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Nucleobase Orientation and Ordering in Films of Single-Stranded DNA on Gold

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Understanding the *structure* of single-stranded DNA (ssDNA) immobilized on surfaces is critical for applications exploiting the molecular recognition *function* of nucleic acids.[1] Here we show how the structure of ssDNA monolayers can be characterized using complementary spectroscopies that probe electronic and vibrational states of nucleobases—X-ray photoelectron (XPS), Fourier transform infrared (FTIR), and near-edge X-ray absorption fine structure (NEXAFS) spectroscopies. XPS reveals core-level shifts sensitive to DNA surface chemistry;[2,3] NEXAFS probes electron transitions between core levels and empty valence orbitals that are sensitive to nucleobase orientation;[4,5] and FTIR provides vibrational fingerprints sensitive to orientation and molecular interactions.[3,6]

Existing approaches for using these surface analysis methods to characterize self-assembled monolayers (SAMs) are not directly applicable to studies of ssDNA monolayers, which share very few properties with prototypical SAMs.[1] Whereas van der Waals *attraction* drives the assembly and ordering in typical SAMs, DNA immobilization is subject to strong electrostatic *repulsion*. Strands of ssDNA are also much longer and more flexible than typical molecules in SAMs; therefore, lateral spacing is length-dependent, and long-range lateral ordering is not observed in DNA monolayers. The main type of local ordering that may be present in a DNA film is nucleobase stacking. Nucleobase stacking largely determines the structure and interactions of DNA (including DNA hybridization); therefore, development of methods to detect the associated orientational ordering is a major objective in characterization of DNA films. Such methods will also help to study other biointerfaces (e.g., surface-bound proteins)[1] and to elucidate the correspondence of structures determined ex situ to those in aqueous solutions.

We use thymine homo-oligonucleotides [oligo(dT)] on polycrystalline Au as model ssDNA films to take advantage of analysis methodologies from extensive prior studies.[2,3,6] To examine the effects of attachment chemistry and ssDNA length on immobilization of oligo(dT), we compared monolayers of unmodified $(dT)_5$ (denoted T5 in the Figures) and 5′-thiol-modified $(dT)_5$-SH and $(dT)_{25}$-SH (T5-SH, T25-SH). IR reflection absorption spectra were measured under a dry nitrogen purge using a p-polarized beam at 75° angle of incidence.[3] Normal emission XPS data were acquired in ultrahigh vacuum (UHV) using a monochromatic Al Kα source.[2] NEXAFS data were collected in UHV with a fluorescence yield detector, and p-polarized synchrotron radiation at variable angle of incidence θ_i.[7] See Supporting Information for experimental details.

In Figure 1, we show how the structure and orientation of immobilized ssDNA can be determined from NEXAFS data at the nitrogen X-ray absorption edge. The two major features in these

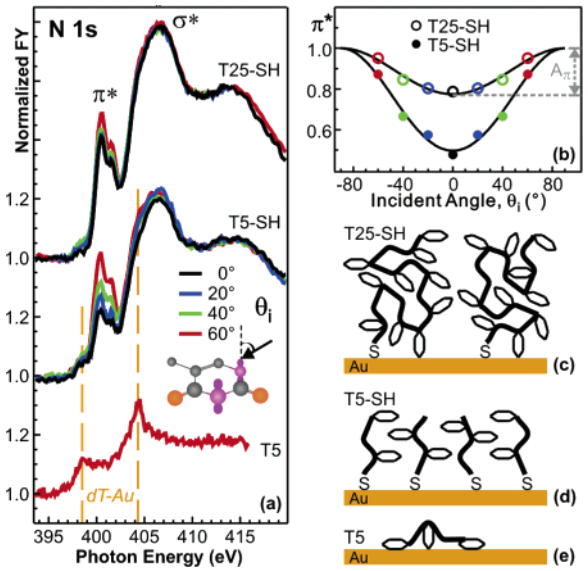

Figure 1. The structure of ssDNA on Au determined using fluorescence yield NEXAFS. (a) The N K-edge spectra of oligo(dT) monolayers on Au. In unmodified $(dT)_5$ films, red-shifted resonances (vertical dashed lines) correspond to dT bases chemisorbed on Au. (b) Polarization dependence of the π^* resonance intensity. A min at $\theta_{min} = 0°$ (p-polarized X-rays normal to the surface, electric field—parallel) indicates that the N π^* orbitals within the nucleobases [(a) inset] are preferentially aligned perpendicular to the surface. (c−e) Structural models of the oligo(dT) monolayers.

nitrogen K-edge spectra of DNA correspond to transitions from N 1s core levels into empty π^* and σ^* orbitals. The spectra in Figure 1a are normalized such that the height of the N π^* peaks above 1 is a semiquantitative measure of the N (and thus the dT *nucleotide*) coverage. In the spectrum of the unmodified $(dT)_5$ film, the π^* and σ^* resonances (vertical dashed lines) are red-shifted,[8] indicating dT chemisorbed on Au (Figure 1e). The two-peak N π^* structure in $(dT)_n$-SH spectra is characteristic of dT nucleobases and likely originates from the two distinct N atoms in dT.[9,10] The orientation of the N π^* orbitals is determined by varying the angle of incidence θ_i of p-polarized X-rays (colors in Figure 1a,b).[4,10−12] The intensity minimum for π^* resonances at normal incidence ($\theta_{min} = 0°$) indicates that dT nucleobases tend to orient parallel to the surface in $(dT)_n$-SH films.[13]

Monolayer structures determined by the complementary XPS and FTIR analysis agree with those determined by NEXAFS (Figure 1c−e). The spectra of $(dT)_5$ monolayers exhibit only features characteristic of dT chemisorbed on Au (vertical dashed lines in Figure 2a−b); that is, corresponding to prone DNA strands (Figure 1e).[3,6] In contrast, the spectra of $(dT)_n$-SH monolayers are dominated by signatures of nonchemisorbed dT; that is, indicating a brush of anchored ssDNA strands (Figure 1c,d).[3]

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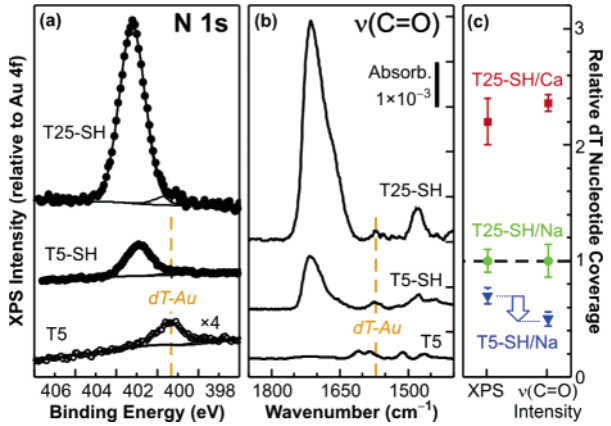

Figure 2. XPS and FTIR characterization of ssDNA on Au. (a) The XPS N 1s intensity normalized by the substrate Au 4f signal. (b) FTIR spectra (peaks ≈ 1700 cm^{-1}) correspond to C═O in dT. Vertical dashed lines in (a and b) indicate features associated with dT chemisorbed on Au. (c) Comparison of relative *nucleotide* coverages determined by XPS and FTIR[2,3] for oligo(dT) deposited from 1 M NaCl and 1 M $CaCl_2$ buffers. The reference *absolute* coverage determined by XPS for T25-SH/Na is 6.3 × 10^{14} dT/cm^2.

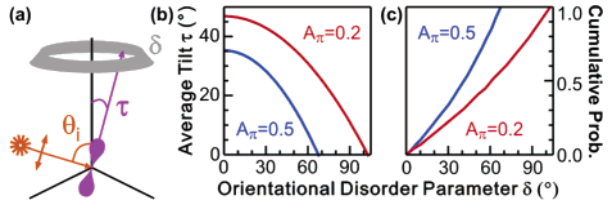

Figure 3. Interpretation of NEXAFS orientation signatures for disordered films. (a) The model assumes that π^* orbitals have random azimuthal orientation and a Gaussian distribution of tilt angles with an *average* tilt τ. The width of this distribution, δ, serves as an orientational disorder parameter; that is, δ is smaller for more ordered systems. The experimental NEXAFS intensity modulation amplitude A_π imposes limits on possible values of τ and δ. Solutions for $A_\pi = 0.2$ and 0.5 (T25-SH and T5-SH in Figure 1b, respectively) are shown in (b), the corresponding cumulative probability distributions for δ are shown in (c). Details in Supporting Information.

The agreement between NEXAFS, XPS, and FTIR on overall film structures provides a basis for comparing respective orientation signatures. Nucleobases can be preferentially oriented by direct interactions with gold only in $(dT)_5$ films which are similar to molecular monolayers. In contrast, $(dT)_n$-SH films more closely resemble multilayers of nucleobases,[14] where intermolecular interactions dominate over interactions with the surface.[10,12] The degree of preferential orientation in $(dT)_n$-SH films can therefore be used to ascertain the presence and to compare the strength of nucleobase interactions in these films.

Molecular orientation is often determined from polarization-dependent modulation of NEXAFS intensity. We augment the standard interpretation[4] of modulation amplitude A_π (Figure 1b) by including effects of orientational disorder, previously shown to be critical in polarization-sensitive spectroscopies.[15] We use the width of a Gaussian distribution of orbital tilt angles as an orientational disorder parameter δ (Figure 3). The standard analysis, which assumes perfect ordering, corresponds to $\delta = 0°$.

For $(dT)_n$-SH films, $\theta_{min} = 0°$, which for orbitals with random azimuthal orientation requires an average tilt $\tau < 55°$. The observed modulation amplitudes A_π further limit the range of τ and δ consistent with the data (Figure 3b,c). Specifically, the narrower range of δ for $(dT)_5$-SH indicates that orientational disorder is significantly lower in films of 5-mers compared to 25-mers (Figure 1c,d).

This difference in orientational disorder is also evident from a comparison of the XPS-determined dT nucleotide coverages and the relative FTIR peak intensities (Figure 2c). NEXAFS and FTIR signals are determined by the same dipole selection rules. In thymine, the N π^* orbitals and carbonyl bonds are roughly orthogonal (Figure 1a inset), so the off-normal *enhancement* of the NEXAFS signal should correlate with a *suppression* of the IR carbonyl peak. XPS is insensitive to molecular orientation, thus for films with similar ordering, the relative FTIR signal correlates with XPS coverage[3]—as shown in Figure 2c for $(dT)_{25}$-SH deposited in NaCl and $CaCl_2$ buffers. In contrast, for $(dT)_5$-SH, the FTIR signal is suppressed by ∼30% relative to the XPS coverage, consistent with preferential orientation of dT bases (Figure 1d).

Preferential orientation of nucleobases in $(dT)_5$-SH monolayers implies correlations between individual bases or, in other words, at least local ordering. This ordering is *not* imposed by direct interactions with Au or by the rigidity of ssDNA backbones. Moreover, hydrogen bonding between oligo(dT) strands is not observed in solution and would not be expected to form during the subsequent rinse in deionized water. Therefore, base stacking is the most likely mechanism of local ordering in $(dT)_5$-SH films.[16] In contrast, the $(dT)_{25}$-SH strands form mostly disordered films similar to polyelectrolyte brushes.[17]

In summary, we demonstrate how the structure, orientation, and ordering in ssDNA monolayers can be quantitatively determined by probing electronic and vibrational states of nucleobases. The simplest interpretation of the agreement between structures observed in UHV and ambient is that both must reflect some aspects of the initial structure in solution. This agreement then strongly suggests that ex situ characterization can be used to study the structure of biosurfaces. This work also establishes a foundation for in situ analysis, as both fluorescence yield NEXAFS and FTIR can be adapted to aqueous environment.

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Supporting Information Available: Materials and Methods; NEXAFS model. This material is available free of charge via the Internet at http://pubs.acs.org.

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