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Chemistry of Cysteine Assembly on Au(100): Electrochemistry, in situ STM and Molecular Modeling

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Cysteine monolayers on Au(100), adsorption dynamics, network assembling, electrochemical interface, DFT

Cysteine (Cys) is an essential amino acid with a carboxylic acid, an amine and a thiol group. We have studied the surface structure and adsorption dynamics of L-cysteine adlayers on Au(100) from aqueous solution using electrochemistry, high-resolution electrochemical scanning tunnelling microscopy (*in situ* STM), and molecular modelling. Cys adsorption on this low-index Au-surface has been much less studied than Cys adsorption on Au(111)- and Au(110)-electrode surfaces. Chronopotentiometry was employed to monitor the adsorption dynamics at sub-second resolution and showed that adsorption is completed in 30 minutes at Cys concentrations above 100 μM . Two consecutive steps could be fitted to these data. Two separate reductive desorption peaks of Cys adlayers on Au(100) with a total coverage of $2.52 (\pm 0.15) \times 10^{-10} \text{ mol/cm}^2$ were observed. *In situ* STM showed that adsorbed Cys is organized in stripes with “fork-like” features which co-exist in (11×2) -2Cys and (7×2) -2Cys lattices, quite differently from Cys adsorption on Au(111)-electrode surfaces. Stripe structures with bright STM contrast in the center suggest that a second Cys adlayer on top of a first adlayer is formed, supporting the dual-peak reductive desorption of Cys adlayers. In addition, monolayers of both pure L-Cys, pure D-Cys and a 1:1 racemic mixture of L- and D-Cys on Au(100) were studied. Virtually identical macroscopic electrochemical features were found, but *in situ* STM discloses many more defects for the racemic mixture than for the pure enantiomers due to structural mismatch of L- and D-Cys. Density functional theory (DFT) calculations combined with a cluster model for the Au(100) surface were carried out to investigate the adsorption energy and geometry of adsorbed monomer and dimer Cys species in different orientations, with detailed attention to the chirality effects. Optimized DFT geometries were used to construct model STM images, and kinetic Monte Carlo simulations undertaken to illuminate the growth of adsorbate rows and the mechanism of the adlayer formation as well as the Cys adsorption patterns specific to the Au(100)-electrode surface.

1. Introduction

Among the 20 essential amino acids, L-cysteine (L-Cys) ($\text{HO}_2\text{CCH}(\text{NH}_2)\text{CH}_2\text{SH}$) is

the only one with a free thiol (-SH) group in addition to the carboxylate and ammonium groups. L-Cys is crucial in protein chemistry

and other biological function,^{1,2} and widely used in food production, cosmetics, pharmaceutical industries,³ and as a linker molecule for immobilization of metal nanoparticles on gold and graphene electrode surfaces.^{4,5}

The reactivity of L-Cys depends on the local environment.¹ Electrochemistry has long been used in order to understand the formation of cystine with a disulfur bridge (-S-S-), and oxidation of the thiol group to higher sulfur oxidation states such as RSO_3^- .⁶⁻¹² L-Cys on metal electrodes gives a number of voltammetric peaks,^{9,11} but is less active on carbon electrodes and here requires mediator molecules or metallic nanoparticles as electrocatalysts for oxidation or reduction,^{7,10} mainly due to strong adsorption of L-Cys and formation of metal-S chemical bonds. Cys can assemble spontaneously on gold surfaces either in vacuum or in aqueous solution. Depending on solution pH, self-assembled monolayers (SAMs) of Cys provide both carboxylate and amine binding groups on a solid surface, onto which biological molecules such as proteins can be immobilized in well controlled orientation and interfacial electron transfer function retained.^{13,14} Structures and properties of Cys SAMs have been investigated using methods that include

scanning tunnelling microscopy (STM), X-ray photoelectron spectroscopy (XPS), the quartz crystal microbalance, electrochemistry, and infrared spectroscopy both in aqueous solution and ultra-high vacuum (UHV).¹⁵⁻²⁹

Well-defined Cys SAM structures are only achieved on atomically flat substrates, single-crystal gold surfaces in particular.^{17,18,20-25} Low-index crystal facets such as Au(111) and Au(110) have been employed, and molecular or even sub-molecular structural information achieved. STM both in vacuum and liquid environment has reached a level of atomic resolution ideally suitable for visualizing molecular packing.³⁰ Notably the organization of Cys molecules in SAMs is sensitive to the environments. In aqueous solutions, Cys is adsorbed chemically on Au(111) forming highly ordered monolayers with $(\sqrt{3} \times \sqrt{3})\text{R}30^\circ$, $(3\sqrt{3} \times 6)\text{R}30^\circ$, and $(4 \times \sqrt{7})\text{R}19^\circ$ cluster-like networks depending on both supporting electrolyte and pH.^{16,18,19,31} Cys SAM formation is accompanied by lift of reconstruction in the metallic surface structure even though the surface structures underneath the adlayers are different. Physisorption of Cys is found in UHV at room temperature and chemisorption at higher temperature.²¹⁻²³ A variety of

molecular surface organization forms can, moreover co-exist on Au(111) in UHV. These can be disordered Cys islands or extended domains of ordered quadratic and trimer Cys adlayers.²³ For example, Cys forms pairs of molecules or clusters of eight molecules in UHV on Au(110),²¹⁻²³ while a highly ordered c(2×2) dense monolayer with two Cys molecules in each unit cell is found in aqueous buffer.²⁵ Sub-molecular resolution was achieved by the visualization of three sub-molecular spots corresponding to the three functional groups of each Cys molecule by *in situ* STM.²⁵

It has been a puzzle that only the L-forms of amino acids are found in nature, even though the chemical nature of L- and D-enantiomers is identical. Chiral effects has been discovered only in pair formation of Cys molecules on the Au(110) surface in UHV.²⁰ Cys dimers on Au(111) in aqueous environment were not found,²⁵ but Cys dimer formation perpendicular to polycrystalline gold surfaces was suggested based on surface plasmon resonance (SPR) spectroscopy.³²

Molecular level understanding of Cys SAM formation on metal surfaces is a challenge. Attempts based on DFT calculations were reported by Kühnle and associates.³³⁻³⁶ A stable double row of cyclic Cys molecular

dimers on Au(110) in UHV was found, supported by UHV STM data.³³ Cys molecules were also found to diffuse freely along the Au(111) surface by combining experimental techniques (XPS, ultraviolet photoelectron spectroscopy, low energy electron diffraction, and STM) with density functional theory (DFT), resembling a 2D-molecular gas.²⁶ No stable cyclic dimers were observed, and formation of single molecular rows was concluded to be more likely. Different types of Cys-Cys dimers (radical forms) on Au(110) were also investigated³⁵. Some dimers could be considered as nucleation centers for the SAM formation. Adsorption of dimers and clusters of Cys molecules at the Au(110) surface studied by DFT have also been reported.^{37,38} Chiral effects on the adsorption of cyclic Cys dimers (L-L, D-D and L-D) were furthermore investigated by Paci, Cordeiro, and associates,^{34,36} but the solvent environment was not addressed.³³⁻³⁶ Solvation is, however, crucial as even the mere identity of the Cys molecule is quite different in UHV (neutral molecule) and in polar solvents (zwitter ion). The energy barrier of the S-H bond cleavage in dissociative adsorption of a Cys molecule on the Au(111) surface was furthermore calculated,^{34,39} based on the method of

reactive molecular dynamics with the solvent incorporated explicitly.

In this report, we address the formation of a Cys adlayer on Au(100) in phosphate buffer, aiming at both dynamic and structural molecular scale mapping, using a combined and comprehensive experimental and theoretical approach. Au(100) was chosen, as Cys adsorption on this surface has been much less studied than on the other low-index Au-surfaces,⁴⁰⁻⁴⁹ but offers clues to new and unique Cys packing modes due to the square symmetry of the Au(100) crystalline surface structure. Reconstruction with “hex” transition on clean Au(100) is commonly found after annealing or under electrochemical potential control.⁴¹ Chemical adsorption and interaction between Cys molecules and surface gold atoms lifts the Au(100) reconstruction, reorganizing the surface Au-atoms into a square pattern.

The Cys coverage on Au(100) was recorded voltammetrically by reductive desorption which is based on cleavage of the S-Au bonds at negative potential in alkaline solution. The macroscopic time dependent adsorption was investigated by chronopotentiometry and framed microscopically, first by high-resolution *in situ* STM mapping the Cys adlayers on Au(100) in phosphate buffer solution. Chiral

effects of Cys adlayers of pure L- and D-Cys as well as a racemic mixture were all included. Secondly, both DFT calculations combined with a cluster model and Monte Carlo simulations were used to elucidate microscopic details of the adsorption process. The work offers a comprehensive view of Cys assembly and packing on Au(100) at the molecular and sub-molecular levels of resolution.

2. Experimental methods and theoretical calculation

2.1 Chemicals. D-Cys (99%) was from Aldrich and L-Cys (99.5%) from Fluka. KH_2PO_4 (99.99 %, CAS 7778-77-0, Aldrich) was supporting electrolyte. Freshly prepared 0.10 M NaOH (99.99 %, Sigma-Aldrich) was used for reductive desorption.

2.2 Electrochemical methods

Cyclic voltammetry (CV) and chronopotentiometry (zero current) were carried out using a PGSTAT12 potentiostat (Metrohm Autolab B.V., Netherlands). An in-house 3-electrode glass cell with working (WE), counter (CE), and reference electrode (RE) was used for CV. Chronopotentiometry was employed to record the potential difference between the WE and RE over time during Cys adsorption. The WEs included single-crystal gold and graphite electrodes. Au(100), Au(111) and Au(110) bead

electrodes were prepared by Hamelin's method,⁵⁰ and checked by CV in 0.10 M H₂SO₄^{18,50}. Annealing at 860 °C and electrochemical polishing were used for cleaning the Au(100), Au(111) and Au(110) surfaces. Prior to electrochemical and *in situ* STM measurements, the gold electrodes were annealed in a hydrogen flame and quenched in Millipore water saturated with H₂. Basal plane (BPG, Ø 5 mm) and edge plane graphite (EPG, Ø 5 mm) electrodes from Pine Instrument Company (Grove, USA) were used as reference WEs in chronopotentiometry. BPG and EPG electrodes were freshly cleaned by mechanical polishing on SiC-papers followed by polishing with 1.0, 0.3 and 0.1 µm Al₂O₃ slurry on a felt disk before ultrasonication twice in Millipore water. A freshly prepared reversible hydrogen electrode (RHE) calibrated vs. a saturated calomel electrode (SCE) was used as RE for all electrochemical measurements. All potentials are referenced to SCE. The in-house setup for chronopotentiometric monitoring of cysteine assembly in 25 mM KH₂PO₄ is described in a previous report⁵¹. CV was used to monitor both adsorption and reductive desorption of Cys adlayers in 25 mM KH₂PO₄ and 0.1 M KOH, respectively.

2.3 *In situ* STM

A PicoSPM STM (Agilent) instrument with an in-house Teflon cell was employed for *in situ* STM. Platinum wires served as both CE and RE. The RE was calibrated vs a SCE after each experiment. Electrochemically etched W tips (Ø 0.25 mm) coated with Apiezon wax were utilized as STM probes. Au(100) disc electrodes (SPL, The Netherlands) were used for *in situ* STM. STM samples were prepared in the same way as for electrochemistry, i.e. the cleaned Au(100) electrodes were annealed in a hydrogen flame and quenched in Millipore water saturated with H₂, then mounted in the STM cell with freshly prepared 25 mM KH₂PO₄. Freshly prepared L-, D-, or L,D-cysteine solution was injected into the cell under potential control. All STM images were recorded under electrochemical control.

Glassware, and electrochemical and STM cells were cleaned by boiling in 15% HNO₃ and washed copiously in Millipore water. All supporting electrolyte and Cys solutions were freshly prepared prior to each experiment.

2.4 Computational Methods

2.4.1 *Molecular structures and Au-surface molecular models*

In comparison to Au(110) or Au(111),²⁵ the *in situ* STM data show completely different Cys lattices at the Au(100)/electrolyte interface. Clear stripes (rows) of bright STM contrasts separated by dark areas are observed, sometimes with a

“fork-like” structure. Using periodical DFT is therefore a complex, combinatorial issue. The nature of the interaction between gold and Cys monomer forms has been theoretically addressed in detail.^{24,37,38,52,53} Our main emphasis was, however on the adsorption of different Cys dimers considered as 2D-“nucleation” centers which trigger further growth of the rows. The Au(100) metal surface was modelled by two-layer clusters of different size (Au₂₅(16+9), Au₃₀(15+15), Au₃₂(20+12)). Primary attention was given to a comparative study that acknowledges the well-known shortcomings of the cluster model.⁵⁴ A merit is, however, that solvent effects can be incorporated both explicitly and implicitly (in terms of a self-consistent reaction field). The quantum chemical calculations were performed at the DFT level with the hybrid exchange-correlation functional b3pw91 as implemented in the Gaussian 09 program suite.⁵⁵ The valence orbitals of the Au atom were described by a basis set of double-zeta (DZ) quality, while the inner electrons were included in the Hay and Wadt effective core potential (LanL2).⁵⁶ The standard basis set 6-31(d) was used to describe the electrons in the S, N, O, C and H atoms. The open shell systems were treated in unrestricted formalism. The geometry of adsorbed species was fully optimized without symmetry restrictions. The natural population analysis (NPA) was employed to calculate the atomic charges. Water molecules were not included directly in the first solvation sphere, but the aqueous medium was addressed by the PCM (Polarized Continuum Model) as implemented in the Gaussian 09 package.⁵⁵ A value of 78.4 was taken for the static dielectric constant and a value of 1.78 for the fast solvent modes. Non-electrostatic (cavitation, dispersion and repulsion) contributions to the solvation free energy were calculated as well. The program ChemCraft⁵⁷ was used for the visualization of the optimized adsorbate geometry.

2.4.2 *In situ* STM contrasts

The STM contrasts (constant current mode) were calculated in the framework of the quantum chemical model previously developed.^{24,25} The electron transfer (tunnelling) process was treated

as a bridge-assisted superexchange process with the electrode/Cys transition adiabatic, and the Cys/STM tip transition diabatic. The STM tip was represented by a tungsten atom, and the optimized geometry of the adsorbed Cys species used. The influence of the metal surface was not addressed directly. In the weak-coupling limit of tip-molecule interaction, the current is proportional to the squared electronic coupling factor V_{if} (resonance integral) calculated by two-level perturbation theory.^{24,25}

2.4.3 Monte Carlo (MC) simulations

A squared lattice including 100×100 surface sites was used in the MC simulations. The total number of steps amounts to 8000000. At first all sites were assumed to be occupied by neutral Cys molecules. In subsequent iterations the weakly adsorbed Cys molecules were transformed to Cys radical and Cys-zwitter ionic forms with specified probabilities (P_{mr} and P_{rz} , see Figure S1). The computation scheme resembles that usually employed in kinetic MC modeling. A random number ξ_1 between 0 and 1 is first generated and compared with the pre-specified P_{mr} value. If $\xi_1 > P_{mr}$, the attempt is considered successful. A second random value ξ_2 is then generated in a second step and compared with the P_{rz} value; if $\xi_2 > P_{rz}$ such an attempt is kept as successful etc. The formation of the zwitterion from the adsorbed Cys radical is modelled similarly. The four neighbors of each site (right, left, up and down) or three (two) neighbors (for the sites on the

boundary) are analyzed in subsequent computational loops. The input probabilities, P_{rm} and P_{rr} (Figure S1) control the formation of rad – mol and rad – rad Cys dimers in the presence of appropriate neighbors in the lattice knots. In turn, the probabilities P_{zz} and P_{zv} describe the appearance of other Cys dimers, zwitter rad – zwitter rad (both species are adsorbed in the first monolayer), and zwitter rad (the first layer) – zwitter mol (the second layer) pairs, respectively. These forms are denoted as planar and vertical Cys zwitter ionic dimers (ZZp and Zv). Single dimers of both types were treated as nucleation centers which initiate the growth of rows consisting of Cys zwitter radicals or vertical dimers Zv, as well as assigning the row direction. Such rows were assumed to grow only in vertical or horizontal directions of the 2D lattice that fits the symmetry of the (100) metal face. The growing rows accumulate all single Cys zwitter rad forms. Additionally, rows that encounter Cys rad–rad and rad–mol dimers as neighbors, break these forms with unit probability and amalgamate with the Cys radicals to single zwitter ions. The parallel rows then form arrays at the surface.

We excluded crossing rows, i.e. when a growing row meets another one, the growth stops. We assumed, however that if a row of

Cys vertical dimers meets a ZZp row (array), growth continues with a certain probability P^*_{zv} . To describe a similar event (i.e. when an array of Cys zwitter ions in planar orientation crosses a strip formed by the Cys vertical dimers and continues its growth), we introduced another probability, P^*_{zz} . Another condition to stop the growth is meeting with a Cys molecule. An original code written in the program suite Matlab was used in all simulations. We varied all input probabilities within certain intervals; in the final simulations probability values were used which yield the most distinct and longest rows, Figure S1. Test simulations were performed also for larger lattices, (150×150 and 250×250 sites) and found to give results very similar to those for the 100×100 ensemble. We note that the different Cys chiral forms (D and L) cannot be unambiguously distinguished in our computational scheme. The influence of the Cys concentration in the solution bulk on the SAM formation was not addressed either.

3. Results and Discussion

3.1 Electrochemistry

Electrochemical techniques are core techniques in exploring solid-liquid interfaces. Voltammetry and chronopotentiometry were used in this work to study Au-S Cys chemisorption, Cys coverage, as well as Cys adsorption

dynamics. Cyclic voltammograms of bare Au(100) give two sharp and small anodic peaks in 25 mM KH_2PO_4 (pH 4.7). The peak at 0.23 V is caused by anion adsorption, whereas the anodic peak at 0.07 V and a broad cathodic peak at 0.04 V are due to lift and formation of surface reconstructions respectively, Figure 1. Similar voltammetric patterns were found for Au(111) and Au(110) as well,^{24,25,58} characteristic of adsorption of anions and formation of a reconstructed low index gold surface.⁵⁹ Cys adlayers give a featureless double layer signal between -0.40 and +0.45 V, (red curve, Figure 1) suggesting stronger adsorption than the electrolyte anions and lift of surface reconstruction, i.e. chemisorption of Cys on Au(100). Peaks caused by oxidation and reduction of Cys adlayers on Au(100) occur at potentials more positive than 0.50 V and more negative than -0.4 V, respectively.

Figure 1. Cyclic voltammograms of bare Au(100) (black) and L-Cys SAM on Au(100) (red) in 25 mM KH_2PO_4 (pH 4.66). Scan rate: 50 mV/s. Au(100) soaked in 1.6 mM L-Cys for 5.75 hours.

Reductive desorption was carried out using 0.10 M NaOH to avoid interference from dihydrogen evolution, Figure 2. Cleavage of

Au-S bonds occurs at negative potentials in basic solution, giving a strong cathodic signal. The charge under the cathodic peak, notably composed of two peaks, at -0.67 V and -0.96 V gives Au-S coverage with charges of $(7.0\text{--}8.8)\times 10^{-6}$ and $(1.65\text{--}1.82)\times 10^{-5}$ C cm^{-2} , respectively, Figure 2. This is very different from the single cathodic Cys peak at -0.705 V on Au(111)¹⁸ $(3.7\pm 0.4)\times 10^{-5}$ C cm^{-2} (coverage $(4.0\pm 0.4)\times 10^{-10}$ mol cm^{-2}) and the -0.71 V $(6.74\pm 0.3)\times 10^{-10}$ mol cm^{-2} Cys peak on Au(110).²⁴ Two cathodic peaks, a sharp peak at -0.70 V (vs Ag/AgCl), and a large but broad peak at -1.05 V were reported for Cys reductive desorption on polycrystalline gold, though the origin of the -1.05 V peak was not clear.¹⁹ In comparison, reductive desorption of *n*-butanethiol on Au(111) and Au(100)⁶⁰ gives peaks at -0.99 V $(78.6\times 10^{-6}$ C cm^{-2}) and -1.03 V $(106\times 10^{-6}$ C cm^{-2}), very similar to the Cys reductive peak on Au(111) and Au(100).^{61,62} A 260 mV difference was reported for reductive desorption of 4-pyridinethiol on Au(111) at -0.50 V (coverage 5.0×10^{-10} mol cm^{-2}) and Au(100) at -0.76 V (coverage 5.7×10^{-10} mol cm^{-2}).^{62,63} All this is different from reductive desorption of Cys on Au(100). The total charge of the two cathodic peaks is $(2.5\pm 0.3)\times 10^{-5}$ C cm^{-2} , which is much smaller than for the network cluster-structure of Cys on Au(111),¹⁸ suggesting stronger

intermolecular interaction on the latter surface. The 290 mV difference between the two cathodic peaks indicates that Cys on Au(100) displays at least two different adsorption modes with significantly different Au-S binding energies. The cathodic peak at -0.67 V is also not a single sharp peak, but a convolution of two peaks, both close to -0.71 V for Cys on Au(111)¹⁸ and Au(110)²⁴. Adsorption reflected by this peak is therefore similar to Cys adsorption on Au(111) and Au(110), i.e. a monolayer of chemisorbed Cys.

Figure 2. Reductive desorption of L-Cys SAMs on (A) Au(100) and (B) Au(111) in 0.10 M NaOH. Scan rate 10 mVs⁻¹. Au(100) and Au(111) soaked in 1.03 mM L-Cys for 5.75 and 4.5 hours, respectively.

Longer-chain thiols normally show the more negative desorption potentials.⁶⁴ The -0.96 V peak supports a model with a Cys bilayer

with Cys molecules on top of each other, very likely through hydrogen bonding between carboxylate and ammonium groups. The excess charges of the two functional groups are partially compensated in the bilayer and would hamper liberation of the molecules into the solution. A similar model was proposed from observations of L-Cys adsorption on (polycrystalline) gold by surface plasmon resonance (SPR) spectroscopy.³² High-resolution *in situ* STM, cf. below further supports this view.

3.2 Chronopotentiometry

As observed by real time SPR monitoring, adsorption of Cys on polycrystalline gold is a fast process that depends on the solution L-Cys concentration.³² We studied the dynamics of Cys adsorption on Au(100) by chronopotentiometry that records the time-dependent Nernstian potential change for redox systems in homogenous solution or/and immobilized on an electrode surface at sub-second time resolution.^{54,64} Among the functional groups in Cys, thiol is a reducing group and lowers the Nernstian potential.

The basis of the chronopotentiometric analysis is that a Cys molecule is first (physi)adsorbed. Adsorption of cysteinate anion is unlikely as this leads to etching of the Au-surface.^{24,25,65,66} There is obviously electron exchange between Cys and the gold electrode as noted by the potential drop. Physisorption is followed by conversion of the Au-S unit through several stages to the final Au(0)-S(0)• form. As conversion progresses, more and more surface Au(0)-S(0)• is formed. With a high and constant Cys concentration in the solution, this leads to gradual Nernstian potential increase in the equilibrium,

Chronopotentiometry requires that the electron exchange between adsorbed Cys species and the electrode is fast compared to all the conversion steps towards final equilibrium between thiol-bound adsorbate and the electrode, so that Nernstian equilibrium is established throughout. With this understanding, the intermediate states in the Cys adsorption process are effectively time evolving (“quasi-”) equilibrium states. This is known from other cases where the time dependent concentration of an electrochemically active species involved in separate, non-electrochemical processes is followed by monitoring the Nernst potential of the species, and the kinetics of the non-electrochemical process determined.^{67,68} Figure 3 shows chronopotentiograms at different Cys concentrations and electrodes. When Cys is added to the solution, the Au(100) potential drops “instantly” by 300-600 mV, followed by increase up to 30 minutes and stabilizing after 60 minutes, Figure 3A. The potential drop and subsequent increase both depend on the Cys concentration, higher Cys concentrations giving lower steady state potentials. Her potential drop is caused by adsorption of the Cys molecule, i.e. with sulfur in the low-oxidation thiolate state. This is followed by conversion to strong Au-S interaction, leading eventually to Au(0)-S(0) gold-thiyl monolayer formation, i.e. with bound sulfur now in a higher formal oxidation state and therefore towards more positive Nernstian equilibrium potentials. Cys adsorption on the three low-index gold substrates, Au(111), Au(100) and Au(111) shows similar but distinct tendencies at given Cys concentration, Figure 3B and S2, suggesting that the details of Cys adsorption on the three surfaces are different. The graphite electrodes EPG and BPG were used as reference, as Cys does not chemisorb on these surfaces. Therefore potential drop was hardly observed when Cys solution was added, Figure 3C.

Figure 3. Chronopotentiometry of L-Cys adsorption in 25 mM KH_2PO_4 (pH 4.66). A 0.2 mL concentrated L-Cys solution is injected at $t = 0$. (A) Adsorption on Au(100) at different L-Cys concentrations. (B) Adsorption on single-crystal gold substrates using 250 μM L-Cys. (C) Adsorption on inert substrates, i.e. a L-Cys adlayer on Au(100) (black), basal-plane graphite (red) and edge-plane graphite (blue) using 250 μM L-Cys.

A small potential drop (50 mV) was, however, still found for graphite electrodes as well as for Cys covered Au(100), Figure 3C suggesting that Cys in solution contributes an additional small potential change, while the main potential changes in Figure 3 are caused by Cys-Au chemisorption. The chronopotentiometry data could be represented as a multi-step process.

Molecular details and intermediate adsorbed species will be addressed in Sections 3.3 and 3.4. The data display approximately exponential time evolution for all the three surfaces, with a minimum of two phases. In a two-phase view the time constants (adsorption rate constants) differ by roughly an order of magnitude. The time constants are similar for Au(100) and Au(111), but smaller for Au(110). Both time constants decrease with increasing Cys concentration. The amplitudes of the fast component are similar for all the Au electrodes, but the slow amplitude for Au(100) is twice that of Au(110) and Au(111) suggesting that this component has contributions from a process occurring solely on Au(100). The fast component amplitude increases weakly with increasing Cys concentration, while the slow component amplitude is independent of concentration. Bi-phasic time evolution was also found by SPR on polycrystalline Au, where the two phases were assigned to bound Cys and a Cys bilayer.¹⁴ The chronopotentiometric data are summarized in Table 1.

Table 1. Biexponential time constants t and amplitudes A , for chronopotentiometric Cys adsorption.

Electrode	C_{cys} [mM]	t_{fast} [s]	A_{fast} [mV]	t_{slow} [s]	A_{slow} [mV]
Au(100)	0.02	52	-87	400	-110
Au(100)	0.10	34	-110	330	-110
Au(100)	0.25	30	-100	270	-120
Au(100)	1.25	20	-140	220	-110
Au(110)	0.25	13	-90	130	-62
Au(111)	0.25	27	-81	270	-60
SAM/Au(110)	0.25	44	43	530	27
BPG	0.25	15	10	230	36
EPG	0.25	62	18	430	39

The simplest, two-phase kinetic scheme, with a single intermediate species, is represented as

$A_{\text{red}}^{\text{sol}}$ is cysteine in solution, $A_{\text{ox},f}^{\text{ads}}$ the adsorbed final Au-thiyl species,^{24,25,65,66} and $A_{\text{ox},i}^{\text{ads}}$ an intermediate species. k_1 , k_{-1} , k_2 , and k_{-2} are the rate constants. Eq.(2) can represent different intermediate species, e.g. N-bound Cys or Cys dimer species

The nature of these cannot be identified from chronopotentiometry, but we have identified likely candidates by DFT calculations on differently oriented solvated Cys monomers and dimers on a Au(100) model surface, Section 3.4. Eq.(2) gives biexponential evolution of the coverage of the final adsorbed Au-thiyl Cys species, $\theta_f(t)$

$$\theta_f(t) = \theta_f^\infty \left[1 + \frac{R_2}{R_1 - R_2} e^{R_1 t} - \frac{R_1}{R_1 - R_2} e^{R_2 t} \right] \quad (3)$$

with the two phases (rate constants)

$$R_1 = -\frac{1}{2} \left(k_{-1} + k_{-2} + k_2 + k_1 \left[A_{\text{red}}^{\text{sol}} \right] \right) + \sqrt{\frac{1}{4} \left(k_{-1} + k_{-2} + k_2 + k_1 \left[A_{\text{red}}^{\text{sol}} \right] \right)^2 - (k_1 k_{-2} + k_1 k_2) \left[A_{\text{red}}^{\text{sol}} \right] - k_{-1} k_{-2}} \quad (4)$$

$$R_2 = -\frac{1}{2} \left(k_{-1} + k_{-2} + k_2 + k_1 \left[A_{\text{red}}^{\text{sol}} \right] \right) - \sqrt{\frac{1}{4} \left(k_{-1} + k_{-2} + k_2 + k_1 \left[A_{\text{red}}^{\text{sol}} \right] \right)^2 - (k_1 k_{-2} + k_1 k_2) \left[A_{\text{red}}^{\text{sol}} \right] - k_{-1} k_{-2}} \quad (5)$$

R_1 and R_2 reduce to the rate constants of the forward steps, when the reverse reactions are disregarded, $R_1 \rightarrow -k_1 \left[A_{\text{red}}^{\text{sol}} \right]$, $R_2 \rightarrow -k_2$ ($R_1, R_2 < 0$). The equilibrium final state coverage is

$$\theta_f^\infty = \frac{k_1 k_2 \left[A_{\text{red}}^{\text{sol}} \right]}{R_1 R_2} = \frac{k_1 k_2 \left[A_{\text{red}}^{\text{sol}} \right]}{(k_1 k_{-2} + k_1 k_2 \left[A_{\text{red}}^{\text{sol}} \right]) - k_{-1} k_{-2}} \quad (6)$$

The recorded quantity is not, however, directly the surface coverage (as in SPR³²) but the reduction potential $E-E_0$, where E_0 is the standard equilibrium potential. Assuming first that $E-E_0$ is determined solely by the final state Cys thiyl chemisorption, $E-E_0$ is given by

$$E - E_0 = \frac{RT}{F} \ln \left(\frac{\text{Ox}}{\text{Red}} \right) = \frac{RT}{F} \ln \left[\frac{\theta_f(t)}{A_{\text{red}}^{\text{sol}}} \right] = 59(\text{mV}) \log \left[\frac{\theta_f^\infty}{A_{\text{red}}^{\text{sol}}} \right] \quad (7)$$

$$+ 59(\text{mV}) \frac{RT}{F} \log \left[1 + \frac{R_2}{R_1 - R_2} e^{R_1 t} - \frac{R_1}{R_1 - R_2} e^{R_2 t} \right]$$

We then notice, **first**, that the recorded $(E-E_0)/t$ correlations **approach** (bi)exponential but approach (bi)exponential behavior, when $|R_1|t > 1$ and $|R_2|t > 1$. Since

$$\left| \frac{R_1}{R_1 - R_2} \right| \ll 1 \text{ and } \left| \frac{R_2}{R_1 - R_2} \right| \approx 1 \quad (8)$$

there are two **approximately** exponential regimes, with a slow phase

$$E - E_0 \approx 59(mV) \log \left[\frac{\theta_f^\infty}{A_{red}^{sol}} \right] + 59(mV) \frac{RT}{F} \left| \frac{R_2}{R_1 - R_2} \right| \exp^{-|R_1|t} \quad (9)$$

when $|R_1/(R_1 - R_2)|e^{|R_2|t} < 1$, and a fast phase

$$E - E_0 \approx 59(mV) \log \left[\frac{\theta_f^\infty}{A_{red}^{sol}} \right] + 59(mV) \frac{RT}{F} \left| \frac{R_1}{R_1 - R_2} \right| \exp^{-|R_2|t}$$

when $|R_2/(R_2 - R_1)|e^{|R_1|t} < 1$, enabling determination of the rate constant combinations R_1 and R_2 .

Secondly, $R_1 + R_2$ and $R_1 \times R_2$ reduce to

$$\begin{aligned} R_1 + R_2 &= -(k_{-1} + k_{-2} + k_2 + k_1 [A_{red}^{sol}]) \\ R_1 \times R_2 &= (k_1 k_{-2} + k_1 k_2) [A_{red}^{sol}] - k_{-1} k_{-2} \end{aligned} \quad (11)$$

The data do in fact follow this $[A_{red}^{sol}]$ dependence, Figure S3. The slopes and intercepts enable determining the rate constants k_1 , k_{-1} , k_2 , and k_{-2} as 1.8 s^{-1} , 0.011 s^{-1} , $4 \times 10^{-3} \text{ s}^{-1}$, and $5 \times 10^{-4} \text{ s}^{-1}$, respectively. These values reflect the $E-E_0$ time evolution from Figure 3, with a fast initial phase where Cys is physisorbed followed by significantly slower conversion to the final, thiol-bound Cys chemisorbed state, both steps shifted in favour of the products. In the following two sections we address the nature of the intermediate state(s).

However, the view of the Nernstian potential as determined solely by Cys thiol adsorption is not compatible with **all** the data. The amplitudes of both phases are roughly the same, Table 1, but eqs.(3)-(10) would assign a significantly smaller amplitude, $|R_1/(R_1 - R_2)| \ll 1$ to the fast phase, R_2

than the amplitude, $|R_2/(R_1 - R_2)| \approx 1$ assigned to the slow phase, R_1 . This must be caused by a potential response not only to final state thiol adsorption of Cys but also to intermediate adsorption. While eqs.(3)-(5) maintain validity, intermediate state potential response must therefore be included in a more complete description of the potential, eqs.(8)-(11). The surface coverage of the intermediate state, $\theta_i(t)$ also displays biexponential time evolution, i.e.

$$\begin{aligned} \theta(t) &= \theta_f^\infty \left[1 - \frac{R_2}{R_1 - R_2} \left(1 + \frac{R_1}{k_{-2}} \right) e^{R_2 t} + \frac{R_1}{R_1 - R_2} \left(1 + \frac{R_2}{k_{-2}} \right) e^{R_1 t} \right] \\ \theta_i^\infty \rightarrow \theta_i^\infty &= \frac{k_1 k_{-2} [A_{red}^{sol}]}{(k_1 k_{-2} + k_1 k_2 [A_{red}^{sol}] - k_{-1} k_{-2})} \end{aligned} \quad (13)$$

As k_{-2} is a reverse rate constant and small, both amplitudes are approximately $1 + (R_1 R_2 / k_{-2})$. Biexponential time evolution of the potential with significantly different phases but approximately equal amplitudes can thus be understood, if both the final state thiol Cys adsorption and an intermediate, N-bound Cys or S-bound bilayer contributes to the potential.

3.3 In situ STM

3.3.1 Bare Au(100) and L-Cysteine on Au(100)

Figure 4. *In situ* STM images (A and B) of bare Au(100) in 25 mM KH_2PO_4 (pH 4.7). $I_t = 0.05 \text{ nA}$, $V_{\text{bias}} = -0.01 \text{ V}$. $E_W = -0.3 \text{ V}$ vs SCE (C) is model of Au(100) (5×20) reconstruction.

Cys monolayers on Au(100) were mapped by *in situ* STM. Bare Au(100) shows a clean surface from -0.30 to +0.25 V vs SCE, with many highly

ordered stripes at a periodic distance 1.40 ± 0.2 nm, Figure 4A and B. Stripes on the same terrace are perpendicular to each other, visible even on small terraces, as indicated by a blue arrow in Figure 4B. This feature accords with (5×20) reconstruction, Figure 4C (as also seen in the CV, Figure 1), with a square pattern of gold atoms in the second layer and a hexagonal pattern in the top layer. (5×20) reconstruction was also found for Au(100) in vacuum.⁶² Other groups have reported the (1×1) Au(100) structure at more positive potentials.^{62,69,70}

Figure 5. *In situ* STM images of L-Cys on Au(100). $I_t = 0.2$ nA, $V_{bias} = -0.4$ V. $E_W = -0.1$ V vs SCE. 200 μ M L-Cys in 25 mM KH_2PO_4 (pH4.66).

In the presence of L-Cys, the (5×20) reconstruction is completely lifted and Au(100) totally covered by L-Cys SAMs (Figure 5), as for chemical thiol (thiyl) adsorption on Au(111) and Au(110).⁷¹⁻⁷⁴ Cys monolayer adsorption on Au(100) does not accord with physical adsorption, such as for metalloporphyrin monolayers, for which both the adsorbed porphyrin monolayer and reconstructed substrate underneath are visible.^{70,75} New stripe-like features along both $[110]$ and $[1\bar{1}0]$ directions following exactly the atomic rows of the Au(100) substrate underneath and parallel to the terrace steps appear on Cys adsorption, Figure 5A-C. The length of the stripes ranges from a few nm to several hundred nm, and the distance between the stripes from 2-3 to 6-10 nm, depending on the adsorption conditions such as soaking time, Cys concentration, and potential. Low and dense coverage of stripes is found at short (3.5 hours, 0.06 mM Cys) and long adsorption times (20 hours, 0.5 mM Cys), respectively, Figure S4.

Notably, both stripes and the space between the stripes are covered by Cys monolayers, Figure S5. Strong STM image contrasts are caused by high electronic conductivity, Section 3.4. These

stripes are very different from the well-defined stripes for alkane or branched chiral thiolates on Au(111).⁷⁶⁻⁷⁸ The most conspicuous features are: (1) 'Fork'-like features among the stripes, indicated by blue circles in Figure 5. Such a feature is not found in L-Cys monolayers on Au(111) or Au(110), and is unexpected and highly notable. Possible reasons will be addressed in Sections 3.4 and 4.

Figure 6. *In situ* STM images of L-Cys on Au(100). $I_t = 0.15$ nA, $V_{bias} = -0.25$ V. $E_W = -0.15$ V vs. SCE. 200 μ M L-Cys in 25 mM KH_2PO_4 (pH4.66). (A) Mismatch (red lines and blue circles). (B) Individual defects (green circles 1 and 2) and defect patch (green circle 3) inside stripes; individual defects between stripes (blue circle 1).

(2) Parallel stripes with two widths, in highly ordered units packed along Au(100) atomic rows underneath. As indicated by blue arrows (Figure 6A), broad (3.2 ± 0.2 nm) and narrow (2.0 ± 0.1 nm) stripes co-exist in L-Cys SAMs.

(3) Mismatch along molecular rows is found in both broad and narrow stripes, as encircled by blue lines in circle 1 and 2 and marked by red lines, respectively, Figure 6A.

(4) Individual defects between stripes (blue circle 1), inside the narrow (green circle 1) and broad (green circle 2) stripes, along with defect patches or domains (green circle 3), Figure 6B.

Figure 7. (A) and (B) *in situ* STM images of L-Cys on Au(100). $I_t = 0.15$ nA, $V_{bias} = -0.25$ V. $E_W = -0.15$ V vs SCE. 200 μ M L-cysteine in 25 mM KH_2PO_4 (pH4.66). (A) Interaction among molecules between the stripes, and molecules in a defect patch are highlighted with green

circles, and blue circle respectively. (B) Unit cells are overlaid on the image. (C) Tentative adsorption model with (11×2) -2 Cys (green) and (7×2) -2 Cys (blue) unit cell. L-Cys molecules in both planar (flat or lying down) and upright (stand up) orientation are suggested.

(5) Orientation of molecules among the stripes (green circles), and stripes with defect patches (blue circle), suggesting strong network interactions, Figure 7A.

(6) The closest periodic distance is the same, 0.55 nm in either broad or narrow stripes, i.e. twice the gold atom diameter underneath. The 3.2 nm and 2.0 nm distances correspond to 11 and 7 gold atoms in perpendicular direction. Unit cells for broad and narrow stripes are therefore described as (11×2) -2Cys and (7×2) -2Cys, Figure 7B.

Models with tentative structures for such unit cells are shown in Figure 7C and Figure S6, resting on both upright and recumbent Cys orientations in both broad and narrow stripes. Mixture of strong and dark contrast in STM images found in 1-butanethiol SAMs on Au(111) was caused by upright and laying down orientation respectively.¹⁸ it is reasonable that vertical Cys here gives strong contrast than planar Cys due to facile tunnelling route. Co-adsorption of water molecules is likely due to the wide space in the broad stripes ((11×2) -2Cys), but not included in the model. Adsorption of a second Cys molecule on top of the upright Cys molecule is possible e.g. by formation of a H-bonded dimer perpendicular to the Au (100) surface, Section 3.4. More detailed insight is provided in Section 3.4 based on quantum chemical modelling and MC simulations, leading to more precise accordance between the modelling and the data.

The *in situ* STM data match the L-Cys coverage from electrochemical reductive desorption, Figure 2. The unit cells (11×2) -2 Cys and (7×2) -2 Cys give 92 and 58.2 Å² per L-cysteine molecule, corresponding to 1.81×10^{-10} and 2.86×10^{-10} mol/cm², respectively. The average coverage of Cys on Au(100) from reductive desorption is $2.52 (\pm 0.15) \times 10^{-10}$ mol/cm², i.e. 68 Å² per L-cysteine molecule, suggesting a mixture of (7×2) -2Cys and (11×2) -2Cys domains. The model with two Cys molecular orientations implies two sets of close lying reductive desorption peaks,

consistent with the double peak at 0.67 V vs SCE in Figure 2. Moreover, a vertical Cys dimer would require a stronger reductive desorption force than monomeric Cys (recumbent), suggesting that the -0.96 V peak, Figure 2 is assigned to Cys dimer.

3.3.2 D-Cys and L, D-Cys adlayers on Au(100)

Figure 8. *In situ* STM images of D-Cys on Au(100). $I_t = 0.56$ nA, $V_{\text{bias}} = -0.15$ V, $E_W = -0.40$ V vs SCE. 200 μM D-Cys in 25 mM KH₂PO₄ (pH4.66).

Monolayers of pure D-Cys and 1:1 racemic D,L-Cys on Au(100) were also investigated by *in situ* STM. Pure L-Cys, D-Cys and D,L-Cys are indistinguishable at the **macroscopic** level, but clearly different at the nanoscale level of *in situ* STM. Similar to L-cysteine, both D-cysteine and D,L-cysteine adlayers are composed of uniform stripes with characteristic ‘forks’, indicated by blue circles in Figure 8 and 9. The main observable difference between the three groups of enantiomers is that while L- and D-Cys give closely similar images, a larger number of nanoscale defects are found in the D,L-Cys adlayers, Figure 9. The defects are either solitary or appear in groups inside the stripes or on either side of the stripes. Defects are caused by molecular mismatching during self-assembly, which points to the importance either of hydrogen bonding or spatial incompatibility among the different Cys molecular enantiomers in the D,L-Cys SAMs. Similar observations were encountered recently for high-resolution *in situ* STM images of the chiral 2-butanethiols^{76,78} We shall return to this issue in Section 4.

Figure 9. *In situ* STM images of L,D-Cys on Au(100). (A) $I_t = 0.12$ nA, $V_{\text{bias}} = -0.20$ V, $E_w = -0.15$ V vs SCE, (B) and (C) $I_t = 2.40$ nA, $V_{\text{bias}} = -0.20$ V, $E_w = -0.20$ V vs SCE. 100 μM L-Cys and 100 μM D-Cys in 25 mM KH_2PO_4 (pH4.66). Defects within stripes (green circles), in fork-like structures (blue circles) and individual missing molecules (purple circles) are highlighted.

3.4 Molecular Modelling

3.4.1 Cys monomer and dimer adsorption on Au(100)

The adsorption energy of a Cys molecule from solution was found to be small (bond energy - 0.19 eV) and comparable to the adsorption energy of a water molecule on Au(100) (-0.3 eV⁷⁹). This can be attributed to physisorption where intermolecular interactions (H-bonds, van der Waals forces) dominate. Chemisorption accompanied by S-H bond cleavage therefore entirely dominates the overall adsorption process, eq. (1). The reaction product is an adsorbed Cys radical $\text{R-S}_{\text{ads}}\cdot$ strongly bound to the Au surface through the sulfur atom. We modelled the first step, i.e. $\text{R-SH}_{\text{ads}} \rightleftharpoons \text{R-S}_{\text{ads}}\cdot + \text{H}_{\text{ads}}$, by DFT calculations.

Figure 10. Potential energy surfaces describing (A) breaking of the S-H bond on dissociative adsorption of an L-Cys molecule at the Au(100) surface (the total energy calculated from the deepest value), and (B) the S-S bond cleavage on dissociative adsorption of an L-Cystine molecule at the Au(100) surface (the total energy of the initial point taken as zero).

The S-H bond was scanned with steps of 0.25 Å, while the rest of the Cys molecule was fully optimized. Representative results are shown in Figures 10A and S7. The energy profiles disclose high barriers, both for the metal/vacuum interface (0.94 eV), and with correction for aqueous

environment (0.64 eV). Chemisorption is therefore likely to be slow. Both overall processes were found to be endothermic: +0.69 eV (vacuum) and +0.27 eV (solution) largely due to the S-H bond breaking step. Additional energy gain comes, however, from the formation of a H_2 molecule by the recombination of the adsorbed H-atoms. We therefore conclude that reaction (1) is feasible, with an estimated reaction heat of -0.26 eV. Simulations of the same process on the Au(111)/water interface using a combination of DFT and Reactive Classic Molecular Dynamics reported recently³⁹ gave an estimated energy barrier of 0.3 eV, substantiating the exothermic character of the overall process.

We investigated also the dissociative adsorption of a cystine molecule (R-S-S-R) at the Au(100) surface, including solvation effects similarly to process (1), Figures 10B and S8. The energy profile, Figure 10B, is exothermic but with a notable barrier (0.82 eV). The entropic factor also favors dissociative adsorption. The reverse process, i.e. recombination, $2\text{R-S}_{\text{ads}} \rightleftharpoons \text{R-S-S-R}_{\text{ads}}$ on Au(100) is therefore both thermodynamically and kinetically unlikely.

Cys dimers turn out to be stable Cys species in both DFT and Monte Carlo simulations, see below. In what follows we therefore take the formation of Cys dimers from the adsorbed products of eq. (1) as an important initial step of the SAM formation on Au(100). With a further view on recent observations of the adsorption of other chiral thiol molecules^{76-78,80} the most stable dimer combinations of L- and D-Cys in all the three stereo-selective combinations, L,L, D,D and L,D were addressed. The most stable radical forms of these species include cyclic dimers, where two Cys radicals are contacted through two hydrogen bonds -O---HO-. The most stable dimer for Cys radicals in zwitterionic form has two -COO---H₃N- contacts.

We define the energy of dimer formation on the Au(100)-surface as:

$$\Delta E_f^{\text{dim}} = \Delta E_{\text{ads}}(\text{Cys dim}) - 2\Delta E_{\text{ads}}(\text{Cys}) \quad (14)$$

where $\Delta E_{ads}(Cys)$ and $\Delta E_{ads}(Cys\ dim)$ are the adsorption energies of a single monomeric Cys species and a cysteine dimer, respectively. Further,

$$\Delta E_{ads}(Cys) = E_{tot}(Au_n - Cys) - E_{tot}(Au_n) - E_{tot}(Cys) \quad (15)$$

$$\Delta E_{ads}(Cys\ dim) = E_{tot}(Au_n - Cys\ dim) - E_{tot}(Au_n) - 2E_{tot}(Cys) \quad (16)$$

Figure 11. Optimized orientation of three different R_Z-R_Z dimers adsorbed at the $Au(100)$ surface in planar (horizontal) orientation; L,L (A); D,D (B); L,D (C).

For brevity, we will refer to thiols as ‘M’, and thiol radicals as ‘R’ with a subscript ‘Z’ to indicate zwitterionic forms. Some results are compiled in Tables S1-S4 and Figures S9-S11. The data in Table S1 do not describe real electrochemical adsorption from the solution, but these results are needed for calculations using eq. (14) and (16). As seen from Tables S1-S4, formation of all Cys dimers at the $Au(100)$ surface is feasible. The deepest ΔE_f^{dim} values (from -3.6 eV to -2.7 eV) were found for the R_Z-R_Z dimers (Figure 11, Table S3). Formation of R-R dimers is less likely. The corresponding ΔE_f^{dim} values (ranging from -1 eV to -0.65 eV) accord with those reported by Chapman and associates³⁶ on the L,L (-1.04 eV) and L,D (-1.14 eV) dimers adsorbed at the $Au(110)$ /vacuum interface. The charge of the adsorbed Cys dimers was found to be small ranging from -0.05 to -0.23 e^- . The chirality of the Cys species significantly affects both the adsorption energy and the

geometry of the dimers, Tables S2, S3. ΔE_f^{dim} for the R_Z-R_Z dimers, increases in the order L,L < L,D < D,D, while a different sequence is observed for the R-R dimers, D,D < L,D < L,L. The adsorption energy of different Cys forms and their dimers with the metal surface thus depends on the interaction of the sulfide-, amino-, and carboxyl groups with the metal surface. In turn, the position and orientation of these molecular groups strongly depend on the geometry of the Cys dimers, which differs noticeably for different enantiomer pairs. This can explain qualitatively the observed trends.

We addressed furthermore two different types of “vertical” Cys dimer. The first form was composed of a Cys radical on the metal surface bound to a Cys molecule from the solution, i.e. (‘R-M’). In the second case, the dimer consists of a zwitterionic radical at the metal surface bound to a zwitterionic molecule from the solution side (‘ R_Z-M_Z ’). Our estimations show that the former structure is not feasible, whereas the second one is stable (Figure 12, Table S4). The formation energy of these dimers is in a narrow range from -1.15 eV to -1.05 eV and slightly chirally sensitive. The normal dipole moment projections calculated for the $Au_n - Cys$ vertical dimer are small and in the range 0. – 1.72 D. This might favour additionally the growth of rows composed of these dimers.

The formation energy of a L-Cys R-M dimer in planar orientation (Figure S11) was estimated to be -0.51 eV. This type of Cys dimer plays the role of an intermediate which might impede SAM formation. Reaction (1) is thus crucial in the SAM formation, but may proceed slowly because of steric immobility of bound Cys molecules. This might stop further initial growth of rows from Cys radicals and favor the appearance of “stripes” clearly observed by *in situ* STM.

Figure 12. Optimized structures of three Different R_Z - M_Z dimers on Au(100) in vertical orientation; (A) L,L; (B) D,D; (C) L,D. R_Z is adsorbed at the metal surface, while M_Z is located on top (from the solution side).

The STM contrasts were calculated for all adsorbed Cys zwitterionic dimers considered. The resonance integral (V_{if}) values were around 10^{-5} Hartree which corresponds to the diabatic (non-adiabatic) limit of electron transfer.^{24,25} As seen from Figures 13-14, the STM images for different types of the adsorbed species are different. “Stereoselectivity” of the STM contrasts is predicted as well. Distinct sub-molecular resolution of the images (which reveal two or three lobes) is observed for the L,L vertical and L,D planar dimers. In the model images for the adsorbed vertical dimers, the isosurface height corresponding to the brightest areas (h_{max}) ranges from 8.5 Å to 11 Å, which is within the 6.4 to 10 Å range for the zwitterionic Cys dimers in planar orientation. Although h_{max} depends on the V_{if} value which is a variable parameter, it can thus be argued that the STM contrasts related to the vertical Cys zwitterionic dimers give the strongest STM contrast.

The following sequence of events can then be proposed from the DFT calculations, Section 3.4.2:

Figure 13. STM contrasts computed for R_Z - R_Z dimers adsorbed on Au(100) in planar (horizontal) orientation; (A) L,L; (B) D,D; (C) L,D. Distances in a.u.

First step

Adsorption of Cys molecules, R-SH at the Au(100)-surface from the bulk solution. As this adsorption bond is weak, fast establishment of adsorption $\leftrightarrow$ desorption equilibrium is expected. Adsorption of Cys zwitterions ($R^{+/-}$ -SH) in molecular form is less likely due to strong hydration effects, even though this species prevails in the aqueous solution. Adsorbed Cys radicals R-S• and H-atoms appear next due to S-H bond dissociation; R-S• forms in turn an adsorbed Cys zwitterion radical $R^{+/-}$ -S• by fast intramolecular reorganization. This is likely to dominate the first chronopotentiometric step.

Second step

This step is the formation of a second Cys (zwitterion) monolayer on top of the first, leading to differently composed and oriented Cys molecular bilayers. These are

dominated by dimers of adsorbed zwitterion radical $R^+-S\cdot$ adjacent to the electrode surface hydrogen bonded to a zwitterion molecular form towards the solution, in horizontal or upright orientation (R_Z-M_Z). Dimer formation is still incomplete and the adlayer composed of a mixed mono- and bilayer. The DFT computations give brighter STM contrasts for the upright than for the horizontal Cys dimer orientation. Different adsorbed intermediate (R-R and R-M) dimer forms might exist as well and affect the dynamics of the SAM formation.

Figure 14. STM contrasts computed for three R_Z-M_Z dimers adsorbed on Au(100) in vertical orientation; (A) L,L; (B) D,D; (C) L,D. Distances in a.u.

Third step

The first and second steps are proposed to lead to formation of a dense zwitterion Cys mixed mono- and bilayer. From reported voltammetric and *in situ* STM studies of the

time evolution of cysteamine⁷² and propanethiol monolayers⁷³ on Au(111)-electrode surfaces, the primary adlayers, however, evolve through several disordered phases towards the ultimate highly ordered adlayers. In the present study these phases would include transition from disordered, vertically or horizontally oriented zwitterionic Cys monolayers and Cys radical/molecular dimer combinations. These act further as directional nucleation sites into the highly ordered surface rows and stripes observed by *in situ* STM. This step may be what is observed as the slow chronopotentiometric phase.

3.4.2 MC simulation of Cys adlayer formation on Au(100)

Some results of kinetic MC simulations for the squared 100×100 surface site lattice illustrating four different stages of the Cys adlayer formation are sampled and shown in Figure 15, where (A) – (D) correspond to different numbers of simulation steps, see Table 2.

Table 2. Fractions of Cys species and structures in the adlayer on Au(100) estimated for different stages of the monolayer formation based on kinetic MC simulations. Stages and corresponding number of steps ($\times 10^4$): (A) 2.25, (B) 9, (C) 27.5, (D) 225. M = molecule, R = radical, R_Z = zwitter ion radical, M_Z = zwitter ion molecule.

Stage	M	R	R_Z	R-M dimer	R_Z-R_Z planar arrays	$[R_Z-M_Z]_n$ vertical rows
(A)	0.86	0.11	0.	0.03	0.	0.
(B)	0.08	0.02	0.14	0.01	0.7	0.05
(C)	0.01	0.	0.03	0.	0.82	0.14
(D)	0.	0.	0.01	0.	0.75	0.24

At the initial stage of adlayer formation, (A), adsorbed Cys molecules, M, clearly dominate. There are also small amounts of Cys radicals, R, and R-M dimers. The number of molecules is then drastically reduced, and rows of adsorbed zwitterionic forms (planar and vertical) appear at the intermediate stage (B). Adsorbed single Cys zwitterions, R_Z , and a very small amount of R-R and R-M dimer is also seen. In the next stages, (C) and (D), approaching the final state we observe further growth of rows of vertical Cys dimers, some of which show a branched structure, similar to the “forked” stripes observed by *in situ* STM. The R-R and R-M dimers have here practically disappeared. Rows of zwitterions in planar orientation, R_Z - R_Z , tend to form arrays in all the stages. Data on the fractions of different Cys species and structures on Au(100) are collected in Table 2.

Figure 15. 2D-pictures illustrating four different stages of the formation of a Cys adlayer on Au(100) built using the data of kinetic MC simulations, cf. Table 2. M, R, R_Z are Cys molecules, radicals, and radical zwitter ions, respectively. R-R, R-M, R_Z - R_Z , R_Z - M_Z are dimer species. $[R_Z-M_Z]_n$ indicates rows of vertical dimers.

The events that emerge from the MC calculations match broadly the voltammetric, chronopotentiometric, and *in situ* STM data. The dominating final state is represented by rows of vertically oriented zwitterion dimers, $[R_Z-M_Z]_n$, but with a number of “fork-like” “irregularities”. The initial fast chronopotentiometric phase is likely to incorporate stages (A) and (B), possibly also partly (C), as these stages cannot be separated. The slow chronopotentiometric phase is likely dominated by stages (C) and (D), i.e. the growth and formation of $[R_Z-M_Z]_n$ from single-molecule zwitterionic forms or from differently oriented (planar) zwitterionic rows. $[R_Z-M_Z]_n$ is most likely what dominates the *in situ* STM images. The abundant “fork-like” structures and other surface structural

irregularities together with the dual voltammetric reductive desorption peaks, however, also suggest that residual single zwitterionic molecules and planar versus vertically oriented zwitterionic dimers are abundant enough to contribute to both voltammetry and *in situ* STM.

For a more rigorous description of the structure and dynamics of molecular adlayers at metal electrodes in contact with electrolyte solution, periodical DFT calculations with CPMD or reactive molecular dynamics would be tempting. Among other advantages such methods can describe the solvent explicitly at the molecular level, which is another challenge for molecular modelling.

The combined approach based on the energy characteristics of different Cys species adsorbed on the Au(100) surface, model STM contrasts, and MC simulation points to the crucial role of the zwitterionic forms in different orientations in the final monolayer. Adsorbed zwitterions are also nucleation centers for the growth of rows of Cys zwitterions (and their dimers) in different directions and likely to be crucial in the initial and intermediate adlayer formation stages. The appearance of “vertical” R_Z - M_Z dimers is favored additionally, as the second Cys zwitterionic molecule enters from the solution bulk with constant high concentration of this species.

The unique organization of Cys dimers in “stripes” and “forks” on the Au(100) surface compared with Cys adsorption on Au(111) and Au(110) where only a single Cys surface species/orientation prevails is at first unexpected. The overall surface adlayer organization is, however, determined by a detailed interplay between lateral intermolecular forces (hydrogen

bonds, van der Waals interactions) and the Au-surface structure. The square Au-atomic Au(100) surface is in fact well suited for unhindered Cys organization in “stripes” along the surface atomic rows, where the dimers are better nucleation centres than the monomers, and which would also trigger further organization in square Cys lattices, Figure 5 and Figure 15. The hexagonal Au(111) structure is much less compatible with “stripe” organization and Cys forms instead highly ordered monolayers with $(\sqrt{3} \times \sqrt{3})R30^\circ$, $(3\sqrt{3} \times 6)R30^\circ$, and $(4 \times \sqrt{7})R19^\circ$ cluster-like networks depending on supporting electrolyte and pH as noted.^{16,18,19,31} The missing row Au(110) structure is clearly visible in high-resolution *in situ* STM imaging²⁵ and might have been expected also to be a favourable template for “stripe” adlayer organization, but such a surface structure seems not to be competitive with the observed highly ordered $c(2 \times 2)$ dense monolayer with two Cys molecules in each unit cell. A recent study of Cys adsorption on polycrystalline gold based on surface plasmon resonance spectroscopy in fact also came to the conclusion that Cys dimers are formed,³² emphasizing again the importance of the local surface structure in the formation of Cys adlayers.

Kinetic MC simulations can shed light on this intriguing observation. As a reference for Cys adlayer formation on Au(100), MC simulations were carried out for Au(110) (Table S5-S6) illustrating the uniqueness of Cys adlayer formation at the Au(100) surface. Indeed, the results on Au(110) look quite different as compared with those obtained earlier for the Au(100) surface. In the case of Au(100), we see a competition between arrays of zwitter ions adsorbed in planar orientation with stripes formed by Cys dimers in vertical orientation (Table 2, Figure

15). In contrast, for Au(110) arrays of “planar” zwitter ions fully dominate at the final stage and the fraction of “vertical” dimers passes a maximum (see Table S6 and Figure S12-13). The latter finding qualitatively agrees with the c(2x2) lattice as disclosed by the experimental *in situ* STM data²⁵

It is important to note that the probabilities in Table S5 were introduced in a “phenomenological” way. To find them from first principles is equivalent to performing fully atomistic molecular dynamics simulations, which remain a complex and challenging problem.

Together the voltammetric, potentiometric, and *in situ* STM data, combined with the DFT and MC calculations thus offer a detailed and coherent view of Cys adlayer structures on electrochemical Au(100) electrode surfaces. This extends to the molecular mechanism with adsorption and nucleation directed growth of Cys adsorbate rows and the Cys monomer and dimer intermediates.

4. Conclusion and Perspectives

As an electrochemical core target, adsorption of the amino acid Cys on single-crystal and polycrystalline Au-electrode surfaces has long been in focus of electrochemical surface science. In the present work we report a comprehensive study of the high-resolution Cys adlayer structure on electrochemical Au(100)-electrode surfaces as well as dynamic

features of the adsorption process. Electrochemical high-resolution *in situ* STM of Cys adsorption on single-crystal Au(111)- and Au(110)-electrode surfaces have been reported before, but very little attention given neither to the third of the low-index surfaces, Au(100), nor to a comparison between the three enantiomeric L-, D-, and racemic L,D-forms, which are the targets of the present study. Cys adsorption dynamics on electrochemical single-crystal Au-electrode surfaces has not been addressed before either. At the same time, the high-resolution structure of the Cys adlayers on the Au(100)-electrode surface has disclosed intriguing features not seen for the other low-index surfaces.

We have used an experimental approach which combines voltammetry, chronopotentiometry, and electrochemical *in situ* STM to single-molecule resolution. The experimental approach was supported and guided by both DFT and MC calculations, to map how the adsorbed solvated Cys species are organized on the Au(100)-electrode surface. Such a combined experimental and theoretical study of the adsorption of solvated enantiomeric chiral Cys monomers and dimers has not been reported before.

The approach used has led to a coherent view of the structure and mechanism of Cys

adlayer formation on the Au-electrode surface. CV displays two reductive desorption peaks instead of the single peak on Au(111)¹⁸ and Au(110) electrode surfaces²⁵. One peak, at ≈ -0.7 V coincides approximately with the Au(111) and Au(110) peaks, while the second peak at 0.3 V more negative potential is unique to Au(100). The peak areas are significantly smaller than for Au(111), and the peak ratio about 1:2 with the -0.7 V peak the smallest. This is a first indication that two adsorbed species co-exist on the Au(100)-surface. The peaks were tentatively assigned to Cys monomer and H-bonded upright dimer species at -0.7 V and -0.97 V.

Dual-structure adlayers was supported by chronopotentiometry, using a generic two-step kinetic model. Chronopotentiometry appears to record signals both from the final, presumably dimeric adsorbed Au-S bound species, and from intermediate possibly monomeric Au-S bound states, but does not offer direct insight into the molecular nature of these (Cys monomer or dimer). Neither can other rapidly equilibrating intermediates such as Cys bound via the ammonium or carboxylate groups be excluded.

Cys adlayer structures on Au(111)-electrode surfaces has been mapped to molecular resolution by *in situ* STM. The surface lattice

structure depends sensitively on both electrolyte and pH. Cys monolayers on Au(110) has also been mapped, even to submolecular resolution by *in situ* STM and show a $c(2\times 2)-2$ lattice with two Cys in each unit cell,²⁵ but no high-resolution imaging has been reported for Cys on Au(100)-electrode surfaces. Neither has comparative *in situ* STM of the different enantiomeric Cys forms been reported.

Molecular scale *in situ* STM resolution in the present study discloses a unique stripe-like pattern of adsorbed Cys molecular species on the Au(100)-electrode surface, with additional “fork-like” structures and defects or domains of defects. The pure enantiomeric D- and L-forms display very similar surface structural patterns, whereas racemic L,D-Cys displays many more defects. Differences such as these were also reported for the two chiral 2-butanethiols among the four isomeric butanethiols.⁷⁶⁻⁷⁸

Enantiomer-specific molecular adsorption can be triggered by substrate Au-surface atoms around defect structures and step edges which can themselves be chiral, leading to different numbers of defects for different enantiomers of chiral adsorbate molecules. Au-surface atoms also become chiral, once adsorbate molecules are bound, and whole adsorbate superstructures

(domains etc.) can be chiral for nonchiral molecular adsorbates and vice versa. Further detailed discussions are given elsewhere^{34,76,78,81-83}. The *in situ* STM images show still another feature to support that the equilibrated final state surface Cys adlayer holds more than a single Cys species, as suggested by voltammetry and chronopotentiometry. Both the stripes and the space between the stripes accommodate adsorbed Cys molecules, with two Cys orientations, as reported for cysteamine adsorption on Au(111)⁷². Upright Cys orientation offers a site for adsorption of a second Cys molecule to form a Cys bilayer on the Au(100)-surface such as suggested by the *in situ* STM images and according with the observation of a second, more negative voltammetric potential peak.

A theoretical frame for the data was, finally provided. This frame included, first DFT calculations of the energetics and *in situ* STM contrasts of a variety of solvated monomers and dimers in different orientations as well as radical, zwitterion and molecular forms in different orientations on a model Au(100)-surface. Secondly, MC computations led to molecular understanding of the adsorption *process* with all the stripe and fork structural elements as well as intermediates in the adsorption process.

DFT was based on a cluster model for the Au(100)-surface, with focus on the different Cys monomers and dimers in radical and zwitterion forms, and in planar or vertical orientation. In contrast to previous reports,³³⁻³⁶ we also included the solvent environment. The results of the DFT modelling were used to calculate model STM contrasts, and chiral effects on adsorption of L- and D-Cys molecules. The quantum chemical results combined with a kinetic MC scheme offer a direct impression of the ultimate adlayer structure as well as of crucial stages in the adlayer formation process. This approach was an immediate support and guide for the *in situ* STM images and the chronopotentiometric data.

Based on the totality of experimental data, and the quantum chemical DFT and MC calculations, we have suggested a sequence of molecular events in the Cys/Au(100) adsorption process. The overarching mechanistic view is that a Cys radical monolayer dominated by the zwitterionic form is formed at first, followed by adsorption of a second layer. The latter is first a disordered bilayer but reorganizes slowly to the final ordered row and stripe adlayer.

As a final observation, the stripe-like *in situ* Cys surface bilayer structure is only observed for Au(100) and is quite different

from the *in situ* Cys c(2x2) monolayer on Au(110)²⁵ and the various monolayer structures on Au(111)^{18,26}. Reasons for this difference cannot be precisely identified, but both the DFT and MC calculations in the present report point to the crucial role of zwitterionic dimers unique to Au(100), and both giving strong *in situ* STM contrasts and acting as nucleation sites for full adlayer formation. The anisotropic charge distribution of a zwitterionic dimer, further supported by the atomic rows of Au-atoms in the square lattice of Au(100) could induce corresponding anisotropic further directional bilayer growth ultimately seen as stripes in the *in situ* STM images and the MC calculations. As noted, stripe-like surface structures of the Cys adlayer are not seen on the Au(111)- and Au(110)-surfaces. It was argued in Section 3.4 that this is associated with the overall surface adlayer organization being determined by a detailed interplay between lateral intermolecular forces and Cys interactions with the quite different Au-surface structures, emphasizing the importance of the local surface structure in the formation of Cys adlayers.

Associated content

Supporting information.

Further characterization of reference experiments and descriptions of

experimental setups are given in the supporting information. This material is available free of charge *via* the Internet at <http://pubs.acs.org>.

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