Conformation And Mechanical Response of Spray–Deposited Single–Strand DNA on Gold

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Abstract

Single–molecule force spectroscopy of DNA strands adsorbed at surfaces is a powerful technique used in air or liquid environments to quantify their mechanical properties. Although the force responses are limited to unfolding events so far, single-base detection might be possible in more drastic cleanliness conditions such as ultra–high vacuum. Here, we report on high-resolution imaging and pulling attempts at low temperature (5K) of a single–strand DNA (ssDNA) molecules composed of 20 cytosine bases adsorbed on Au(111) by scanning probe microscopy and numerical calculations. Using electro-spray deposition technique, the ssDNA were successfully transferred from solution onto a surface kept in ultra-high vacuum. Real–space characterizations reveal that the ssDNA have an amorphous structure on gold in agreement with numerical calculations. Subsequent substrate annealing promotes the desorption of solvent molecules, DNA as individual molecules as well as the formation of DNA self–assemblies. Furthermore, pulling experiments by force spectroscopy have been conducted to measure the mechanical response of the ssDNA while detaching. A periodic pattern of $\sim 0.2–0.3$ nm is observed in the force curve which arises from the stick-slip of single nucleotide bases over the gold. Although an intra-molecular response is obtained in the force curve, a clear distinction of each nucleotide detachment is not possible due the complex structure of ssDNA adsorbed on gold.
Introduction

Nucleic acids (NA) are one of the four essential ingredients in all known forms of life (alongside with proteins, lipids and carbohydrates). They occur in nature in two chemically different molecules: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), both possessing the ability to form complementary double helix-chains. Due to their biological relevance and the increasingly growing interest in their application to nanodevices or computing, NA are among the most studied bio-molecules nowadays. Interestingly, the control of the wide variety of nucleotide sequences as well as the knowledges of folding properties has enabled the rational design of highly elaborated two- and three-dimensional DNA structures, so-called "DNA origami", programmed by Watson–Crick complementarity. These remarkable advances have been made possible by the accurate determination of the DNA nucleotide sequences beforehand using non-invasive manipulation techniques of single-molecules such as on optical tweezers, magnetic tweezers or their confinement into nanopores. These techniques have further shed lights into their mechanical properties, the force of the base pairing or the interplay between force and lifetime during unfolding or unzipping processes of DNA in liquid and air environments. Beside these approaches, single-molecule force spectroscopy using atomic force microscopy (AFM) also offers the ability to directly measure forces while manipulating DNA adsorbed on surfaces and allows real-space imaging. In contrast of the aforementioned techniques, AFM-based single-molecule force spectroscopy can tackle new issues related to the DNA-surface interactions such as their adhesion properties, self-assembly processes as well as their tribological properties. Extensive investigations in this spirit might open new avenues towards the integration of DNA in solid nanodevices.

With the advent of scanning tunneling microscopy (STM) and AFM, imaging of DNA have been a long-term challenge with the ultimate goal of imaging single nucleotides. Numerous works successfully visualized DNA branches with impressive spatial resolution in liquid or air environments. Inherent to the experimental conditions, among the highest spatial resolutions of DNAs have been obtained using STM in ultra high vacuum (UHV)
conditions and low temperature by avoiding surface contaminants and tip instabilities.\textsuperscript{27,28} These experiments have required developing efficient deposition techniques to successfully transfer those macromolecules from solution onto a surface kept in UHV, and this while maintaining the UHV cleanliness standards. A pulse-injection method\textsuperscript{28} and electro-spray deposition techniques\textsuperscript{29,30} have recently addressed this by demonstrating the depositions of large organic molecules or oligomers onto surfaces compatible with such scanning probe microscopy investigations.\textsuperscript{31–35} However, the investigation at the intra-molecular level of such biomolecules in those conditions, and more particularly DNA, still remains unexplored. Notably, the recent advances of tuning fork sensors operated at low temperature have pushed the AFM spatial resolution of molecules to the intra-molecular level during imaging,\textsuperscript{36,37} sliding or pulling experiments at surfaces.\textsuperscript{38–41} Experiments in this line might open a new platform towards the fundamental investigation of the structural, mechanical and tribological properties of single DNA molecules adsorbed at surfaces down to the atomic scale.\textsuperscript{21}

Here we report the investigation of electro spray-deposited single-strand DNA (ssDNA) on Au(111) combining AFM imaging, force spectroscopy and numerical calculations. The adsorption of ssDNA composed of 20 cytosine bases is observed using high-resolution STM and AFM in agreement with numerical calculations. Depending on annealing temperatures of the surface, single-DNA oligomers are found either as individual molecules or in self-assemblies. Molecular dynamic (MD) simulations further reveal that, although the ssDNA diffusion can be thermally activated at $\sim 500$K, the ssDNA conformation is not modified by those thermal treatment remaining in an amorphous structure. The structure is defined at the first step of the adsorption process with most of nucleotide bases $\pi$–stacked to the surface. Pulling experiments were attempted using force spectroscopy which show a clear periodicity of $\sim 0.2$-$0.3$ nm. According to the numerical calculations, this modulation arises from the stick-slip motion of concerted cytosine bases during single-base detachment and is intrinsically related to the ssDNA conformation.
Results and Discussion

The DNA Structure

A single stranded DNA consists of a sequence of nucleotide bases (dark-blue hexagons in Figure 1), such as adenine, cytosine, guanine and thymine, that are attached to a phosphate backbone (cyan ribbon in Figure 1) through a deoxy-ribose sugar (cyan pentagon in Figure 1). In nature, DNA is most commonly found as a double-stranded molecule (Figure 1(a)) where two complementary single-strands, held together by inter-molecular hydrogen bonds, wrap around each other forming a helical structure. This structure has very well defined parameters such as helical-rise and helical-twist, among others, which are key features in understanding both mechanical properties of DNA as well as its interaction with proteins.

The helical rise corresponds to the distance between consecutive base-pairs along the longitudinal DNA axis. Assuming the helical rise of $\sim 0.33$ nm obtained from numerical calculations for the ssDNA in its canonical B-form, the sequence considered in this work, i.e. 20-cytosine ssDNA, should have a total length of $\sim 6.3$ nm (Figure 1(a)). Nevertheless, this is without considering the impact of the surface on the mechanical stability of the DNA helical structure upon adsorption. To estimate the maximum base-pairs distance, we numerically elongate the ssDNA molecule along its longitudinal axis to form an almost straight structure. In that case, the total length of the oligomers increases to 13.5 nm corresponding to a base separation of 0.675 nm, as compared to the $\sim 0.33$ nm of its helical counterpart.

The helical-twist refers to the rotation around the helical axis of consecutive base-pairs. In the B-form dsDNA, the helical twist is equal to 18° corresponding to a twist period of 10.5 bases. This parameter is an intrinsic property endowed by the phosphate backbone as well as the sugar connecting the nucleotides to the backbone. Therefore, the helical twist is also present in ssDNA and further impacts its folding properties. Another important parameter of ssDNA is that each nucleotide step carries one negative charge which makes
Figure 1: Schematic Representation of a Single-Strand DNA (ssDNA) Composed of 20 Cytosines in its Canonical B-form. (a) Side-view of a canonical B-form double stranded DNA (dsDNA). The 20 cytosine-mer ssDNA (20C-ssDNA) used as a starting configuration in our simulations is represented using an opaque texture. We also represented its complementary strand (not included in our simulations) using a transparent texture. (b) Close up side view showing the helical rise of 0.33 nm. (c) Top view of a single cytosine base. The atoms are represented as liquorice with the following color scheme: red (oxygen), cyan (carbon), blue (nitrogen), brown (phosphorous). The hydrogens are not represented for clarity reason.

the ssDNA a charged molecule. These two effects (charge and twisting) coupled with the non uniform distribution of charges lead to a complex folding energy landscape of ssDNA molecules. Altogether, these parameters have a fundamental role into the ssDNA adsorption, its folding as well as its mechanical properties.

**ssDNA On Au(111)**

To investigate the interplay between the mechanical properties of ssDNA and the Au(111) surface, we systematically conducted constant-current STM imaging at 4 K of spray-deposited ssDNA on gold. Upon deposition, the substrate was kept at room temperature and subsequently annealed step-by-step. Figure 2 shows the evolution of the surface morphology
after those temperature treatments. As received samples are shown in the Supplementary Information Figure S6a and b. The herringbone reconstruction of the Au(111) is clearly observed in Figure S1b, thus we conclude that the contamination of the sample by H$_2$O solvent molecules is low, well-below a monolayer coverage. To activate the desorption of the remaining solvent molecules, we annealed the surface from 340 K to more than 500 K. Figure 2(a) shows a STM overview of the 340 K-annealing. In comparison to Figure S6a, a decrease of the cluster size is observed while their density remains similar. On terraces, we observed the appearance of bright protrusions in Figure 2(a) that we associate to single H$_2$O molecules.$^{44,45}$ We thus conclude that the decrease of the cluster size observed at 340 K is directly related to the partial desorption of water molecules from the surface. This assumption is confirmed after annealing above 440 K as shown in Figure 2(b). The overview STM images shows the cluster disappearance in favour to single oligomers at the surface. Though few water molecules still remain at elbows of the reconstruction, single DNA strands individually adsorbed at valleys. They have a characteristic shape with a typical length of about 3-4 nm. At temperatures slightly larger than 440 K, we observe the appearance of large unidimensional structures with typical lengths of $\sim$ 30 nm and width of 4 nm. These structures are most likely obtained because the annealing temperature favours the surface diffusion of the ssDNA and their self-assembly.

Strikingly, none of these aforementioned theoretical lengths of the ssDNA provide a satisfactory agreement with the conformations observed on Au(111). Indeed, the expected dimensions of 20-Cytosine ssDNA in a double helix ($\sim$6.3 nm) or fully stretched structure ($\sim$13.5 nm) does not match the experimental ones ($\sim$ 4 nm). This discrepancy between theory and experiments underlines the fundamental difference between ssDNA helical structure in solution and when adsorbed at surfaces. It also questions the way ssDNA reaches the surface, its most relaxed structure when adsorbed in such conditions and its diffusion over the surface. In order to obtain a better atomistic understanding of the DNA conformation with respect to the experimental data, we have thus performed MD simulations that
Figure 2: Evolution of The Sample Morphology As a Function of The Sample Temperature. (a) STM overview of the spray-deposited ssDNA on Au(111) after annealing at 340 K, (b) 440 K and (d) above 500 K (\(I_t = 2\) pA, \(V = -1.5\) V), respectively. Scale bars = 20 nm.

attempt to reproduce the whole scenario, meaning from the ssDNA spray deposition up to the subsequent annealing at 340 K, 440 K and >500 K, respectively.

**Room Temperature Electro-Spray Deposition.** The life-cycle of the molecule in the electro-spray (Figure S2) can be assumed as three stage process: 1- several ssDNA molecules in solution; 2- A single ssDNA confined in a small water droplets entering the vacuum; 3- The adsorption of the ssDNA surrounded by an hydration layer to the Au(111) surface.

To elucidate the oligomer conformational change during these steps, we first performed a MD simulation of the canonical B-form 20-cytosine ssDNA fully embedded in water without surface and with all water molecules explicitly included (Figure S3). Using this relaxed ssDNA conformation in water, we further simulated the relaxed structure only considering the three first surrounding hydration layers (i.e. within \(\sim 1\) nm around the molecule, see Figure S4). Finally, this “nano-droplet” containing the ssDNA is employed to run simulations in the vicinity of Au(111) surface as shown in Figure 3(a-h).

Interestingly, the 20-cytosine ssDNA structure embedded in water (Figure S3) slightly deviates from the helical structure in its canonical B-form (Figure 1), which can be imputed to the absence of the complementary strand as in the dsDNA. As a result of this slight structural change, the stacking between consecutive nucleotide bases is improved as compared to
Figure 3: Molecular Dynamic Simulation of Electro-Spray Deposition of ssDNA on Gold. (a)-(d) Side- and (e)-(h) top views of a small water droplet containing a ssDNA adsorbing on the gold surface. The color scheme used here is the same as in Figure 1. The water molecules are represented using a transparent-cyan surface. (i-j) Perspective and top view of the final ssDNA conformation. The topographic representation shown in (j) was obtained using a Connoly surface and colored according to the distance from the surface (z-color scale bare is also represented in Å units). Scale bars = 1 nm. (k) STM topographic image of the ssDNA on gold (white protrusions). The gray protrusions are water molecules.

its helical form. Although the molecule tries to wrap itself, all these attempts are frustrated (Figure S3).

The second stage of our simulation considers the ssDNA confined into the "nano-droplet". In contrast to ssDNA embedded in water, the simulations reveal that the ssDNA is folded into a wrapped structure within the first 10 ns of the simulation reducing the droplet size
(Figure S4). After folding, no major structural change is reported. The final molecule structure had a total extension of $\sim 3$ nm which tend to coincide with the ssDNA length observed at surface by STM experiments (Figure 3(k)). Regarding the hydration layer, it is worth mentioning that most of the water molecules initially surrounding the molecule still remained in contact with it after the folding and 100 ns of the simulation. This indicates that the number of water molecules considered is initially sufficient to hydrate the ssDNA.

The last stage of our simulation tackles the adsorption of the ”nano-droplet” to an unreconstructed Au(111) surface (Figure 3). As shown in Figure 3(a), the droplet containing the strongly folded ssDNA is initially located at $\sim 2$ nm above the surface and left free to adsorb. Water molecules are the first to reach the gold surface (Figures 3(b) and (f)) and then form a meniscus between the ssDNA hydration layer and the surface (Figures 3(c) and (g)). At the final stage of the simulation (Figures 3(d) and (h)), the ssDNA adsorbs and is surrounded by water molecules. The final conformation of the ssDNA (Figures 3(i) and (j)) has a total length of $\sim 4$ nm. The comparison between MD results and experimental STM data (Figure 3(k)) reveals a good agreement in term of overall ssDNA topography and length of the molecule ($\sim 3\text{--}4$ nm). A non-negligible amount of water is also observed in both experiments and calculations which can be efficiently desorbed by thermal treatments (Figure 2).

Thermally-Activated Desorption of Solvent Molecules And The DNA Conformation. After annealing at 440 K, the ssDNA STM topography on gold (Figure 2(b)) appears more homogeneous with an average molecule length of $\sim 4$ nm (Figure 4(f) and (h)). The STM data shows the thermal desorption of most of the water molecules surrounding the ssDNA adsorbed on gold. This process can however impact the DNA structure. In order to better understand this point, we performed a series of MD simulations starting from the relaxed conformation of the hydrated ssDNA on gold shown in Figure 3. By comparing the initial and final ssDNA conformation in the simulations after annealing (Figure 3), another
Figure 4: **Single–strand DNA on Gold at 440K.** (a-b) Initial and final configuration of the ssDNA structure after a 100-ns MD simulation of its adsorption on gold at 440 K without considering water molecules. The initial configuration corresponds to the one obtained in Figure 3. (c-d) Initial and final configuration of a ssDNA in the canonical B-form after a 100-ns MD simulation of its adsorption on gold at 400 K. Both molecules were initially placed 1 nm away and parallel to the surface. Scale bars=1 nm. (e) STM topographic image of the Au(111) surface with ssDNA after 440 K annealing with an Au tip (I = 1 pA, V = 1.2 V). (f) STM Zoom on a ssDNA with a CO terminated-tip (I = 1pA, V = 0.2 V), (g) Corresponding AFM image obtained at constant-height. (h) and (i) Profiles along the longitudinal axis of the ssDNA.

Conclusion is the negligible variation of the ssDNA conformation. Its length remains $\sim 4$ nm as before the temperature treatment and the average corrugation is quite similar ($\sim 0.18$ nm).

Given the impossibility of completely removing the water molecules from the simul-
tion box during the dynamics, we also run a 100 ns-long simulation of the adsorption of the structure previously obtained in Figure 3 at a constant temperature of 440 K and in vacuum conditions after having removed all the H$_2$O molecules (Figure 4(a)). In such dehydrated configuration, we observed similar conformations than the hydrated cases. Therefore, we conclude that the water desorption weakly affects the final conformation of the ssDNA molecule. Instead, we think that the amorphous ssDNA conformation on gold is obtained during the first step of its adsorption at room temperature which remains at elevated temperatures. This observation suggests a strong adhesion of the ssDNA on gold which prevents its rearrangement.

To confirm this assumption, we also performed MD simulations with identical parameters using the ssDNA in its canonical B-form placed 1.5 nm above the surface (Figure 4(c)). In despite of its crystalline structure, the final results shown in Figure 4(d) reveals a random folding of the conformation in analogy to the sprayed and dehydrated ssDNA configurations. Even though the folding of the ssDNA is slightly different, the total length of the molecule is also $\sim 4$ nm. These observations confirm the systematic compaction of the ssDNA molecule when adsorbed over the gold surface which is independent on its initial configuration prior to adsorption.

By comparing with experimental data (Figures 4(e) and (i)), the simulations are in good agreement in terms of molecule lengths ($\sim 4$ nm, Figure 4(h)). However, the STM contrasts shown in Figures 4(e) and (f) does not entirely capture the structural details of the ssDNA molecule at the surface as in the simulations. We think that the nucleotides which are stacked over the gold are observed in the STM image Figure 4(f) as a gray halo around the molecule. The bright contrast in the image has a relative height of $\sim 0.18$ nm (Figure 4(h)) and might correspond to the ssDNA backbone. To improve the real-space resolution, we further imaged by AFM with CO terminated tips at constant height (Figure 4(g)). As observed in the simulations, the ssDNA systematically adopted a rather corrugated and amorphous conformation which avoids intra-molecular imaging similar to flat aromatic molecules at surface.\textsuperscript{36}
Figure 5: **Thermally-Activated Diffusion of ssDNA Molecules on Gold.** (a-c) 500 ns-MD simulations of two ssDNA at 300K, 400K and 500 K respectively. The initial configuration is the one obtained in Figure 4(c) from the canonical B form adsorption. The representation used here is the same as the one used in the previous figures. At 500K, the ssDNA start diffusing to favor the formation of intermolecular interactions and self-assembly. (e) STM topographic image of the ssDNA self-assembly (I =2 pA, V = -1.3 V). (f) Corresponding constant-height AFM image with a CO-terminated tip.

The AFM contrast is richer than the STM one (Figures 4(g) and (i)) but a clear assignment of ssDNA structural details such as nucleotide bases is rather difficult.

**Thermally-Activated ssDNA Diffusion on Gold.** In this part, we focus on the effect of the surface annealing over 500 K on the adsorbed ssDNA. Indeed, STM images show in that case the formation of long structures (Figure 2(c)) and the coalescence of molecules at step edges in contrast to 440 K annealing. These observations suggest an enhancement of the ssDNA mobility at such high temperature leading to these super-molecular structures composed of several ssDNA molecules and stabilized by intermolecular interactions.
In order to validate this hypothesis, we compare the diffusion behaviour of two ssDNA adsorbed on gold obtained from 500 ns–MD simulations at temperatures of 300 K, 400 K and 550 K, respectively. The starting configuration of ssDNA is the one obtained by the free adsorption of B-form ssDNA at 440 K (Figure 4(c)). The distance between the two ssDNA molecules is \( \sim 3 \text{ nm} \) which corresponds to the average distance between the ssDNA molecules experimentally observed at 440 K (Figure 4(e)). The final results as well as an intermediate stage are shown in Figure 5(a), (b) and (c), respectively. Note that in these MD simulations a much longer simulation time was required, i.e. 0.5 \( \mu \text{s} \), as the final configuration of the simulation at 550 K did not converge after 100 ns.

Up to 400 K, simulations show no major structural rearrangements of both ssDNA molecules and no diffusion after 500 ns. In contrast, ssDNA diffusion is clearly observed at 500 K favouring the formation of intermolecular interactions and their self-assembly. It is also interesting to observe that the structure of the ssDNA is slightly altered at 550 K compared to previous temperature treatments since all the strands are now lying flat on the surface. We emphasize that these flattening does not show any structural rearrangements, meaning that the overall ssDNA remains in an amorphous form. A close inspection at the STM image of Figure 5(e) shows a slightly higher resolution of each ssDNA molecule than in the case of Figure 5(e). The MD simulations explain this increased resolution as a consequence of the flattening of the molecule obtained at such high annealing temperature. Aiming to visualize each of the nucleotide bases with atomic resolution, we have further performed AFM measurements with CO terminated tips. The image shown in Figure 5(f) still reveals an irregular topography without enabling a clear resolution of each base. This is still the consequence of the important corrugation on the order of \( \sim 0.3 \text{ nm} \) in most parts of the molecule thus complicating the AFM imaging at constant-height.
Figure 6: Mechanical Response of a ssDNA During a Pulling Attempt. (a) Force-and stiffness-distance spectroscopic curves, $\Delta f(z) \propto k_{TS}$, showing the pulling of a ssDNA from the Au(111). Red and black lines refer to approach and retraction curves, respectively. During the approach at the blue dots of the inset, numerous jumps in the curve occur which are related to conformational changes of the ssDNA induced by the tip. After picking the ssDNA to the tip at $\sim 160$ kHz, the retraction curve reveals a periodic patterns of $\sim 0.2$ nm arising from the step-like detachment of six cytosine bases of the ssDNA. The pulling of the whole DNA structure were never experimentally achieved.

Pulling ssDNA From The Gold Surface

Experimental Pulling With AFM. To explore the mechanical properties of single ss-DNA, we experimentally attempted to pull off single oligomers from the surface using the AFM tip. Figure 6 shows a typical approach–retract curve of such pulling experiments (further details are provided in the methods section). The approach and retraction path are shown in red and black, respectively and display the variation of frequency shift, $\Delta f(z)$, as a function of the tip-sample distance $z$. Thank to the small oscillation amplitude of the force sensor ($\sim 50$ pm), the $\Delta f$ signal arising from the tip-sample interaction forces can be approximated by the tip-sample stiffness $k_{ts}$ (blue scale in Figure 6) using the formula: $k_{ts} \approx 2k\Delta f/f_0$, with $k$ is the sensor stiffness and $f_0$ its resonance frequency. Many attempts were done with ssDNA strands as well as self-assembled ssDNA shown in the inset of Figure 6. In analogy to our previous manipulation experiments with single polymers/molecules, 38,39
we always approached the tip apex at the very end of the ssDNA in order to establish a
tip/molecule contact which is usually observed as an abrupt jump in the current or the force
channel. The approach curve is shown in red in Figure 6. Between $Z = 1.4$ nm and $Z = 0.1$
nm, important variations of the $\Delta f \propto k_{ts}$ are observed that reflect conformational changes
of the ssDNA under the tip action. The tip/molecule junction is experimentally obtained
at $Z = 0.05$ nm in Figure 6. From this point, the ssDNA molecule is attached between
the tip and the surface. During the tip retraction, the ssDNA is then pulled off the surface
while recording the variations of the tip-sample interaction forces (black curve in Figure 6).
As show in the black curve, an abrupt jump occurs at $\sim Z = 1.5$ nm coinciding with the
ssDNA detachment since the stiffness of the contact becomes null. The retraction path does
not exceed $\sim 2$ nm in total. This value is twice smaller than the ssDNA molecule length of
$\sim 3$–$4$ nm obtained by both experimental and theoretical data on gold. As a consequence,
the ssDNA is only partially lifted from the surface by the AFM tip before its re-adsorption.
This assumption is further confirmed by STM imaging after the pulling experiments which
always shows the presence of the oligomer over the surface (see inset Figure 6) in contrast to
previous pulling experiments showing the disappearance. We also emphasis that among all
the pulling attempts we never succeeded to fully extract a ssDNA from the surface suggesting
a weak tip/molecule interaction compared to the surface/molecule interactions. This can be
explained by the absence of a well-defined anchoring site in the ssDNA structure for the tip
as well as a strong adhesion of the ssDNA on gold.

Interestingly, the retraction curve also reveals a monotonically decrease of $\Delta f$ and a
clear periodic pattern of 0.2–0.25 nm. The monotonically decrease further reveals that the
stiffness of the tip/molecule contact decreases as the molecule is detached. This observation
again contrasts with previous lifting experiments of long polymeric chains over surfaces which
shows a constant tip/sample stiffness in average. As the stiffness of a ssDNA molecule is
an intrinsic property, i.e. independent of its length, the diminishing of the effective stiffness
while lifting indicates that the quality of the contact (either tip-molecule or molecule-surface)
is changing during retraction. Knowing that the ssDNA always remains at the surface, the ssDNA is thus slipping away from the tip apex.

The most relevant aspect of the curve likely concerns its periodicity. In previous pulling experiments,\textsuperscript{38,39} periodic patterns were observed and found related to the step-by-step detachment of molecular units from the surface as well as stick-slip behaviour of the molecule while sliding. However in our case, the stiffness is decreasing while lifting the ssDNA due to the molecule detachment from the tip. Note first that the period of the helical rise is \( \sim 0.33 \) nm in the ssDNA B-form (Figure 2b) which is larger than the experimental value. Adsorbed on gold, the ssDNA adopts a rather amorphous structure. The best match of such periodicity seems to be the minimum distance between gold atoms of \( \sim 0.28 \) nm that would act as atomic pinning sites of the nucleotide bases. Fundamentally, we think that as soon as the tension cumulated in these pinning sites is high enough a stick-slip event occurs to the neighboring pinning site located \( \sim 0.2-0.25 \) nm thus resulting in a weaker contact (e.g. with a smaller number of nucleotide bases on the tip) and also accounting for the observed periodicity.

**MD Simulations of The Pulling Experiment.** To better unravel those asp, we have performed long Steered-MD (SMD) simulations. In these simulations, we lift the phosphorous atom located in the backbone (between the nucleotides 1 and 2, see Figure 7(a)) along the \( Z \) direction with a constant velocity of 0.1 nm/ns at a temperature of 5 K. As we do so, we record the applied force as well as the displacement along the \( Z \) direction. The resulting force-distance curve is plotted in Figure 7 and The initial configuration considers the adsorption configuration obtained at 550 K (see Figure 5). Despite the fact that we are lifting only one ssDNA molecule (in Figure 5 550K/500 ns, the up-most molecule) we have included in the simulations the two molecules assembled on the gold surface as in the experiments. Note also that during the simulations we observed that the neighboring ssDNA molecule had no effect on the lifting dynamics. The detachment observed when the lifting force becomes zero
Figure 7: ssDNA Force-Distance Curve Obtained From SMD Simulations. (a) Lifting force (average computed in windows of 100 ps) in black and the corresponding FFT low pass filter using a maximum frequency of 2.7 Hz in gray. Blue and green areas correspond to the successive lifting of single nucleotide base. The index is shown at the bottom. The purple lines are linear fits of segments of the lifting force, and are represented with the corresponding slope. (b-c) Snapshots at the marked in (a) above to highlight the importance of stick-slip events to the lifting force of ssDNA over gold. The nucleotide bases are coloured according to their \( Z \) position. (a) To better identify the pining sites that participate/prevent lifting of the first two nucleotides we have signalled those nucleotides with a red push-pin. The nucleotides that moved from one image to the other are signalled with an arrow with the same direction as the movement.

occurs at a length of 12.4 nm. This value correspond to the full detachment of ssDNA which corresponds to the length of a fully stretched ssDNA molecule (see Figure S1). Furthermore, the simulations indicate that the ssDNA detachment mostly occurs in a sequential manner. Note that in the initial conformation of the ssDNA over gold (Figure 7(a)) we observe some folding and loops that if lifted as a whole would certainly look smaller than the corresponding
sequential detachment. A priori one could say that the short detachment length obtained in the experiments could arise from the detachment of these compact loops/domains. Nevertheless, the SMD simulations clearly show that this cannot be the case.

Regarding the stiffness values, i.e. the slope of the lifting force shown in purple, a close agreement with the experimental data of Figure 6 is found particularly in the corresponding region (from 0 to 2 nm). The SMD simulations in Figure 7 also display the monotonic decrease of the stiffness values as a function of the tip/sample distance. The graph further reveals that the lifting force fluctuates around an average value of \( \sim 1.5 \) nN. Furthermore, these fluctuations have a characteristic sawtooth modulation with a variable amplitude, thus bearing similarity to those obtained in conventional stick-slip motion. It is important to remark, that the slip phase, i.e. when we observe a decrease in force, is extremely sharp.

When computing the stiffness, we observe that the slopes of both the first peak (between (a) and (b) in Figure 7 (©FromCoauthors to Guilherme: Please make a zoom plot in this region)) and the second peak (between (b) and (c)) are in excellent agreement with the ones obtained in the experiments (see Figure 6). A close inspection of the SMD simulation allows us to see that each of these peaks can be traced to a set of combined stick-slip events of the ssDNA on gold as shall be explained latter in greater detail.

The reduction in the effective stiffness during the lifting results from the fact that as we lift the molecule, less and less nucleotides contribute to the pinning of the ssDNA to the gold surface and therefore it becomes easier to lift it, inducing a smaller stiffness. In fact, we can observe that when lifting the last nucleotide (the 20\(^{th}\) shown in Figure 7(a), the stiffness is of 1.8 N/m which compares well the one obtained with graphene nanoribbons of 1.5 N/m.\(^{38,39}\) Therefore, the cooperative effect of nucleotide pining explains the decrease of the effective stiffness as we lift. Both experiments and simulations show the appearance of periodic peaks 0.2–0.25 nm throughout the force lifting curve. To better understand the mechanism responsible for this, we analyse in detail two of such events. In the plot shown in Figure 7, on the X-axis we highlight with two red bars two stick-slip (lift) events with a periodicity
of 0.2 nm. We also mark the beginning/end of each of these events with a different letter so to associate with their corresponding atomic configurations, shown in the second row of Figure 7. Initially, most nucleotides (nuc) are lying flat on top of the surface and 9 out of 20 are aligned/pinned to the gold lattice (Figure 7(a)). The lifting force along z direction (out of plane) is applied at the phosphorous atom shown in pale red located in the backbone near nuc#1 and nuc#2 whose axis passes through the gold atom. From (a) shown in Figure 7, we observe that the force starts to increase meaning that the tension in the backbone starts to increase. This tension is induced because nuc#1, nuc#3 and nuc#4 are pinned to the gold surface thus anchoring the backbone around there. As a result, lifting the molecule along the axis at the pale red position (Figure 7(a)) becomes impossible. Nevertheless, having cumulated enough tension at stage (b), i.e. just after the first slip/lift event, we observe a repositioning of these pinning sites. In particular nuc#1 and nuc#3 slip over the surface thus allowing the backbone to move upwards. Simultaneously, we observe the appearance of new pinning sites which lead to new anchoring and thus an increase of the tension as we lift from stage (b) to (c). During this period, the tension on the backbone starts to lift the nuc#2 pinned site. This can be seen by the change in its color from green (planar and close to the surface) to blue (detached from the surface). Once the tension is high enough to peel nuc#2, another slip (lift) event is observed that almost completely lifts the first two nucleotides. Nevertheless, this slide (lift) event is again stopped by a new pinning site at nuc#3. This process happens throughout the whole lifting process of the ssDNA molecule as shown in the supplementary movies SIM1 and SIM2 and leads to the appearance of those force peaks every 0.2-0.25 nm. Note also that the periodicity of the gold substrate does not have to be necessarily reproduced since we are lifting the molecule having a complex and non-homogeneous conformation. Due to different angle with the surface, all the slipping events cannot be easily translated into a lifting movement. More precisely, the periodicity of the gold surface is in fact revealed in the force lifting curve due to the concerted sliding of many different nucleotides during a lifting event. As the ssDNA is not linearly straight on
the substrate, such pinning configuration depends on the local arrangement of neighboring nucleotides which are not equivalent along the ssDNA conformation. As a result, this gives rise to the rich casuistic observed in the lifting force curve.

Trying to exploit ssDNA lifting experiments as a possible method to sequence ssDNA molecules, it is however important to enable distinguishing in the force curve the characteristic contribution of single nucleotide detachment. In Figure 7(a), we coloured regions related to such detachments as also shown in the supplementary movies of the detachment SIM1 and SIM2. A close inspection reveals that even though certain nucleotides have well-defined detachment peaks in the force curve that could be used as relevant information (e.g. 6,7,8,9,11,12,13), some others do not (e.g. 4,5,10,11). As aforementioned, the reason is related to the fact that the ssDNA is not linearly straight over the substrate and does not have equivalent pinning centers along its structure. As a consequence, although most of the bases are lying flat (Figure 7(a)), the ssDNA is wrapped which leads to peculiarities such as almost consecutive lifting events. An exemplary case can be seen in the force curve while lifting nuc#10 (Figure 7(a)). There, the twisting tension accumulated in the backbone via the adsorption results in an effortlessly lifting of nuc#11 as soon as nuc#10 is desorbed. Another case where almost simultaneous detachment of two nucleotide bases during lifting are observed is between nuc#13 and nuc#14. In this case, nuc#14 is adsorbed on top of nuc#15 and they can be detached together as shown in the force curve.

With the prospect of using force spectroscopy with intra-molecular resolution to sequence ssDNA, several conclusions of our study can be made. The period of \( \sim 0.2 \) nm arising from the concerted sliding of many different nucleotides during lifting event is always reproducible and depends on the ssDNA adsorption configuration. Since those pining configurations are not equivalent for each nucleotide detachment along the ssDNA structure, some detachment events are not distinguishable in the force curve. Our approach is thus a priori failing to sequence ssDNA in this particular frame. To find a more appropriated surface/molecule system to address this issue, few considerations can be made. First, the adsorption in a
straight fashion of the ssDNA on the surface as reported in the case of ssDNA deposited on copper by Tanaka et al.\textsuperscript{28} seems an important prerequisite to avoid force signal from unfolding events of the ssDNA. An alternative would be, for instance, to employ patterned surfaces where the ssDNA adsorption can be directed at the atomic scale or to use thermally activated diffusion to favour the ssDNA adsorption in a straight fashion. As shown in our work, this last strategy fails in our case since the adhesion of ssDNA on gold is too important to allow a significant structural rearrangement after substrate annealing. Another fundamental aspect to enable ssDNA sequencing is to obtain equivalent pinning configurations all along the ssDNA molecule. This will allow to have reproducible and well-distinguishable force signals arising from atomic stick-slip or nucleotides detachment events in analogy to the lifting of polymeric chains.\textsuperscript{38,39} Therefore, in addition to the straight ssDNA adsorption configurations, the adsorption configuration further requires to have equivalent nucleotide adsorption sites. Regarding the lifting experiments, the MD simulations clearly show that the ssDNA nucleotides detach after sliding over only few atomic sites of the surface which suggest a strong adhesion compared to polymeric chain units sliding over long distances before detachment.\textsuperscript{38,39} This ssDNA detachment similar to the peeling of an adhesive tape reveals the strong ssDNA adhesion on gold avoiding structural rearrangement, easy-pulling experiments as well as potential friction experiments. Clearly, one could use surfaces where ssDNA has less adhesion such as the graphene surface.\textsuperscript{21} By choosing more appropriate system addressing the ssDNA conformation and adsorption characteristics, one could expect that the interpretation of force-lifting of ssDNA molecules from surfaces could provide a direct and successful method to sequence DNA molecules.

**Conclusion**

In summary, the adsorption of single-strand DNA (ssDNA) composed of 20 cytosine bases on Au(111) using electro spray-deposition is investigated combining AFM imaging, force
spectroscopy and numerical calculations. High-resolution STM/AFM images and molecular dynamic simulations unambiguously reveal that single-DNA oligomers are found either as individual molecules or self-assemblies depending on the annealing temperature of the surface. In terms of conformation, the ssDNA has a amorphous structure on gold with most of its nucleotide base stacked to the surface which is insensitive to surface post-annealings. In fact, the structural adsorption peculiarity results from the first step of the uncontrolled adsorption process. To measure the mechanical response of ssDNA, pulling experiments using force spectroscopy were also conducted in order to discriminate nucleotide base detachment from the surface. Due to the strong adhesion on gold, such experimental attempts have only shown partial ssDNA detachments revealing periodic pattern of $\sim 0.2$-$0.3$ nm. MD simulations elucidate such period to arise from a stick-slip motion across the Au(111) atomic potential of concerted sliding nucleotide base prior to their detachment. The ssDNA detachment is similar to the peeling of an adhesive tape due to the strong molecular adhesion on gold. Although intra-molecular details are systematically observed in the force curves, a clear assignment of single-nucleotide base detachment fails in our system. This is partly due to the complex adsorption conformation of the ssDNA as well as the too large variety of conformations at the surface. We believe that addressing those particular parameters, i.e. the adsorption characteristics in term of structure and adhesion strength, will enable AFM-based force spectroscopy to sequence down to the intra-molecular level single-nucleotide bases in DNAs through the force response of their detachment from a surface.

Methods

Sample Preparation. Au(111) single crystals purchased from Mateck GmbH were cleaned by several sputtering and annealing cycles in a ultra-high vacuum (UHV) preparation chamber (base pressure of $5 \times 10^{-10}$ mbars). The single-strand DNA molecules, purchased from Microsynth®, were dissolved with a concentration of around $1\text{nmol.mL}^{-1}$ in a mixture of
water and methanol (ratio of 4:1).

**ElectroSpray Deposition (ESD).** The MolecularSpray® apparatus connected to the preparation chamber allows the introduction of molecules contained in a solution from air to UHV through a capillary and three differential pumping stages separated by leak orifices.\textsuperscript{32,35} The formation of the spray requires to drive the solution through the capillary (inner diameter 750 µm) by applying a high voltage to it. High voltage is applied between the solution and the capillary leading to the formation of charged droplets that are accelerated through the differential pumping system. The droplets undergo pumping combined with coulomb fission resulting to the diminution of their size down to single ionized molecules. During ESD, the base pressure of the preparation chamber reaches $p \approx 10^{-7}$ mbar. Typical high voltage values are $\sim 1.5$ kV which must be adjusted to maintain the spray quality during deposition. The deposition time varies from 1 to 30 min at constant solution flux controlled by a syringe pump with a speed of $2 - 10 \times 10^{-6}$ L.min$^{-1}$.

**STM/AFM Experiments.** The STM/AFM experiments were carried out at 4.8 K with an Omicron GmbH low-temperature STM/AFM operated with a Nanonis RC5 electronics. We used commercial tuning fork sensors in the qPlus configuration ($f_0 = 26$ kHz, $Q = 10000-25000$, nominal spring constant 1800 Nm$^{-1}$). Unless mentioned, the AFM images were acquired with CO-terminated tips using the non-contact mode. All voltages refer to the sample bias with respect to the tip. The pulling experiments were performed with oscillation amplitude of $\sim 50$ pm without bias voltage applied at 4.8 K.

**Atomic Level Models and Force Fields** In our simulation we use a Au(111) surface three atomic layers thick, where the positions of the atoms in the lowest layer are fixed during the MD runs using a harmonic restrain of 5 Kcal/mol. Here we considered surfaces of two different sizes, i.e. 16×16 nm$^2$ (for the data reported in Figure 3 and Figure 4) and 18×22 nm$^2$ (for the data reported in Figure 5 and Figure 7). For the molecule we choose a
20-cytosine mer as in the experiments. The initial structure was generated using the software NAB ref\textsuperscript{7} thus obtaining a double helix with the canonical B-form as shown in Figure 1. Then we removed the complementary sequence and used only the single-stranded 20-mer cytosine. Given that the phosphate groups in the backbone of the ssDNA are charged we added 19 sodium counter-ions per each ssDNA molecule. The ssDNA atoms were described using the parmbsc0\textsuperscript{7} and the $\chi$OL3 modification ref\textsuperscript{7} of the Cornell ff99 force field\textsuperscript{7} was used to describe ssDNA. This force field has been shown to accurately reproduce the mechanical properties of DNA ref. The sodium counter-ions were described using the recently improved Joung/Cheatham parameters ref\textsuperscript{7}. As for the gold atoms, we have resorted to CHARMM-METAL force-field which simultaneously describe the intrinsic properties of gold, while retaining thermodynamical consistency with all the other force fields used here ref. Furthermore, this force field has been extensively tested to study the adsorption of different peptides (charged and uncharged) and the results are in good agreement with both density-fucntional-theory simulations as well as with available experimental results ref. Although most of the simulations presented here were performed in vacuum, some were performed in water (Figure 3 and the first row of Figure 4). In these cases, the water molecules are explicitly considered and modeled using TIP3P force field.

**Molecular Dynamic (MD) Simulations.** MD simulations were carried out using AMBER14 software suiteref with NVIDIA GPU accelerationref. Periodic boundary conditions and Particle Mesh Ewald (with standard defaults and a real-space cutoff of 2 nm) were used to account for long-range electrostatic interactions. Van der Waals interactions were truncated at the real space cutoff, and Lorentz-Berthelot mixing rules were used to determine the interaction parameters between different atoms. In all vacuum simulations the volume of the system was kept fixed and the temperature was kept constant by means of a Langevin thermostat. SHAKE algorithm was used to constrain bonds containing hydrogen, thus allowing us to use an integration step of 2 fs. Coordinates were saved every 1000 steps. In
all our simulations we observed that the final configuration was stable as it did not change during the last 40 ns of simulations (which was corroborated by the low, i.e. < 0.2 nm, root-mean-square deviation). In the simulations reported in Figure 7, the forces were evaluated using steered-molecular-dynamics simulations in a similar fashion in previous works. In particular, the phosphorous atom in between the nucleotides 1 and 2 (Figure 7(a)) is displaced along the z-direction with a constant velocity of 0.1 nm/ns. A thorough discussion of this method can be found in the Supplementary Information of reference ref.

**Associated Contents**

**Supplementary Information**
The Supporting Information is available free of charge on the ACS Publications website at DOI: !!!.

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**Notes**
The authors declare no competing financial interest.

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E.M., R.P., R.P. and J.G.V. conceived the experiments. A.H. performed the electrospray—deposition of the ssDNA. R.P. performed the STM/AFM measurements. J.G.V. performed the numerical calculations. R.P. and J.G.V. analysed the data and co–wrote the manuscript. All authors discussed on the results and revised the manuscript.
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