the discussion in this Chapter will focus on SAMs formed on Au(111) surfaces.^{5, 75-77} These systems have been extensively studied owing to the following reasons. Firstly, the ease of preparation of Au(111) and the inert nature of Au enables its cleanliness, stability and popularity in application. Secondly, the Au-S bonding interaction in the thiolate is sufficient to keep the thiolate on the surface.⁵ In addition, the structure of Au(111) is well studied.

2.1.2 Growth mechanism and structure of SAMs on Au(111)

The growth mechanism, structure and properties of SAMs have been extensively studied by using many different and complementary surface analysis techniques, both *ex-situ* and *in-situ*, for instance, ultra-high vacuum (UHV) techniques (XPS, AES, LEED, SEM, TEM,

TPD, GIXD, etc.^{*}), spectroscopies (IR,[†] Raman, etc.), and scanning probe microscopies (STM, AFM, SNOM, etc.[‡]).^{5, 66, 78-80} Among all techniques utilized, scanning probe microscopy (SPM) and its combination with average surface analysis techniques provides valuable local and detailed information with high spatial resolution, even down to the single-molecule scale. SPM has been employed as an essential tool to investigate surface structures at the molecule level.^{75, 81} These techniques can be performed in UHV, in liquids and in ambient conditions. Important advantages are the possibility to probe non-periodic structures and defects of SAMs and to perform real time measurements.⁸² There have been numerous studies regarding the structure of alkanethiol SAMs on Au substrates. The structure of the SAMs depends on the chain length, surface coverage, temperature and substrate morphology.^{71, 83-94} The main problem, however, is related to the poor time resolution. The strategies to improve the time resolution will be addressed later in this Chapter. In the coming parts, the structure and growth mechanism of SAMs on Au(111) will be introduced. Before showing the structure of the SAMs, the structure of the Au(111) will be presented.

2.1.2.1 Au(111)

Based on an uniaxial compression of the topmost atomic layer along one of the three $\langle 1\bar{1}0 \rangle$ directions, the Au(111) surface exhibits a peculiar reconstruction with a large unit cell referred to as herringbone reconstruction (see Figure 2.2). The result is a $(22 \times \sqrt{3})$ unit cell where 23 atoms of the top layer are placed on 22 atoms of the second layer.⁹⁵ Along the direction of the compression, the stacking sequence changes from *fcc* (face-centered-cubic) to bridge to *hcp* (hexagonal-close-packing) and again followed by a bridge, with a periodicity of 6.3 nm.⁹⁵ These bridging rows, also termed as soliton walls (introduction see the coming part), are shown in scanning tunneling microscopy (STM) image as elevated ridges aligned with substrate $\langle 121 \rangle$ directions. The orientation of the reconstruction depends on the surface stress, which can be interpreted as topological solitons.⁹⁶ A soliton can be described as a wave that maintains its shape while propagating. For the Au(111) reconstruction, it is the balance between two potential contributions. The top layer favors a uniform contraction owing to a variation in electronic structure as compared to that of the bulk, whereas the underlying bulk layer tends to bind the surface atoms to the bulk positions.⁹⁷ The compromise between these two contributions is the creation of the soliton. Two bright lines, *i.e.* the aforementioned ridges, are visible in the STM image with their

^{*} Full name of those abbreviations: X-ray photoelectron spectroscopy (XPS), auger electron spectroscopy (AES), low-energy electron diffraction (LEED), scanning electron microscopy (SEM), transmission electron microscopy (TEM), thermal desorption spectroscopy (TPD), grazing incidence X-ray diffraction (GIXD).

[†] Full name: infrared (IR).

[‡] Full name of those abbreviations: scanning tunneling microscopy (STM), atomic force microscopy (AFM) and scanning near field optical microscopy (SNOM).

height equal to the amplitude of the soliton. The height difference between the *fcc* domains and the *hcp* domains is only 15 pm. STM image of herringbone reconstructed Au(111) surface is shown in Figure 2.3.

Figure 2.2 Schematic of the herringbone reconstruction. Blue parallelogram shape indicates the $(22 \times \sqrt{3})$ supercell. Reproduced from Ref. 98 with permission from The Royal Society of Chemistry.

Figure 2.3 STM image (image size = 50 nm $\times$ 50 nm) of herringbone reconstructed Au(111) surface showing periodically arranged stress domains separated by soliton walls and a secondary structure of the solitons (herringbone) with partial dislocations (elbow sites). Reprinted with permission from Ref. 99. Copyright (2005) Springer Science+Business Media, Inc.

During the self-assembling process, the sulfur atoms of the thiols interact so strongly with the herringbone reconstruction on Au(111) that two atoms per $(22 \times \sqrt{3})$ primitive unit cell are released from the plane of the surface layer, resulting in the occurrence of one net vacancy per primitive unit cell.^{69, 73} The released Au adatoms diffuse rapidly on the surface. Subsequently, these Au adatoms are incorporated at neighboring step edges while the vacancies nucleate and combine into vacancy islands in the terraces. Upon the removal of these atoms, the compressive surface stress is relieved, and consequently the average

separation between herringbones increases until the herringbone reconstruction is lifted,¹⁰⁰ depending on the molecule-Au interaction strength. The strong interaction between the Au and the sulfur atoms of the thiols leads to the herringbone reconstruction to be completely lifted during thiol SAM formation. A typical topographic image, showing the vacancy islands and different domains characteristic for an decanethiol SAM is shown in Figure 2.4.

Figure 2.4 Topographic image of the decanethiol SAM (image size = 100 nm $\times$ 100 nm). Sample bias voltage +325 mV, tunneling current 20 pA. Adapted with permission from Ref. 101. Copyright (2004) American Chemical Society.

2.1.2.2 Lattice structure and growth mechanism of SAMs on Au(111) determined by STM

SAMs on Au(111) can be prepared either by immersing the gold substrate into a solution of thiol molecules for several hours, called solution phase deposition, or by gas phase deposition, where the gold substrate, which is placed in UHV, is exposed to a vapor of the corresponding thiol molecules.⁵ In the latter method, advantages lie in substrate cleanliness in UHV and the availability of traditional *in-situ* surface characterization techniques. Utilizing STM, Poirier and Pylant studied the growth of vapor-deposited thiols on single crystal Au(111) surfaces.⁶⁹ Studying the formation process of C6-C10 alkylthiols, both methyl and hydroxy terminated, they reported a two-step process which started with the nucleation and growth of islands of striped phases from a lower density lattice-gas phase. As reported by Poirier in 1999, gas phase deposited self-assembled decanethiol monolayers on Au(111) exhibit six different phases, four ordered (β , δ , ϕ and χ) and two disordered (α and ε) phases.⁶³ Figure 2.5 displays a sequence of phases with increasing coverage of decanethiol on Au(111). Four of these six phases are stable: the two-dimensional gas phase

(α phase), the $(23 \times \sqrt{3})$ striped phase (β phase), the $(15 \times \sqrt{3})$ striped phase (δ phase) and the densely packed $(4\sqrt{3} \times 2\sqrt{3})R30^\circ$ upright phase (ϕ phase). The two metastable phases are the striped χ phase, which consists of alternating $(15 \times \sqrt{3})$ and $(23 \times \sqrt{3})$ stripes, and the disordered dynamic two dimensional liquid phase (ϵ phase). The χ^* phase, which comprises β and δ domains of varying size, has recently been observed by Toerker *et al.*¹⁰² All the ordered phases (β , δ , χ and χ^*) consist of flat-lying molecules.

Figure 2.5 Sequence of monolayer phases with increasing coverage of decanethiol on Au(111).⁶³

Monomolecular films obtained from solutions have advantages over vapor-deposited SAMs in terms of lower cost, screen printing, inkjet printing, possibility to operate at lower temperature and over large areas.^{103, 104} Subsequently, preparing SAMs by immersion of a freshly prepared or clean substrate into a dilute ethanolic solution of thiols for ~12-18 h at room temperature has become the most common protocol.^{5, 76} There are a number of factors that can affect the structure of the consequent SAM as well as the rate of formation, such as solvent, temperature, concentration of molecule, immersion time, purity of the molecule, concentration of oxygen in solution, cleanliness of the substrate, and structure of the molecule.⁵ Yamada *et al.*, in a sequence of two papers, performed *in-situ* STM experiments tracking alkanethiol growth on Au(111) from μM heptane solutions.^{90, 105} The self-assembling process of alkanethiols via solution adsorption, as concluded by these authors, seems to proceed as follows. Initially, patches of adsorbed molecules without periodic structures were observed. Vacancy islands of the gold surface are created at this stage. In

the final stage of growth, the islands on which molecules arrange in the $(\sqrt{3} \times \sqrt{3})R30^\circ$ structure grow and the subsequently monolayer formation is completed.

Despite the fact that the gas phase deposition is fundamentally different from liquid phase deposition, both result in the saturated coverage with a $c(4 \times 2)$ superlattice (the sketch is shown in Figure 2.6, notation of superlattice see Appendix of Chapter 2) of the hexagonal $(\sqrt{3} \times \sqrt{3})R30^\circ$ lattice.^{67, 106} The differences and similarities of methyl-terminated n-alkanethiol SAMs formed from liquid and vapor phases has been studied by Chailapakul *et al.* in terms of the structure and chemistry.¹⁰⁷ According to their study, thickness, structure and packing densities of the SAMs obtained from the two different deposition methods are identical. However, the mass and electron transfer properties were dependent on the phase from which the SAM was assembled as well as the chain length of the molecules. In addition, the structure of the Au vacancy islands was different.

Figure 2.6 Schematic of the model for alkanethiolate SAMs on Au(111). White circles represent for the Au atoms while the orange circles represent the S atoms in the thiolate molecules. The rectangle refers to a unit cell of the $c(4 \times 2)$ superlattice, containing 4 molecules. In this sketch, it is assumed that the S atoms adsorb at a threefold hollow sites for the Au(111) substrate. Adapted with permission from Ref. 67. Copyright (1994) American Chemical Society.

Since the SAMs are thin and homogeneous, they have frequently been used as model systems in research applications such as molecular electronic devices, adhesion and wetting regulators and tuning wetting properties of various surfaces.¹⁰⁸ Subsequently, the dynamics of the SAMs attracted tremendous attention owing to the importance of the reproducibility and the stability of the devices as well as the possibility to control and tune the properties of the devices.^{82, 109} In this case, time resolved technique is needed. The coming part will focus on the dynamics of the SAMs investigated by time-resolved SPM, in particular, STM.

2.2 Dynamics of SAMs surfaces studied by scanning tunneling microscopy

2.2.1 Scanning tunneling microscopy

In the early 1980s, Binnig and Rohrer at IBM Zurich invented a novel type of microscope, termed as STM, for which they received the Nobel prize in 1986.¹¹⁰ With the invention of STM, exploration of the atomic scale realm of surfaces in real space has become feasible. The STM can be used not only in UHV but also in air, water, and various other liquids or gas ambient, and at temperatures ranging from near 0 K to a few hundred degrees. What sets STM apart from most other surface analysis techniques, is its ability to investigate the topographical as well as the electronic properties (*e.g.* local density of states) of flat surfaces with a high spatial resolution. A variety of interesting things can also be done using STM such as imaging, manipulation and investigating dynamics of solid surfaces on the length scale of individual atoms and molecules.

The development of STM has triggered the invention of a whole family of SPM which make use of almost every kind of interaction between a tip and sample of which one can think of. STM can now provide information about nanometer scale properties of matter which is often not accessible by any other experimental technique.

2.2.1.1 Basic principles of STM

The working principle of STM is based on quantum mechanical tunneling. The tip and the conducting sample serve as electrodes across which a voltage difference can be applied. The corresponding voltage is termed as bias voltage. An atomically sharp metal tip, which is mounted on three orthogonal piezoelectric transducers, is brought at a distance less than 1 nm away from a conducting surface under investigation and scanned over it at an applied sample bias. In this way, the quantum mechanical wave functions of the conducting surface and tip exhibit some overlap and an electron can tunnel from an occupied state of one electrode to an unoccupied state another electrode through the vacuum barrier. The tunneling current depends strongly on the amount of overlap of the wave functions of the tip and the surface and thereby on the gap between tip and surface. For low sample bias voltage range, the tunneling current, I , can be described using the Wentzel-Kramer-Brillouin approximation:

$$I \propto \frac{eV}{z} e^{-2kz} \quad (2.1)$$

where V is the applied voltage, z is the separation between the tip apex and the substrate, and k is the decay constant.¹¹¹ Usually, a sample bias voltage (~ 1 V) is applied across the tip and the sample giving rise to a tunneling current (~ 1 nA) which decrease exponentially

with the increase of the barrier width (the separation between the tip apex and the surface). An schematic of an STM is depicted in Figure 2.7.

Figure 2.7 A schematic of an STM with an atomically sharp tip placed at a distance about 1 nm away from a conductive surface. The sample bias applied through the tip and the surface gives rise to a tunneling current. The movement of the tip is recorded as a function of the lateral position on the surface which gives rise to a contrast in the z-scan image.

There are two modes of operations for STM: the constant current mode (see Figure 2.8a) and the constant height mode (see Figure 2.8b). In the constant current mode, the distance between the apex of the tip and the surface of the sample is kept constant with the feedback loop on (for keeping the tunneling current constant) while the tip is scanning across the surface. Since the tunneling current varies exponentially with the gap between the tip apex and the surface, this mode of operation keeps the gap width essentially constant. The vertical displacement of the tip as a function of the lateral position at the surface is recorded and this distance variation is converted to a value of the z coordinate in the image. Alternatively, in the constant height mode, the tip can be scanned across the surface at nearly constant height and constant voltage while the current is recorded. This current is then converted to a z -coordinate of the image. Considering the potential risk to crash the STM tip, the constant height mode is not as popular as the constant current mode for imaging non-atomically flat surfaces.

When the sample is negatively biased, the image z -coordinate is related to the filled electron density of states. In this case, the electrons tunnel from the sample to the tip. For positive sample bias, the electrons will tunnel in the opposite direction, *i.e.*, from tip to sample. Meanwhile, the empty electron density states of the surface imaged are recorded.

Figure 2.8 Schematic representations of the two scanning modes in STM: (a) constant-current topography mode and (b) constant-height topography mode. Reprinted with permission from Ref. 112. Copyright (2010) by the American Physical Society.

2.2.1.2 Scanning tunneling spectroscopy

The STM is a very powerful tool for imaging surfaces at the atomic scale. With a lateral resolution of about 0.1 nm and a z -resolution in the pm range, this instrument is capable of imaging single atoms. Moreover, in addition to topography imaging with high spatial resolution, the STM can also be used for spectroscopic measurements, providing information on chemical, electronic and dynamical properties of a given surface (conductive and semiconductor). This spectroscopic technique is commonly known as scanning tunneling spectroscopy (STS). STS is essential for studying of electronic transport properties of single molecules or molecular assemblies. There are three well-known STS techniques as described briefly below.

Current-voltage (I-V) spectroscopy is a frequently used method to determine the electronic properties of a sample. At a fixed tip-surface distance, the tunneling current is recorded as the sample bias is ramped from a set minimum value to a set maximum value with small increments (as is illustrated in Figure 2.9a). While performing these measurements, the feedback loop is disabled, keeping the distance between the tip and the sample constant. This is done by a sample-and-hold circuit present in the STM electronics. The I-V curve in this case departs from the setpoint, defined by (the pre-adjusted) bias voltage and the setpoint tunneling current. The current values are thereby expressed relative to the setpoint. Most substrates show characteristic I-V curves (example see Figure 2.10). Metals show a linear I-V curve with a finite slope at zero bias (Fermi energy of the surface; semiconductors and molecules display a vanishing differential conductivity (for the definition see below) around zero bias because of the presence of a band gap. The I-V spectroscopy is used to numerically determine the differential conductance ($\frac{dI}{dV}$). The differential conductivity is closely related to the local density of states ($\frac{dI}{dV}/I$, LDOS) of a

sample, which provides information about the electronic and chemical properties of the sample. At negative sample bias, electrons tunnel from the surface to the tip which allows to investigate LDOS of the filled states; while at positive sample bias the electrons tunnel into the sample allowing one to investigate LDOS of the empty states of the surface.

Figure 2.9 Illustration of current-voltage spectroscopy (a, I-V) and current-distance spectroscopy (b, I-Z).

Figure 2.10 I-V curves of the tunnel junctions recorded at 77 K before and after the STM tip has picked up an octanethiol molecule. For both traces we have used a setpoint value of 0.5 nA at 1.5 V. Reproduced with permission from Ref. 113. Copyright (2012) by the IOP Publishing.

Current-distance (I-Z) spectroscopy is used to estimate the barrier height of the junction and the work function of a surface by measuring dependence of the tunneling current on the tip-sample separation Z while the applied tip-sample bias is kept constant.¹¹⁴ Within one I-Z measurement the feedback loop is switched off and the current is recorded while the STM tip is moved toward the sample or away from the sample with respect to initial tip height (as is illustrated in Figure 2.9b). After each I-Z measurement the feedback

loop is switched back on again to allow the tip to move back to its initial position. At this point the junction returns to the original configuration defined by its setpoint value. The barrier height between the tip and the surface is extracted from the exponent of the tunneling current plotted against the tip-surface distance. I-Z traces have been successfully applied to study the transport properties of the molecules. Figure 2.11 shows two different I-Z curves recorded with (solid line) and without (dashed line) molecule as an example for depicting differences with and without an octanethiol molecule trapped within the STM junction.

Figure 2.11 Example of I-Z curves without (dashed line) and with (solid line) an octanethiol molecule attached to the STM tip. Both traces were recorded at 77 K, at a fixed setpoint (tunneling current 0.5 nA and sample bias +1.5 V). Reprinted with permission from Ref. 115. Copyright (2012) by the American Physical Society.

In addition to the topographic image and the aforementioned spectroscopic techniques, chemical analysis of a single molecule on surfaces is possible by the application of inelastic electron tunneling spectroscopy (hereafter referred to as IETS), which has been employed for studying organic molecules buried within a junction, in the STM. STM-IETS was first demonstrated by Stipe, Rezaei and Ho in 1998,^{116, 117} seventeen years after the development of the STM. In this so called STM-IETS arrangement, the metal-oxide-metal tunnel junction is replaced by an STM tunnel junction: a sharp metal tip, a vacuum gap of several angstroms and a surface with the adsorbed molecules. In this case the insulating oxide layer is fulfilled by the vacuum gap between the tip and the adsorbed molecules. As STM is capable of imaging surfaces with atomic resolution, the molecule's bonding environment with respect to an ordered substrate can be determined precisely. Keeping the

tip of a STM at fixed position above the surface and sweeping the bias voltage, one can record a I-V curve. The first derivative (dI/dV) provides information about the LDOS of the substrate, presuming that the tip has a constant density of states. The second derivative (d^2I/dV^2) gives information on vibrations of the adsorbate as in IETS.¹¹⁸ By probing individual molecules, it should be possible to explore how vibrational properties are affected by neighboring co-adsorbed species or surface defects. This technique can probe the bonds of a single molecule and can thereby serve as fingerprint tool for chemical analysis. STM-IETS has been applied to a wide range of systems and has led to a better understanding of the vibrational properties at the single molecular level in the adsorbed state.^{101, 116, 119, 120} In an STM-IETS measurement (see Figure 2.12) for a SAM of decanethiol at 77 K one could distinguish two peaks, that could be assigned to the Au-S or S-C stretch mode and the C-C stretch mode or a CH₂ wag or twist mode, respectively.¹⁰¹

Figure 2.12 Inelastic electron tunneling spectrum of the decanethiol SAM recorded at room temperature. The solid line represents an average over 2400 curves. (Inset) I-V curve recorded simultaneously as the IET spectrum. Adapted with permission from Ref. 101. Copyright (2004) American Chemical Society.

2.2.2 Development of the time resolution of STM

The strength of STM comes from its capability of imaging with high spatial resolution as well as its versatile spectroscopic possibilities. This has led to new insights into, for instance, diffusion, reaction, nucleation and reconstruction phenomenon on surfaces at the

atomic scale which play an essential role in important fields such as catalysis, thin-film growth and sensor technology.^{121, 122} However, the time resolution for the standard STM is limited, usually in the range from seconds to minutes.¹¹² In this case, processes that occur on a much faster timescale will not be accessible. In order to visualize and probe the dynamic phenomena on the surfaces, it is necessary to significantly enhance the temporal resolution. Strategies that have been applied to improve the time resolution of STM will be reviewed in the following section.

2.2.2.1 High speed STM

The first approach we discuss for speed enhancement is recording the STM images at sufficiently high scanning rate. Since about 1990, several research groups start to explore the possibility of using STM to visualize dynamics on surfaces by recording time-lapse series of images for the same area.¹²³⁻¹²⁶ For one-dimensional diffusion processes, a time resolution in the range from 50 to 250 ms has been achieved by repeatedly scanning the same line.¹²⁷⁻¹³⁰ However, this approach cannot be applied to study two-dimensional surface processes with a higher than conventional time resolution. In order to investigate 2D processes, one needs to modify the STM quite substantially. Several research groups have reported that STM images can be recorded sequentially at 1-100 frames per second by optimizing mechanical construction and electronics of conventional STM.¹³¹⁻¹³⁵ In order to obtain the required high mechanical resonance frequency, these high speed STMs which are also referred to as video rate STMs have a rigid and compact design. Additionally, a high band width I-V converter, fast analog-to-digital converters and fast feedback electronic are applied. By utilizing video STM, Besenbacher *et al.*^{134, 136, 137} have unraveled an adsorbate-mediated diffusion mechanism of O vacancies on a rutile surface. These sequential STM images obtained by these authors reveal that diffusion is accomplished by the presence of O molecules on the surface, and that the diffusion pathway is perpendicular to the bridging O rows on the TiO₂(110) surface, as shown in Figure 2.13. To improve the maximum scan speed, Rost *et al.*¹³⁵ implemented a hybrid mode between constant height and constant current modes. A better resolution is achieved at lower scanning speed by using this hybrid mode.

So far, most of video STM investigations have all been performed in clean, well-controlled UHV environments. However, electrochemical STM studies by Magnussen *et al.*¹³⁸⁻¹⁴⁵ have provided new insight into the electrochemical deposition and dissolution of metals, which are important processes in metal corrosion, coating and metal refinement. Magnussen *et al.*¹⁴⁴ presented video STM observations of the dynamic behavior of five-atom-wide, hexagonally ordered strings of Au atoms embedded in the square lattice of the (1×1) domain of Au(100) surface that reveal quasi-collective lateral motion of these strings perpendicular to as well as along the string direction (see Figure 2.14).

Figure 2.13 Ball model of the TiO_2 (110) surface. A bridging O vacancy is marked by a circle. The arrow denotes the observed vacancy diffusion pathway. (A) and (B) are two consecutive images extracted from an STM movie. (C) The corresponding difference image shows that the vacancies jump perpendicular to the Ti/O rows. Reprinted from Ref. 134, Copyright (2005), with permission from Elsevier.

Figure 2.14 Motion of 'hex' strings perpendicular (a) and parallel (b) to the string direction. (a) video-STM sequences, showing a, positional fluctuations of an isolated string (image size = $23 \text{ nm} \times 23 \text{ nm}$, 20 frames per second), video-STM sequence (image size = $18 \text{ nm} \times 29 \text{ nm}$, 15 frames per second). Adopted by permission from Macmillan Publishers Ltd: [Nature Materials] Ref. 144, Copyright (2003).

Although there are high speed STM in several laboratories all over the world, there are not yet convenient and simple techniques available to substantially improve the time-resolution of the conventional STM.

2.2.2.2 Atom-tracking STM

The limited time resolution of STM is due to the fact that the cutoff frequency of the feedback loop is usually of the order of a few kHz, implying that dynamic processes that occur on a time scale of a millisecond or less are averaged out in the scanning process. In the mid-1990s, a novel technique, which is called atom-tracking STM, with an improved time resolution was introduced by Swartzentruber.¹²⁶ In the atom tracking mode, the STM tip is maintained at a preselected atom or vacancy by applying a two-dimensional feedback. In order to acquire the lateral feedback, a circular motion is imposed on the STM tip (see Figure 2.15a). Generally, this circular motion is a few angstroms in radius with a frequency higher than the cutoff frequency of the z -feedback electronics. A lock-in amplifier is usually employed to measure the derivative of the tunneling current with respect to the lateral coordinates x and y (see Figure 2.15b). These derivatives are translated into independent x and y integrating feedback circuits that maintain a position of zero local slope. In this way, the lateral feedback forces the STM tip to continuously climb uphill, following the local surface gradient and remaining on the top of the atom. By a simple inversion of the phases of the x - and y -feedback circuits, the atom tracker can be forced to run downhill in order to maintain a position on a vacancy.

In the atom-tracking mode, the STM spends all of its time measuring the kinetics of the selected atom, molecule or vacancy rather than acquiring a two-dimensional image of its neighborhood. The data collection thus shrinks from a two-dimensional matrix to a continuous single point, *i.e.*, zero-dimensional, data set. By utilizing the atom tracking mode, the capability of the STM to monitor individual dynamic events has been improved by about 3 orders of magnitude (in regards of time resolution) as compared to conventional STM imaging techniques. In addition, the measurement of every diffusion event eliminates the need to assume random walk statistics as is the case in mean square displacement measurements.

After its advent, atom tracking has been successfully applied to study the dynamics of atoms and molecules.^{125, 126, 146-149} Using atom tracking, Borovsky *et al.* determine the corresponding energy barrier by measuring the average hopping rate as a function of temperature over the range 360–460 K.¹⁵⁰ In another experiment, the rotation of a Si ad-dimer on a Si(001) substrate dimer row was tracked.¹²⁵ Si ad-dimers can have their dimer bond aligned either parallel (A) or perpendicular (B) to the dimer bonds of the underlying substrate dimer row. The presence of these two stable configurations is found in conventional STM images (see Figure 2.16a and Figure 2.16b).

Figure 2.15 Schematic representation of the atom tracking mode. The tip is dithered above the adsorbed atom in (a) and the lateral feedback [shown schematically in (b)] responds to the local slope, forcing the tip to climb uphill. For example, when the tip is offset to the left, the slope is positive and the tip is pushed back to the right. Reprinted with permission from Ref. 112. Copyright (2010) by the American Physical Society.

Figure 2.16 STM images (image size = 4 nm × 3 nm) of a Si (001) surface with a Si ad-dimer with its dimer bond aligned (a) parallel or (b) perpendicular to the dimer bonds of the underlying substrate dimer row. In (c) the measured z signal is shown as a function of time. The transitions between A and B positions show up as sharp changes in the measured z signal. Reprinted with permission from Ref. 125. Copyright (1996) by the American Physical Society.

Apart from its strengths, there are also several drawbacks of the atom tracking mode. First, owing to the low speed of STM movement and the necessary feedback time, tracking must be performed under low diffusion speed conditions. Second, the STM spends all of its time in the proximity of the particle under study during the atom tracking mode. As the electric fields and the current densities can be large, there is a possibility that the tunnel process itself can affect the experiment. The effect of the tunneling conditions on tracking mode imaging was studied by Carpinelli *et al.*¹²⁴ They found that the electric field barely has influence on the diffusion kinetics, affecting the diffusion activation barrier by less than a few percent. A third drawback is that, when an object diffuses to a neighboring site on the surface, the tracking tip is supposed to quickly relocate to the atom's new position. However, it can happen that the tip will be locked onto to another object that travels nearby. Fortunately, this could be excluded by applying a simple approach, which is to frequently record normal STM images of the region in the proximity of the object under study.

2.2.2.3 Close feedback loop STM

To improve the time resolution further, the challenge is to image processes on surfaces in real time, *i.e.* to depict the surface at rates so high that no diffusion jumps, reaction events, etc. are missed. However, on the time scale of imaging, each object under study is imaged for only in the range of μs per image, depending on the image resolution. To broaden the dynamic range for measuring the object, in real-time, fixed lateral position topographic measurements have been performed.⁸ As the feedback loop is active, thereby drift is minimized while recording the topographic height of the object in question (z vs. t). In 1996, Swartzentruber *et al.* monitored the rotation of an adsorbed silicon dimer on a dimerized Si(001) surface by recording z -piezo-voltage traces as a function of time (z - t , see Figure 2.16c).¹²⁵ The ratio of the averaged residence times tells the energy difference between both states (preciously mentioned A and B configurations for Si ad-dimer on a Si(001) substrate dimer row) if the attempt frequencies of both states are same. If the latter condition is not satisfied, one should measure the temperature dependence of the residence times. The attempt frequency and the activation barrier can be determined by plotting the logarithm of the averaged residence time versus the reciprocal temperature. Sato *et al.* demonstrated that the tunneling current recorded above one of the atoms of a dimer of the Ge(001) surface exhibited telegraph like noise.¹⁵¹ Moore *et al.* measured the real-time conductance switching and place-exchange for 4-(2-nitro-4-phenylethynyl phenylethynyl)benzenethiol molecules and the host SAM by recording height versus time with the feedback loop active.⁸ Their temporal resolution is limited by the bandwidth of the feedback loop; thereby, some conductance switching and motion events may not be observed.

2.2.2.4 Open feedback loop STM

There is another alternative approach to substantially improve the time resolution of a standard STM. In this alternative route, the tunneling current is measured as a function of time with the feedback loop disabled (I vs. t , see Figure 2.17).^{112, 118, 152} Therefore the separation distance between the tip and the surface of the sample was kept constant during these experiments. So if there is a change in the geometry of the adsorbed object, which is in between the tip and the surface of the sample, this should be immediately visible in the measured current. In this way, the time resolution is determined by the bandwidth of the I - V converter instead of the cut-off frequency of the feedback electronics.^{42, 112, 153-156} Typically, the bandwidth ranges from 50-600 KHz, which enables the time resolution of the time resolved STM goes up to 2 to 20 μ s. One should realize that such an approach requires a stable microscope and goes at the expense of spatial resolution.

Figure 2.17 Illustration of current-time spectroscopy (I - t).

There are several quantities that can be studied by the I - t traces, for instance the distribution of residence times and the rates. The energy difference between different molecular configurations studied can be acquired from the I - t traces. By positioning the tip of an STM over single flip-flopping dimers and measuring the tunneling current as a function of time, van Houselt *et al.* studied the dynamic behavior of surface dimers on Ge(001).¹⁵⁷ They monitored the flip-flop motion of dimers ordered in (2×1) and $c(4 \times 2)$ domains, as shown in Figure 2.18b. In the middle of the image (Figure 2.18b) two missing dimer defects are visible (indicated by white circles). The missing dimer defect on the left induces buckling of the nearby dimers, while the right one leads to dimers with a symmetric appearance. The dimer rows that contain the missing dimer defects have a noisy appearance, corresponding to a rapid flip-flopping motion of the dimers. Figure 2.19a shows typical current traces measured above various dimers. The distribution of the residence times of the dimers in each of the two buckled states was measured and is shown

in a histogram $H(t)$ for the flickering symmetric dimers in Figure 2.19b. State (1) here means a dimer is buckled such that the ‘left’ atom is higher; state (2) means the ‘right’ atom is higher. The ratio of these average residence times of the dimers with asymmetric look immediately gives the energy difference between two buckled configurations, by using the following equation,

$$\frac{\tau(1)}{\tau(2)} = \exp(\Delta E/k_B T) \quad (2.2)$$

where k_B is Boltzmann’s constant and T is the temperature provided that the attempt frequencies for both states are the same. The energy difference between the buckled dimers labeled A–F is 22 ± 2 meV.¹⁵⁷ The averaged residence times of both states give the kinetic barriers that separate both states, *i.e.*,

$$\langle \tau_{1,2} \rangle = \tau_0 \exp(-\Delta E/k_B T) \quad (2.3)$$

where $\tau_0 = 1/\nu_0$ and where ν_0 is the attempt frequency. (ν_0 is typically on the order of 10^{12} – 10^{13} Hz.) As speculated from Equation 2.3, the open loop I-t measurements open up the possibilities to monitor dynamic events with high frequencies.

Figure 2.18 (a) Filled-state room temperature STM image of Ge(001). The sample bias is -1.5 V, and the tunneling current is 0.4 nA. The local $c(4 \times 2)$ and (2×1) reconstructions are indicated. At the phase boundaries between the domains, white color straight lines have been added as a guide to the eye. Note that the domain boundary has a finite width that shows up as a reduced buckling amplitude in dimer rows directly adjacent to the domain boundary. Inset: Schematic representation of a buckled dimer. The tilt angle of the dimer is about 10° – 20° . (b) Filled-state STM image of Ge(001) ($V = -1.5$ V, $I = 0.4$ nA). The flickering in some of the substrate dimer rows is due to the flip-flop motion of dimers during imaging. The flickering occurs in rows that contain a missing dimer defect. Note that this flickering occurs both in a symmetric dimer row (right defect) and an asymmetric dimer row (left defect). Labels 1–4 refer to the different types of dimers (a flickering asymmetric dimer 1, a flickering symmetric dimer 2, a nonflickering symmetric dimer 3, and a nonflickering asymmetric dimer 4) over which the tunneling current is measured as a function of time. (c) The dimer positions where the current is measured as a function of time are labeled A–F. Reprinted with permission from Ref. 157. Copyright (2006) by the American Physical Society.

Figure 2.19 (a) Current traces measured on a flickering asymmetric dimer [curve (1)], a flickering symmetric dimer [curve (2)], a nonflickering symmetric dimer [curve (3)], and a nonflickering asymmetric dimer [curve (4)]. The sampling rate is 50 kHz and the total sampling time is 20–80 ms. (b) Histogram of the residence times in the two buckled states of a symmetric appearing dimer. The line is the theoretical fit for a random process (Poisson distribution). $\tau(1)$ and $\tau(2)$ are the counts for the residence times in the two different states, respectively. Reprinted with permission from Ref. 157. Copyright (2006) by the American Physical Society.

Since 2000, numerous studies of atoms, single molecules and molecular assemblies, utilizing time resolved STM, have been reported.^{8, 108, 119, 154, 157–216} One example is the single-molecule rotation in the tetra-tert-butyl zinc phthalocyanine ((*t*-Bu)₄-ZnPc)/Au(111) system. It is pointed out by Gao *et al.* that single (*t*-Bu)₄-ZnPc molecules rotate at 80 K without lateral diffusion.¹⁶⁴ Figure 2.20c shows a typical I-t spectrum corresponding to this research. In this study a tip with a large apex was used. Discrete stepwise tunneling current values were observed with time because of changes in the molecular configurations. Figure 2.20d shows the corresponding frequency-counting analysis, where four different current values could be recognized. Therefore, the variation of tunneling current could be attributed to the contribution from four different molecular configurations (shown in Figure 2.20g). Another example for I-t traces used in molecular dynamics studies encompasses lateral diffusion of FePc (Pc stands for phthalocyanine) on Au(111).¹⁶⁵ Due to its flat structure and low diffusion barrier, FePc molecules begin to diffuse all over the surface and cannot be distinguished in the STM images. As seen from Figure 2.21, I-t traces reveal that there are four typical configurations.¹⁶⁵ In addition, Kockmann *et al.* have successfully applied this technique to study the dynamics of a single octanethiol molecule.¹⁵⁴ As seen from Figure 2.22, open-loop current time traces reveal that the molecule wags its tail and attaches to the STM tip resulting in a dramatic increase of the current.

Figure 2.20 A $(t\text{-Bu})_4\text{-ZnPc}/\text{Au}(111)$ system exhibiting rotational motion. (a) Ball and stick model of the $(t\text{-Bu})_4\text{-ZnPc}$ molecule. (b) Topography STM image of the $(t\text{-Bu})_4\text{-ZnPc}$ molecule on the elbow site of the Au(111) surface obtained with a blunt tip. Experimental parameters: image size = 3 nm $\times$ 3 nm, $V = -1.67$ V, $I = 0.037$ nA. (c) I-t spectrum measured at the site marked with a bright spot in (b). (d) Frequency-counting statistical analysis of the I-t spectrum according to current values in (c). (e) A typical two level I-t spectrum with a sharp tip. (f) Frequency-counting statistical analysis of the I-t spectrum according to current values in (e). (g) Adsorption configurations provided by DFT calculations. Reprinted with permission from Ref. 165. Copyright (2010) by the American Physical Society.

Figure 2.21 An FePc/Au(111) system exhibiting both rotational and diffusional motions. (a) Ball and stick model of a FePc molecule. (b) Topography STM image of FePc/Au(111). Experimental parameters: image size = 14 nm $\times$ 14 nm, $V = 0.1$ V, $I = 0.1$ nA. (c) I-t spectrum measured at the site marked with a blue spot in (b). (d) Frequency-counting statistical analysis of the I-t spectrum according to current values in (c). (e) Adsorption configurations provided by DFT calculations. Reprinted with permission from Ref. 165. Copyright (2010) by the American Physical Society.

Figure 2.22 (A) I-t spectrum recorded on an octanethiolate molecule that is adsorbed on a Pt chain. The trace is recorded at constant height. The sample bias is 1.5 V. The current jumps back and forth between the current setpoint (1 nA) and a much higher current of 11 nA. We have measured residence times up to 40 s and currents in the range of 10-15 nA. The current jumps are caused by the octanethiolate molecule that wags its tail and subsequently contacts the STM tip, as indicated by the schematic drawings in panels B and C. Reprinted with permission from Ref. 154. Copyright (2009) American Chemical Society.

2.3 Dynamics of organic layers studied by STM

Dynamic processes play an essential role in important fields like catalysis, thin-film growth and sensor technology.^{122, 134} The structural and dynamical properties of organic thin layers are of interest from both the fundamental and the application points of view. The ultimate utility of SAMs for the fore-mentioned applications will be critically dependent on their structural and dynamical properties, for instance, conformational changes upon stimuli (light, pH, temperature and electrochemical potential etc.) and phase transition.

2.3.1 RS-Au adatom-SR complex

Given the ubiquitous applications of the gold-sulfur interface, it is surprising that, until recently, detailed information on the atomic structure and dynamics of this interface has largely been missing. In spite of the apparent simplicity of the alkanethiols, there has been some debate on how the alkanethiolates are actually bound to the Au(111) substrate.^{217, 218} Previously, one assumed that the sulfur atom of the alkanethiol formed a covalent bond with one of the Au atoms of the Au(111) substrate.⁶⁶ However, the fact that alkanethiolates are surprisingly dynamic at room temperature is difficult to be understood in the presence of strong Au-S covalent bonds.^{93, 94, 219} The thiolate-gold (RS-Au) bond has a strength close to that of the gold-gold bond, so it can significantly modify the gold-gold bonding at the gold-sulfur interface.²¹⁷ A scenario where the sulfur atom forms a covalent bond with a Au adatom instead of a Au atom of the Au(111) substrate, seems therefore much more likely.²²⁰⁻²²² As the diffusion barrier of a Au adatom on Au(111) is rather low, it is very probable that the observed dynamics of the SAM is caused by diffusion of the Au adatom-alkanethiolate complex. The Au adatoms are supplied by lifting the herringbone reconstruction of the Au(111) surface²²⁰ and etching of the monatomic steps and terraces²²³ upon thiolate self-assembly.

In a low-temperature STM study, Maksymovych *et al.*²²⁰ showed that methylthiolates are even dynamic at cryogenic temperatures. Since the covalent Au-S bond cannot be broken at these temperatures, these authors put forward the elegant idea that Au adatoms are involved in the formation of methylthiolate complexes. Based on density functional theory calculations and STM observations they provided compelling evidence that the methylthiols form Au adatom-methyldithiolate complexes (RS-Au-SR). Figure 2.23 and 2.24 show the adatom-bonded structural model of the stripe phase unit. Alkanethiol SAMs with longer carbon chains, for instance decanethiol SAMs, on Au(111) are very dynamic as well and thereby it is very likely that also here Au-adatom-decanethiolate complexes are formed.²²⁴⁻²²⁹

Figure 2.23 STM images of some selected, adsorbed molecular species. (a) A single $\text{CH}_3\text{S-SCH}_3$ molecule. (b) Two CH_3S fragments formed by pulsing $\text{CH}_3\text{S-SCH}_3$ with a 1.0 V pulse at 5 K. (c) Close-up of a single stripe-phase unit. The asymmetrical boundaries of the CH_3S species are marked by dashed white lines. (d) Chains of the CH_3S stripe phase after heating $\text{CH}_3\text{S-SCH}_3$ on $\text{Au}(111)$ to 300 K. (e) Top-view model of the CH_3S stripe phase (Au_a is the adatom). Reprinted with permission from Ref. 220. Copyright (2006) by the American Physical Society.

Figure 2.24 Structure of $\text{CH}_3\text{S-Au-SCH}_3$ on $\text{Au}(111)$. (a) and (b) are calculated structures of the adatom-bonded pair unit, which forms the CH_3S stripe phase. Reprinted with permission from Ref. 220. Copyright (2006) by the American Physical Society.

Due to the presence of the long alkyl chains, the Au atom bound to the S atom in alkanethiol becomes invisible for STM. Thus, there is not so much direct experimental evidence for the existence of RS-Au-SR on Au(111) for alkanethiols with long carbon chains. By exposing octanethiolate SAMs to gas-phase hydrogen atoms to remove the monolayer, Kautz *et al.* detected the amount of gold atoms released from the SAM on Au(111) utilizing STM.^{230, 231} Figure 2.25 shows the observation of gold atoms that remain behind after an octanethiol SAM on Au(111) is removed from the surface. It is confirmed that gold atoms were incorporated into octanethiol monolayers at a 1:2 gold adatom/octanethiol ratio.²³¹ However, diffusion of the gold occurred upon the SAM removal, implying that Kautz *et al.* did not observe the exact structure of the adatom layer. Indirect evidence of the dynamics of the decanethiolate molecules can be provided by time-resolved STM, *i.e.* I-t spectroscopy.^{94, 112}

Figure 2.25 A octanethiol monolayer (a) before exposure to hydrogen atoms and (b) after 576 s hydrogen atom exposure, image size = 129 nm $\times$ 132 nm. (c-e) A central area at higher resolution, image size = 32 nm $\times$ 31 nm. As the monolayer is removed, bright island features appear and increase in size while the gold vacancy islands shrink (c-e). Reprinted with permission from Ref. 231. Copyright (2008) American Chemical Society.

2.3.2 Phase transitions of thiolate SAMs

Discussions of dynamics in SAMs obviously involve discussions of their phases. Dynamics reflects the stability of the molecular structure for a certain phase. Understanding the molecular structure of SAMs is one of the most important issues for practical applications,

as the structure is of paramount importance for their properties, such as optical and electronic properties,²³² molecular interactions, and surface reactions.⁵¹

Using STM, phase transitions and corresponding structures of annealed decanethiol monolayers on Au(111) surfaces were systematically investigated under ambient,²³³ UHV^{71, 100, 234, 235} and in solution^{223, 236-240} conditions. During self-assembling, the decanethiol monolayer, which is a model system for alkanethiol, sequentially displays six different structural phases: α , β , χ , δ , ε , and ϕ .^{63, 71} The question of great importance from a fundamental as well as a technological point of view is whether these phases are stable or metastable under a set of given conditions. Poirier *et al.* proposed a two-dimensional phase diagram using variable temperature UHV STM.⁷¹ They determined the phase stability of the decanethiol monolayer as a function of temperature and they showed that at the temperature of 300 K three phases, δ , ε and ϕ , coexist. At a still higher temperature (306 K), χ , δ and ε phases are present. These phases change to χ , ε and β at 308 K and finally, at 328 K, ε , β and α phases are present.⁷¹ The sketches of these phases are shown and explained in Figure 2.5. By annealing of densely packed films, Toerker *et al.* report the formation of the mesh like δ phase (see Figure 2.26) and $(15/23 \times \sqrt{3})$ stripe phase, which they termed as the δ^* phase.¹⁰² Frizzy appearance is visible in Figure 2.26, indicating the presence of dynamics; which was not discussed in their original paper. Yamada *et al.* have shown that the temperature of the solution affects the size of the domains and of the vacancy islands.^{90, 105, 241} As reported by Seo *et al.*, the octanethiol SAMs formed at 343 K in an octanethiol solution resulted in the formation of molecular rows at ordered domain boundaries.²³⁷ This is explained to be the result of excess molecules inserting into the monolayer in solution, which also results from the increased lateral movement, for example diffusion of thiol-gold complexes, of alkanethiol molecules due to the Oswald ripening process in a high temperature solution. By performing thermal annealing in a high temperature solution, the formation of only one lattice structure as $c(4\sqrt{3} \times 2\sqrt{3})$ on Au(111) occurred. As a control experiment, Seo *et al.* investigated the thermal annealing process as well.²³⁷ The thermodynamically driven Ostwald ripening process of the SAMs resulted in long-range ordered domains with few pits or defects (Figure 2.27).²³⁷ Moreover, with the increase of the annealing temperature and time, the number of pits decreased drastically. Therefore, elevated temperature and longer time could be utilized to fabricate molecular devices with less defects. Terán Arce *et al.* demonstrated the transitions $p(6 \times 1) \rightarrow \sqrt{3} \times \sqrt{3}$ $R30^\circ \leftrightarrow c(4 \times 2)$ in the butanethiol and dodecanethiol SAMs on Au(111).²⁴⁰ They attributed this reversible transition to the alternating displacements of thiol molecules from hollow to bridge and *vice versa*. The annealing method is often applied in the spirit of an accelerated time test. However, there is a potential risk of converting the system to another phase that is only stable near the annealing temperature.

Figure 2.26 STM current image ($V = 1.9$ V; $I = 0.21$ nA), image size = 100 nm $\times$ 100 nm, showing the mesh like phase in coexistence with the $c(2\sqrt{3}\times 4\sqrt{3})R30^\circ$ structure. (c) Proposed real space model of the arrangement of the sulfur groups (dark grey spheres) on Au(111) (light grey spheres) in the mesh like phase. The mesh dimensions of $a = 2.18$ nm, $b = 3.75$ nm, $\alpha = 8.12$ nm, $\gamma = 6.6^\circ$ and $\omega = 2.2^\circ$ are in good agreement with the observed values. Reprinted from Ref. 102, Copyright (2000), with permission from Elsevier.

Figure 2.27 STM images of an octanethiol SAM on Au(111) thermally annealed in vacuum. (a) The SAMs were imaged after increasing the temperature from room temperature to 343 K, followed by annealing for 2 h in vacuum. (b) The SAM was imaged after elevating the temperature from room temperature to 353 K, followed by annealing at 353 K for 5 h in vacuum. Initially, the SAM was prepared in a 1.0 mM ethanol solution for 2 h at room temperature. Images were obtained with a set-point of 20 pA and a sample bias of 1.0 V. Adapted with permission from Ref. 237. Copyright (2011) American Chemical Society.

Actually, this phase transition was even observed without thermal annealing. As shown in Figure 2.28, Noh *et al.* found that the structural transitions of octanethiol SAMs from the $c(4\times 2)$ superlattice to the $(6\times \sqrt{3})$ superlattice takes place after long-term storage.^{233, 242} It is proposed that this is induced by both the dynamic movement of the adsorbed sulfur atoms on several adsorption sites of the Au(111) surface and the change of molecular

orientation. It would be of great interest to apply time resolved STM to study the dynamical behavior at the domain boundary between $c(4\times 2)$ superlattice and the $(6\times \sqrt{3})$ superlattice. In this way, the energy barrier one needs to overcome for the transition to take place could be determined in a very precise way.

Figure 2.28 STM images showing the phase transitions of octanethiol SAMs on Au(111) from the $c(4\times 2)$ phase to the new $(6\times \sqrt{3})$ phase depending on the storage duration at room temperature: (a) the $c(4\times 2)$ superlattice obtained from a SAM sample after 1 day of deposition (image size = $50\text{ nm} \times 50\text{ nm}$, $V = 0.43\text{ V}$, and $I = 0.25\text{ nA}$); (b) two mixed phases consisting of the $c(4\times 2)$ superlattice and of the $(6\times \sqrt{3})$ phase that appeared after 3 months (image size = $60\text{ nm} \times 60\text{ nm}$, $V = 0.83\text{ V}$, and $I = 0.12\text{ nA}$); (c) the $(6\times \sqrt{3})$ phase appeared after 6 months (image size = $60\text{ nm} \times 60\text{ nm}$, $V = 0.60\text{ V}$, and $I = 0.19\text{ nA}$); (d) high-resolution STM image of the $(6\times \sqrt{3})$ phase (image size = $8\text{ nm} \times 8\text{ nm}$, $V = 0.60\text{ V}$, and $I = 0.19\text{ nA}$). The lattice constants of the rectangular unit cell are $a = \sqrt{3}a_h = 0.49\text{ nm}$ and $b = 6a_h = 1.75\text{ nm}$, where $a_h = 0.289\text{ nm}$ denotes the interatomic spacing of the Au(111) lattice. Reprinted with permission from Ref. 233. Copyright (2006) American Chemical Society.

The migration of a single molecule or a collection of molecules plays an important role in the phase transitions. To understand these dynamical processes, a high time resolution is thus required. Typical time resolution in these studies ranges from seconds to minutes. However, as is pointed out in our group's recent work, the dynamical events of the molecules in the SAM could happen in a very narrow interval, *e.g.* within μs .^{42, 153, 155}

Therefore, the dynamical events that are occurring in a short time interval will be averaged out when SAMs are observed by conventional STM. Meanwhile, relatively few molecular level dynamical studies for SAMs with high time resolution have been performed. To understand the dynamics of the SAMs at the molecular level, current-time STM would be the ideal candidate.

2.3.3 Molecular conformational changes in thiolate SAMs

The structure and conformation of a molecule dictate its chemical and physical properties. The conformation of molecules adsorbed on surfaces is determined by the subtle balance between the molecule-substrate interplay and the internal mechanics of the molecules.²⁴³ Understanding this complex interaction and controlling the conformational structures of adsorbed molecules would offer a deeper understanding of chemical dynamics on surfaces and enable the construction of molecule-based devices. It is also of fundamental scientific interest to examine the conformational dynamics for molecules.

The formation of SAMs is a promising route towards functional surface nano-architectures. Our understanding of associated dynamical processes occurring on the SAM surface has been significantly advanced by STM investigations.^{112, 116, 235, 244, 245} Intramolecular interactions are widely accepted to influence ordering of complex adsorbates. Albeit molecular conformation has been identified²⁴⁶ and even manipulated²⁴⁷⁻²⁴⁹ by STM, the detailed dynamics of spontaneous conformational changes for adsorbed molecules in SAMs has not been addressed to date.

High speed imaging has put the dynamic monitoring capability of STM forward. One example is stereochemical effects for so-called prochiral molecules which adsorbed on the substrate physically.^{244, 250} In addition to truly chiral molecules, molecular surface structures often show important stereochemical effects as a large class of so-called prochiral molecules become chiral, *i.e.* losing their symmetry, once confined on a surface. Weigelt *et al.* investigated a model system in which adsorbed molecules switched between enantiomeric forms as they underwent thermally induced conformational changes.²⁴⁴ Busse *et al.* investigated chiral ordering and conformational dynamics for a class of three conjugated organic molecules (oligo-phenylene-ethynylenes) using high speed STM imaging under UHV conditions.²⁵⁰ The molecules are found to be able to undergo a thermally induced conformational change which displays as rotation of a bulky molecular end group. Accordingly, the molecules switch between chiral and non-chiral forms. Kinetic studies performed in the temperature ranging from 150 K to 220 K show that the conformational switching arises with an activation energy of approximately 0.3 eV.²⁵⁰ The associated kinetic parameters are quantified from high speed STM images and relevant insights can be subsequently obtained from modelling. However, STM image acquisition is limited by the rate at which dynamic events can be resolved.

Figure 2.29 STM images of self-assembled structures of methylthiolate at low coverage on Au(111), produced by heating the gold crystal predosed with CH_3SSCH_3 above 200 K for ~ 10 min (a). Triangulation of two *trans*-(CH_3S) $_2\text{Au}$ complexes (b) and adjacent *cis*- and *trans*-adatom complexes (c), and their schematic models (d) and (e), respectively. Reprinted with permission from Ref. 251. Copyright (2009) American Chemical Society.

Figure 2.30 STM images of (a) intermediate coverage of methylthiolate on Au(111) revealing the coexistence of stripes, single *cis*- and *trans*-complexes, and tetramers ($T > 200$ K during self-assembly). (b) Striped phase of propylthiolate with $(11 \times \sqrt{3})$ unit cell, formed by thermal dissociation and subsequent self-assembly of $\text{C}_3\text{H}_7\text{SH}$ molecules at $T > 250$ K. The tetramer unit (c) and its schematic structure (d) correspond to the outlined area in panel (a). Reprinted with permission from Ref. 251. Copyright (2009) American Chemical Society.

DFT computations yield *cis*- and *trans*-complexes (RS-Au-SR complexes for methylthiolate (CH_3S) SAM on the Au(111) surface) as thermodynamically almost equivalent. Thus the difference in the adsorption energy is only of the order of 0.1 eV. The calculated activation barrier for *cis*–*trans* switching is around 0.5 eV for low-coverage conditions in which the switching is not affected by interactions from close by RS-Au-SR complexes.²⁵¹ The low activation barrier indicates facile *cis*–*trans* isomerization at typical temperatures (200–300 K) at which thiolate-SAMs are formed. As depicted in Figure 2.29 and Figure 2.30, both isomers are also typically found in the STM images of low- or intermediate-coverage phases. Accurate energy barriers can be potentially determined by analyzing time bin counting in the I-t spectra. Nearly all time resolved STM studies, where

current-time spectroscopy was applied, related to dynamical and conformational events for organic molecules, focus on the a single molecule on a surface.^{112, 118} However, there have been only a few investigations applying time-resolved STM on molecular self-assemblies.

2.4 Conclusions

In this Chapter, fundamentals of SAMs and their dynamics as studied by STM were discussed. Firstly, fundamental aspects, potential applications, growth mechanisms and structure of SAMs, especially SAMs on Au(111), which are extensively studied owing to their applications in various fields, were introduced. Secondly, the basics and spectroscopic methods of STM, which are powerful tools for probing surfaces at the atomic scale, were introduced. Despite the attractive high spatial resolution, STM also suffers from a number of disadvantages, one of which is the rather poor temporal resolution. Strategies that have been applied to enhance the temporal resolution of STM are also reviewed. These strategies include high speed STM, atom-tracking STM, close feedback loop Z-t and open feedback loop STM I-t. Finally, dynamics on SAM surfaces investigated with STM are surveyed. The structural and dynamical properties of the SAMs are of interest from both fundamental and application points of view. However, there have been only few investigations applying time-resolved STM on molecular self-assemblies. The work in this thesis provides novel insights into dynamical processes occurring in SAMs on the Au(111) surface.

2.5 References

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Appendix of Chapter 2

A superstructure is a periodic structure of, for instance, an adlayer on a substrate.¹ An adsorbed adlayer often exhibits a periodicity and symmetry that deviates from the underlying substrate. Usually, the new periodicity is ‘commensurate’ with the (1×1) substrate lattice, *i.e.* there is a relationship between these two lattices. Such a ‘superlattice’ can be described by the notation,²

$$c(v \times w)R\alpha \text{ or } p(v \times w)R\alpha \quad (2A.1)$$

Here, v and w are elongation factors of the (1×1) lattice vectors of the substrate, *i.e.* the unit vectors of the non-centered $(v \times w)$ lattice are v and w times the lengths of the (1×1) substrate unit cell edges, respectively. α is an angle by which the (1×1) lattice must be rotated ($R\alpha$ is omitted when $\alpha=0$). The prefix p denotes a primitive lattice (it is often omitted), while c represents a centered lattice.

Figure 2A.1 Structural model depicting the arrangement the commensurate decanethiolates adlayer on the Au(111) lattice when saturation coverage of the thiolates is accomplished. The arrangement shown is a $(\sqrt{3} \times \sqrt{3})R30^\circ$ structure where the sulfur atoms (dark gray circles) are positioned in the 3-fold hollows of the Au(111) lattice (white circles, $a = 0.288$ nm). The light gray circles with the dashed lines indicate the approximate projected surface area occupied by each alkane chain; the dark wedges indicate the projection of the CCC (C stand for carbon atom) plane of the alkane chain onto the surface. Note the alternating orientation of the alkane chains defines a $c(4 \times 2)$ superlattice. The formal $c(4 \times 2)$ unit cell is marked (long dashes); an equivalent $(2\sqrt{3} \times 3)$ unit cell is marked by lines with short dashes. The alkane chains tilt in the direction of their next-nearest neighbors. Adapted with permission from (Ref. 3). Copyright (2005) American Chemical Society.

In the case of SAMs of alkanethiolates on Au(111), the thiolate adlayer and the Au(111) often have a different periodicity. As depicted in Figure 2A.1, Au(111) surface has a *fcc* (face-centered-cubic) lattice, termed as (1×1) with a lattice constant of $a = 0.288$ nm. For simplicity, the sulfur atoms of the molecules self-assembled on Au(111) are placed in the threefold hollow sites of the Au(111) lattice (see Figure 2A.1). At the saturation coverage of the alkanethiol SAMs, the sulfur heads of the chains in the alkanethiols are pinned to a hexagonal overlayer, leading to a $(\sqrt{3}\times\sqrt{3})R30^\circ$ lattice. As seen from Figure 2A.1, the alkane chains tilt in the direction towards their next-nearest neighbors. The alternating orientation of the alkane chains results in a $c(4\times 2)$ superlattice structure.^{4, 5} As is illustrated in Figure 2A.1, this unit cell contains four thiol molecules. The formal $c(4\times 2)$ unit cell is marked (long dashes); an equivalent $(2\sqrt{3}\times 3)$ unit cell is marked by lines with short dashes.

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Dynamics and energy landscape of decanethiol self-assembled monolayers on Au(111) studied by time-resolved scanning tunneling microscopy

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In this Chapter, the energetics and the dynamics of decanethiol self-assembled monolayers (SAMs) on Au(111) surfaces are studied and discussed using time-resolved scanning tunneling microscopy at room temperature. Four ordered (β , δ , χ^* and ϕ) phases and a disordered phase (ε) are observed. Current-time (I-t) traces recorded on the sulfur end group of the decanethiolate molecule exhibited a stochastic two-level switching process when the molecule was adsorbed in a (local) β phase registry. This two-level process is attributed to the diffusion of the Au-thiolate complex between two adjacent adsorption sites. The irregular current jumps in the current-time traces recorded on the tails of decanethiolate molecules in the ordered β , δ and χ^* phases are ascribed to wagging of the alkyl tails. The disordered phase is characterized by even larger current jumps, which indicates that the tail of the decanethiolate flips up occasionally and makes contact to the tip. It is revealed in this Chapter that the massive dynamics of the SAM is due to diffusion of decanethiol-Au complexes, rather than the diffusion of decanethiolate molecules. In addition, the boundary free energies between the disordered and order phases are discussed by analyzing the thermally induced meandering of the domain boundaries. On the basis of these results, it is possible to accurately predict the two-dimensional phase diagram of the decanethiolate/Au(111) system. These work improved our understanding of the energetics and dynamics of decanethiol SAMs, demonstrating that I-t spectroscopy is a powerful tool.

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3.1 Introduction

Molecular self-assembly is omnipresent in nature: it is of key importance for many biological processes.¹⁻⁴ Self-assembly of organic molecules into highly ordered monolayer films on metal or semiconductor surfaces has potential applications in molecular electronics, biosensing, corrosion inhibition and biomimetics.⁵⁻⁸ It is well-known that alkanethiols form well-ordered self-assembled monolayers (SAMs) on Au(111) surfaces.⁹⁻¹³ SAMs of alkanethiols on metals have been extensively studied because of their potential applications, such as corrosion inhibition, surface patterning, wetting inhibition, molecular device fabrication and in the field of biosensing.¹⁴⁻¹⁷ In order to understand the formation of these well-ordered SAMs, it is essential to comprehend the interaction of the molecules with the substrate as well as the interaction between the molecules. Many techniques, including scanning probe microscopy, second harmonic generation, near-edge X-ray absorption fine-structure, quartz crystal microbalance and several diffraction techniques have been applied to investigate the formation process of the SAMs of alkanethiolates.^{1, 4, 14-30} Of all the techniques employed, scanning tunneling microscopy (STM) is the only technique that provides a real space image at the molecular level.^{1, 16-19}

In particular, decanethiol SAMs have been extensively studied as a model system of alkanethiols.^{1, 11, 13, 18, 20-24, 31-42} Upon adsorption the decanethiol molecule is deprotonated and thus becomes a decanethiolate. Alkanethiols have been adsorbed on Au(111) both via vacuum deposition and via an organic solution.^{1, 11, 21-25, 36} The formation of SAMs is governed by a number of parameters, such as temperature, substrate morphology, coverage and crystal structure.^{4, 42} The formation of decanethiolate SAMs on Au(111) was extensively studied by Poirier *et al.*^{1, 20, 22, 24} It was found by Poirier²² that the ordering of decanethiol SAMs depends strongly on the coverage. In his investigations, Poirier reported the formation of six different phases of decanethiol on Au(111): four ordered (β , δ , ϕ and χ) and two disordered (α and ε) phases.²² Schematic diagrams of these phases are depicted in Figure 3.1. The various phases that were shown later in this Chapter were extensively discussed by Poirier *et al.*²² Four of these six phases are stable: the two-dimensional gas phase (α phase), the $(23 \times \sqrt{3})$ striped phase (β phase), the $(15 \times \sqrt{3})$ striped phase (δ phase) and the densely packed $(4\sqrt{3} \times 2\sqrt{3})R30^\circ$ upright phase (ϕ phase). The two metastable phases are the striped χ phase, which consists of alternating $(15 \times \sqrt{3})$ and $(23 \times \sqrt{3})$ stripes, and the disordered dynamic two dimensional liquid phase (ε phase). In addition, it was shown that structural changes of the SAMs occur upon annealing.^{13, 33, 43} On the basis of a detailed experimental study, these authors have put forward a two-dimensional phase diagram of the decanethiolate/Au(111) system.¹ They found that decanethiolates can order into different phases: a phase where the tails of the decanethiolate molecules are in an upright position and tilted 30° from the surface normal (ϕ phase), a disordered liquid- or gas-like phase (ε),⁴³ and three phases where the decanethiolate molecules are lying flat-down on the surface (β ,

δ and χ). The most densely packed phase, the ϕ phase, is dominant at high coverage ($>1/3$ monolayer) and exhibits a $c(3\times 2\sqrt{3})R30^\circ$ registry, whereas the flat lying down and disorder phases are found at a coverage lower than $1/3$ monolayer.^{13, 22} The β phase is a striped phase where the decanethiolate molecules lie flat down on the surface in an alternating head-to-head and tail-to-tail registry (see Figure 3.1).³¹ The upper image in Figure 3.1 shows a schematic diagram of the β phase. The cartoons shown in Figure 3.1 are simplified and the Au-S complex is just represented by the light yellow circle while the white circles refer to the regular Au atoms. The white and grey circles refer to hydrogen and carbon atoms, respectively. The head-to-head pairs align themselves in kink-less rows that form the higher part of the striped phase. The tails of the molecules form the lower part, or troughs, of the striped phase. The corrugation measured along the row direction is 0.5 nm, whereas the periodicity perpendicular to the stripes is 3.3 nm, leading to a $c(23\times\sqrt{3})$ periodicity.³¹ Since the length of decanethiol molecules is 1.695 nm,⁴⁴ the alkyl chains exhibit a small inclination of approximately $4\text{--}5^\circ$ with respect to the in plane normal of the sulfur atom chains.³¹ The effective molecular area per decanethiol molecule in the β phase is $82.8 \text{ \AA}^2$.^{22, 31, 33} The δ phase which has an out-of-plane interdigitation configuration is very comparable to the β phase; however, the tails of the molecules are lying partly on-top of each other, leading to a smaller stripe width of only 7.5 times the nearest neighbor Au-Au distance, *i.e.* 2.2 nm, resulting in a $(5\sqrt{3}\times\sqrt{3})R30^\circ$ periodicity (see Figure 3.1).^{22, 43} The χ phase consists of a perfect array of alternating β and δ stripes.²² Toerker *et al.*,³³ and later also Li *et al.*,⁴⁵ found in the low coverage regime a new phase, which was referred as the χ^* phase. The χ^* phase (see Figure 3.1) is also composed of β and δ domains; however, in contrast to the χ phase, the width of the β and δ domains varies from location to location. The χ^* phase shows switching behavior between the stripe segments, suggesting that this phase is highly dynamic at room temperature.³³ The disordered ε phase does not exhibit any structure.

As the vast majority of the alkanethiols SAMs are produced via the liquid deposition method, it is very important to clarify the self-assembly process of the alkanethiols SAMs in solution and compare these results with the ultra-high vacuum (UHV) deposition method. Poirier *et al.*²³ mentioned that striped phases of decanethiol molecules are found when Au(111) substrates were dipped into dilute solutions for a short period. *In-situ* STM observations of the self-assembly process of decanethiol in solution have been carried out by Yamada *et al.*^{11, 36} They found the formation of $(\sqrt{3}\times\sqrt{3})R30^\circ$ ordered domains in the upright configuration.

Although numerous studies have been performed, a full understanding of the decanethiolate/Au(111) system has not been achieved. Meanwhile, the time resolution of Poirier's and other prior studies is limited to time resolution of a standard STM, *i.e.* seconds to minutes. Due to this limitation in temporal resolution, dynamic events are often averaged

Figure 3.1 Schematic representation of the top and side views of the β , γ^* and δ phases. The exact nature of the Au-decanethiol complex is not yet known and for the sake of simplicity represented here by a simple bond between a sulfur atom and a Au atom of the Au(111) substrate.

out. Only a limited number of studies have been performed with a substantially higher time resolution, see for instance Ref. 46 and Ref. 47.^{46, 47} The time resolution of a standard STM instrument can be improved by 6-7 orders of magnitude by recording current-time spectroscopy (I-t traces) at a preselected position with the feedback loop disabled. In this Chapter, the bandwidth of the current-voltage converter (≈ 50 kHz) limits our temporal

resolution to 20 μs .⁴⁷ This is the motivation for the first part of this Chapter, in which the dynamics on various phases were studied by recording the I-t traces with a temporal resolution of 20 μs .

Another issue that is still not completely solved, deals with the thermodynamic stability of different domains.¹⁷ Considering this issue, the goal of the second part of this Chapter is to investigate the occurrence and thermal stability of the different ordered phases of a decanethiolate SAM on Au(111) by analyzing the domain boundaries between the phases. In the second part of this Chapter, particular attention has been paid on the domain boundaries between the low-coverage ordered phases and the disordered phase. By a statistical analysis of the thermally induced domain boundary meandering, the two-dimensional phase diagram for this system was derived. The boundary free energy between the χ^* and ε phases and between the β and ε phases vanishes at 295 K and 325 K, respectively. These transition temperatures are in very good agreement with the two-dimensional phase diagram proposed by Poirier *et al.*¹

3.2 Results and discussion

3.2.1 Dynamics of decanethiol SAMs on Au(111) studied by TR STM

First, the different structures observed will be presented. The various phases of the decanethiol SAM are briefly summarized in the introduction of this Chapter and visually depicted in Figure 3.1. After immersion of Au(111) in a 1 mM solution of decanethiol for 24 hours, various phases were observed at room temperature, as shown in Figure 3.2a-d. In addition to the different phases (β , δ , χ^* and ε), the SAM also exhibits several characteristic defects, such as substrate steps, domain boundaries and vacancy islands.²¹ Figure 3.2g shows a vacancy island⁴⁸ in the decanethiol SAM. The depth of the vacancy island is exactly one atomic Au layer, *i.e.* 2.5 Å, revealing that the vacancy island is actually a vacancy in the Au(111) substrate and is not a defect in the SAM. Additional evidence in favor of this assignment comes from the fact that ordered decanethiol domains were found at the bottom of the vacancy islands, see Figure 3.2c-d.

As shown in Figure 3.2c, there are three striped phases (β , δ and χ^*) and one disordered phase (ε). Occasionally, several small ϕ phase patches were observed (see Figure 3.2h).⁴⁹ According to the cross-sectional profile (Figure 3.2e), the width of the β phase strip is $11.5a$, *i.e.* 3.3 nm (a is the Au(111) lattice constant of 2.884 Å),⁴³ while the width of the δ phase is $7.5a$, *i.e.* 2.2 nm (Figure 3.2f). The bright stripes of the β phase (see Figure 3.2) correspond to double sulfur atom chain, whereas the dim stripes correspond to the well-ordered alkyl tails.²² In Figure 3.3, the ordering of the tails of the decanethiolate molecules in the δ phase is shown. The nearest neighbor distance between the sulfur ends of the decanethiolate molecules in the δ phase is $\sqrt{3}a$, *i.e.* 0.5 nm (see lower image in Figure 3.1).

These findings are in perfect agreement with Poirier's results.^{1, 20, 22-24} As shown in Figure 3.2, a series of consecutive images reveal the dynamics of the decanethiolate SAM at room temperature. Well-ordered phases undergo significant changes, implying the transition between various phases which includes the disordered phase.

Figure 3.2 (a)-(d): STM image with various phases and showing dynamics of the decanethiol monolayer at room temperature, image size = 120 nm × 120 nm, the time interval between each frame is 21 min. The tunneling parameters are 190 pA and 1.20 V. (e), (f) and (g) cross sectional height profiles from corresponding lines in A, B and C in (c): A is for the β phase and B is for the δ phase while C is for the defect on the surface. (h) STM image of a decanethiolate self-assembled monolayer on Au(111) showing small domains consisting of decanethiolate molecules aligned in an upright configuration (ϕ phase), image size = 100 nm × 100 nm.

Figure 3.3 STM image on δ phase at room temperature, image size = 11.5 nm × 11.8 nm. The tunneling current and sample bias are 190 pA and 1.20 V, respectively.

The vacancy islands are also quite dynamic; they grow and shrink in size and occasionally they merge together. For more details about the dynamics ongoing on the decanethiolate/Au(111) surfaces, a movie is available in the following link: <http://pubs.acs.org/doi/suppl/10.1021/la304902y>. The strong dynamics of this system reveals that the decanethiolate molecules attach and detach easily to the Au(111) surface. Since the covalent Au-S bond is strong (2 eV), it is very unlikely that this bond breaks at

room temperature.^{50, 51} A much more plausible scenario is that the Au-thiolate complex diffuse as a unit rather than the thiolate molecule alone.^{28, 52-55} Yu *et al.*²⁸ showed that the thiolate bonds to a Au adatom and not to the Au atoms of the Au(111) surface. Maksymovych, Sorescu and Yates Jr⁵⁵ performed low temperature STM experiments and density functional theory calculations and demonstrated that the Au adatoms bind to two alkanethiolate species, rather than one alkanethiolate.

In order to confirm the presence of the decanethiol monolayer, current-voltage (I-V) spectroscopy was carried out at different locations on the surface. I-V spectra recorded at the β , δ and ϕ phases (see Figure 3.4) are very comparable and reveal a typical shape that is expected for an insulating molecule like a decanethiol which has a HOMO-LUMO gap (HOMO: highest occupied molecular orbital, LUMO: lowest unoccupied molecular orbital) of about 8 eV, see for instance Liu *et al.*^{32, 56} In order to compare the I-V curves of the three phases properly, we have selected a fixed setpoint ($V = 1.2$ V and $I = 90$ pA). All three I-V curves intersect at this setpoint and hence any asymmetry in the I-V curves shows up most prominently at negative sample biases. However, it seems as if it is not possible to record a noise-free I-V curve at the disorder phase. As it will be discussed later, this turns out to be fully consistent with the speculation for the disordered phase.

Figure 3.4 I-V spectroscopy performed on top of β , δ and ϕ phase. The tunneling current and the sample bias are 90 pA and 1.2 V, respectively.

The δ phase has an out-of-plane interdigitation ordering (see lower image in Figure 3.1), leading to an effective molecular area per molecule of $54.0 \text{ \AA}^2$.²² In the ϕ phase (Figure C in Table 3.1), the alkyl chains are oriented upright and tilted 30° from the surface normal.^{14, 22, 57} The effective area per molecule is lowest in this phase and amounts to $21.6 \text{ \AA}^2$.²² Poirier^{17, 18, 22} estimated that the density of the two-dimensional liquid phase, ε , lies somewhere between the densities of the δ and ϕ phases. The χ phase (see middle image in

Figure 3.1 and Figure D in Table 3.1) has a molecular packing area of $64.8 \text{ \AA}^2$ and comprises a perfectly ordered array of alternating β stripes and δ stripes.^{22, 33} The χ^* phase is also composed of β stripes and δ stripes, however the domain sizes vary from location to location.³³

In order to better understand the dynamics of the various phases, I-t traces were recorded at various locations.⁵⁸ The setpoints were fixed at 200 pA and 1.2 V. Figures E-G in Table 3.1 show the I-t traces performed on various well-ordered phases (β , δ and ϕ phase), while Figure H in Table 3.1 shows the I-t traces performed at the disordered phase. The most remarkable feature in these I-t traces of the disordered phase is the high current level. As pointed out by Kockmann *et al.*⁵⁹, such high current values can only occur if the tail of the decanethiolate molecule flips up and makes contact with the tip. In this case, the electrons tunnel through the molecular backbone rather than through the vacuum.^{60, 61}

For the β phase, we recorded I-t traces on the bright stripes, *i.e.* the sulfur atoms, as well as the dim stripes, *i.e.* the alkyl tails. The I-t traces taken on the top of the bright stripes of the β phase reveal a two-level switching process (Figure E in Table 3.1). In Figure 3.5a, another I-t trace is shown with a setpoint current of only 50 pA. An analysis of the distribution of residence time (see Figure 3.5b for a semi-log plot) reveals that the switching process is fully random.

We suggest that this switching process is due to the fact that the Au-S complex diffuses back and forth between two adjacent lattice positions of Au(111) surface. It should be noted here that the cartoons shown in Table 3.1 are simplified and the Au-S end is just represented by the single light yellow filled circle.

Due to the intermolecular van der Waals forces the alkyl tails in the densely packed β phase do not have many degrees of freedom, thus the I-t traces only exhibit relatively small jumps in the current (see Figure F in Table 3.1). The I-t trace (not shown) of the δ phase is very comparable, but the current jumps are on average a little lower than that in the β phase. This result is easy to understand since the alkyl tails in the δ phase experience an even larger van der Waals force as compared to the alkyl tails in the β phase.

The I-t traces recorded at the densely packed ϕ phase (Figure G in Table 3.1) have a substantial noise band, but well-defined current jumps are absent. We anticipate that this time dependent response is due to dynamics occurring on a time scale that cannot be resolved by our instrument due to the limited bandwidth (50 kHz) of the current-voltage converter.

Table 3.1 (A)-(D): List of structures of various phases in decanethiol SAMs on Au(111) that were observed in previous studies. (E)-(H): representative I-t traces performed on different phases of decanethiol on Au(111). The setpoints are 200 pA and 1.20 V. *c* refers to centered rectangular and *h* refers to hexagonal. The orange triangle stands for the STM tip. The cartoons shown in Table 3.1 are simplified and the Au-S complex is just represented by the light yellow circle while the dark yellow circles refer to the regular Au atoms. The white and grey circles refer to hydrogen and carbon atoms, respectively.

Phase ²²	Schematic	I-t traces
β phase $c(23\times\sqrt{3})$	A 	E
δ phase $h(5\sqrt{3}\times\sqrt{3})R30^\circ$	B 	F
ϕ phase $(4\sqrt{3}\times2\sqrt{3})R30^\circ$ Also referred as $c(3\times2\sqrt{3})$	C 	G
Disordered phase	-	H
D	χ phase is a linear combination of β and δ . Unit cell $c(19\times\sqrt{3})$ 	

However, the I-t traces on top of the disordered phase exhibit a rich dynamical behavior and extremely high current values (see Figure H in Table 3.1). The molecules diffuse very rapidly within the disordered phase. When the alkyl tail of molecule flips up and makes contact with the apex of the tip, the current jumps to a value of several nA. As shown by Kockmann *et al.*⁵⁹, the only viable explanation for these high current values is a physical contact between the substrate and the tip. The current values for the decanethiol are somewhat lower than Kockmann's⁵⁹ current values because they used octanethiol molecules, whereas the slightly longer decanethiol molecules is used here in this Chapter. Finally, one should realize that it is virtually impossible to record decent I-V traces of the disordered phase due to the large current jumps (the feedback loop is disabled during recording I-V traces).

Figure 3.5 I-t trace on the sulfur atom of β phase stripes (a) and histogram of residence time for the high current states (b). The tunneling current and sample bias are 50 pA and 1.20 V, respectively.

The I-t traces shown in Table 3.1 should be considered as fingerprints for the different phases found on decanethiol SAM on Au(111). For a better understanding of the dynamics of the SAM, I-t traces were also recorded at the boundaries between the ordered (β , δ , ϕ and χ^*) and disordered (ε) phases. Figure 3.6 shows an I-t trace recorded at the boundary between a β domain and a disordered domain. During the first 3.3 seconds no large current jumps are observed and the measured time dependence is characteristic for the β phase. However, in the time interval 3.3 seconds to 3.36 seconds large current jumps are observed which is a hallmark of the disordered phase. So, although there is no spatial resolution during the open-feedback loop measurements, the I-t trace response could tell which phase is underneath the STM tip.

Figure 3.6 I-t trace recorded at the boundary between a β domain and a disordered domain. The tunneling current and sample bias are 200 pA and 1.20 V, respectively.

3.2.2 Energetics of various phases of decanethiol SAMs on Au(111) by analyzing the thermally induced meandering of the domain boundaries

In Figure 3.2, four consecutive STM images of a decanethiolate SAM on Au(111) are shown. The SAM consists of different phases, three ordered phases (β , δ and χ^*) and one disordered phase (ε). The only low density ordered phases adjacent to the ε phase are the β and χ^* phase. The absence of domain boundaries between the δ phase and ε phase at room temperature suggests that this boundary free energy vanishes at a temperature below room temperature. This does not necessarily imply that the δ phase is completely absent on the surface, since δ phases encapsulated between other ordered domains and/or steps can still be stable.

The lower terrace in Figure 3.2 is completely covered with the δ phase and remains stable during the whole experiment. The upper terrace shows several β and χ^* domains. In the middle of the upper terrace a large β domain, surrounded by several smaller χ^* domains, is present. Consecutive images recorded with a time interval of 21 minutes (Figure 3.2a-d) of the same region reveals that the lower terrace remains unaltered during imaging, while the upper terrace is very dynamic. Larger regions convert from a β phase to a χ^* phase or *vice versa*. The vacancy islands also exhibit quite some dynamics. In agreement with earlier observations by, for instance, Poirier *et al.*,²⁰ the vacancy islands prefer a triangular shape and have a depth of exactly one Au layer. In the vast majority of cases, one finds a well-ordered β phase in the vacancy islands.

A straightforward way to characterize an order-disorder phase transition is via the boundary free energy. At zero temperature, it costs energy to create a domain boundary. With increasing temperature, kinks will be thermally generated in the domain boundary, leading to an increase of the meandering of the boundary. The creation of a kink will cost energy, but the kink can be generated in many different ways, resulting in an increase of the entropy and thus a decrease of the free energy. The order-disorder phase transition occurs at a temperature where the boundary free energy vanishes. As shown below, the domain boundary free energy can be extracted from a statistical analysis of the thermally induced meandering of the domain boundary.

The Au(111) surface has a hexagonal structure with a threefold symmetry. This threefold symmetry shows up in all ordered phases, and therefore the meandering of the domain boundaries within the framework of simple triangular lattice with a nearest neighbor distance a_{nn} and an isotropic nearest neighbor interaction energy E will be analyzed.^{62, 63}

The first thing to do is to derive an expression for the partition function, Z , of an elementary boundary segment. The total partition function of the boundary consisting of N elementary boundary segments is $Z_{total} = Z^N$. The boundary free energy is defined as $F_{total} = -k_b T \ln(Z_{total}) = -k_b T \ln(Z^N) = -N k_b T \ln(Z) = NF$, where F is the boundary free energy per elementary boundary segment, k_b is the Boltzmann constant, and T the temperature.

The formation of an elementary boundary segment with unit length $a_{nn}\sqrt{3}/2$ along one of the three high symmetry directions involves the breaking of two nearest neighbor bonds (see Figure 3.7). For the sake of simplicity, we take from now on $a = a_{nn}\sqrt{3}/2$ as the elementary length unit. The formation energy of an elementary boundary segment is therefore $2(E/2) = E$. The formation energy of single kink in the boundary also costs energy E . A double kink costs energy $2E$, a triple kink $3E$ etc. (see Figure 3.7 for a schematic diagram). The partition function for an elementary boundary segment unit with length a is given by summing over all possible boundary configurations:⁶³⁻⁶⁵

$$Z = \sum_i e^{-E_i / k_b T} = 2e^{-E / k_b T} + 2e^{-2E / k_b T} + \dots = \sum_{n=1}^{\infty} 2e^{-nE / k_b T} = \frac{2}{e^{E / k_b T} - 1} \quad (3.1)$$

The factor of 2 comes from the degeneracy of the pathways since kinks can point to the right or to the left. The boundary free energy per unit length $a = a_{nn} \sqrt{3} / 2$ is given by

$$F = -k_b T \ln \left(\frac{2}{e^{E / k_b T} - 1} \right) \quad (3.2)$$

The order-disorder phase transition temperature can be found by setting the boundary free energy equal to zero, resulting in $T_R = E / (k_b \ln 3)$. As shown by Wannier,⁶⁶ this is precisely the phase transition temperature of the isotropic triangular 2D Ising spin-lattice.

Figure 3.7 Solid line: Schematic diagram of a domain boundary of a triangular lattice with isotropic nearest neighbour interactions (ε). The nearest neighbour distance of the atoms is a . The length of an elementary boundary segment along the horizontal direction is $\frac{a\sqrt{3}}{2}$. Dashed line: Domain boundary at $T = 0$ K.

The probability to find a kink depends on its length, the longer the kink is, the lower its probability of occurrence is. In order to analyze the thermally induced meandering of the boundaries, it is very helpful to introduce the mean-square kink length,⁶⁷ $\langle n^2 \rangle$, which is defined as,

$$\langle n^2 \rangle = \sum_{n=-\infty}^{\infty} n^2 P_n = \frac{\sum_{n=-\infty}^{\infty} 2n^2 e^{-nE / k_b T}}{\sum_{n=-\infty}^{\infty} 2e^{-nE / k_b T}} = \frac{e^{-E / k_b T} (e^{-E / k_b T} + 1)}{(1 - e^{-E / k_b T})^2} \quad (3.3)$$

where P_n is the probability of finding a kink with a length n . The summation runs over all the possible kink configurations. The mean-square kink length is the expectation value of

the square kink length and can be considered as the diffusivity of the domain boundary.^{68, 69} The value $\langle n^2 \rangle$ can be immediately extracted from the distribution of kinks and kink lengths. At the phase transition temperature, $e^{-E/k_b T_R} = 1/3$ and thus the mean-square kink length will be exactly 1.

The mean-square displacement or deviation-deviation correlation function of a boundary is given by⁶⁶

$$\langle (h_r - h_0)^2 \rangle = \langle n^2 \rangle \frac{r}{a} \quad (3.4)$$

where r is the position along the boundary and h_r the deviation measured in a direction perpendicular to the boundary. The mean-square displacement, $\langle (h_0 - h_r)^2 \rangle$, can be extracted from the STM images by determining the mean-square displacement of the step with respect to its “mean” direction. This mean direction is always aligned along one of the three high symmetry directions of the underlying Au(111) substrate (see Figure 3.2). One thing should be emphasized here is that there is no need to measure the domain wall position, r , and the mean-square displacement with atomic or molecular resolution, in units of a . Here the unit chosen to measure these positions is nanometers, which is perfectly fine since the mean-square displacement scales linearly with the position measured along the domain wall (see Figure 3.8). The slope of the curve is just the mean-square kink length, $\langle n^2 \rangle$.

Figure 3.8 Mean-square displacement, $\langle (h_0 - h_r)^2 \rangle$, of a β - ϵ domain boundary (circles) and χ^* - ϵ domain boundary (triangles) versus the position measured r along the domain boundary.

Figure 3.9 Domain boundary free energy of the β - ϵ domain boundary (solid line) and the χ^* - ϵ domain boundary (dashed line) versus temperature. The phase transition temperatures are 295 K and 325 K, respectively.

In Figure 3.8, plots of the mean-square displacement as a function of r for both the β and χ^* domain boundaries are shown. The mean-square length, $\langle n^2 \rangle$, at room temperature is 0.83 and 0.97 for a β and χ^* domain boundary, respectively. The experimental data reveals an linear relationship, which implies a random distribution of kinks in the domain boundaries. In addition, the slopes of both curves are slightly smaller than 1, suggesting that the actual temperature is very close to the phase transition temperatures. From the slopes in Figure 3.8, a nearest neighbor interaction energy of $E_\beta = 30$ meV/nm and $E_{\chi^*} = 28$ meV/nm are extracted. The boundary free energies are plotted in Figure 3.9. The phase transition temperatures are 325 K and 295 K for the β and χ^* domain boundaries, respectively. The phase transition temperature of the χ^* phase is close to room temperature, which means that at room temperature, domain boundaries can easily be generated since their formation free energy is almost 0. For the β phase, the phase transition temperature is somewhat higher, and therefore, this phase appears more stable at room temperature. These findings are consistent with previous observations of Poirier *et al.*²⁰ and Toerker *et al.*³³ Both observed that the χ^* phase is metastable at room temperature, while the β phase remains stable at temperatures up to 325 K.

3.3 Conclusions

The dynamics of decanethiol SAMs on Au(111) surface was investigated by time-resolved STM at room temperature. We found, in agreement with Poirier *et al.*,²² five different

phases, four ordered phases (β , δ , χ^* and ϕ) and one disordered phase (ε). I-V traces recorded on the ordered phases look very similar, whereas it is impossible to record I-V traces at the disordered phase because of the dynamics of the molecules. I-t traces recorded on the Au-sulfur end of the decanethiolate display a fully stochastic two level switching process. The two level process is attributed to the diffusion of the Au-thiolate between two adjacent adsorption sites. I-t traces recorded on the tails of decanethiolate molecules in the ordered β , δ and χ^* phases exhibit rich dynamics. The disordered phase is characterized by large current jumps which indicates that the tail of the decanethiolate flips up and makes contact to the tip. I-t traces provide additional evidence for models of the various phases that have been put forward by Poirier *et al.*²²

The domain boundaries between various low coverage ordered phases (β and χ^*) and the disordered phase (ε) of a decanethiolate SAM on Au(111) were studied in the second part of this Chapter. By analyzing the thermally induced meandering of the domain boundaries, the domain boundary free energies and extract the phase diagram of this system is determined. The boundary between the χ^* - ε domains and the β - ε domains vanish at 295 K and 325 K, respectively. These findings are in very good agreement with the phase diagram put forward by Poirier *et al.*¹

The work performed in this Chapter improve our understanding of the dynamic of decanethiol SAMs, demonstrating current-time spectroscopy is a powerful tool in studying the dynamic of SAMs surfaces. And we believe that the I-t traces reported here can also be applied to other molecular systems study the dynamics of the their surfaces.

3.4 Experimental section

Materials

Decanethiol (purity $\geq 99\%$) was purchased from Sigma-Aldrich (Steinheim, Germany) and used without further purification. Au substrates (11 mm $\times$ 11 mm, 250 nm Au on 2 nm Cr on borosilicate glass) for STM measurements were purchased from Arrandee (Werther, Germany). Au(111) samples were obtained by annealing these substrates in a high purity H_2 flame for 5 minutes.⁷⁰ Prior to use, these substrates were cleaned in a piranha solution (7:3 $H_2SO_4:H_2O_2$ (30%) by volume), followed by rinsing with Milli-Q water and ethanol and dried in a nitrogen stream. (**Caution: Piranha solutions should be handled with extreme caution: it has been reported that these solutions can detonate unexpectedly**).

Preparation of self-assembled monolayers

Following the protocol described by Love *et al.*,⁷¹ decanethiol SAMs are obtained at room temperature by chemisorption from a 1 mM ethanolic solution onto freshly prepared Au(111) substrates for 24 hours. It is common knowledge that this immersion step leads to a densely packed SAM where the decanethiolate molecules form a standing up phase.^{5,71} In

order to reduce the coverage we immersed our sample in a pure ethanol solvent for one hour. After thorough rinsing of the decanethiol SAMs with pure spectroscopy grade ethanol, the samples were loaded into UHV STM for imaging (see Figure 3.10). As shown in Figure 3.2, the SAM mainly consist of decanethiolate molecules arranged in flat lying phases.

Figure 3.10 Preparation of decanethiol SAMs on Au(111)

Contact angle

To pre-check the presence of the SAMs on the Au(111) surface, contact angle measurements were carried out. The contact angle measurements were performed by the sessile drop technique using an optical contact angle device equipped with an electronic syringe unit (OCA15, Dataphysics, Germany) at ambient and room temperature. Contact angles were measured using ultrasonically degassed Milli-Q water. Advancing contact angles were determined automatically by drop shape analysis during the growth of the droplet at a constant flow rate ($1 \mu\text{L/s}$). The values of the advancing and receding contact angles were 92° and 80° , respectively. The volume of the water droplet used for the measurements was $2.0 \mu\text{L}$. At least three different measurements of each sample were performed. This result is in agreement with the observations of Li *et al.*⁴⁵

Figure 3.11 RHK STM. Inset (left upper corner): A scanner is place on top of the sample holder.

Scanning tunneling microscopy

All the STM measurements were performed with an UHV STM (see Figure 3.11, RHK Technology, Inc.) with a base pressure of 1×10^{-10} mbar at room temperature. STM tips were prepared from tungsten wire using electrochemical etching.⁷² STM topography for the freshly annealed Au surface showed the herringbone reconstruction characteristic of clean Au(111).²⁵ In order to prevent damage of the SAMs, STM images were recorded at sample biases less than 1.2 V and tunneling currents less than 190 pA.^{24, 38} The temperature in the laboratory was kept constant at a temperature of 293 ± 1 K.

Tips used for scanning tunneling microscopy

Tungsten (W) tips were employed for all measurements in this Chapter. STM tips were prepared from tungsten wire with a diameter of 0.25 mm using electrochemical etching.⁷² A 2 M NaOH solution was used as the electrolyte and a Pt ring as the counter electrode.

Time-resolved scanning tunneling spectroscopy

In this Chapter, time-resolved STM measurements were carried out on decanethiol SAMs on Au(111) to obtain a further understanding about the dynamics within the molecular system. For I-t measurements, the tunneling current was recorded as a function of time, while the feedback loop was switched off. Sampling frequencies in the range of 1-50 kHz were used. Each I-t trace was acquired for 10-20 s.

3.5 References

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Ordering and dynamics of oligo(phenylene ethynylene) self-assembled monolayers on Au(111)

Ordering and dynamics of two kinds of oligo(phenylene-ethynylene) (OPE) self-assembled monolayers (SAMs) on Au(111) were investigated by time-resolved scanning tunneling microscopy (TR STM). We found a hitherto unknown SAM phase of 4-[4'-(phenylethynyl)-phenylethynyl]-benzenethiolate (denoted as monothiol OPE hereafter) molecules on Au(111) surface. This phase consists of a fine-striped pattern that is aligned along the close packed $\langle 1\bar{1}0 \rangle$ directions of the Au(111) surface. The fine-striped pattern has a $\sqrt{3}$ periodicity and is separated by narrow vacancy lines which are oriented perpendicular to the fine-striped pattern. Surprisingly, the herringbone reconstruction of the underlying Au(111) substrate is not fully lifted by the SAM. Spatially resolved STM and current-time spectroscopy experiments revealed that the monothiol molecules at the edges of the vacancy lines exhibit dynamic behavior and frequently jump back and forth between neighboring stripes.

For 2,5-bis(4'-mercapto-phenylethynyl)benzene (termed as dithiol OPE) SAM on Au(111), the dithiol OPE molecules are adsorbed in a flat-lying orientation. TR STM measurements reveal that the molecules continuously switch back and forth between two nearly degenerate configurations. The energy difference between the two configurations is 22 ± 5 meV and the activation barrier for the transition from the low-energy configuration to the high-energy configuration is 0.65 ± 0.03 eV. A statistical analysis of the residence times revealed that the switching process is stochastic. We propose that the two level switching is due to a torsional mode of the central phenyl ring of the dithiol OPE molecule.

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4.1 Introduction

Self-assembled monolayers (SAMs) have been extensively studied due to their potential application in molecular electronics.¹⁻²⁰ Molecular electronics is a research field that aims at the use of single molecules as active components in electronic devices and a research area of increasing interest.^{16, 21-26} Design and operation of future molecular electronic devices requires a proper understanding of the electronic properties, dynamics and interactions of the interfaces and molecules utilized.

SAMs of alkanethiols containing saturated C-C bonds have served as model systems in many experimental and theoretical studies.^{14-17, 27-30} However, alkanethiols have a rather large HOMO-LUMO (highest occupied molecular orbital – lowest unoccupied molecular orbital) gap resulting in a rather modest molecular conductance, which makes them less suited for molecular electronic applications. For electronic devices, conjugated molecules with alternating double and single bonds or delocalized π electrons are much more suitable candidates. Fully conjugated oligo(phenylene-ethynylene) (OPE) molecules with tunable functionalities have attracted great attention because of their potential applications in molecular electronics.³¹⁻³³ Owing to the better rigidity and stronger intermolecular interactions of OPE molecules as compared to their aliphatic counterparts, OPE molecules enable the possibility of a much better control over their ordering in SAMs. Compared to alkanethiols, OPE molecules have rather small HOMO-LUMO gaps (~ 3 eV),³⁴ possess synthesis flexibilities,³⁵⁻³⁷ and some OPE molecules exhibit even negative differential resistance (NDR) characteristics.³⁸⁻⁴⁰

Characterization of the ordering and packing of conjugated OPE SAMs is essential for the understanding of the corresponding electronic and optical properties of materials and devices based on these systems. The structural and electronic properties of OPE SAMs on Au(111) substrates have been extensively investigated by STM.^{23, 38, 41-48} 4-[4'-(phenylethynyl)-phenylethynyl]-benzenethiol (hereafter referred as monothiol OPE) molecules embedded in a dodecanethiolate monolayer were found to exhibit stochastic conductance switching behavior at room temperature.⁴² In an STM study performed by Donhauser *et al.*, spontaneous changes in height of the inserted monothiol OPE molecules between subsequent topographic images have been reported.⁴² These height fluctuations were interpreted in terms of a stochastic conductance switching, caused by the conformational changes of the inserted monothiol OPE molecules.⁴² It is argued by Ramachandran *et al.* that this switching is caused by changes in the thiol bond between the molecules and the gold substrate.⁴³ The switching behavior was referred by Ramachandran *et al.* as ‘the blinking of a thiol–gold bond’.⁴³ Hallbäck *et al.*, however, related the ‘blinking’ effect of the monothiol OPE molecules to lateral diffusion and exchange of conjugated molecules, which are embedded as small bundles within the SAMs.⁴¹ In addition to these UHV studies the dynamics of molecular layers have also been extensively studied with

scanning probe microscopy at solid-liquid interfaces.^{49, 50} Despite the fact that the dynamics of OPE molecules embedded in alkanethiols SAMs has been intensively studied, the dynamics of monothiol OPE SAMs has, to the best of our knowledge, not been studied yet.

As mentioned in the previous Chapter, part of this lack of understanding in the structure and dynamics of OPE SAMs is due to the limited time resolution of standard scanning probe microscopes (typically seconds to minutes). Because of this limitation in temporal resolution, dynamic events are often averaged out. Scanning probe microscopes with a substantially higher temporal resolution are thus required in order to obtain more insights into these processes. A simple, but elegant, method to improve the temporal resolution of an STM is to perform current-time measurements (as is illustrated in Chapter 2) with the feedback loop disabled.⁵¹

In the first part of this Chapter, we present a high resolution STM study on a simple and well-studied monothiol OPE SAMs on Au(111) substrates. In two previous studies structural models for the monothiol OPE SAMs have been proposed.^{23, 46} Both articles reported the formation of densely packed SAMs, where the molecules are ordered in several rotational invariant domains.^{23, 46} Dhirani *et al.* used STM to resolve the structure of the monothiol OPE SAMs on Au(111).⁴⁶ These authors found a unit cell with lattice vectors of $a = 5.2 \pm 0.4$ Å and $b = 9.7 \pm 0.9$ Å, respectively. The angle between both lattice vectors was $\gamma = 55^\circ \pm 5^\circ$. Dhirani *et al.* pointed out that this unit cell is very close to a $(2\sqrt{3} \times \sqrt{3})R30^\circ$ periodicity. In a later study Yang *et al.* showed that there are six different

domains and these authors proposed a $\begin{pmatrix} 9 & 4 \\ -2 & 2 \end{pmatrix}$ unit cell (in terms of the lattice vectors of the underlying Au(111) substrate).²³ The measured domain orientations deviate from the hexagonal symmetry of the Au(111) substrate, suggesting that there is no simple commensurate relationship between the lattices of the SAMs and Au(111) substrate. Interestingly, we found that the monothiol OPE molecules arrange themselves in a hitherto unknown striped phase. Due to an additional substrate treatment step the packing density of this novel phase is slightly lower than the packing density of the already known SAMs phases.²³ The striped pattern is composed of fine stripes and vacancy lines that are oriented perpendicular to these fine stripes. The fine stripes consist on the average of 5 standing up monothiol molecules, with the actual number fluctuating between 4-6 as the monothiol OPE molecules or monothiol OPE molecule-Au adatom complexes at both ends of the fine stripe jump frequently back and forth between fine stripes in neighboring stripes. The surface unit cell involves two fine stripes and exhibits $\sqrt{3} a$ periodicity, where a is the nearest-neighbor distance between the Au atoms.

In the second part of this Chapter, current-time spectroscopy is utilized to explore the dynamic events of a dithiol OPE, standing for 2,5-bis(4'-mercapto-phenylethynyl)benzene, SAMs on Au(111) surface. We found that the dithiol OPE molecules switches back and

forth between two well-defined levels, which we ascribe to a torsional mode of the central phenyl ring.

4.2 Results and discussion

4.2.1 Ordering and dynamics of monothiol OPE SAMs on Au(111)

Before the formation of the SAM on the Au(111) substrate we recorded STM images of the bare flame annealed substrate. In Figure 4.1a a typical STM image of the bare Au(111) substrate is shown. The Au(111) surface exhibits the characteristic herringbone reconstruction. This herringbone reconstruction has a $(22 \times \sqrt{3})$ unit cell where 23 atoms of the top layer are placed on 22 atoms of the second layer (see Figure 4.1b).⁵² Along the direction of the compression, the stacking sequence changes from *fcc* to bridge to *hcp* and again followed by a bridge, with a periodicity of 63 Å.⁵² The height difference between the *fcc* domains and the *hcp* domains is only 15 pm. We have used the Au(111) herringbone reconstruction and the height of the step edges to check the calibration of our piezo scanner.

Figure 4.1 (a) STM image of bare Au(111) with herringbone reconstruction pattern, setpoints are 200 mV and 300 pA. (b) Schematic of the herringbone reconstruction. Blue parallelogram indicates the $(22 \times \sqrt{3})$ unit cell.

In addition to the earlier reported densely packed monothiol OPE SAM phases on Au(111),^{23, 46} we found a striped phase. We want to emphasize here that we have obtained this result for three different samples. As shown in Figure 4.2, the herringbone reconstruction pattern underneath the striped phase of the SAMs is still faintly visible, indicating that the formation of this SAMs does not completely lift the Au(111) herringbone reconstruction. It should be pointed out here that the densely packed monothiol OPE SAMs phases lifted the herringbone reconstruction of the underlying Au(111) substrate as reported in Ref. 23 and Ref. 46.^{23, 46} Figure 4.3a shows a large scale STM image of the monothiol OPE SAMs. There are three aspects that we want to highlight here. Firstly, high quality layers can be observed; *i.e.*, the molecules pack densely and exhibit perfect crystalline packing over large areas, albeit the packing density is not as high as reported in refs.^{23, 46} Secondly, the individual fine stripes of the striped phase are well-resolved. It is clear that the monothiol OPE SAMs exhibits a long range ordering. In addition, the vacancy lines between the adjacent striped domains are not perfectly straight, but wander a little bit. Thirdly, the STM images shown in Figure 4.2 and Figure 4.3a reveal an angle of 30° for orientation of the fine stripes (indicated by red arrow in Figure 4.3a) with respect to high ridges of the Au herringbone reconstruction (indicated by blue arrow in Figure 4.3a); while the angle between the herringbone reconstruction and the broad stripes (indicated by the purple arrow in Figure 4.3a) is 60°. In addition, the fine stripes are always out-of-phase with respect to their neighboring fine stripes at the other side of the vacancy line. Interestingly, Noh *et al.* recently found that the densely packed $c(4\times 2)$ phase of octanethiol SAMs eventually converts to a striped ($6\times\sqrt{3}$) phase that is very comparable to the striped phase that we found for the monothiol OPE SAMs.⁵³ Figures 4.3c and 4.3d display the line scans along lines of A and B (shown in Figure 4.3b), respectively. The spacing between the vacancy lines is 3.46 ± 0.05 nm, which is approximately 12 times the Au(111) lattice constant. It should be pointed out here that we also found slightly different vacancy line separation values, as well. The line scan in Figure 4.3c reveals that the apparent depth of the trough between the broad stripes is only ~ 20 pm. We should emphasize here that the actual depth of the troughs is much deeper. The vacancy lines are so narrow that the tip of the STM is unable to probe the bottom of the troughs. The stripes are composed of fine stripes which aligned in a direction perpendicular to the vacancy lines (see Figure 4.3b). The separation between these fine stripes (see Figure 4.3d) is 0.50 ± 0.02 nm. As shown in Figure 4.2, the domain boundary (indicated by the area outlined by the dashed green line in Figure 4.2) is most probably the disordered domain of monothiol OPE molecules. The vacancy lines (see Figure 4.3a and 4.3b) exhibit a frizzy appearance, indicating the presence of dynamics.

Figure 4.2 STM image of monothiol OPE SAMs on Au(111). Image size = 50 nm $\times$ 43.4 nm. The tunneling parameters are 191 pA and 1.0 V.

Figure 4.3 (a) and (b) STM images of monothiol OPE SAMs on Au(111). Image size = 36.0 nm $\times$ 35.5 nm (a) and 30.0 nm $\times$ 10.3 nm (b). The tunneling parameters are 191 pA and 1.0 V. (c) and (d) are line scans along lines of A and B [shown in (b)], respectively.

Figure 4.4 A representative current-voltage spectrum of monothiol OPE SAMs on Au(111).

I-V measurements were performed by the standard method of switching off the STM feedback loop during the time of the bias voltage sweep. A representative current-voltage spectrum of monothiol OPE SAMs on Au(111) is shown in Figure 4.4, for a voltage range of 1.5 V to -1.5 V. As we can see from Figure 4.4, a metallic characteristic I-V curve is observed, implying transport through a conductive medium, which in our case is the conjugated monothiol OPE molecule.

Next we propose a unit cell of the SAMs based on our high resolution STM images. We refer to these unit cells in units of $a = 0.288$ nm, which is the nearest-neighbor distance of bulk Au atoms. Figure 4.5a shows the proposed sulfur lattice and the phenyl ring lattice for SAMs of monothiol OPE on Au(111). The blue oval represents the top view of the phenyl ring lattices of monothiol OPE, while the orange circles represent sulfur lattices. The size of the phenyl ring, which has van der Waals dimensions of $6.4 \text{ \AA} \times 3.3 \text{ \AA}$, is substantially larger than that of a sulfur atom.⁵⁴ Considering this size difference between the S atom and the phenyl ring, it is very likely that the phenyl ring lattice will experience some lateral compressive strain. We believe this strain causes the sulfur atoms to skip a row in order to release the strain at well-defined separations of about 3.0-3.5 nm, resulting in row defects, *i.e.* vacancy lines, between the striped domains (Figure 4.5). We would like to emphasize here that the actual bonding of the S atoms to the Au(111) is more complex than shown in our schematic diagram. By performing low temperature STM experiments and density functional theory calculations, Maksymovych, Sorescu and Yates Jr⁵⁵ demonstrated in a convincing way that alkanethiolate species bind to Au adatoms rather than to the regular Au atoms of the Au(111) substrate. The Au adatoms are most probably provided by the pre-existing step edges. Bearing this in mind it is very plausible that also monothiol OPE molecules bind to Au adatoms. The occurrence of these monothiol OPE-Au adatom

complexes might also explain why the herringbone reconstruction of the underlying Au(111) surface is not fully lifted. For the sake of simplicity, however, we assume that the S atoms are positioned at threefold hollow sites, see Figure 4.5a (density functional theory calculations have shown that the Au adatom is not exactly located at a hollow site, but slightly shifted towards a bridge site⁵⁶⁻⁵⁸). Considering the diameter of the phenyl ring, the monothiol OPE molecules align themselves in such a fashion that they are standing upright. We would like to emphasize here that we are not able to extract from our STM data the exact orientation or angle of the long axis of the molecule with respect to the normal of the Au(111) substrate.

Figure 4.5 (a) The proposed model for SAMs of monothiol OPE: an example in which each fine stripes contains 4 or 5 molecules. The blue oval represents the top view of the phenyl ring lattices of monothiol OPE, while the orange circles represent sulfur atoms. Regarding the molecular sketch, the grey circles refer to the carbon atoms and the white circles to the hydrogen atoms. (b) Side view (red line in figure a) of the phenyl ring plane and the S plane. The blue rod shape stands for the monothiol OPE molecule lying parallel to the surface.

We propose that the phenyl rings of the monothiol OPE molecules within a fine stripe are aligned in a plane as depicted in Figure 4.5b. The phenyl rings of the monothiol OPE molecules in a stripe are therefore parallel to the phenyl rings of the monothiol OPE molecules in the adjacent stripes. In this way intermolecular interactions, such as π - π interactions, can stabilize the fine-stripes of the monothiol OPE SAMs on the Au(111) surface.^{59, 60} The effective molecular area per monothiol OPE molecule in the stripe phase is $\sim 35 \text{ \AA}^2$.

Our model fits the STM images very well. Firstly, the angle between the herringbone pattern and the fine stripes agrees with the observations. As shown in the STM images, the herringbone reconstruction is aligned along the high symmetry direction of the Au(111) surface. The angle between the fine stripes (orientation indicated by the red arrow in Figure 4.3a) and the herringbone reconstruction pattern (orientation indicated by the blue arrow in Figure 4.3a) is 30° . Secondly, both the width of the broad stripes and the spacing between the adjacent fine stripes agree with the measured values. In addition, as displayed in Figure 4.5a, the fine stripes are out-of-phase with respect to their neighbors locating at the other side of the vacancy line.

Figure 4.6 A representative example of an I-t trace recorded on the dim line of an monothiol OPE SAM on Au(111). The tunneling current and sample bias are 200 pA and 1 V, respectively.

As shown in the STM images, some regions exhibit a frizzy appearance. These frizzy features hint to the occurrence of dynamic events during imaging and mainly occur at the edges of the vacancy lines. In order to study the dynamics, current-time (denoted I-t hereafter) spectroscopy is performed on various locations on the surface. The I-t traces are recorded with the feedback loop of the STM electronics disabled resulting in a temporal resolution of about 5-10 μs .⁵¹ This spectroscopic mode allows us to investigate relatively fast dynamic processes at any predefined position of the surface. Figure 4.6 shows a typical

example of a I-t trace recorded at a vacancy line. The current flips back and forth between two well-defined levels. The relative current change is substantial, suggesting that a major change takes place at the edges of the vacancy lines. Since the dynamics is only found at the edges of the vacancy lines, we propose that monothiol OPE molecules located at the edges of the fine stripes jump back and forth between adjacent stripes. Since the sulfur atom of the monothiol OPE molecule is most probably bound to a Au adatom, the energy barrier for these rearrangement events is rather low. The latter point, combined with the observation that the herringbone structure of the underlying Au(111) surface remains intact, provides strong indication in favor of the model that the sulfur atom binds to a Au adatom rather than to a regular Au surface atom as originally proposed by Maksymovych, Sorescu and Yates Jr.⁵⁵ Interestingly, we recently found a two-level switching behavior for a decanethiol self-assembled monolayer that has a packing density which is somewhat lower than that of the most densely packed standing up phase.⁶¹

4.2.2 Dynamics of dithiol OPE SAMs on Au(111)

STM measurements for dithiol OPE [represents for 2,5-bis(4'-mercapto-phenylethynyl)benzene] (Figure 4.7) SAMs on Au (111) were performed in UHV at room temperature. As seen in Figure 4.7, the surface exhibits several characteristic defects, such as substrate steps and vacancy islands.^{17, 28, 62} The depth of the vacancy islands⁶³ is exactly one atomic Au layer, *i.e.* 2.5 Å, revealing that the vacancy islands are actually vacancies in the Au(111) substrate rather than defects in the SAMs. Islands with a diameter of several nanometers show up as bright spots on top of the SAMs. The height of these islands is the same as the height of Au(111) monoatomic steps, *i.e.* 2.5 Å, indicating that the islands are Au islands covered with a dithiol OPE SAM. The SAMs appear as a flat and structure-less layer^{41, 48}. The resolution of our STM images is in the vast majority of cases rather poor. We believe that this is due to the fact that the apex of the STM tip is contaminated with some dithiol OPE molecules. A dithiol OPE molecule that diffuses underneath the STM tip can easily be picked up by the STM tip.²⁷ However, STM images with high spatial resolution are obtained, as well. A striped phase with a well-defined width of 1.9 ± 0.2 nm is observed frequently (inset Figure 4.7). We propose that for the striped phase the dithiol OPE molecules are lying flat down on the Au(111) surface. The observed stripe width is somewhat smaller than the length of the dithiol OPE molecular and therefore we propose that either the end groups of the dithiol OPE molecules are interlaced or that the long axis of the dithiol OPE molecules is not exactly orthogonal to the stripes. We suggest that an attractive intermolecular interaction between the dithiol OPE molecules make them line up in stripes.⁶⁰ We have found three rotational domains, indicating that the adsorbed dithiol OPE monolayers adapt themselves to the threefold symmetry of the underlying Au(111) substrate.

Figure 4.7 STM image of dithiol OPE SAMs on Au(111). Image size = 196 nm $\times$ 180 nm. The tunneling parameters are 188 pA and 1.20 V. The stripes have a width of 1.9 ± 0.2 nm (inset).

I-t spectroscopy measurements were carried out at different locations on the surface to obtain more information on the various dynamic processes that take place within the dithiol OPE SAMs.^{28, 51} The I-t traces are recorded with the feedback loop of the STM electronics disabled resulting in a temporal resolution of 10 μ s. This spectroscopic mode allowed us to investigate relatively fast dynamic processes at any predefined position of the surface. In total we have recorded more than 3000 I-t traces.

In Figures 4.8a and 4.8b, two typical time traces are depicted. In both cases the current flips back and forth between two well-defined levels. In order to check whether the current fluctuations are induced by electrons that tunnel inelastically or by the applied electric field, we have systematically varied the setpoint tunnel current as well as the sample bias. We did not observe any trend in our data and therefore we must conclude that the observed dynamic events are thermally activated. Another, and more direct, way to determine whether or not we are dealing with thermally induced processes would be a temperature dependent time-resolved STM study. Such an approach would, in principle, allow us to extract the energy barriers and attempt frequencies via an Arrhenius plot.⁶⁴ There are, however, a number of reasons why this approach is not viable in our case: (1) it is not trivial to find back the same location after a temperature step and (2) the temporal resolution of time-resolved STM is improved by about 6-7 orders of magnitude, but this goes at the expense of the spatial resolution and thus even a small thermal drift can substantially affect our current-time traces. Therefore, it is far from trivial to perform a temperature dependent time-resolved STM study of the various dynamical processes that take place in the dithiol OPE SAMs.

Figure 4.8 Two representative examples of I-t traces recorded on a dithiol OPE SAM. The average residence time in the low current state is substantially larger than the high-current state. (a) The tunneling current and sample bias are 150 pA and 1 V, respectively. (b) The tunneling current and sample bias are 220 pA and 1 V, respectively.

Despite the fact that a temperature dependent study is not within reach, we can still extract valuable information from our room temperature current-time traces. From Figure 4.8, it is immediately clear that the average residence time of the low current level is substantial larger than the average residence time of the high current level. An analysis of many current-time traces reveals that the ratio of residence times of the low-current level versus the high-current level is about 70:30. By using Boltzmann's equation, we find an energy difference between the low-current level configuration and the high-current level configuration of about 22 meV. In Figure 4.9, the distribution of residence time of the low-current configurations is plotted in a semi-log manner. For a random process, the

probability of finding a residence time t decays as $e^{-t/\langle\tau\rangle}$, where $\langle\tau\rangle$ is the average residence time. In order to understand this we consider a system that consists of two states. The probability, after τ_0 seconds, to reside in state 1 is given by $(1-p)$ and the probability to jump from state 1 to state 2 is given by p . The probability to jump from state 1 to state 2 after $2\tau_0$ seconds is $(1-p)p$. The probability to jump from state 1 to state 2 after $3\tau_0$ seconds is $(1-p)^2p$ etc. (please note that $\sum_{n=0}^{\infty} (1-p)^n p = 1$). Hence the linear dependence found in Figure 4.9 reveals that we are dealing with a fully random, *i.e.* stochastic, process.

Figure 4.9 Semi-log plot of the distribution of residence times for a low-current level. The residence times are binned in bins of 5 ms. The linear dependence reveals that the switching process is stochastic.

We propose that the two-level switching is due to a torsional mode of the most flexible, *i.e.* central, phenyl ring of the dithiol OPE molecule. The low-current would then correspond to the low-energy configuration where the central phenyl ring is lying flat down on the substrate. In the high-current and also high-energy configuration the central phenyl ring makes an angle with the substrate. An alternative explanation would be that one of the outer phenyl rings exhibits a torsional mode. We believe that the latter interpretation is less likely since it would require a torsional motion with respect to a S atom, which is firmly attached to the Au(111) substrate.

In comparing Figure 4.8a and Figure 4.8b it is obvious that the activation energy barriers are not exactly the same (the barriers are somewhat lower in Figure 4.8b as compared to Figure 4.8a). This result is typical for self-assembled monolayers²³ and reflects the fact that the exact value of the activation barrier depends on the local environment of the molecule under scrutiny. If we assume an atomic vibration frequency of 10^{13} Hz (this is the typical value for atomic processes) we find an activation barrier for the transition from the low-energy configuration to the high-energy configuration of 0.65 ± 0.03 eV. The

activation barrier for the transition from the high-energy configuration to the low-energy configuration amounts 0.63 ± 0.03 eV.

4.3 Conclusions

SAMs of monothiol OPE on Au(111) surfaces were investigated in UHV by STM. The SAMs were prepared by immersion in a solution followed by annealing at 40 °C for 5 hours in a pure solvent. By applying this annealing step, a very stable striped phase composed of standing up monothiol OPE molecules was formed. The width of the stripes was 3.0-3.5 nm, while the distance between the adjacent fine stripes was 0.50 ± 0.02 nm. A tentative model for the striped phase is proposed in which each fine stripe consists of 4-6 upright standing monothiol OPE molecules. I-t traces recorded at the edges of the vacancy lines reveal a two-level switching process, indicative for dynamics of the monothiol OPE molecules located at the edges of the vacancy lines. It is very likely that sulfur atoms of the monothiol OPE molecule form a complex with a Au adatoms.

SAMs of dithiol OPE on Au(111) were studied by STM. We observed that the molecules lie flat down on the Au(111) substrate and are arranged in stripes. The molecules switch back and forth between two nearly degenerate levels. A statistical analysis of the current-time traces reveals that the switching process is stochastic. We suggest that the dynamics is due to a torsional mode of the central phenyl ring. We anticipate that in the low-energy configuration the central phenyl ring is lying flat down on the substrate, whereas the phenyl ring makes an angle with the substrate in its high-energy configuration. The energy difference between configurations is about 22 ± 5 meV and the activation barrier for the transition from the low (high)-energy configuration to the high (low)-energy configuration is $0.65(0.63) \pm 0.03$ eV assuming an atomic vibration frequency of 10^{13} Hz.

4.4 Experimental section

Substrate

Au substrates (11 mm $\times$ 11 mm, 250 nm Au on 2 nm Cr on borosilicate glass) were purchased from Arrandee (Werther, Germany). Au(111) samples were obtained by annealing these substrates in a high purity H₂ flame for 5-10 minutes.⁶⁵ After flame annealing the Au substrates were cleaned in a piranha solution (7:3 H₂SO₄:H₂O₂ (30%) by volume), followed by rinsing with Milli-Q water and ethanol and dried in a nitrogen stream.

Preparation of monothiol OPE SAMs on Au(111)

S-[4-[2-[4-(2-Phenylethynyl)phenyl]ethynyl]phenyl] thioacetate (OPE-SAc, see Figure 4.10) was purchased from Sigma-Aldrich and further purified by preparative thin-layer chromatography in dichloromethane-hexane (1:1). OPE-SAc is used as a precursor of 4-[4'-

(phenylethynyl)-phenylethynyl]-benzenethiol (monothiol OPE, see Figure 4.11). Solutions of 50 μM of a OPE-SAc were prepared in freshly distilled, deoxygenated tetrahydrofuran (THF) to which 5-10 μL of a 25% aqueous solution of ammonium hydroxide was added. The acetyl groups of OPE-SAc are removed during self-assembly by adding small amount of ammonia. This de-protection activates the thiolate end groups, which then react in situ with the Au(111) surface and form a self-assembled monolayer.⁴¹ The thioacetate precursor was used instead of the free thiol to prevent side reactions with functional groups in the SAM precursor backbone.

Subsequently Au(111) substrates were placed in the OPE-SAc/THF solution for ~ 1 min allowing the conjugated molecules to assemble on the Au surface. After the modification, the substrate was annealed at 40 $^{\circ}\text{C}$ for 5 hours in ethanol solvent to remove any physisorbed and weakly bound molecules from the surface of the substrate. Finally the substrate was rinsed with pure ethanol and dried with N_2 gas. The sample preparation was performed under oxygen-poor conditions to prevent disulfide formation of the monothiol OPE molecules. The SAMs were immediately inserted into UHV for STM measurements.

Figure 4.10 Chemical structure of *S*-[4-[2-[4-(2-Phenylethynyl)phenyl]ethynyl]phenyl] thioacetate (OPE-SAc).

Figure 4.11 Chemical structure of 4-[4'-(phenylethynyl)-phenylethynyl]-benzenethiol (monothiol OPE)

Preparation of dithiol OPE SAMs on Au(111)

S, S'-[1,4-Phenylenebis(2,1-ethynediyl-4,1-phenylene)]bis(thioacetate) (OPE-2SAc) was purchased from Sigma-Aldrich and further purified by preparative thin-layer chromatography in dichloromethane-hexane (1:1). The chemical structure of the OPE-2SAc molecule used in this study is shown in Figure 4.12. OPE-2SAc is used as a precursor of 2,5-bis(4'-mercapto-phenylethynyl)benzene (dithiol OPE, see Figure 4.13). SAMs were prepared by incubating freshly annealed Au(111) for 3 to 24 hrs in a 1 mM solution of the OPE-2SAc molecules in freshly distilled, deoxygenated THF to which a 5-10 μL of a 25% aqueous solution of ammonium hydroxide was added.^{41, 48} In this solution the thiolate groups are deprotected. The sample preparation was performed under oxygen-poor conditions to prevent disulfide formation of the molecules in question. After preparation the samples were quickly loaded in the UHV system.

Figure 4.12 Chemical structure of *S, S'*-[1,4-Phenylenebis(2,1-ethynediyl-4,1-phenylene)]bis(thioacetate) (OPE-2SAc).

Figure 4.13 Chemical structure of 2,5-bis(4'-mercapto-phenylethynyl)benzene (dithiol OPE).

Scanning tunneling microscopy and spectroscopy

All STM measurements were performed with an UHV STM (RHK Technology, Inc.) with a base pressure of 1×10^{-10} mbar at room temperature. STM tips were prepared from tungsten wire using electrochemical etching.⁶⁶ For I-t traces, the tunneling current was recorded as a function of time, while the feedback loop was switched off. Sampling frequencies in the range of 1-100 kHz were used. Each I-t trace was acquired for 10-20 s. In total we have recorded more than 3000 I-t traces on both the monothiol and dithiol OPE SAMs on Au(111). For I-V curves, the tunneling current is recorded as the sample bias is ramped from -1.5 V to +1.5 V. While performing spectroscopy measurements, the feedback loop is disabled, keeping the distance between the tip and the sample constant.

4.5 References

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Variable temperature study of the transport through a single octanethiol molecule

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Knowledge on a true molecular level is essential in order to advance in the field of molecular electronics. Techniques need to be perfected and new approaches have to be developed in order to characterize the properties of a single molecule. In this Chapter, we report temperature-dependent transport studies of a single octanethiol molecule trapped between the apex of a scanning tunneling microscopy tip and a substrate. At each temperature the molecule is brought into contact by decreasing the gap between tip and substrate in a controlled way. At a positive sample bias the molecule jumps into contact upon approaching the substrate by 0.16 ± 0.01 nm with respect to a fixed reference point defined by a sample bias of +1.5 V and a tunneling current of 0.5 nA. The conductance of octanethiol is temperature independent, demonstrating that either tunneling or ballistic transport is the main transport mechanism.

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5.1 Introduction

The field of molecular electronics, emerging from the potential use of single molecules as elementary building blocks for functional devices, has attracted much attention over the last few decades.¹ A prerequisite for the advancement of this research field is the ability to measure the properties of an individual molecule. From an application point of view the conductance of a molecule is by far the most important property. But how can one measure the conductance of a single molecule? This, at first glance, very simple and elementary question turns out to be tough to answer properly. Contacting a single molecule is a very delicate process that requires great care and ultimate precision. Although several successful and elegant approaches to contact a single molecule have recently been put forward,²⁻²¹ there is still a need to come up with alternative and more flexible routes to contact a single molecule.

In order to obtain a better understanding of the charge transport through a single molecule, it is of key importance to perform transport experiments in which the length and the temperature of the molecules are varied in a controlled way. Lafferentz *et al.* demonstrated conductance measurements while changing the length of a single polyfluorene molecule.¹³ The molecule was continuously pulled up from a Au(111) surface using the tip of a scanning tunneling microscopy (STM).

A rather straightforward method to contact a single molecule is to trap it between a substrate and the apex of the STM tip. Once contacted, the conductance of the molecule can easily be measured. However, this method does not allow for a measurement of the temperature dependence of the conductance of the molecule because even the slightest change in temperature will lead to a change of the substrate-tip separation and thus to a compressed, stretched, or detached state of the molecule. This is presumably the main reason why there are only a few examples in literature where single molecule conductance has been measured as a function of temperature. An exception is a recent study by Sedghi *et al.* where the temperature dependence of the conductance of short chains of porphyrin molecules was measured in a temperature range from 300 to 375 K.¹⁵

Using STM to create a well-defined substrate-molecule tip junction that can actively be opened and closed in an accurate and controlled way will diminish the problem of a thermally induced varying substrate-tip separation. Recently, we put forward a method that relies on a combination of current-voltage (I-V) and current-distance (I-Z) spectroscopy to open and close an electrode-molecule-electrode junction in a well-defined way.¹⁷ Using this recipe we are able to trap a single octanethiol between two electrodes at a temperature of 77 K, one electrode being the tungsten tip of the STM and the other an atomic platinum chain on a Ge(001) substrate. The controllability of this junction, *i.e.*, the ability to manage the cessation and initiation of contact with high accuracy, allows for a feedback mechanism that can be sustained over a wide temperature range. This approach differs significantly

from methods that use static contacts for the molecule, *e.g.*, break junction methods.^{4, 5, 22} In our work, at every measurement the contact was freshly established, and after the I-Z/I-V traces were recorded the contact was broken again, so that the junction could rearrange itself to its reference point. Therefore, the junction itself remained uniform, *i.e.*, in particular the electrode-electrode separation, throughout the whole experiment.

Several possible molecular transport mechanisms have been proposed, including thermionic emission, hopping, Fowler-Nordheim tunneling, and direct tunneling. Thermionic emission and hopping are temperature dependent, whereas tunneling is temperature independent. Direct tunneling usually occurs at biases smaller than the work function, while Fowler-Nordheim tunneling takes place at biases that exceed the work function. In addition, Fowler-Nordheim tunneling in the STM setup will lead to field emission resonances, also referred to as Gundlach oscillations, which show up as well-defined oscillations in I-V and I-Z traces.²³

In this Chapter, we present an approach to measure the conductance of a single octanethiol molecule as a function of temperature in a well-defined and accurate way. The transport measurements reveal that the conductance of an octanethiol molecule is temperature independent, indicating that the transport mechanism through the octanethiol molecule is direct tunneling or ballistic transport.

5.2 Results and discussion

In Figure 5.1 STM images of Pt modified Ge(001), before and after the exposure to 10 Langmuir (L) of octanethiol, are depicted. The vast majority of octanethiol molecules prefer to adsorb on the self-organizing atomic chains. We subsequently pick up a single octanethiol molecule by placing the tip over the molecule, opening the feedback loop and bringing the electrochemically etched tungsten STM tip several angstrom closer to the surface than the normal tunneling distance. Since the success rate of this pick up experiment is not very high, we often had to repeat this procedure before a single molecule was picked up from an atomic Pt chain. After every attempt we recorded I-V and I-Z spectra to affirm if we had indeed an octanethiol molecule dangling at the apex of the STM tip.¹⁷ In Figure 5.2 an illustration of an octanethiol molecule attached to the apex of the STM tip is shown. A successful attachment of an octanethiol molecule to the STM tip can be proven either via an I-Z measurement (see Figure 5.3a) or an I-V measurement. In case of attachment, the I-V curves change from a symmetric shape to a rectifying behavior (see Ref. 17). Once the molecule is attached to the tip, the configuration is very stable and remains unchanged over a large number of experiments. Figure 5.3a shows two different I-Z curves recorded at a bias voltage of 1.5 V and a setpoint current of 0.5 nA. The dashed line shows the response of a tunneling junction in case no molecule is attached to either substrate or STM tip. The solid line displays the I-Z response of a molecule trapped within

the STM junction. After a small, but well-defined, tip displacement, the current suddenly jumps to a much larger value. At this point the molecule bridges the gap between substrate and STM tip. The attachment process is judged successful when the characteristic jump into contact can be reproduced on random locations of the substrate (*e.g.*, positions where no molecule could previously be found). Furthermore, contact of the molecule never occurs at negative bias voltage.¹⁷ Therefore, when changing the polarity no jump should appear and the current-distance curve follows an exponential behavior (as seen by the dashed line in Figure 5.3).

Figure 5.1 (a) STM image (image size = 8 nm × 8 nm) of a Pt modified Ge(001) substrate with a 2D image of the same location in the inset. (b) STM image (image size = 8 nm × 8 nm) of the same surface as in A after the exposure to 10 L octanethiols with a 2D image of the same location in the inset.

Figure 5.2 Cartoon of an octanethiol molecule attached to the apex of an STM tip with an initial separation Z_0 to the surface. The tip displacement, ΔZ , is the distance the tip moves closer to the surface during an I-Z measurement. For the sake of simplicity we assume that only the sulfur atom of the octanethiol molecule binds to the apex of the scanning tunneling microscope.

Figure 5.3 (a) Example of an I-Z curve without (dashed line) and with (solid line) an octanethiol molecule attached to the STM tip. Both traces were recorded at 77 K, at a fixed setpoint (tunnel current 0.5 nA and sample bias +1.5 V). (b) Tip displacement at which the molecule jumps into contact versus temperature. The displacement (point of contact) is measured with respect to the setpoint values (tunnel current 0.5 nA and sample bias +1.5 V).

With the molecule attached to the tip of the STM, we constantly scanned the surface for many hours. We did not refill the cryostat with liquid nitrogen (LN_2) and after some time the LN_2 was fully evaporated, causing the STM to slowly heat up. The temperature was carefully monitored during the experiment. Every few degrees (typically 1-2 K) a series of I-Z curves were measured. All the traces were recorded at a positive bias voltage of +1.5 V and a setpoint current of 0.5 nA to ensure that the molecule made contact upon approaching the substrate. Within one I-Z measurement the feedback loop was switched off and the current was recorded as the STM tip moved a total of 0.4 nm towards the surface. After every I-Z measurement the feedback loop was switched back on again to allow the tip to move back to its initial position, thus breaking the contact again. At this point the junction returns to the original configuration defined by its setpoint value, *i.e.*, a tunnel current of 0.5 nA and a sample bias of 1.5 V, after every single measurement and thereby providing us with a well-defined and fixed reference point. A series of I-Z traces measured for temperatures ranging from 77 to 172 K. Beyond 180 K the thermal drift of the instrument started to affect the measurements and therefore we were unable to achieve accurate and well reproducible experimental data.

We have corrected all the I-Z measurements to compensate for the temperature-dependent response of the piezo crystals by applying a factor of the expansion coefficient. This changes the distance value to the actual value that the tip has moved at the specific temperature of the measurement. The measurements are very comparable to the results obtained at 77 K in a previous experiment, where the switching behavior of a single octanethiol molecule was studied.¹⁷ First, the jump into contact occurs at the same tip-substrate separation (after approaching the substrate by $\Delta Z = 0.15\text{-}0.17$ nm with respect to our fixed and well-defined setpoint, *i.e.*, $I = 0.5$ nA and $V = +1.5$ V). The variation in ΔZ is comparable to the measurements that were recorded at 77 K. Second, the current value that

is reached after the molecules makes contact is the same as previously recorded at 77 K, *i.e.*, ≈ 45 nA. Since we never detected a second jump in the I-Z traces, we have to conclude that only a single molecule is trapped between tip and substrate.

Figure 5.3b shows a plot of the tip displacement values at which the molecule jumps into contact versus the temperature. One can easily see that within the recorded temperature window the point of contact remains amazingly constant. The mechanism for the molecule to jump into contact is thus temperature independent. This definitely supports the idea that the cause for the jump into contact is the electric field applied between STM tip and substrate, as has been put forward previously.¹⁷

Figure 5.4 Conductance of an electrode-single octanethiol molecule-electrode junction versus temperature. $G_0 = 2e^2/h$ is the conductance quantum.

The conductance of the molecule has been determined at a sample bias of +1.5 V, *i.e.*, well below the reported tunneling barrier of an octanethiol molecule (see Figure 5.4).²⁴ Throughout the experiment the conductance through the molecule remains constant at a value of 30 nS, a value that is consistent with reports from literature.²⁴ In theory, the transport mechanisms that are temperature independent are ballistic transport and quantum-mechanical tunneling. In principle there are two tunneling processes: direct tunneling or Fowler-Nordheim tunneling.^{25, 26} Fowler-Nordheim tunneling only occurs at high voltages, *i.e.*, voltages larger than the work function ($V > \Phi/e$). Since the voltages applied in our experiments are significantly smaller than the 4 eV barrier (assuming that the Fermi edge lies somewhere in the middle of the 8-9 eV gap between highest occupied and lowest unoccupied molecular orbitals of the octanethiol molecule²⁷), we have to exclude Fowler-Nordheim tunneling. The conductance of a tunnel barrier decreases exponentially with the

width (L) of the barrier, *i.e.*, $G \propto e^{-\beta L}$. The total conductance of the substrate-molecule-tip junction can be written as

$$G_{\text{total}} = \frac{2e^2}{h} T_{\text{total}} \quad (5.1)$$

where T_{total} is the total transmission coefficient of the substrate-molecule-tip junction. If we assume that the conductance of the octanethiol molecule is much smaller than the contact conductance we find that the conductance of a single octanethiol molecule is ≈ 30 nS, which agrees well with large area junctions data.²⁷ However, hybridization of the electronic states of the molecule and the electrodes will also affect the transport mechanism. Since our experiments reveal a single molecule conductance, that is about a factor of 3 larger than obtained previously by a method where the sulfur atom of the octanethiol binds to the substrate rather than to the tip, we propose that in our experiments not one but two carbon atoms of the tail of the octanethiol molecule are involved in bonding with substrate. The latter conclusion is supported by the observation that we have to move the tip 0.16 ± 0.01 nm, which compares favorably with the projected C-C bond distance (0.155 nm), closer to the substrate as compared to the experiments reported in Ref. 17.

5.3 Conclusions

In summary, we have shown how a substrate-octanethiol-STM tip junction can be used to study the conductance of a single octanethiol molecule. In a well-defined manner we were able to extract the transport properties of this molecule as a function of temperature. By making use of the ability of the junction to be controlled by the applied electric field, we were able to set a contact, well defined in respect to the reference point for each measurement. The immense advantage of this method is that we can very accurately adjust the gap between the two electrodes, which is an absolute prerequisite for precise single molecule transport measurements. The conductance of an octanethiol molecule is temperature independent, demonstrating that the main transport mechanism is dominated by either direct quantum-mechanical tunneling or ballistic transport. The amount of control over a single molecule junction that we have demonstrated here will open yet another door into the understanding and realization of molecular electronics.

5.4 Experimental section

Low-temperature scanning tunneling microscopy

All experiments are performed in an UHV Omicron low temperature STM (UHV LT-STM, see Figure 5.5). The LT-STM is a commercial low temperature STM procured from Omicron NanoTechnology GmbH, Germany. The STM consists of a main chamber where the STM scanner is located, a home-built preparation chamber for processing of the

samples and a load lock unit which could be used to quickly introduce new samples and/or tips. The preparation chamber is equipped with the following components: a sputter gun, a heating stage, a Pt evaporator, a Knudsen cell evaporator, a leak valve for depositing molecules and a commercial mass spectrometer. The system is also equipped with two magnetic transfer rods to transfer samples from the load lock to the preparation chamber and then to the STM chamber.

The base pressure in both the main chamber and the pressure chamber is maintained below 5×10^{-11} Torr. The purity of the gaseous contents is monitored by the mass spectrometer. The STM works at UHV conditions at low temperatures. In the experiment performed for this Chapter, LN_2 was used to keep the temperature down to 77 K.

The STM consists of a single piezo tube scanner (shown in inset in Figure 5.5) which enables z-resolution better than 0.002 nm. The z-axis refers to the direction normal to the sample surface. The whole scanner stage is enclosed inside two concentric bath cryostats. The outer one is filled with LN_2 for thermal shielding while the inner one for cooling the scanner stage which is filled with LN_2 for measurements at 77 K.

Figure 5.5 Omicron LT-STM. Inset (right lower corner): A single piezo tube scanner from the Omicron LT-STM.

Tips used for low-temperature scanning tunneling microscopy

Tungsten (W) tips were used for all the measurements in this Chapter. STM tips were prepared from tungsten wire with a diameter of 0.37 mm using electrochemical etching.²⁸ A 2 M NaOH solution was used as the electrolyte and a Pt ring as the counter electrode.

Materials and sample preparation

The molecules were deposited on Pt/Ge(001) substrates that have been cleaned and prepared in UHV. Flat, n-type Ge(001) substrates (4 mm × 10 mm), were cleaned using 500 eV Argon ion bombardment followed by annealing at 1100 K until atomically clean and flat (001) surfaces were obtained. Well-ordered arrays of atomic Pt chains were produced by deposition of half a monolayer of Pt onto the substrate and annealing at 1100 K.^{29, 30} Subsequently, octanethiol molecules (98.5% pure, purchased from Sigma-Aldrich) were introduced into the chamber for 20-60 sec at a pressure of 2.5×10^{-7} Torr via a leak valve that allows the sample to be exposed to a precise dose of molecules. The atomic Pt chains are very helpful in locating and immobilizing the molecules on the substrate for the attachment process.¹² The sample was then transferred to the LT-STM, cooled down to 77 K, and subsequently imaged.

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Potential stimulated height changes of Cu-azurin studied by *in-situ* electrochemical atomic force microscopy

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The redox responsive behavior of Cu-azurin molecules immobilized on bare gold electrodes was assessed by cyclic voltammetry. The voltammetric response of the adsorbed proteins revealed a reversible, well-defined and stable signal, indicating surface confinement with a redox potential being in good agreement with the literature values. These results clearly show a preserved redox functionality of adsorbed molecules and wiring to the underlying gold electrode. *In-situ* electrochemical atomic force microscopy (EC-AFM) studies revealed a height difference of 0.32 nm between the oxidized and reduced states for Cu-azurin as derived from the histogram of height changes between the oxidized state (+0.45 V) and the reduced state (-0.10 V) by imaging single molecules. Height changes of the Cu-azurin upon electrochemical redox switching are thought to originate from conformational changes of the protein and the variation in the orientation of immobilized proteins between the oxidized and reduced states. As expected, no large height changes have been detected for Zn-azurin which is non-redox active and served as a control. In conclusion, the height of the Cu-azurin could be modulated by the applied potential.

6.1 Introduction

6.1.1 Metalloprotein film

Metalloproteins account for almost half of all proteins in nature and are involved in a plethora of fundamental biological processes on the molecular level, including the highly efficient and well controlled electron transfer (ET) in photosynthesis and the respiration chain.¹⁻⁶ Metalloproteins hold great promise for applications in biotechnology in industrial catalysis, biomolecular electronic devices and biosensing including their miniaturization, which justifies the growing interest in these materials.⁷⁻⁹ Biological ET proceeds between donor and acceptor sites via chemically and orientationally well defined pathways, that have been optimized continuously throughout biological evolution.¹⁰ Metalloproteins also possess a significant technological relevance for the application of biofuel cells.^{11, 12} In particular, biofuel cells require electron-transfer between the catalyzing metalloproteins and electrodes. Here Ni-Fe hydrogenases found widespread application catalyzing the oxidation of molecular hydrogen to water and the reverse reaction. Various immobilization and wiring strategies of the enzyme have been established to enhance efficiency and durability of biofuel cells.¹³

Applications of metalloproteins in nanobiotechnology requires knowledge of the structural and ET properties of the redox metalloproteins assembled on metallic surfaces.^{8, 11, 12, 14, 15} For successful utilization strategies it is of fundamental importance to assemble the molecules in a controllable fashion and to bring them into reliable and efficient communication under electrode potential control between the redox-active cofactor and a suitable macroscopically addressable electrode component. Many redox proteins can directly transfer electrons to an electrode, but oriented protein immobilization without the loss of its native confirmation and redox activity still remains a challenge. In this regard protein film voltammetry (PFV) significantly contributed to the understanding of the thermodynamic and kinetic aspects of metalloprotein electron transfer including enzyme immobilization to appropriately modified and designed surfaces.^{16, 17} This confinement eliminates diffusion limitations in cyclic voltammetry (CV) and enables spectroscopic measurements with small amounts of samples. Despite the fundamental contributions of PFV, the observed voltammograms are usually non-ideal and show broad peaks and variations from experiment to experiment, as a result of thermodynamic and kinetic dispersion of the wired metalloproteins. This behavior can originate from structural variations within the confinement, such as the variation of molecular orientations, the electronic coupling to the electrode, or surface charges.¹⁸ This results in uncertainties of electron transfer pathways and contributes to significant heterogeneity within a protein film. To exploit the full potential of metalloproteins, these heterogeneities must be

understood at the single molecule level. Hence there is a fundamental need for the establishment of experimental techniques enabling these types of studies.

In one approach, Canters and coworkers introduced a very elegant and extremely sensitive method for the optical detection of the redox state of metalloproteins Cu-azurin (introduction see the coming section) via the resonance energy transfer from an attached fluorescence label (FRET donor) to the prosthetic group (FRET acceptor) of Cu-azurin.¹⁹ When a redox event occurs, the fluorescence intensity switches from high to low (or *vice versa*) as a consequence of the exchange of electrons with the electrode. In this way redox activity is observed by following the intensity of fluorescence, providing the inherently enhanced sensitivity of optical detection. This method enables one to distinguish between reduced and oxidized states of Cu-azurin and to perform potentiometric titrations at sub-micromolar concentrations at different prosthetic groups (type-1 copper, heme, flavodoxin).¹⁹ In this manner a highly efficient FRET process was established, enabling optical monitoring of the redox switching down to a zeptomolar protein level. Strikingly, with this surface-potential controlled cycling of emission, the redox potential of clusters of a few hundreds of molecules could be determined. Recently an extension of this approach termed fluorescence cyclic voltammetry (FCV) was introduced.²⁰

In another approach, metalloproteins like cytochrome *c*²¹ and azurin²²⁻²⁵ were investigated at the single molecule level by *in-situ* electrochemical scanning tunneling microscopy (EC-STM) and novel types of single molecule fluorescence techniques. STM studies revealed apparent height changes of the cytochrome *c* and azurin of ~0.5 nm with complete reversibility.²²⁻²⁵ In addition, non-redox active reference molecules such as Zn-azurin did not show any height changes.²²⁻²⁵ However, it is important to mention that these were apparent height changes due to resonant tunneling of the metalloprotein and thus it remains unclear if a topographical (*i.e.* true height) height change occurred due to conformational or charging effects. Furthermore, these STM based approaches suffer from a drawback as they are not controlled by force, hence the force during the probing of a protein is unknown and cannot be controlled, thereby a significant perturbation of the observed behavior cannot be excluded.

It was demonstrated that AFM (introduction available in section 6.1.3) imaging enables observations of enzymatic activity, which involves height fluctuations, at the single molecule level. For instance, Radmacher *et al.* observed conformational changes of lysozyme during catalysis by liquid tapping mode AFM (TM-AFM).²⁶ For this purpose the enzyme was electrostatically adsorbed to mica surfaces, subsequently the height was recorded by AFM. After addition of the enzyme substrate occasionally height fluctuations of ca. 1 nm were recorded. The fluctuations were interpreted as conformational structural changes assigned to the hydrolysis reaction. In a recent work Ulstrup and coworkers²⁷ investigated nitrite reductase adsorbed to a gold substrate by AFM imaging under electrochemical control arriving at similar observations as Radmacher.²⁶

To the best of our knowledge, AFM based observations of the variation of molecular dimensions that would accompany changes in the redox state for metalloproteins under controlled force have not been reported. Such observations would not require any specific fluorescence labeling of the metalloprotein which might significantly alter its redox properties. Here we propose to utilize AFM based mapping techniques with simultaneous electrochemical control to detect the redox states of single Cu-azurin molecules on gold substrates as a function of the electrode potential for the investigation of dimensional variations within Cu-azurin layers.

6.1.2 A redox active metalloprotein - azurin

Azurin from *Pseudomonas aeruginosa* has been selected for the current study. Azurin is a small blue single-copper protein (128 amino acids, molecular mass 14.6 kDa) which acts as an electron transfer agent in the respiratory chain of denitrifying bacteria.²⁸ Azurin from *Pseudomonas aeruginosa* has been extensively studied and explored as model for the study of electron tunneling through metalloprotein molecules.^{21, 22, 28-49} Due to its high stability and electrochemical properties (~500,000 fully reversible redox cycles have been reported⁵⁰) it has been identified as a superior target for nanoscale bioelectronic devices. Azurin displays a strong opto-electrical coupling⁵¹; in its oxidized state (Cu^{2+}) it shows an intense ($\epsilon = 5700 \text{ M}^{-1}\text{cm}^{-1}$) ligand-to-metal-charge transfer transition in the visible region at ~600 nm, conferring to Cu-azurin's blue color. Azurin can be self-assembled directly on gold substrates by chemisorption via its solution exposed disulfide group,^{30, 46} or alternatively, by binding via a hydrophobic patch around the copper center to alkanethiol self-assembled monolayers on gold.⁵²

Figure 6.1 Cartoon representation of Cu-azurin from *Pseudomonas aeruginosa*. Structural information from PDB file 1E5Y. The active redox site is located about 0.8 nm below the surface and a solvent accessible disulfide bridge is formed between Cys3 and Cys26 side chain thiols.

The three dimensional structure of Cu-azurin from *Pseudomonas aeruginosa* based on crystallographic data is shown in Figure 6.1. As reported earlier, Cu-azurin molecules could be well organized to form monolayers on metal surfaces, due to its disulfide group (Cys3Cys26).^{23-25, 32, 46, 53}

A disulfide bridge locates in a region of the protein opposing to the copper active site and is fully accessible for chemisorption onto a gold electrode, as schematically represented in Figure 6.2. The azurin molecules immobilized on bare Au(111), evaluated by TM-AFM, have reduced vertical size compared to the value derived from crystallographic data.³⁸

Figure 6.2 Cu-azurin molecular orientation on bare Au(111) when protein is anchored via disulfide group.

6.1.3 Atomic force microscopy

Before we discuss our own work for this Chapter, basic principles of AFM, peak force tapping mode and EC-AFM will be briefly introduced.

6.1.3.1 Basic principles of AFM

AFM was introduced in 1986⁵⁴ and soon hereafter the first results using AFM on biological samples were published.^{55, 56} It is important to mention that the invention of the optical lever detection principle for AFM^{57, 58} was pivotal for measurements in liquid environment which is a fundamental prerequisite to retain biological function. This makes the AFM a versatile tool which allows imaging the sample surface with nanometer resolution. Biological systems can be studied by AFM under physiological conditions without labeling or staining of the samples.⁵⁹⁻⁶³

In AFM an ultrasharp tip, with a radius of a few to tens of nanometers, is scanned with nanometer precision over the sample, giving a three-dimensional topography image of the sample surface (sketch shown in Figure 6.3). In general, the AFM tip is supported on a 100-200 μm long force-sensing cantilever. Interactions between tip and sample induce bending of the cantilever which is measured typically through a laser deflection according to the

above mentioned optical lever detection principle.^{57,59} This laser deflection is measured via a 4-quadrant photodetector enabling the detection of height changes with nanometer spatial resolution. Accordingly, the feedback loop electronics respond to detected height changes. In this way, a deflection of the cantilever remains constant. The vertical movement of the tip, which occurs through feedback loop operation, is translated into a topographical image of the surface with an accuracy of ~ 1 nm. The above described AFM imaging mode is termed as contact mode, as the tip is in permanent contact with the sample and scans with constant force. Lateral forces can occur during scanning, resulting in severe distortion and even mechanical damage of the sample. Obviously, this can create severe problems for the imaging of soft and fragile structures, such as individual isolated nucleic acids adsorbed to surfaces. In this regard, the invention of the intermittent contact mode or tapping mode (TM) AFM has been a significant improvement of the AFM instrument.⁶⁴

Figure 6.3 Functioning principle of AFM.

TM-AFM operates by vibrating the cantilever at a frequency close to its resonance frequency. A piezoelectric modulation with an amplitude in the range of nanometers is applied. In this manner, there is only intermittent contact with the sample. When interaction between the tip and the sample varies, both amplitude and phase of the cantilever oscillation change as the tip approaches or retracts from the sample surface.⁶⁴⁻⁶⁶ TM-AFM imaging is performed under a constant vibration amplitude of the cantilever. Lateral forces are significantly reduced in TM-AFM, resulting in a much gentler mode of AFM imaging compared to contact mode.⁶⁷ The phase signal depends on adhesive and dissipative properties and provides a qualitative approach for surface mechanical properties of soft sample surfaces, for instance, biological sample surfaces.⁶⁸

TM imaging was a major development in AFM technology. It has been widely applied to address numerous biological questions. In particular, it has been successfully applied to image isolated and soft objects such as single biomolecules, biological membranes and lipid bilayers.^{67, 69} However, it has not allowed one to obtain quantitative mechanical maps because the phase signal is related to the energy dissipation of the tapping tip.⁷⁰

6.1.3.2 AFM based mechanical mapping – peak force tapping

Despite the tremendous progress in AFM technology development, it has remained difficult to obtain quantitative mechanical maps of soft matter with high resolution. Traditionally, for this purpose force volume imaging has been utilized for soft matter and biological samples.^{71, 72} In principle, force curves are recorded across a sample surface at respective locations to which these are assigned. Thus, a force volume image contains discrete force curves corresponding to locations at which these force curves were probed. From these force curves, mechanical parameters can be derived in a quantitative fashion utilizing appropriate mechanical models. Severe drawbacks of force volume imaging are related to the low time resolution and poor lateral resolution. There is an ongoing effort in enabling quantitative mechanical imaging with high resolution, including high temporal resolution and high spatial resolution. Various approaches are under development, including higher harmonic frequency analysis, contact resonance imaging and peak force tapping.^{73, 74}

Peak force tapping may be considered as a novel type of force volume imaging with enhanced time and lateral resolution. In peak force tapping mode, the sample is oscillated at a typical frequency of 2 kHz with amplitudes in the regime of 10-150 nm.⁷⁵ Each time the AFM tip taps the sample surface, force curves are recorded, averaged and processed in real-time to derive mechanical mappings. While in TM-AFM the feedback loop keeps the cantilever vibration amplitude constant, peak force tapping controls the maximum force on the tip. This has the additional advantage that tip and sample are protected from damage by minimizing the tip-sample contact time.

Fundamental breakthroughs in AFM based mechanical property mapping have been recently achieved.⁷³ Real-time simultaneous topographic and mechanical mapping with high spatial resolutions down to the molecular level have been demonstrated on short DNA nucleotides and various membrane proteins, such as bacteriorhodopsin.^{76, 77} In addition to topography of sample under detection, mechanical properties such as stiffness, adhesion and energy dissipation can be simultaneously mapped.^{78, 79}

6.1.3.3 Electrochemical AFM

Stimulus responsive surfaces, which switch their physical and chemical properties upon changes in the external environment (temperature, pH, solvent, radiation and

electrochemical potential etc.), offer great possibilities in many technological areas.⁸⁰⁻⁸² Redox processes offer an effective way to control the interfacial properties (such as morphological and mechanical properties).^{27, 83} The combination of AFM with electrochemistry (EC-AFM) allows one to simultaneously monitor *e.g.* voltammetric behavior and the variations of surface structure and properties triggered by electrochemical response. For this purpose, the substrate surface under detection serves as working electrode in an electrochemical circuit.⁸⁴ The EC-AFM measurement cell is further equipped with working and counter electrodes to enable potentiostatic control like in a conventional three electrodes configuration. In this manner, changes in topography and mechanical properties with respect to an electrochemical process can be studied down to the molecular or even atomic scale.⁸⁴ Figure 6.4 shows the schematic setup of EC-AFM.

Figure 6.4 Functioning principle of EC-AFM.

EC-AFM has been applied to study the responsiveness of redox active materials, like redox active SAMs, redox active polymer layers, single redox active molecules and biological films.^{83, 85-89} For instance, Fukui and Umeda studied the redox state dependent reversible local structural change of ferrocenyl-terminated molecular islands by electrochemical frequency modulation AFM.^{83, 90} This AFM mode utilizes very small amplitudes and thus operates at extremely low imaging forces with minimal perturbation of any fragile structure. Interestingly, these authors found that the apparent height of the Fc islands increased by about 0.44 nm accompanied by the oxidation of the terminal Fc groups.⁹⁰ They explained this observation by formation of an ionic double layer where ClO_4^- ions are strongly bound to the Fc groups. In accordance the energy dissipation displayed significant changes upon oxidation, as well.

Vancso and coworkers performed intensive studies on the responsive behavior of Poly(ferrocenylsilane) (PFS) polymer layers, grafts and single redox molecules by EC-AFM.^{88, 89, 91-94} It was found that the height depends on the potential applied, revealing a ~10% height expanded morphology (swelling) of PFS upon oxidation.⁹² The change of adhesive properties was observed upon oxidation. The change in single molecule mechanical properties was detected *in-situ*, establishing one of the first concepts of a redox-driven electromechanical motor.⁹²

Hao *et al.* studied the electrochemical behavior of the redox responsive metalloenzyme copper nitrite reductase on Au(111)-electrode surfaces modified via a cysteamine monolayer.²⁷ They found that the enzyme showed no voltammetric signals in the absence of nitrite substrate. In contrast, a strong reductive electrocatalytic signal appeared in the presence of nitrite. This was interpreted by improving the enzyme/electrode contact upon substrate binding, or opening intramolecular electron-transfer channels between the redox center for electron inlet and the catalytic site for nitrite reduction. Interestingly, by using EC-AFM the authors found that the voltammetric signals were in concert with a clear increase (swelling) of the molecular height when the electrochemical potential traversed the region from resting to the electrocatalytically active redox enzyme function in the presence of nitrite. Importantly, no change in size was observed in the absence of nitrite over the same potential range.

In a number of studies Facci and coworkers investigated the metalloprotein azurin on gold substrate electrodes.²²⁻²⁵ By using EC-STM, these authors found that the apparent height of a single azurin depended on the redox state of the protein.²³ In contrast, using contact mode AFM in an EC-AFM combination they did not observe any height changes for single protein molecules as function of its redox state.²² This is probably due to the relative high imaging force which was adjusted to 1 nN (at 2–4 Hz scan rate) which lead to compression of the molecules, making the detection of a potential-driven height change beyond reach.

As mentioned earlier, peak force tapping enables one to map soft biological samples with high spatial resolution. To measure the true height in dependence of the redox potential of azurin immobilized on Au(111), we use peak force tapping in combination with electrochemistry. CV of Cu-azurin on bare gold electrodes was performed to assess the functionality of protein molecules adsorbed on gold substrates and their proper wiring with the electrode surface. Using EC-AFM (AFM operated in peak force tapping mode), azurin metalloprotein films on bare gold electrodes were studied down to the molecular level. This combination of techniques indicated that Cu-azurin molecules adsorbed on bare gold electrodes preserved their morphological characteristics and redox properties. Moreover, *in-situ* EC-AFM provided information on their morphological characteristics, focusing on how apparent height changes upon the variation of the surface potential.

6.2 Results and discussion

Azurin molecules were immobilized to Au substrates as described in the experimental section (see Figure 6.7). Layers consisting of single molecules were then imaged by AFM under electrochemical control.

The voltammograms obtained exhibited anodic (+0.250 V) and cathodic (+0.187 V) peaks, which were separated by 0.063 V. This value is close to the value reported for an ideal one electron transfer reaction (0.060 V) (for electrochemical results see Figure 6.5).⁹⁵ The two peaks that can clearly be observed correspond to the reduction and oxidation of Cu-azurin. This indicates that the electron transfer process is not influenced by direct adsorption on gold and thus the protein is wired to the electrode surface.

The heights of the anodic and cathodic peak current for the CV curves shown in Figure 6.5b were plotted as a function of the scan rate (see Figure 6.5c). The plotted peak current against the voltage scan rate shows a linear dependence. This characteristic indicates that the redox process is controlled by charge-transfer kinetics⁹⁶ and confirms that Cu-azurin were immobilized on the gold surface (for a diffusion limited process a dependence on the square root of the scan rate is expected).⁹⁷ An estimate of the surface coverage (Γ) with electroactive Cu-azurin molecules can be obtained from Equation 6.1,

$$\Gamma = \frac{Q}{nFA} \quad (6.1)$$

where Q is the charge passed for the oxidation and reduction of Cu^{2+} centers, n is the number of electrons involved in the electron transfer process ($n = 1$), F is the Faraday constant and A is the geometric area of the electrode. The charge passed in the redox reaction was determined by integrating the areas under the redox peaks. By analyzing the CV curves, the estimated surface coverage for Cu-azurin is 1.7×10^{12} molecules/cm², which is similar to the reported values.²⁹

The morphology of Cu-azurin molecules electrostatically adsorbed on mica was characterized by AFM to obtain first morphological information on the protein at molecular level. This SPM technique enables on the one hand precise determination of the height of biomolecules adsorbed on the substrate, on the other hand for the determination of lateral dimensions, the broadening effects introduced by the dimension of the tip (tip convolution) must be taken into account. The typical protein lateral dimension, as evaluated from the full width at half maximum of the molecular cross-section profile (Figure 6.6b), is in the range 25 - 35 nm, considerably larger than the crystallographic data,³⁸ due to the above mentioned tip broadening. Multiple-molecular aggregation into structures of such size cannot be excluded but discrepancies of the order observed have been noted elsewhere for AFM imaging of soft biological material.^{34, 47}

Figure 6.5 (a) CV for Cu-azurin immobilized on Au(111) (prepared by incubating the Au(111) electrode in 0.1 mM Cu-azurin in 50 mM NH_4Ac (pH = 4.6) for 1 hour followed by gently rinsing with Milli-Q water) at a scan rate of 50 mV/s. (b) CVs for Cu-azurin immobilized on Au(111) at different scan rates $v = 10, 20, 30, 40, 50, 60, 70, 80, 90$ and 100 mV/s. (c) Plot of peak currents versus scan rate for the CV curves in (b). (d) CVs for Zn-azurin immobilized on Au(111) (prepared in a similar fashion as in (a)) at a scan rate of 50 mV/s. (e) CVs of Cu-azurin and Zn-azurin in the same potential window. All CVs were recorded in 50 mM NH_4Ac aqueous electrolyte (pH = 4.6), reference electrode Ag/AgCl, counter electrode Pt.

Figure 6.7a-b show AFM images of single Cu-azurin molecules adsorbed on Au(111) in oxidized (potential = 0.45 V) and reduced (potential = -0.10 V) states, respectively; the molecules remained stable adsorbed on the substrate upon repetitive scans. According to the counts of single particles in the AFM images for Cu-azurin on Au(111), the surface coverage is 5×10^{11} molecules/ cm^2 , and this is in a similar regime as the coverage calculated from the peak area of the CV curves as mentioned before. We assume that the slight discrepancy between CV and AFM particle values found in our case might originate from

surface heterogeneities, which can only be detected by AFM, while voltammetry gives one signal over the entire surface.

Figure 6.6 (a) Cu-azurin on mica imaged by peak force tapping mode AFM image 50 mM NH_4Ac buffer and (b) corresponding height profile on the protein particle indicated with the white line.

Figure 6.7 AFM images of Cu-azurin immobilized on Au(111) (made by incubating the Au(111) electrode in 0.1 mM Cu-azurin in 50 mM NH_4Ac for 1 hour followed by gently rinsing with Milli-Q water) in oxidized (potential = 0.45 V) (a) and reduced (potential = -0.10 V) (b) states, followed by another image obtained in the oxidized state (c). The *in-situ* electrochemical AFM measurements were carried out in 50 mM NH_4Ac aqueous electrolyte (pH = 4.6), reference electrode Ag, counter electrode Pt.

Experimental evidence that indicated the increase in the apparent height of Cu-azurin immobilized on Au(111) by electrochemical oxidation of the copper ion was reported by using various techniques, including STM.²²⁻²⁵ However, STM operating in constant current mode almost always underestimates the physical height of the assembled proteins on Au(111) surface with respect to other techniques (crystallography, AFM and ellipsometry).^{22, 30, 32, 33, 95, 98-101} To measure the ‘true height’, height changes (height change = height in the oxidized state – height in the reduced state) between the oxidized state (+0.45 V) and the reduced state (-0.10 V) for both Cu-azurin and Zn-azurin were detected by AFM. The increase in apparent height by oxidation to Cu-azurin (Cu^{2+}) can be derived from the height histogram (see Figure 6.9a). The average height of Cu-azurin increased by 0.32 nm as a result of the oxidation. The origin of the height change of the Cu-azurin can be attributed to the following factors: conformational changes, and the variation in the orientation of immobilized proteins between the oxidized and reduced states. To verify that height changes indeed originate from the redox states of azurin, Zn-substituted azurin was utilized as a control. Figure 6.8 shows AFM images of single Zn-azurin molecules adsorbed on Au(111) at a surface potential of 0.45 V (Figure 6.8a) and -0.10 V (Figure 6.8b), respectively. The protein layer on Au(111) was stable upon repetitive scans. As is shown in Figure 6.9b, control experiments with non-redox active Zn-azurin show negligible or minimal height modulation with applied potential, confirming that the difference found for Cu-azurin are due to its redox properties. The Zn-azurin reference gives us strong support that double layer charging effects are neglectable, as both macromolecules, Zn-azurin and Cu-azurin, have the same structure (amino acid sequence – secondary and tertiary structure are exactly the same, including the disulfide bridges) and all the over charge on exposed protein domains is not changing as the redox centers are buried inside the macromolecules and shielded with the same protein domains. The remaining explanations are indeed conformational and orientational changes which are due to the redox induced physiological conformational change and “non-physiological” which may as well be a result of the strong electrical wiring and thus coupling of Cu-azurin with the electrode. This results in strong electro-mechanical coupling which might as well induce orientational changes (only for Cu-azurin) and explain the observed apparent height changes and *vice versa* the surface potential control thereof.

Figure 6.8 AFM images for Zn-azurin immobilized on Au(111) (prepared by incubating the Au(111) electrode in 0.1 mM Zn-azurin in 50 mM NH_4Ac for 1 hour followed by gently rinsing with Milli-Q water) in ‘oxidized’ (potential = 0.45 V) (a) and ‘reduced’ (potential = -0.10 V) (b) states. The *in-situ* electrochemical AFM measurements were carried out in 50 mM NH_4Ac aqueous electrolyte (pH = 4.6), reference electrode Ag, counter electrode Pt.

Figure 6.9 Histograms of the height changes (height change = height in the oxidized state – height in the reduced state) between the oxidized state (+0.45 V) and the reduced state (-0.10 V) for (a) Cu-azurin and (b) Zn-azurin. To compare the height in different redox states at a similar tip condition, two AFM images at the same area were used for the analysis for (a) Cu-azurin (Figure 6.7) and (b) Zn-azurin (Figure 6.8), respectively. Data were obtained from individual cross section profiles over 120 molecules.

Figure 6.10 Histograms of the height changes of Cu-azurin in Figure 6.7a compared to Figure 6.7c. Data are obtained from individual cross section profile over 100 molecules.

Another point we would like to address here is that the height of the Cu-azurin could be modulated by changing the applied potential of the sample, as indicated by the experimental data shown above. To demonstrate if the height could be tuned reversibly via the potential, another AFM image in the oxidized state (see Figure 6.7c) was recorded after completing one redox cycle [see oxidized (potential = 0.45 V) Figure 6.7a and reduced state (potential = -0.10 V) Figure 6.7b]. The height difference (see histogram shown in Figure 6.10) between the Cu-azurin molecules in the two oxidized state images (Figure 6.7a and Figure 6.7c) has been calculated. As seen from the histogram, essentially no height difference for the Cu-azurin molecules in those two images in oxidized states (Figure 6.7a and Figure 6.7c) was found, indicating that the height of the Cu-azurin immobilized on Au(111) could indeed be modulated by the surface potential.

6.3 Conclusions

The redox functionality of Cu-azurin molecules immobilized on gold electrodes was assessed by CV. The voltammetric response of the adsorbed proteins was well defined and stable, demonstrating wiring of the protein to the electrode surface. The measured redox potential agrees with the formal potential reported in the literature. *In-situ* EC-AFM study revealed a height difference of 0.32 nm between the oxidized and reduced states for Cu-azurin. The origin of the height change of the Cu-azurin could be attributed to conformational changes and redox driven molecular orientation effects. No height changes were detected for Zn-azurin which is non-redox-active, thus confirming that the height variation originates from the redox response. We also found that the height of the Cu-azurin immobilized on Au(111) could be modulated by the applied potential. *In-situ* EC-AFM (peak force tapping mode) could then be a tool for studying the stimulus responsiveness (height and mechanical properties) and heterogeneity of metalloprotein film at electrodes at the single molecule level.

6.4 Experimental section

Materials

Cu-azurin and Zn-azurin (both with a molecular mass of 14.6 kDa) from *Pseudomonas aeruginosa* were provided by the research group of Prof. dr. Gerard W. Canters from Leiden University and used without further purification. More information of Cu-azurin can be found in a research work reported by Gerard W. Canters and co-workers.⁴⁰ Zn-azurin was prepared according to reported procedures.³⁷ Cu-azurin (isoelectric point pI = 4.6) is electrochemically active while Zn-azurin is not. The amino acid sequence of the protein and secondary and tertiary structure are exactly the same for both proteins, referring to Cu-azurin and Zn-azurin.^{32, 53, 102, 103} All electrochemical measurements including *in-situ* EC-

AFM were done in 50 mM NH_4Ac (ammonium acetate, Sigma) buffer, pH = 4.6. The buffer was degassed with N_2 prior to use. Milli-Q grade water (resistivity 18.2 $\text{M}\Omega$) was used throughout all the experiments.

Substrates

Au substrates (11 mm $\times$ 11 mm, 250 nm Au on 2 nm Cr on borosilicate glass) for STM measurements were purchased from Arrandee (Werther, Germany). Au(111) samples were obtained by annealing these substrates in a high purity H_2 flame for 5 minutes.¹⁰⁴ Prior to use, these substrates were cleaned in a piranha solution (7:3 H_2SO_4 : H_2O_2 (30%) by volume), followed by rinsing with Milli-Q water and ethanol and dried in a nitrogen stream. **(Caution: Piranha solutions should be handled with extreme caution: it has been reported that these solutions can detonate unexpectedly).** Mica substrates, with a diameter of 15 mm, for AFM measurements were purchased from SPI supplies (West Chester, USA) and cleaved with Scotch tape just before azurin adsorption.

Sample preparation

The sample preparation procedure is shown in Figure 6.11. Freshly prepared Au(111) substrates were incubated in 10^{-4} M Cu-azurin or Zn-azurin solution for 1 hour and then rinsed in abundant NH_4Ac buffer directly in the measuring cell, leaving always an aqueous layer on the top of the substrate to prevent protein exposure to water surface tension. After several rinsing cycles the measuring cell was filled with the same buffer and immediately installed in the microscope for imaging (or for CV measurements).

Figure 6.11 Sample preparation procedure.

Cyclic voltammetry

CV measurements were carried out with metalloprotein films on gold substrates in aqueous 50 mM NH_4Ac (pH = 4.6) buffer, using a Model 800D Series electrical Analyzer (CH instrument Inc., USA). Cyclic voltammograms were recorded between -0.5 V and $+0.7$ V at different scan rates, using a Ag/AgCl reference electrode and a Pt counter electrode.

Atomic force microscopy

A Multimode AFM with a NanoScope V controller and NanoScope version 8.15 software (Bruker Nano Surfaces, Santa Barbara, CA) was operated in peak force tapping mode to obtain the surface morphology of the protein layers.

***In-situ* electrochemical atomic force microscopy**

AFM measurements were performed in a combined electrochemistry AFM setup using a Multimode 8 AFM (Bruker Nano Surfaces, Santa Barbara, CA, USA) with a NanoScope V controller and an electrochemical liquid cell in combination with a Model 800D Series electrical Analyzer (CH instrument Inc., USA). Commercially available ScanAsyst Fluid+ probes with silicon tips on silicon nitride cantilevers (Bruker AFM probes, Camarillo, CA, USA) were used for the measurements. The electrochemical AFM was operated in peak force tapping mode. Cantilever spring constants were determined using the thermal tune method¹⁰⁵ and showed values in the range of ~0.6–0.7 N/m. The electrochemical measurements were performed in a three electrode arrangement in aqueous 50 mM NH₄Ac (pH = 4.6) buffer solution. The gold substrates with immobilized protein layers were used as the working electrode, respectively. The Ag wire and Pt wire functioned as the reference and counter electrodes which were mounted to the AFM electrochemical liquid cell. Cyclic voltammograms were recorded between –0.5 V and +0.7 V vs. Ag wire with a scan rate of 50 mV/s. Prior to the experiments, the electrolyte was degassed by purging the solution with nitrogen gas for 20 min.

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Outlook

This Thesis describes my research during the four years period I spent at the University of Twente as PhD student. The focus of this work is placed on the dynamics of organic thin layers as explored by scanning probe microscopy (SPM) techniques, including time-resolved scanning tunneling microscopy (TR STM) and electrochemical atomic force microscopy (EC-AFM). Dynamic processes play an essential role in important fields like catalysis, thin-film growth and sensor technology.^{1, 2} The structural and dynamical properties of organic thin layers are of interest from both, a fundamental as well as an application's point of view. The ultimate utility of SAMs for the aforementioned applications will be critically dependent on their structural and dynamical properties, for instance, conformational changes upon stimuli (light, pH, temperature and electrochemical potential etc.) and phase transitions. The time scales of the dynamical events that can be detected by STM cover a range from a few minutes to a few microseconds. In our work by performing current-time spectroscopy, the time resolution of STM has been improved to ~10 μ s. Below, we discuss a few follow up directions for further explorations of surface dynamics.

As addressed in Chapter 2, it is of fundamental interest to examine the dynamics of molecules. Using TR STM, the dynamics of three kinds of SAMs on Au(111) have been studied. Molecular conformational changes and phase transitions of other SAMs on Au(111) surfaces could be traced with TR STM measurements to further elucidate understanding of these systems.

Similarly, dynamics of the mixed SAMs could be explored by TR STM as well. For instance, 4-[4'-(phenylethynyl)-phenylethynyl]-benzenethiol (hereafter referred as monothiol OPE) molecules embedded in a dodecanethiolate monolayer were reported to exhibit stochastic conductance switching behavior at room temperature.³ Donhauser *et al.* reported the spontaneous changes in height of the inserted monothiol OPE molecules between subsequent topographic STM images.³ These height fluctuations were interpreted in terms of a stochastic conductance switching, caused by the conformational changes of the inserted monothiol OPE molecules.³ It is argued by Ramachandran *et al.* that this switching is caused by changes in the bond between the thiol of the inserted molecules and the gold substrate.⁴ This switching behavior was referred to as 'the blinking of a thiol-gold bond' by Ramachandran *et al.*⁴ Hallbäck *et al.*, however, related the 'blinking' effect of the monothiol OPE molecules to lateral diffusion and exchange of conjugated molecules, which are embedded as small bundles within the matrix SAMs.⁵ Despite the fact that the

‘blinking’ effect of the monothiol OPE molecules embedded in alkanethiols SAMs has been intensively studied, the mechanism of this phenomenon remains unclear. Open loop STM enables one to position the tip apex above the protruding islands, which are monothiol OPE molecules embedded in alkanethiols SAMs. Thus we expect that such additional experiments would further help an enhanced understanding as dynamics occurring during the ‘blinking effect’ could be monitored by performing current-time spectroscopy. Thus, the mechanism behind this ‘blinking’ phenomenon could be revealed.

In addition, dynamics of stimulated responsiveness of surfaces (temperature and light etc.) can be monitored by TR STM. As dynamic processes play an essential role in important fields like catalysis, thin-film growth and sensor technology,^{1, 2} it is essential to understand the dynamics in these processes. I-t spectroscopy is a potential tool to track the catalytic reaction and thin film growth.

It is a common recognition that the understanding of three-dimensional structures of protein molecules is essential for revealing their function and pave the way for their potential future applications. In Chapter 6, the morphological changes of molecular metalloprotein layers stimulated by redox potential was studied by EC-AFM in peak force tapping mode. EC-AFM in peak force tapping mode also offers the possibility to study the mechanical properties and potential changes thereof induced by stimuli. Moreover, the EC-AFM work elucidated in Chapter 6 could be applied to other redox active soft matters, including organic layers, polymers and other biological molecules which are potential candidates for nano-devices.

Compared to the high time resolution that has been achieved with STM, the time resolution of the AFM is significantly lower. Yet by delivering complementary information to STM, also this technique has been useful to better understand surface dynamics. To further enhance this understanding, it is essential to track the real time height fluctuations and variation of the interactions between the AFM tip and the sample surface down to the single molecular level in various environments. Therefore, the time resolution of the AFM should be improved. Time resolution of a standard STM has been improved to 10 μ s – 20 μ s by carrying out open loop STM measurement, *i.e.* current-time spectroscopy.⁶ A similar strategy used for enhancing the time resolution of STM could be applied to AFM. In this approach, the AFM tip can be positioned above the particle of interest with the feedback loop disabled. As such, the height fluctuations of the particle under detection, Cu-azurin for instance, can be obtained. Another bio-molecular system of interest would be ferritin. It was observed with high-speed AFM imaging that ferritin molecules, which are not densely immobilized on substrates, are moving freely on the surface.⁷ Molecular imaging could be applied to localize the active site of this biomolecule. By parking the tip above the active sites, dynamical events of the ferritin can be detected. In TR STM measurements, there are several quantities that can be studied by the I-t traces, for instance the distribution of residence times and rates. In addition, the energy difference between different molecular

conformations studied can be acquired from the I-t traces. Similar attempts could be made with the data from AFM measurements with high temporal resolution.

As described in Chapter 6, the height of the redox active Cu-azurin molecules could be triggered by the electrochemical potential. Following this concept, EC-AFM could then be a tool for molecular recognition. By checking the heterogeneity of the mixed SAMs, Cu-azurin and Zn-azurin, recognition of the molecules can be accomplished. These concept can then be adapted to many other similar molecular systems in which one molecule is stimuli-responsive (electrochemical potential, pH and light etc.) while the other molecule is not. This strategy will open up the possibility of real time and *in-situ* dynamical studies from both fundamental and application's perspectives. Regarding the fundamental aspect, the diffusion, aggregation and nucleation of particles as well as their interactions with the surroundings can be examined. With respect to the application's aspect, this concept can be employed in searching the site of interest in many research fields, for instance catalysis.

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Summary

Organic thin layers, especially self-assembled monolayers (SAMs), on well-defined solid surfaces have attracted tremendous attention owing to their interesting physical and chemical behavior as well as potential applications. The aim of this PhD thesis was to study the structural properties, electronic properties and dynamical process of SAMs. The objective was achieved by using scanning probe microscopy techniques, like time-resolved scanning tunneling microscopy (TR STM) and electrochemical atomic force microscopy (EC-AFM), with high spatial resolution and time resolution. Employing a current-voltage (I-V) converter with a large bandwidth, current-time spectroscopy, while the feedback loop disabled, can be recorded (hereafter referred as I-t traces). In this way, a high temporal resolution for STM can be achieved. This makes STM a versatile tool for the characterization of the structure, electronic properties and dynamics of SAMs. Recently developed peak force tapping AFM enables one to measure soft biological samples with high spatial resolution and minimal damage for the sample. With the combination of peak force tapping mode in EC-AFM, morphological responsiveness of redox-active metalloprotein azurin molecular layers were investigated.

The motivation and the introduction of the topics that were covered in this thesis are presented in **Chapter 1**.

In **Chapter 2**, a literature overview of SAMs and their dynamics as studied by TR STM were described. Firstly, fundamental aspects, potential applications, growth mechanisms and structure of SAMs on Au(111) were introduced. Secondly, the basics and spectroscopic methods of STM were briefly introduced. More importantly, developments of the temporal resolution of STM and its application in studying the dynamics of SAMs were surveyed.

Based on TR STM measurements, energetics and dynamics of decanethiol SAMs on Au(111) surfaces were ascribed in **Chapter 3**. It was revealed that the massive dynamics of the decanethiol SAMs were due to diffusion of decanethiol-Au complexes, rather than the diffusion of individual decanethiolate molecules. In addition, the boundary free energies between the disordered and order phases were studied using a statistical analysis of the thermally induced meandering of the domain boundaries. On the basis of these results, it is possible to accurately predict the two-dimensional phase diagram of the decanethiolate/Au(111) system.

Chapter 4 focused on the ordering and dynamics (including phase transition and conformational changes) of monothiol oligo(phenylene ethynylene) (termed as OPE

hereafter) SAMs and dithiol OPE SAMs. It was revealed that the monothiol OPE molecules at the edges of the vacancy lines exhibited dynamic behavior and frequently jump back and forth between neighboring stripes. I-t traces recorded on dithiol OPE SAM on Au(111) suggested that the molecules continuously switch back and forth between two nearly degenerate configurations.

In **Chapter 5**, current-voltage (I-V) spectroscopy and current-distance (I-Z) spectroscopy were applied to study the transport properties of a single octanethiol molecule in the temperature range from 77 K to room temperature. The conductance of octanethiol is temperature independent, demonstrating that either quantum mechanical tunneling or ballistic transport is the main transport mechanism.

In **Chapter 6**, stimuli responsiveness (via changing the electrochemical potential) of the redox-active metalloprotein Cu-azurin on Au(111) surface was investigated by *in-situ* EC-AFM. It was revealed that the height of the Cu-azurin can be modulated by the surface potential.

Finally, in the **Outlook**, directions for future research were provided. For instance, TR STM could be employed to study dynamics of mixed SAMs, which is under debate to date. Another future direction, related to the dynamics of organic layers on surfaces, would be to improve the time resolution of AFM.

The bulk part of this Thesis describes the dynamic of SAMs from a true molecular perspective by using TR STM. Deep insights discovered in the dynamics of the SAMs, down to the single molecular level, were obtained. In addition, monitoring molecular dynamics of responsive systems with TR STM, EC-AFM was also attempted with success to investigate the responsiveness, for instance the redox responsiveness of redox active molecules like azurin. We documented that by single molecular imaging, electrochemical potential stimulated apparent height changes can be resolved. Our work in this thesis further enhances knowledge of dynamics of SAMs and other molecular systems, which will find applications in molecular devices.

Samenvatting

Organische dunne films, en in het bijzonder zelf-organiserende monolagen (self-assembling monolayers, of SAMs), op goed gedefinieerde oppervlakken hebben enorm veel aandacht getrokken door de interessante fysische en chemische eigenschappen alsmede de potentiële toepassingen. Het doel van dit proefschrift was het bestuderen van de structurele en elektronische eigenschappen alsmede de dynamische processen van zelf-organiserende monolagen. Dit doel is bereikt door middel van het gebruik van scanning probe technieken, zoals tijd-opgeloste scanning tunneling microscopy (TR STM) en elektrochemische atomic force microscopy (EC-AFM). Stroom-tijd spectroscopie (hierna genoemd I-t) kan een veel hogere tijd resolutie halen dan de standaard STM, doordat de feedback wordt uitgeschakeld. De resolutie in de tijd wordt dan bepaald door de bandbreedte van de stroom-voltage (I-V) omvormer waardoor de STM een veelzijdig gereedschap wordt voor de karakterisatie van zowel de structurele als elektronische eigenschappen alsook dynamische processen. De recent ontwikkelde peak force tapping AFM (PF Tapping-AFM) geeft de gelegenheid om een zacht biologisch monster te meten met hoge ruimtelijke resolutie en met minimale schade. De combinatie van PF Tapping- en EC-AFM maakt het mogelijk om de morfologische responsiviteit van een dunne film van het redox-actieve metalloproteïne azurin te bestuderen. De motivatie en een introductie van de verscheidene onderwerpen die worden behandeld in deze dissertatie zijn te lezen in **Hoofdstuk 1**.

Hoofdstuk 2 geeft een literatuur overzicht van het onderzoek aan de dynamische processen van SAMs door middel van TR STM. Eerst worden de fundamentele aspecten, potentiële toepassingen, groeimechanismen en de structuur van SAMs op Au(111) oppervlakken geïntroduceerd. Vervolgens worden de theorie en de spectroscopische methoden van STM belicht. De nadruk ligt hierbij op de verbetering van de tijd resolutie van de STM en de toepassing hiervan in het bestuderen van de tijdafhankelijke dynamica van SAMs.

Aan de hand van de TR STM metingen van decaanthiol SAMs op Au(111), worden de energetica en dynamica van dit systeem behandeld in **Hoofdstuk 3**. In dit hoofdstuk wordt beschreven hoe de dynamica van de decaanthiol SAMs wordt veroorzaakt door diffusie van decaanthiol-Au complexen in plaats van de diffusie van individuele decaanthiolen. Daarnaast worden de vrije energieën van de grens tussen de ongeordende en geordende toestand bestudeerd met behulp van een statistische analyse van de thermisch geïnduceerde slingeren van de domeingrenzen. Op basis van deze resultaten is het mogelijk om een

nauwkeurige voorspelling te geven van het tweedimensionale fase diagram van het decaanthiol/Au(111) systeem.

Hoofdstuk 4 richt zich op de ordening en dynamica (inclusief faseovergangen en conformatie veranderingen) van monothiol en dithiol oligo(phenylene ethynylene) (hierna aangeduid als OPE) SAMs. Hieruit blijkt dat de monothiol moleculen op de randen van de vacaturelijnen dynamica vertonen, waarschijnlijk veroorzaakt door het heen en weer diffunderen tussen naastgelegen strepen. I-t metingen opgenomen op de dithiol OPE SAM suggereren dat de moleculen continu heen en weer switchen tussen twee bijna ontaarde configuraties.

Hoofdstuk 5 beschrijft het transport door een enkel octaanthiol molecuul in het temperatuurgebied van 77K tot kamertemperatuur met behulp van een combinatie van stroom-voltage (I-V) en stroom-afstand (I-Z) spectroscopie. De geleiding van het octaanthiol is temperatuur onafhankelijk, hetgeen bevestigt dat het transportmechanisme quantum mechanisch tunnelen is.

In **Hoofdstuk 6** wordt de impuls responsiviteit (wanneer de chemische potentiaal wordt veranderd) van het redox-actieve metalloproteïne Cu-azurin bestudeerd door in-situ EC-AFM. Hieruit is gebleken dat de hoogte van een azurin molecuul kan worden gemanipuleerd door de oppervlaktepotentiaal.

Tenslotte worden er in de **Outlook** enige aanbevelingen gedaan voor toekomstig onderzoek. Bijvoorbeeld het bestuderen van de dynamica van gemengde SAMs door middel van TR STM, iets waar tot op de dag van vandaag nog over gedebatteerd wordt.

Het merendeel van deze dissertatie beschrijft de dynamica van zelf-organiserende monolagen vanuit een moleculair oogpunt door gebruik te maken van TR STM. Diepere inzichten zijn verkregen op het gebied van de waargenomen dynamica in SAMs. Naast de tijdafhankelijke metingen aan de dynamica van SAMs is er ook succesvol gebruik gemaakt van EC-AFM om de responsiviteit, bijvoorbeeld de redox responsiviteit van redox actieve moleculen zoals azurin. Door het afbeelden van enkele moleculen, zijn elektrochemische potentiaal afhankelijke hoogte veranderingen waargenomen van de azurin moleculen. Het werk zoals beschreven in deze dissertatie vergroot de kennis van dynamische processen in SAMs en andere moleculaire systemen, hetgeen relevant is voor technologische toepassingen.

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吴海荣

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Curriculum Vitae

Hairong Wu was born on April 21st, 1984 in Shandong, P. R. China. After graduating from No.1 Middle School in Feicheng, she entered the Faculty of Chemical Engineering and Materials Science, Shandong Normal University (Jinan, China) in 2003. Four years later, she obtained her Bachelor degree in Chemistry. In 2008, she started her master study in Graduate University of Chinese Academy of Sciences (Beijing, China), which is now known as University of Chinese Academy of Sciences. In 2009, she joined the research group of Prof. dr. Qinling Bi to pursue her Master degree in the State Key Laboratory of Solid Lubrication, Lanzhou Institute of Chemical Physics (Lanzhou, China), Chinese Academy of Sciences. With a thesis entitled “Tribology performance of Babbitt alloys and copper-based bearing materials under sea water environment”, she graduated in 2011 with the Master degree in Physical Chemistry. From September 1st, 2011, she joined Material Science and Technology of Polymers group and Physics of Interfaces and Nanomaterials group in University of Twente, the Netherlands, as a Ph.D. student under the supervision of Prof. dr. G. Julius Vancso, Dr. Peter M. Schön and Prof. dr. ir. Harold J. W. Zandvliet. Her Ph.D. research subject is surface dynamics of self-assembled monolayers (SAMs) studied by scanning probe microscopy techniques, including scanning tunneling microscopy (STM) and atomic force microscopy (AFM).

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