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Comment

Wonderful world of single biopolymer thermodynamics Comment on "Biophysical characterization of DNA binding from single molecule force measurements" by K.R. Chaurasiya et al.

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The technologies to probe and manipulate individual biopolymers are some of the most outstanding achievements in modern physics. With optical tweezing or atomic force microscopy at our disposal, we can essentially open a textbook on polymer physics and experimentally analyse the fundamental thermodynamic laws of a single, long (bio)polymer, one-by-one. This unprecedented possibility to a large extent relies on the fact that biopolymers, DNA in particular, are relatively long (several μm and longer), and can be produced at high fidelity and at reasonably large amounts. Importantly, by protein linkers, they can be attached to the tweezers bead or the AFM cantilever. While the chain remains invisible its resisting force in optical tweezers can be measured with sub-pN resolution. Forces of up to 300 pN can be exerted in dual beam optical tweezers setups, which is considerable, given the value of around 65 pN to overstretch a DNA double-strand. Covalent bonds, however, typically rupture in the nN range and protein domains unfold at forces of 150 to 300 pN. For such experiments AFM is the preferred method.

Examples for DNA-related *biophysical* experiments include DNA unzipping due to lateral pulling, during which the force is applied to the two single-strands at the same end of the DNA chain; and longitudinal stretching of DNA, the force applying to the ends of complementary DNA single-strands at the opposite ends of the DNA, leading to force-induced DNA melting. These types of experiments allow one to study the associated phase transitions and thermodynamic stability as well as the mechanical response of DNA quantitatively described by the worm-like chain model. In particular such experiments are also relevant to the calibration of force fields used in molecular dynamics descriptions and computationally efficient coarse-grained simulations methods [1]. Over the recent years optical tweezers techniques have been pretty much standardised to perform such unzipping and overstretching experiments. Complementarily, AFM methods allow one to directly measure conformations of DNA chains, for instance, to determine the localisation behaviour of DNA-knots or the formation of denaturation bubbles in supercoiled, topologically constrained DNA plasmids [2].

However, as beautifully demonstrated in the review by Chaurasiya et al. [3], these techniques also allow one to explore additional properties that are unique to biopolymers, in particular, their *biochemical* interactions with other molecules. I was thrilled when I originally heard about the overstretching experiments in the presence of single-stranded DNA binding proteins and their mutants reported by Pant et al. [4]. In this work the long-standing question on the thermodynamic stability of DNA in the presence of the single-strand binders was proved to indeed be a kinetic

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issue. Without the direct measurement using a destabilising mechanical force instead of a thermal melting experiment (during which the single-strand binders simply denature) one would not be able to finally answer this puzzle. Similarly, optical tweezers techniques allowed one to prove that, in the considered system, the single-strand binders locate newly denatured zones on the overstretched DNA predominantly by one-dimensional diffusion along the double-strand while non-specifically bound [5]; or, a dual tweezers setup was used to confirm the role of DNA conformations in gene regulation [6]. Moreover, optical tweezers provide both biochemical and physical insight into the binding and associated phase diagrams of DNA intercalators [7]. Similarly AFM methods have been used to unzip DNA [8], rupture protein domains [9], or break covalent bonds [10]. Mechanical single-molecule experiments have become almost indispensable in the investigation of the biochemical properties of DNA and their interaction with other biomolecules and chemicals.

At the same time one should keep in mind that mechanical DNA overstretching and the resulting denaturation of the double-strand is not strictly equivalent to thermal melting of DNA. Indeed we could show [11] that the critical exponent associated with the force-induced melting is different from the one found for thermal melting. This is due to the fact that the presence of the stretching force changes the entropic degrees of freedom of the denatured base-pairs. Away from the thermodynamic limit this difference is not expected to be significant. Thermodynamic stability parameters are available from thermal melting experiments [12], and are now routinely used to validate simulations results of DNA stability. It would be interesting to explore whether the mechanical denaturation techniques could be improved to obtain complementary information on the DNA stability on a single base-pair level.

What else would I, as a theorist, have on my wish-list if I were asked which experiments should be pursued? One of the intriguing results from statistical mechanics and simulations of overstretching [11] and unzipping [13] is the observation of a "re-entrance" behaviour in the force–temperature phase diagram: while the increase of the force at a given temperature drives the denaturation of the DNA chain across the phase boundary, this boundary forms an inverted U-shape in the force-versus-temperature diagram, such that at a lower temperature a transition occurs at the same critical force. It would be very interesting to find experimental evidence for this behaviour.

The second point of interest is the more detailed analysis of poly-AT domains in DNA. It is known that their thermodynamic and mechanical, but also dynamic, behaviour differs from random B-DNA. However, it would be nice to see such effects directly in overstretching experiments. In particular it would be interesting to explore its effects with respect to the interactions with binding proteins or intercalators.

Finally an important point would be to gain more insight into the behaviour under conditions resembling those in living cells. In particular, there will be significant consequences stemming from molecular crowding that prevails in cells. The presence of a high concentration of large biopolymers should influence the entropic gain of denatured bases, and thus impact the denaturation transition. Secondly natural DNA suffers from topological constraints, similar to magnetic tweezers experiments in which the torsional strain cannot relax [14]. It would be valuable to probe these effects experimentally, and see in how far current thermodynamic models are able to grasp the effects of crowding and fixed topology.

I am still thrilled each time I learn about fundamental new results being reported from optical tweezers or atomic force methods. Not at least such a feeling is due to the directness of the mechanical method, i.e., it is physically very intuitive. I am convinced that the readers of the review article by Chaurasiya et al. will share this thrill about the new, and wonderful, world of probing biopolymers mechanically and thereby measuring their thermodynamic behaviour.

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