Orientation of Ordered Structures of Cytosine and Cytidine 5′-Monophosphate Adsorbed at Au(110)/Liquid Interfaces

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It is demonstrated using reflection anisotropy spectroscopy that the adsorption of cytosine and cytidine 5′-monophosphate at the Au(110) 1 × 2/electrolyte interface gives rise to ordered structures in which the base is oriented vertical to the surface and parallel to the $[1\bar{1}0]$ axis of the Au(110) plane.

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An understanding of the interaction between DNA and Au surfaces and the coupling this induces between the optical response of the molecule and the surface would advance the exploitation of surface plasmon resonance [1], DNA labeled nanoparticles [2], and bioarray technology [3,4].

In this work we show that the orientation of ordered structures formed by the adsorption of the DNA base cytosine (C) and cytidine 5′-monophosphate (CMP) at Au(110)/electrolyte interfaces can be determined from reflection anisotropy spectroscopy (RAS). For each molecule the plane of the nucleic acid base is oriented vertically to the surface and parallel to the $[1\bar{1}0]$ axis of the Au(110) plane. The adsorbed molecules increase the RAS over a wide spectral range by reducing the intensity of contributions to the optical response of the Au(110) associated with steps and by the coupling of dipole transitions in the base to the bulk dielectric constant of Au.

An Au(110) single crystal of 99.999% purity in the form of a disc of diameter 10 mm and thickness 2 mm was orientated to an accuracy of 0.1° by x-ray diffraction and prepared and transferred to the electrochemical cell as described previously [5]. The electrochemical cell was a typical three electrode cell with a platinum counter electrode, a saturated calomel electrode as the reference and a silica strain free disc as an optical window. The potentiostat was an Autolab PGSTAT 30. Solutions were prepared from NaH_2PO_4 and K_2HPO_4 (BDH, Analar grade) with millipore ultrapure water (18 $M\Omega \cdot cm$) purged with Ar. The RAS of the Au(110) electrodes was measured, at *p*H 7, with an instrument of the Aspnes design [6,7] at a potential of 0.0 V, which preserves the 1 × 2 reconstruction [8]. The measured RA signal is given by

$$\mathrm{Re}\left(\frac{\Delta r}{r}\right) = \mathrm{Re}\frac{2(r_{[1\bar{1}0]} - r_{[001]})}{r}. \tag{1}$$

The RA spectra of the Au(110) disc prepared in the two separate experiments prior to the deposition of the molecules are shown in Fig. 1. The spectra of the two surfaces show small differences in detail, that we attribute to differences in surface morphology [9], and are similar to RA spectra obtained previously in electrochemical environments [5,9–11] and in UHV [5,7,12,13]. The strong positive contribution to the RAS beyond 4 eV suggests that the surfaces have a large concentration of monatomic steps aligned along $[1\bar{1}0]$ directions [5], a conclusion confirmed by STM.

In separate experiments C and CMP (Aldrich: Analar grade) were added to the solution, to give concentrations of 0.1 mM, with the Au electrodes held at 0.0 V. The addition of the molecules increases the RAS across a wide spectral range and although C gives rise to a slightly more intense signal than CMP, there are only slight differences in spectral profile (Fig. 1). The two molecules enhance the negative RA signal of the Au(110) surface between 2.5 and

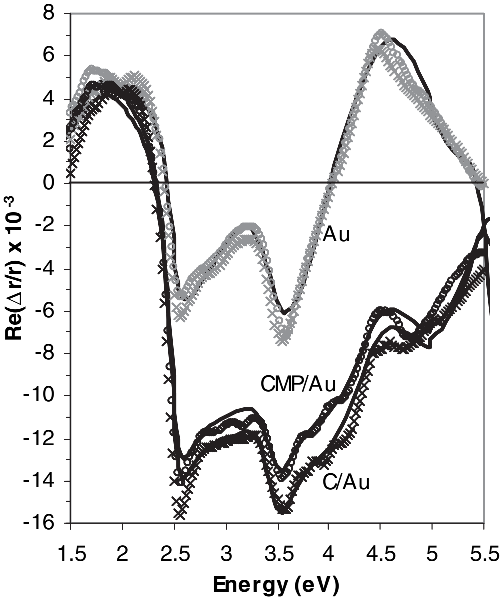

FIG. 1. The RAS of the two Au(110) 1 × 2 surfaces prior to the addition of the molecules are shown by × and ○ gray symbols. They are almost indistinguishable. The simulation of these spectra is shown by the upper line. The RAS of C/Au and CMP/Au are shown by the × and ○ bold symbols, respectively. The simulations of these spectra are shown by the lower lines.

3.5 eV, but produce a markedly different spectral shape by contributing a strong negative signal in the range 3.5 to 5.5 eV. The results of STM studies show that both molecules produce a saturation coverage of the surface.

The changes induced in the RAS of the Au(110) surface establish that both molecules are adsorbed and form ordered structures. Since the ribose sugar and the phosphate group do not contribute to the adsorption spectrum of CMP, the RAS of both molecules will arise from transitions involving the base. The close similarity of the RAS of the two molecules coupled with the sensitivity of RAS to the orientation of dipole moments indicates that the bases of the two molecules adopt similar orientations on the Au(110) surface. When combined with a consideration of the molecular structures (Fig. 2), this suggests that the two molecules bond to the Au surface through the lone pairs on the N and O atoms, as found for C/Au(111) [14].

The absorption spectra of these molecules in the gas phase and in solution consist of a small number of π to π^* transitions, polarized in different directions in the plane of the nucleic acid base, and a few n to π^* transitions, polarized at right angles to the plane of the base, which are very weak and are ignored in what follows [15–18]. The optical transitions of free molecules of C have recently been calculated in quantum chemical multiconfigurational [17] and gradient-corrected density functional theory approaches [18]. The results from the two theoretical approaches are in good agreement and also agree with experiment for the transition energies. They lead us to expect that the cytosine base will give rise to two π-π^* transitions in this spectral range with energies of 4.7 ± 0.3 eV and 5.6 ± 0.3 eV. Unfortunately, unambiguous information on the directions of the transition dipoles with respect to the molecular axes of C is not available, since there are major differences between experimental [15,16] and theoretical [17] results for the directions of the transition dipoles and, in addition, the directions and amplitudes of the transitions are sensitive to the bonding environment [17]. For example, theory predicts angles of 60° and −5° for the directions of the 4.7 eV and 5.6 eV transitions, respectively, relative to the N_1-C_4 axis of C while experiment finds 6° and −46°. However, the fact that the two transitions are expected and found to have alignments in the plane of the cytosine base that differ by 50° to 60° is a useful result to which we return below.

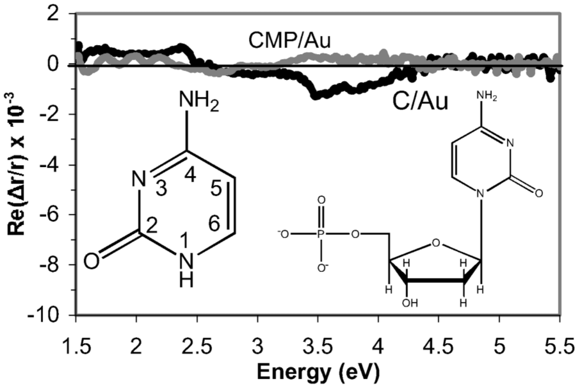

FIG. 2. The structures of C and CMP and the RAS of C/Au and CMP/Au obtained at $\theta = 45°$.

We now consider the relationship between the optical axes of the transitions arising from the adsorbed molecules and the optical axes of the Au(110) substrate. The angular variation of the intensity of the RA spectrum observed from the surface plane of a cubic single crystal varies as $\cos 2\theta$, where θ is the angle between the plane of polarization of the incident light and a crystal axis in the surface plane [7]. We confirmed this result for the RAS of the Au(110) surfaces. Macdonald and Cole have shown [19] that, when a RAS signal arises from a system with more than one set of optical axes, the angular dependence of the RAS shows a more complicated dependence on θ. For example, if the plane of the C molecule is adsorbed parallel to the Au(110) surface, then the intensity of the RAS observed from each of the two molecular transitions polarized in the plane of the molecule would vary as $\cos 2\theta_1$ and $\cos 2\theta_2$ where θ_1 and θ_2 are the angles between the plane of polarization of the incident light and the direction the dipole transition in the surface plane. Since θ_1 and θ_2 differ by $\sim 50°$, the contributions of the two transitions to the RAS would be almost optimally out of phase and there will be no angle at which the RA spectrum observed from the molecular plane will go to zero across the whole spectral range. If, instead of being orientated parallel to the Au(110) surface, the molecular plane is tilted with respect to the surface then the effect of this phase difference between the contributions of the two molecular transitions on the angular dependence of the RAS will decrease as the angle of tilt increases. If the molecule is orientated vertically on the surface, then the projections of the directions of the two dipole transitions onto the Au(110) surface plane will be equal, $\theta_1 = \theta_2$, and the contribution of the molecular transitions to the RAS will vanish across the whole spectral range when these angles equal 45°. However, the RAS observed from both the Au(110) surface and the vertically adsorbed basal plane of the molecule will only vanish across the whole spectrum at the same angle if the vertical plane of the molecule is orientated along one of the crystal axes of the Au(110) surface. The dependence of the RAS on θ is thus a powerful indicator of the orientation of the basal plane of these molecules with respect to the Au(110) surface. We find for each molecule that the total RAS from the Au(110) substrate and the adsorbed molecule vanishes across the whole spectrum for $\theta = 45°$. These results are shown in Fig. 2 and indicate that both molecules are orientated with the base vertical and aligned along one of the principal axes, [001] or $[1\bar{1}0]$, of the Au(110) surface. The angular sensitivity of our instrument, in the surface plane, is ±4°. This translates into a tilt angle sensitivity of at worst ±10° which occurs when the two transitions are 50° apart and when the transitions are in the directions ±25° from a principal axis of (110). Thus the plane of the molecule is orientated to within ±10° degrees of the normal to (110) and within 4° of either [001] or $[1\bar{1}0]$. If one of the transitions is along [001] or $[1\bar{1}0]$ and the other at 50° to this direction, then the tilt angle sensitivity is 3.5°. Actually in the absence of accurate results for the orientations of the dipole transitions in the basal plane it is difficult to exclude the possibility that one of the transitions is oriented in a near vertical direction to the surface, thus reducing its contribution to the RAS spectrum, and masking a slight tilt of the molecule away from the vertical. This would mean that only one molecular transition contributed to the RAS, a point discussed below. However, the results of Fig. 2 confirm that, whether tilted or not, the basal planes of both molecules are coincident with one of the principal axes of the Au(110) surface.

We now analyze the RAS profiles of the Au(110) 1 × 2 surface and the adsorbed molecules using the empirical approach of Cole *et al.* [20,21]. The bulk dielectric response of Au, ε_b, is represented by

$$\frac{1}{(1-\varepsilon_b)} = A(\omega) - iB(\omega). \tag{2}$$

In this work we employ the $A(\omega)$ and $B(\omega)$ functions deduced from the measurements of Blanchard *et al.* [13] which provide high resolution data up to 5 eV. For the region beyond 5 V we use the lower resolution data of Palik [22]. We specify an optical transition arising from light polarized along an x axis in the surface by a Lorentzian contribution:

$$\varepsilon_s^x(\omega) = 1 + \frac{S/\pi}{(\omega_t - \omega + i\Gamma/2)}; \qquad \varepsilon_s^y(\omega) = 1 \tag{3}$$

where S is the amplitude, ω_t the energy and Γ the full width at half maximum for the transition. For a thin surface layer of thickness $d \ll \lambda$, the wavelength of light, we have [5,19]

$$\mathrm{Re}\left(\frac{\Delta r(\omega)}{r}\right) = -\frac{2\omega d}{c}[A(\omega)\Delta\varepsilon_s'' + B(\omega)\Delta\varepsilon_s'] \tag{4}$$

where c is the velocity of light, and $\Delta\varepsilon_s' = (\varepsilon_s^{x'} - \varepsilon_s^{y'})$ and $\Delta\varepsilon_s'' = (\varepsilon_s^{x''} - \varepsilon_s^{y''})$ are the differences between the real and imaginary parts of ε_s^x and ε_s^y.

Using this approach Sheriden *et al.* [5] reproduced the spectral shape of the RAS of the Au(110) surface in the range 1.5 eV to 4.5 eV using three transitions with energies, 1.6 eV, 3.7 eV and 4.5 eV polarized along [001], $[1\bar{1}0]$ and [001] directions. The interpretation of the RAS of Au(110) in terms of this and other empirical models has been extensively discussed [7].

Here we obtain an empirical simulation of the Au(110) 1 × 2 RA spectrum in good agreement with experiment as shown by the full line in Fig. 1. This simulation was obtained with a least squares fitting procedure which yielded the parameters shown in Table I [Row (a)], the errors arising from the variations in the parameters needed to fit both Au(110) spectra simultaneously. The simulation, over this extended spectral range, of the contribution to the RAS associated with steps required two transitions of 4.7 and 5.6 eV polarized along [001].

While the simulation of the Au(110) RAS cannot be guaranteed to be optimum it serves as a basis on which to interpret the very large changes induced in the RAS by the adsorption of the molecules. The theoretical studies lead us to expect two transitions from the adsorbed molecules in this spectral range, so we sought to model the changes induced in the RAS by the adsorbed molecules by varying the parameters used to simulate the Au spectrum and including two additional transitions with energies of 4.3 and 5.5 eV. These simulations, which are in good agreement with the experimental results, are shown by the full lines in Fig. 1 and were obtained with the parameters shown in Table I [rows (b) and (c)]. These simulations required negligible changes in the 1st and 3rd transitions used in the simulation of the Au spectrum and reductions in the amplitude and widths of the 2nd Au transition, by factors of 5 and 2, respectively, to simulate the changes in the spectral range 2.5 to 3.5 eV. An important finding is that almost all the changes in the RAS of the Au surfaces induced by the adsorbed molecules can be simulated by reducing the intensities of the two high energy transitions

TABLE I. Parameters used to calculate the simulated RAS.

	Transition	1	2	3	4	5	6	7
	Direction	[001]	[001]	$[1\bar{1}0]$	[001]	[001]	$[1\bar{1}0]$	$[1\bar{1}0]$
(a) Au	ω_t/eV	1.60 ± 0.20	3.40 ± 0.10	3.90 ± 0.15	4.65 ± 0.15	5.60 ± 0.15	···	···
	Γ/eV	0.97 ± 0.15	0.74 ± 0.15	2.00 ± 0.20	2.00 ± 0.15	0.50 ± 0.05	···	···
	S^a	1.00 ± 0.10	0.26 ± 0.06	1.12 ± 0.09	1.11 ± 0.17	0.12 ± 0.03	···	···
(b) C/Au	ω_t/eV	1.60 ± 0.15	3.40 ± 0.08	3.90 ± 0.10	4.65 ± 1.00	5.60 ± 1.00	4.29 ± 0.05	5.50 ± 0.10
	Γ/eV	0.96 ± 0.30	0.38 ± 0.20	2.00 ± 0.20	2.00 ± 0.30	0.70 ± 0.40	0.70 ± 0.15	1.80 ± 0.10
	S^a	1.00 ± 0.16	0.06 ± 0.02	0.76 ± 0.14	0.31 ± 0.05	0.09 ± 0.02	0.11 ± 0.03	0.69 ± 0.06
(c) CMP/Au	ω_t/eV	1.60 ± 0.20	3.40 ± 0.10	3.90 ± 0.10	4.65 ± 0.05	5.60 ± 1.00	4.29 ± 0.11	5.50 ± 0.30
	Γ/eV	0.89 ± 0.30	0.39 ± 0.30	2.00 ± 0.30	2.00 ± 0.35	0.60 ± 0.32	0.60 ± 0.12	1.75 ± 0.15
	S^a	1.00 ± 0.15	0.05 ± 0.03	0.58 ± 0.14	0.27 ± 0.05	0.08 ± 0.04	0.07 ± 0.02	0.49 ± 0.04

[a] Relative intensities. Absolute intensities of transition 1 are 8127 (Au), 7702 (C/Au), 8240 (CMP/Au).

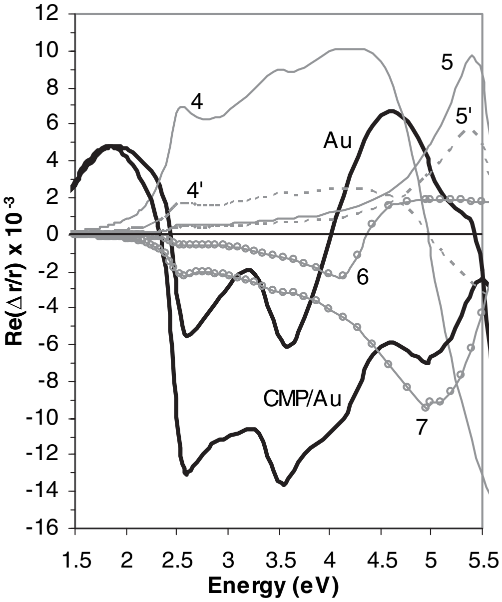

FIG. 3. The simulations of the RAS of Au and of CMP/Au are shown by the bold lines. The contributions of the two high energy transitions to the simulation of the RAS of Au are shown by 4 and 5. In the simulation of the RAS of CMP/Au these two transitions reduce in intensity to 4′ and 5′ and the two additional spectral profiles are associated with transitions 6 and 7.

in the simulation of the Au spectrum and adding two transitions attributed to the molecules. A very clear result was that two additional transitions are required and both have to be polarized along the $[1\bar{1}0]$ direction in order to simulate the RAS of C/Au and CMP/Au (Fig. 3). Comparison of the contributions of the high energy transitions to the simulation of the Au and CMP/Au RAS shows that the two high energy transitions polarized along [001], represented by curves 4 and 5, give a net positive contribution to the spectrum. The shift to negative values of the RAS across the whole spectrum caused by the adsorption of CMP can be simulated by reducing the intensities of the Au transitions as shown in curves 4′ and 5′, and assuming the transitions associated with the molecules are polarized along $[1\bar{1}0]$. Although the two molecular transitions have high energies the coupling to the dielectric constant of Au gives them a major influence on the optical response of the surface over the whole spectral range.

The results of the simulations establish that the basal plane of both molecules is orientated along the $[1\bar{1}0]$ direction on the Au(110) surface. An important finding is that it is necessary to include a significant contribution from each of the two molecular transitions to simulate the RAS spectra of adsorbed C and CMP. This indicates that neither transition is orientated vertically to the surface and consequently any tilting of the basal plane from the vertical must be small and certainly less than 10°. It is possible that the need to reduce the intensities of the two transitions that simulate the feature in the Au RAS associated with steps is caused by the adsorbed molecules interacting with, or reducing the number of, steps.

The simulations of the RAS of the adsorbed molecules require the molecular transitions to have the same energy and similar widths for the two cases but reduced amplitudes for CMP/Au. This reduction in the transition amplitudes could be due to a lower concentration of absorbed CMP. Alternatively, it could arise from a rotation of the CMP base in the plane normal to the surface, relative to that of C, or from a weakening of the coupling of the CMP transitions to the Au caused by the sugar and phosphate groups.

In addition to the determination of the orientation of these ordered structures this work demonstrates the power of RAS to determine molecular orientations at metal-liquid interfaces. We have also shown that the coupling between the molecular dipole transitions and the dielectric response of Au leads to an enhancement of the optical response across a wide spectral region.

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