Supplement Material

Interaction of self-assembled monolayers of DNA with electrons: HREEL and XPS studies

Experimental

Sample preparation

DNA Preparations and Adsorption. Self-assembled DNA monolayers on gold films were prepared by deposition of 3' end thiolated single-stranded (ssDNA) and double-stranded DNA (dsDNA) of different sequences (Table 1). A solution of 10μM DNA (in 20mM Tris-HCl, pH 7.5, 0.4M NaCl) was spotted on the clean gold slide for a given time and humidity was controlled to avoid dryness. After adsorption at room temperature, the slide was rinsed in 20mM Tris-HCl, pH 7.5, 0.4M NaCl to wash away excess DNA. The slide was then soaked in the same buffer while it was shook for 15 min. This wash was then followed by shaking it for 15 min in 20mM Tris-HCl, pH 7.5, 0.2M NaCl, and soaking it 15 min in 20mM Tris-HCl, pH 7.5, and subsequently, by a thorough rinse in 20mM Tris-HCl, pH 7.5, and a quick rinse in sterile deionized (Millipore) water to remove any excess salt left on the surface. The slide was then dried by a stream of N_2. A 26 nuecleotides (nt) DNA oligomer was incubated with its complementary oligomer in 20 mM Tris-HCl, pH 7.5, 0.2M NaCl. This mixure was incubated at 75°C for 10 min, after which the reaction was cooled down slowly to room temperature. For hybridization, we used a thiolated oligo with a C3 thiol linker at its 3' end and a non-thiolated oligo, which was complementary to the thiolated one. The molar ratio between the thiolated oligo and its non-thiolated complementary oligo in the hybridization mixture was 1: 1.1 to ensure that no thiolated oligo remained as a single-strand. Thiolated single-stranded and double-stranded DNA were kept in their oxidized form $-(CH_2)_3-S-S-(CH_2)-OH$ in order to protect the thiol group from undesired oxidation products or dimerization. Prior to adsorption, the amount of DNA needed for adsorption was incubated with 10 mM of the reducing agent Tris(2-Carboxyethyl) Phosphine (TCEP) in 100 mM Tris-HCl, pH 7.5. The mixture was incubated at room temperature for several hours or at 4°C overnight to allow for complete reduction of the disulfide bond. The DNA samples

were then loaded on a BioSpin 6 (Bio Rad), equilibrated in the adsorption buffer (25mM Tris-HCl, pH 7.5, 0.4M NaCl). The high molecular weight DNA molecules were collected in the flow through in adsorption buffer, while small molecular weight species (TCEP and the reduction product HS-(CH_2)-OH, the latter may compete with the DNA for binding to the gold) were captured on the spin columns. The flow-through samples were spotted immediately on the clean gold slides.

Adsorption of adenine: Solution of 1 mM adenine (sigma) was prepared by dissolved in an 85% acetonitrile/water solution. The clean wafers were deposited in the adsorption solution, under dry nitrogen. The container with the samples was then sealed and kept for overnight adsorption, at room temperature. Following the adsorption, excess molecules were removed by washing while shaking for 1 minute with DMSO.

Preparation of gold slides

Both silicon and glass were used for the HREEL measurements. Polished n-type single crystal (111) silicon wafers and glass wafers were cleaned in a plasma asher (March, plasmod) for 5 min in an atmosphere of oxygen and argon. Immediately after having been cleaned, the wafers were placed in an electron beam evaporator. The deposition was performed at a base pressure of $5x10^{-6}$ mbar. A 10 nm chromium layer was deposited at a rate of 0.1nm/s, and then a 150-nm gold layer was deposited at the same deposition rate. The wafer was cut into 0.8x1.2 cm samples using a diamond pen. The gold samples were cleaned for 20 minutes in an ultra violet ozone cleaner (UVOCS, model no. T10X10/OES/E), and then immersed for 20 min in absolute ethanol (Merck). Finally, they were rinsed with ethanol and were immersed immediately in the adsorption solution. Glass wafers were cut into 1x1 cm samples using a diamond pen. A 1-nm chromium layer was deposited at a rate of 0.1nm/s, and then a 100-nm gold layer was deposited at the same deposition rate. The DNA was adsorbed immediately after evaporation.

Characterization Techniques

The XPS spectrometer used was an XSAM800 (KRATOS) operated in the Fixed Analyser Transmission (FAT) mode, with a pass energy of 20 eV, the non-monochromatised Al *K*α X-radiation (hυ = 1486.7 eV), and a power of 120 W. Samples were analyzed in an ultra-high-vacuum (UHV) chamber (~10^{-7} Pa) at room temperature, using Take-Off-Angles (TOA) of 90 and 30º, as measured from the

surface. Spectra were recorded by a Sun SPARC Station 4 with Vision software (KRATOS) using a step of 0.1 eV. A Shirley background was subtracted and curve fitting for component peaks was carried out using Gaussian-Lorentzian products. X-ray source satellites were subtracted. No charge compensation (flood-gun) was used. Binding energies were corrected by using as reference the peak of aliphatic carbon at 285 eV.[1] For quantification purposes, the sensitivity factors were 0.42 for N 1s, 0.66 for O 1s, 0.25 for C 1s, 0.39 for P 2p, 0.54 for S 2p, 2.3 for Na 1s, 0.73 for Cl 2p, and 4.95 for Au 4f.

Three different HREELS spectrometers were used:

a Leybold-Heraeus ELS-22 spectrometer. Sweeping voltage steps of 2.7 cm^{-1} (0.34 meV) were used in all spectra. Only the energy loss or gain corresponding to a spectral range < 0.5 eV was studied.

HREELS vibrational spectra were also recorded for comparison using a LK Technologies ELS3000. A specular geometrical arrangement was used with incidence and analysis angles of 60°. The value E_p ranged from 1.5 to 5 eV. The analyzer acceptance angle is ± 1.5°. In this case, resolution in direct beam is around 1 meV and on the sample the *FWHM* of the elastic peak after reflection on the sample was about 3 meV. HREELS spectra obtained exhibited exceptional resolution.

Electron energy losses corresponding to electronic transitions were recorded using a LK2000R spectrometer. The value E_p ranged from 0 to 10 eV. Sweeping steps were smaller than 4 meV for all spectra. Spectral resolution was around 13 meV for all samples. Incident and analysis angles ($\alpha_{inc.}$ and $\alpha_{ana.}$, respectively) are obtained by rotating both the sample holder and the electron energy analyzer. An off-specular geometry corresponding to 60º of incidence and 30º of analysis was used here (angles refer to the normal to the film surface).

Results

For the detailed study, three ss-DNA samples are particularly interesting, those that contain a single base type, i.e. poly(C), poly(A) and poly(T). The main difference between XPS spectra of those oligomers is seen in the N 1s region (Error! Reference source not found.(a)). N 1s region of the cytosine-base oligomer is composed of two peaks centered at 398.6 and 400.1eV. Peak 1 is assigned to the conjugated N(sp^2) and peak 2 has the contribution from amine group, $–NH_2$, and of non-conjugated N(sp^3). The area of peak 2 is twice the area of peak 1. The N 1s

region of adenine oligonucleotides is also composed of two peaks centered at 399.0 and 400.2 eV, with the same assignment as for cytosine. However in this case the area of peak 1 is 1.4 times the area of peak 2. This region also presents an extra peak at 401.4 eV assigned to NH_3^+. In thymine-base oligomer N 1s is presented as a single peak centred at 400.0 eV attributed to N-(C=O)-N. This peak could include an extra component at lower binding energies. The presence of two peaks in N 1s region of thymine, adsorbed on gold surfaces, has been reported before. In these studies the component shifted to lower binding energies was attributed to the interaction between nitrogen N3 of thymine and the substrate. However, in the present case, this type of interaction cannot be inferred from this N 1s region.

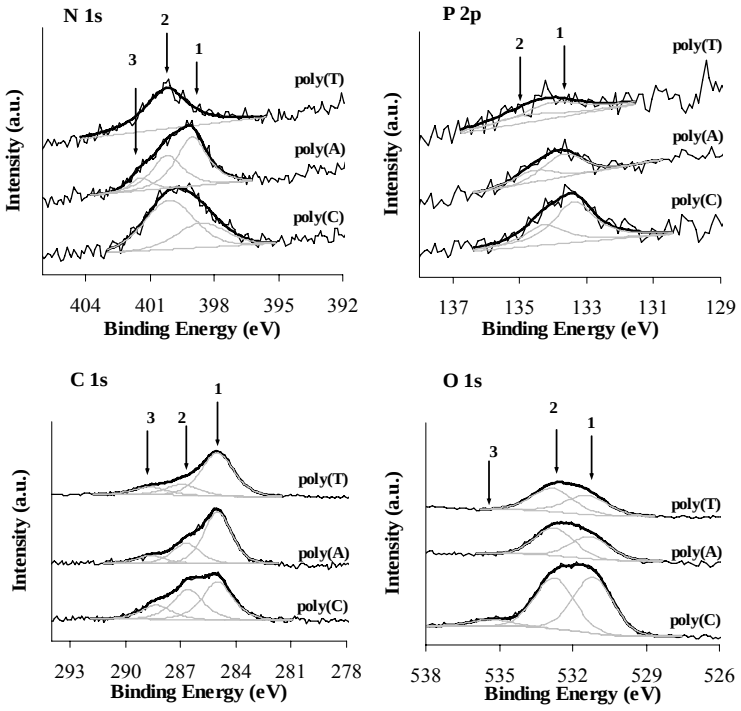
Another important feature that attests undoubtedly the presence of the adsorbed oligomers on gold is the P 2p region (Figure 1S (b)). In spite of its poor signal-to-noise ratio it is possible to detect a peak around 133.8 eV associated with phosphorus (P 2p is a doublet due to the spin orbit splitting, ΔB.E.=0.87 eV [Error! Bookmark not defined.]). Lee et al. reported a peak centred at 133.9 eV for pure DNA monolayers deposited on gold and proposed that it arises from non-thiol interactions of the DNA polyanions with the gold substrate. [Error! Bookmark not defined.] However, this binding energy is lower than the one reported for phosphoric acid (B.E. of P $2p_{3/2}$ in H_3PO_4=135.2 eV [Error! Bookmark not defined.]), being closer to the B.E. of phosphorus in sodium phosphate (NaH_2PO_4), 134-134.2 eV. [Error! Bookmark not defined.] In fact, sodium is detected in some samples but not in all of them. It is missing in the case of *ss*-poly(T) and *ss*-CGAC. The sample richer in Na^+, also present a chlorine peak, indicating that salt is present. In these samples, also the O 1s region presents a component at high binding energies assignable to water (Figure 1S (d)). In some samples, the amount of sodium is much lower than the amount of phosphorus and hence it is not enough to assure neutrality. Since no other candidate to counter-ion was detected, we must conclude that the large exchange should have been with hydrogen.

C 1s regions were fitted with a minimum of three components (Figure 1S (c)). Each component is the sum of several contributions. Peak 1, centred at 285 eV, includes aliphatic carbons of the mercapto-propane terminal group $–(CH_2)_3$-SH, carbons of the sugar group and carbons of aliphatic contamination. All these contributions are common to all samples. In *ss*-poly(T), peak 1 also contains components attributed to carbons of thymine base, namely from the methyl group and

the aromatic carbon C5. *ss*-poly(C) also presents here a contribution of the aromatic carbon C5. Peak 2 of *ss*-poly(A), centred at 286.7 eV, has a strong contribution of carbon bound to nitrogen in adenine, in addition to C-O bonds of the sugar groups and of sugar-phosphate bonds that, obviously, are present in all samples. The peak centred at higher binding energies, between 288.3-288.7 eV (Peak 3), is assigned to carbonyl groups in thymine and cytosine bases that do not exist in *ss*-poly(A).

The O 1s regions were fitted with two components (Figure 1S (d)). One at 531.3 ± 0.2 eV is assigned to oxygen present in the bases and sugars. The other one at 532.7 ± 0.2 eV is assigned to the backbone phosphate group. In the samples ss-poly(C) there is a third component around 535.4 ± 0.1 eV assigned to water, as mentioned above.

Sulphur, in principle, is at the same level as gold since it binds directly to it. However its detection is not obvious firstly because it is located at the interface Au-CH_3, *i.e*. deep in the layer, secondly because its photoionisation cross section is very low compared to that of Au 4f and finally because its atomic density is much lower than the gold atomic density. In *ss*DNA traces of sulphur are detected (samples *ss*-GTA-OH, *ss*-poly(C), *ss*-GCAT and *ss*-CGAC), S $2p_{3/2}$ is centred at 161.4 ± 0.4 eV (not shown) typical for sulphur bound to a gold surface.



Error! Reference source not found.S: XPS spectra of single strands *ss*-poly(C), *ss*-poly(A) and *ss*-poly(T): (a) N 1s, (b) P 2p with ΔB.E.=0.87 eV, (c) C 1s and (d) O 1s. Spectra were acquired with Al *Kα* at TOA=90º. Source satellites were subtracted. For peak assignment see Table 1S.

Table 1S –Corrected Binding Energies (B.E.) (eV) using as reference C 1s = 285 eV and assignments. Error = ± 0.2 eV. C: Cytosine; A: Adenine; T: Thymine. Atoms are labelled as presented in Figure 2S.

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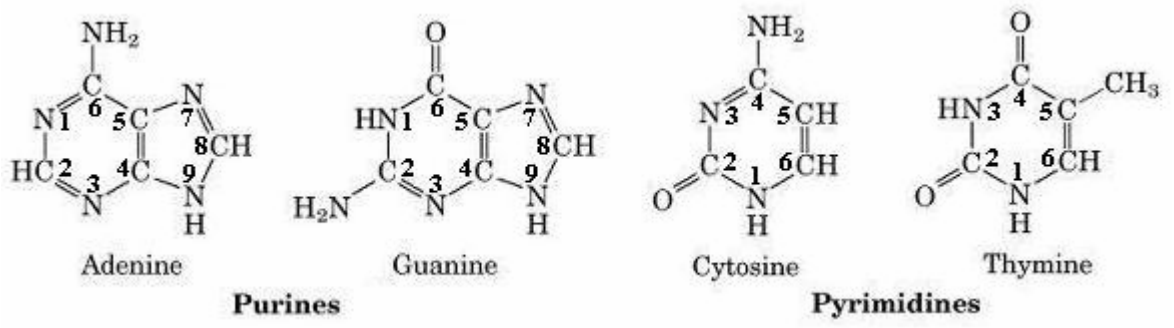
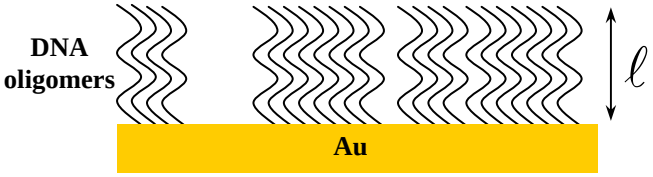
		poly (C)	poly (A)	poly (T)	**Literature** [Error! Bookmark not defined.,**2,3,4, 5,6,7]**	**Assignments**
N 1s	**1**	398.6	399.0		399.0-399.3	-**N**= conjugated -NH_2 (C and A) (399.4)
	2	400.7	400.4	400.0	399.9-401.0	-**N**H- non conjugated; **N**-(CO)-**N** (C and T).
P 2p	**1**	133.9	133.6	133.8	133.9-134.2	**P** $2p_{3/2}$ -**P**$O4^-$ (backbone)
	2	134.8	134.5	134.7	+0.87	**P** $2p_{1/2}$
C 1s	**1**	285.6	285.1	284.8	284.7-286.2	-**C**-**C**- (deoxyribose); -**C**= (C5 in C) -**C**H (alkyl chain -$\mathbf{C}_3$SH, methyl group in T and aliphatic contamination); (CO)-**C**= (C5 in T).
	2	287.1	286.7	286.7	286.0-287.0	**C**-N and N-**C**=N (A and C6 in C and T); **C**-O-**C** cyclic ether (deoxyribose); -$\mathbf{C}H_2$-O- (sugar-phosphate bond)
	3	288.9	288.3	288.5	287.8-289.1	**C**-NH_2 (C6 in A and C4 in C); N-(**C**O)-C (C4 in T); N-(**C**O)-N (C2 in C and T)
O 1s	**1**	531.7	531.0	531.2		C-**O**-C cyclic ether ?
	2	533.3	532.8	532.6	532-534	P**O**4^- (backbone); N-(C**O**)-N and N-C=**O** (T) ? N-C=O ~534 [May] N-C=O ~532 [Database]
	3	535.9				H_2O
Au 4f	**1**	83.98	83.98	83.98		**Au** $4f_{7/2}$ (substrate)
	2	87.63	87.64	87.64		**Au** $4f_{5/2}$
Na 1s	**1**	1071.7	1071.4			Counterion
Cl 2p	**1**	198.8				**Cl** $2p_{3/2}$
	2	200.4				**Cl** $2p_{1/2}$


Adenine
Guanine
Cytosine
Thymine
Purines
Pyrimidines

S: Structure of the four bases of DNA.

Thickness estimation from XPS measurements

Thicknesses were estimated fitting a model of an island-like layer (see Scheme IS) described by Equation (1S). The fitted ratios were: C 1s/Au 4f, N 1s/Au 4f and P 2p/Au 4f for two different TOA (90º and 30º).



Scheme IS

$$\frac{X}{Au} = \frac{n_X}{n_{Au}} \frac{f\left(1-\exp\left(-\frac{\ell}{\lambda_X \sin\theta}\right)\right)}{(1-f)+f\exp\left(-\frac{\ell}{\lambda_{Au4f}\sin\theta}\right)} \qquad \text{(Eq. 1S)}$$

X is C 1s, N 1s or P 2p. θ is the take-off-angle (TOA = 90º or 30º) and λ are the photoelectrons inelastic mean free paths (imfp) of C 1s, N 1s, P 2p and Au 4f. The imfp are determined with the Wagner's expression $\lambda=kE^m$, where E is the photoelectron kinetic energy and k and m are parameters attributed to DNA equal to 0.130 and 0.792, respectively. [8] $\frac{n_X}{n_{Au}}$ is the atomic density ratio, where n_X and n_{Au} are the carbon, nitrogen or phosphorus density in the film and the gold density in the substrate, respectively. The thickness, ℓ, and the covered surface fraction, f were fitted parameters (see table 2S).

Table 2S – Estimated thicknesses and covered surface fraction.

	ss-poly(C)	*ss*-poly(A)	*ss*-poly(T)
ℓ (nm)	6	5	5
f	0.9	0.7	0.8

Nevertheless, Equation (1S) does not take into account shadow effects and heterogeneous distributions of the elements P and N as it should be the case for highly organized layers and obtained values should be taken as merely indicative ones.

References

[1] Beamson, G.; Briggs, D. *High Resolution XPS of Organic Polymers. The Scienta ESCA300 Database*. Ed. John Wiley & Sons: New York, 1992.

[2] Wagner, C. D.; Naumkin, A. V.; Kraut-Vass, A.; Allison, J. W.; Powell, C. J.; Rumble, J. R. *NIST X-ray Photoelectron SpectroscopyDatabase, NIST Standard Reference Database 20*, Version 3.3 (Web Version), http://srdata.nist.gov/xps/ [2003].

[3] Saprigin, A. V.; Thomas, C. W.; Dulcey, C. S.; Patterson Jr., C. H.; Spector, M. S. *Surf. Interface Anal.*, **36**, 24 (2004).

[4] Roelfs, B.; Bunge, E.; Schröter, C.; Solomun, T.; Meyer, H.; Nichols, R.J.; Baumgärtel, H. *J. Phys. Chem. B*, **101**, 754 (1997), and references therein.

[5] May, C. J.; Canavan, H. E.; Castner, D. G. *Anal. Chem*., **76**, 1114 (2004).

[6] Lee, C. Y.; Gong, P.; Harbers, G. M.; Grainger, D. W.; Castner, D. G.; Gamble, L. J. *Anal. Chem*., **78,** 3316 (2006).

[7] Laiho, T.; Leiro J. A. *Appl. Surf. Sci*., **252**, 6304 (2006).

[8] Tanuma, S.; Powell, C. J.; Penn, D. R. *Surf. Interface Anal.*, **1994**, *21*, 165.