New Insight into the Interface Chemistry and Stability of Glutathione Self-Assembled Monolayers on Au(111)

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Cyclic voltammetry of GSH SAMs on Au(111) prepared in water (pH = 3.5)

In the case of SAMs prepared from 5 mM GSH aqueous solutions (pH ≈ 3.5) for t_{SAM}=1 h, electrodesorption peaks are very similar (both in potential and charge density (Q)) to those obtained for incubation in GSH solutions at pH = 7 (Fig. 1, green line). For larger incubation times, however, Q values do not increase significantly: for t_{SAM} = 24 h they are very similar to that for 1 h incubation, while for t_{SAM} = 48 h Q only increases to 40-50 μC cm^{-2}. Therefore, while some GSH desulfurization takes place which is favored by increasing t_{SAM} (as revealed form CV and also from the shape of the S 2p XPS spectra in the literature),[1-2] it can concluded that this reaction is largely inhibited at low pH values, whereas it is favored by the increase in pH (as found at pH = 13).
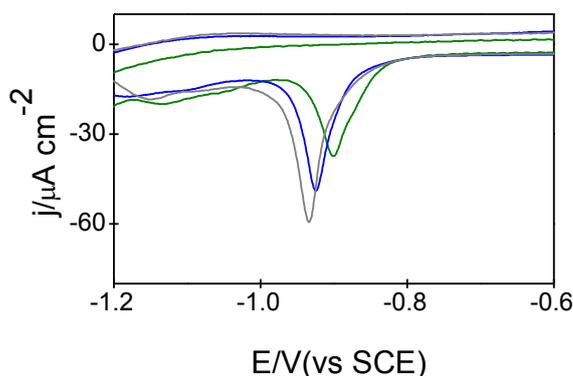

Fig. S1. *Cyclic voltammograms of GSH SAMs on Au(111) prepared by immersion in 5 mM GSH solution in water (pH = 3.5) for different incubation times: 1 h (green), 24 h (blue) and 48 h (gray). Scan rate: 0.05 Vs^{-1}. Electrolyte: 0.1 M NaOH.*

Cyclic voltammetry of S SAMs on Au(111) prepared at pH = 7

Au(111) substrates were incubated for 1 h in 5 mM Na_2S solutions in 10 mM PB (pH = 7). In this condition the predominant sulfide species are H_2S and HS^- (pK_{a1}≈7). Cyclic

voltammograms performed in 0.1 M NaOH solutions of S-modified substrates show broad peaks at $\approx$–0.95 V and Q = 300-500 μC cm^{-2} (see Fig S2(a)). This charge density is considerably larger than that expected for the electrodesorption of a S submonolayer with coverage θ = 1/3 on Au(111) according to:

$$S\text{-}Au(111) + 2e^- \rightarrow S^{-1} + Au(111) \qquad \text{(eq. 1)}$$

which yields a sharp peak with Q = 150 μC cm^{-2}.[3-4] The difference in Q can be attributed to the presence of additional adsorbed polysulfide species formed at OCP at pH = 7, which are reduced according to:

$$Au\text{-}(S_{x+1})^{-2} + 2(x\text{-}1)e^- \rightarrow Au\text{-}S + xS^{-2} \qquad \text{(eq. 2)}$$

As reported by Woods et al[5], at pH = 13 both the reduction of the S submonolayer and of the adsorbed polysulfides take place at the same potential, giving rise to broad, asymmetric peaks as those in Fig. S2.
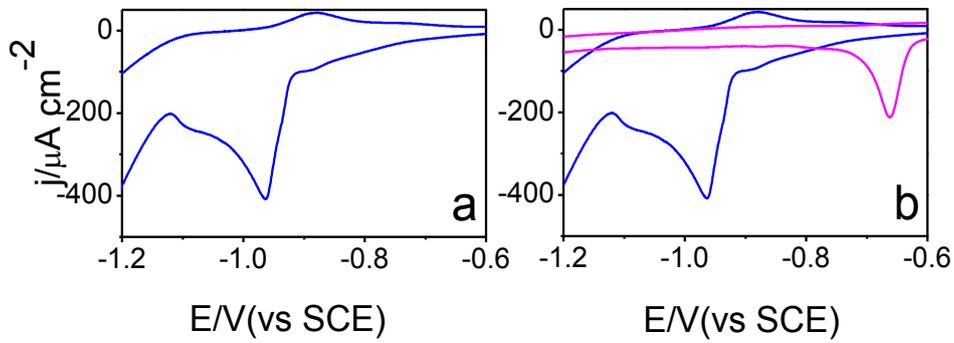

Fig. S2. *Cyclic voltammograms (scan rate: 0.05 Vs^{-1}; electrolyte: 0.1 M NaOH). (a) Au(111) immersed in 1 mM Na₂S solution (pH = 7) for 1 h. (b) (blue line) 6MP SAM exposed to 1 mM Na₂S solution (pH = 7) for 1 h. CV corresponding to blank 6MP SAM is shown (magenta line).*

Moreover, electroreduction in 0.1 M NaOH solutions of 6MP SAMs on Au(111) that were exposed for 1 h to Na$_2$S solutions in PB (pH =7) (Fig S2(b)) show large, broad peaks similar to that in Fig S2(a). This shows that, as expected, sulfide species completely displace adsorbed 6MP molecules.

STM imaging of GSH SAMS on Au(111)

In air STM of GSH SAMs on Au(111) was performed to further understand the nature of the interface. Experiments were done in the constant current mode with an ECM microscope from Veeco Instruments (now Bruker, Santa Barbara, CA) controlled by a Nanoscope IIIA unit, also from Veeco Instruments. Mechanically cut Pt-Ir tips were used and typical bias voltages (E$_{bias}$), setpoint currents and scan rates were 0.4 V, 0.7 nA, and 3 Hz, respectively. The scanner calibration was checked by imaging highly oriented pyrolytic graphite (HOPG) with atomic resolution.

Images of fresh SAMs (t$_{SAM}$ = 1 h) show a total lack of vacancy islands ("pits") (Figure S3(a)), in resemblance to what has been previously observed by our group for 6-mercaptopurine[6] and 4-mercaptobenzoic acid[7] and by others[8] for the case of different aromatic thiols. In the light of the adatom models for alkanethiol SAM on Au(111), it is accepted that the presence of "pits" is a direct consequence of the reconstruction of the gold surface. The absence of these surface features suggests that GSH is unable to induce this surface reconstruction (which involves "staple" motif formation) and thus adsorption takes place on the Au(111)-(1×1) surface. Moreover, it should be mentioned that no long range order was observed from high resolution imaging of the GSH SAMs, even though in some cases a few rows with 1.5-1.7 nm separation were imaged which

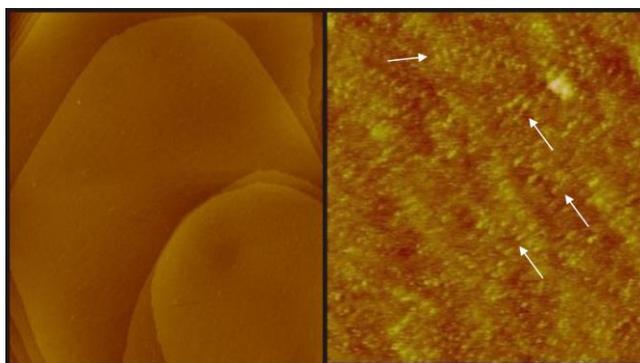can be assigned to ordered molecules (see Figure S3(b) for 1 h GSH incubation at pH = 3.5).

Figure S3. *In air STM images of GSH SAM on Au(111). (a) Sample corresponding to t_{SAM} = 1 h in fresh 1 mM GSH solution at pH = 7 (300 × 300 nm^2). The gold surface is free of "pits". (b) Sample corresponding to t_{SAM} = 1 h in fresh 1 mM aqueous GSH solution (pH = 3.5) (50 × 50 nm^2). Some rows separated 1.5-1.7 nm can be distinguished.*

Thiol competitive adsorption experiments

Mixed GSH-6MP SAMs were prepared by incubation of Au(111) in binary 1 mM GSH-6MP solutions (pH = 7) of different molar fractions (X_{GSH} = 0.15, 0.5, 0.85), either for t_{SAM} = 1 h or 24 h. Samples were rinsed with water, dried with N_2 and then placed in an electrochemical cell for cyclic voltammetry measurements. The reductive desorption cycles of the mixed SAMs are shown in panels (b) and (c) in Fig. S4 for 1 h and 24 h incubation times, respectively. In panel S4(a) we have included CV of typical blank GSH (t_{SAM} = 1 h) and 6MP SAM for comparison. Results are discussed in the text.
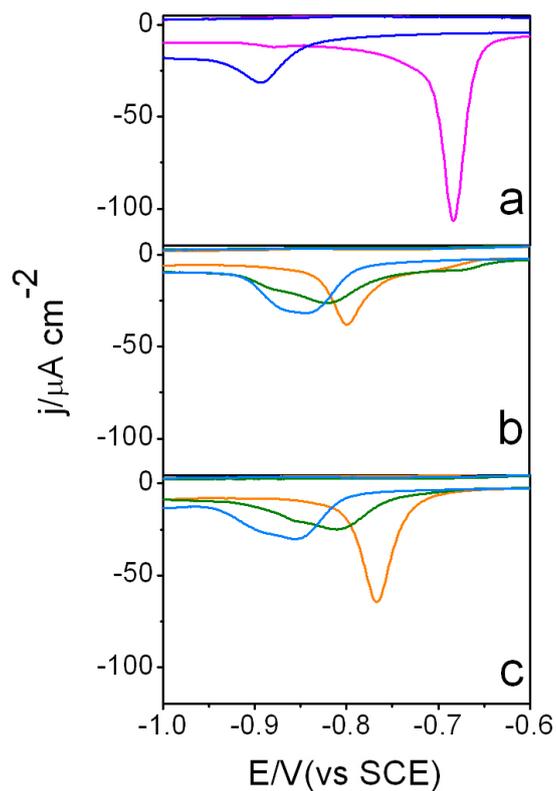
Figure S4. *Cyclic voltammograms (scan rate: 0.05 Vs^{-1}; electrolyte: 0.1 M NaOH). (a) Curves for blank 6MP (magenta line) and GSH (blue line) SAMs on Au(111) (prepared from 1 mM solutions in PB, pH =7; t_{SAM} = 1 h). (b) and (c) Curves for different mixed GSH/6MP SAMs: X_{6MP} = 0.85 (orange line); X_{6MP} = 0.5 (green line); X_{6MP} = 0.15 (light blue line). Curves in (b) and (c) correspond to 1 h and 24 h incubation, respectively.*

Additional DFT results

(See Materials and Methods for details on the DFT calculations).

(a) Physisorbed GSH and zwitterionic GS on Au(111)

Table S1. Energetic data for GSH species on Au(111)

Adsorbate	GSH	GS$_{zwitt}$
unit cell	$(3\sqrt{3}\times3)$	$(3\sqrt{3}\times\sqrt{3})$
θ	0.055	0.11
E_b/eV	-3.48	-4.40
γ/meV. Å^{-2}	-25.81	-65.23

Table S1 and Figure S5(a) and (b) show the optimized structure and the most relevant energetic data corresponding to the form of anionic GSH with a protonated thiol group, which we will call physisorbed GSH (θ = 0.055). In this case the molecule is not bound to the Au surface through the S atom but by two oxygens of the carboxylate groups of Gly and Glu. In a similar way, Table S1 and Figure S5(c) and (d) show the structure and energetic data of adsorbed zwitterionic GS, which differs from anionic GS in the protonation of Gly.
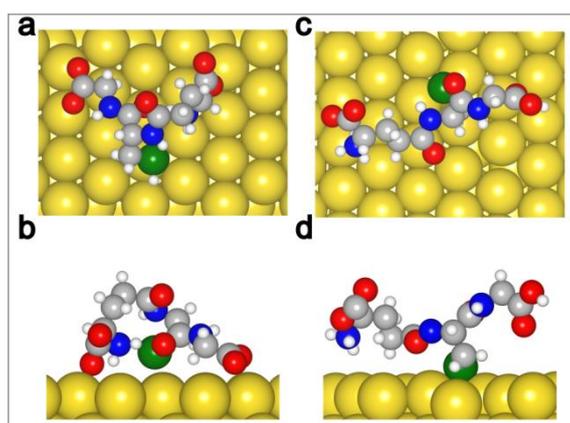

Figure S5. *Optimized structures of (a)-(b) $(3\sqrt{3}\times3)$ physisorbed GSH (θ= 0.055) and (c)-(d) $(3\sqrt{3}\times\sqrt{3})$ zwitterionic GS (θ= 0.11) on the unreconstructed Au(111) surface. (a)-(c): top views; (b)-(d) lateral views. Color of the atoms: yellow, Au; green, S; gray, C; white, H; red: O; blue: N.*

(b) Sulfur adlayers on Au(111)

Table S2. Energetic data for S surface structures on Au(111)

unit cell	(3√3×√3)	(2√3×√3)	(√3×√3)
θ	0.11	0.17	0.33
E_b/eV	-4.77	-4.60	-4.54
γ/meV. Å^{-2}	-70.74	-102.34	-201.96

DFT calculations have been also performed for S adlayers on Au(111) at different coverages which show that surface free energies (γ) significantly increase (in absolute value) for S coverage > 0.11 (see Table S2) compared to adsorbed GS species.

(c) 6-Mercaptopurine on Au(111)

STM imaging has revealed the existence of rectangular molecular structures 0.75×0.6 nm^2 in size, which have been modeled as a (3√3×2) lattice with two molecules oriented along the [110] direction per unit cell (θ = 0.17). [9] However, images also show a variety of coexisting molecular structures (like dimers, trimers and larger aggregates of closely packed molecules) which appear in a disordered arrangement, as already reported.[6] In order to perform the calculations we have only considered the ordered structure, that is, the (3√3×2) structure with 2 molecules per unit cell (θ = 0.17).
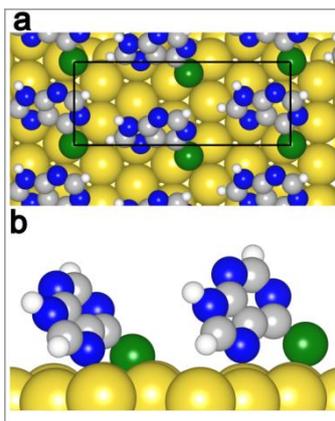
Figure S6. *Optimized structure of the (3√3×2) 6MP lattice on the unreconstructed Au(111) surface: (a) top view; (b) lateral view. Color of the atoms: yellow, Au; green, S; gray, C; blue: N; white, H.*

The structure corresponding to the most stable configuration is shown in Figure S6: 6MP molecules are bonded by the S and N(7) atoms at nearly top- bridge and top sites, respectively, with a 60° tilt with respect to the surface normal.[6] The relevant parameters are shown in Table 2 in the text for comparison with the GSH lattices.

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