Stretching DNA Using Cryo-Force Spectroscopy

Cryo-Force Spectroscopy Detects Sub-Molecular Mechanics of DNA

Mechanical properties of micrometer long DNA have mainly been probed at room temperature with a force resolution limited by thermal fluctuations. Cryo-force spectroscopy combined with computer simulations now enables to quantify adhesion and intra-molecular mechanics at the sub-nanometer level by stretching up to several nano-newton tensile loads spray-deposited single-strand DNA oligomers on surfaces in ultrahigh vacuum.

Single-molecule force spectroscopic experiments on bio-molecules, such as Deoxyribonucleic acid (DNA), are usually conducted under ambient conditions in solutions using few tens of pico-Newton loads [1]. The mechanical response is then hampered by thermal motions of the molecule and only folding/unfolding of soft parts are detectable when pulling the molecules [2-4]. To suppress thermal fluctuations in such measurements [5], a solution is to apply large tensile loads in the nano-newton range. Using an atomic force microscope (AFM), few investigations have reached such force levels with long polymers by strongly binding their ends using specific chemical end-groups. These bulky anchoring groups however limit the experiments to several hundreds of nanometer long bio-polymers. As a result, and to our knowledge, sub-nanometer structural details of such molecules have remained elusive in force versus extension curves recorded under ambient conditions.

ssDNA Imaging with Sub-Molecular Precision

Although imaging of DNA using scanning probe techniques has also been a long-term challenge [7-13], frequency-modulation AFM under cryogenic conditions has surprisingly never been exploited for such purpose. Low-temperature AFM provides spatial resolution of adsorbed molecules down to the single-bond level [14] and enables complex tip-assisted manipulations at surfaces [15]. To address biomolecular systems in general using such technique, we developed an electrospray deposition techniques [16-17] enabling the transfer of oligomers contained in solution to an atomically-clean gold substrate prepared in ultrahigh vacuum (UHV). As prototypical bio-molecule, we choose a 20-Cytosine single-strand DNA (ssDNA)
oligomer having an expected length of about 6 nm in solution according to molecular dynamic (MD) simulations.

Upon deposition, we observed by scanning tunneling microscopy (STM) at 4.8K clusters of various dimensions which always appeared much larger than this expected size. Additional simulations indeed revealed that the sprayed ssDNA molecule reaches the surface surrounded by a hydration layer. Experimentally, successive annealing of the substrate above 100°C in UHV provoked the desorption of the solvent molecules leading to the observation of single-ssDNA oligomers as well as assemblies (fig. 2a and b). Sub-nm resolution could be obtained by STM as well as associated constant-height AFM with CO-terminated tips on both morphologies (fig. 2c and d). In spite of the potential resolution below the molecular level, the determination of the overall ssDNA conformation remains rather difficult due to the complex folded conformations of the oligomers on the Au(111) surface as confirmed by computer simulations.

**ssDNA Cryo-Force Spectroscopy**
We recently showed pulling experiments of long polymeric chains or graphene nano-ribbons with the tip of an AFM in cryogenic conditions [18-19]. Experimentally, the tip is first approached to one end of a selected molecule until a tip-molecule contact is being formed, and then retracted lifting the molecule off. We applied the same method to investigate the mechanical response of the ssDNA adsorbed on Au(111). In contrast to polymeric chains, only partial lifting of oligomers could be however achieved since the folded ssDNA conformation and its strong adhesion on gold appeared to be limiting factors. Complementary MD simulations further revealed that desorbing a single-folded oligomer with the AFM tip requires not only peeling off the structure from the gold surface, but also unfolding part of the backbone prior to detachment. Each peculiarity of the folding configuration can thus cause an abrupt increase of the required force to lift the molecule and the rupture of the tip-molecule bond.

**Softer as it Detaches**

Looking at the experimental and theoretical lifting curves, we noticed that the variations of the tip-sample stiffness $k$ as a function of the tip separation $Z$ are very different from our previous works on polymeric chains [18]. In the chain case, the $k$ maxima (~0.4 N m$^{-1}$) were constant during retraction and the lifting process ended at a distance corresponding to the number of monomers initially identified by STM imaging. These synthetic chains weakly adhere to the substrate allowing nearly friction-less sliding and also a complete chain detachment. In contrast, the ssDNA oligomer backbone is more flexible with most bases strongly bonded to the gold surface leading to a much stronger adhesion on the substrate. We thus explained the gradual decrease of $k$ (fig. 3b) by the decrease of stiffness of the lifted segment as it becomes longer, being strongly pinned between the tip and the surface. We further rationalize the detachment mechanism using a springs-in-series model that includes the stiffnesses $k_{\text{tip}}$ and $k_{\text{pin}}$ of the segment ends anchored to the tip and to the adsorbed part of the oligomer (fig. 3a). By fitting the envelope of $k(Z)$ traces assuming $k_{\text{tip}} = 35$ N m$^{-1}$, we extracted $k_{\text{pin}} = 18.3 \pm 3.3$ N m$^{-1}$ and $k_1 = 32.6 \pm 3.9$ N m$^{-1}$. The latter corresponds to the stiffness per repeat distance in the lifted segment. As a consequence, $k_{\text{DNA}} = k_1/19 = 1.7$ N m$^{-1}$ is the stiffness of the fully stretched 20-cytosine oligomer with one base at each end subject to an average tension of 2 nN. Noteworthy, this load is one to two orders of magnitude larger than the maximum values attained in typical room-temperature investigations of ssDNA in ambient conditions.

**Conclusion**
Cryogenic force spectroscopy [20] detects the mechanical properties of biomolecules, only few nanometer long, strongly adsorbed on surfaces under tensile loads up to a few nano-newtons, i.e., 10–100 times higher than hitherto applied in most force spectroscopy studies under ambient conditions. Such complementary techniques implemented at ultra-low temperature might better characterize the adhesion properties or enable single-base distinction in future experiments.

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More on cryo-force spectroscopy

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