POLYMER PHYSICS

DNA off the Hooke

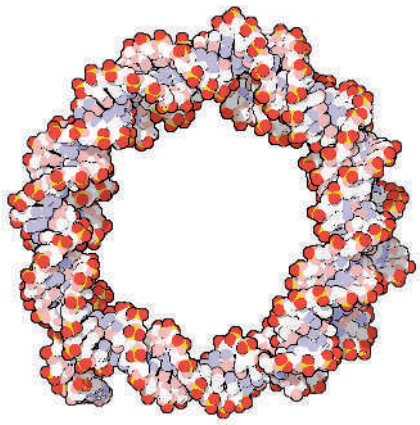
Most models of DNA elasticity ignore the details of how it bends on short length scales. Now, high-resolution atomic force microscope images of DNA on a surface suggest that it is much more flexible than previously thought.

Rudolf Podgornik
is at the Laboratory of Physical and Structural Biology, NICHD, National Institutes of Health, Bethesda, MD 20892-0924; the Faculty of Mathematics and Physics, University of Ljubljana and the Department of Theoretical Physics, J. Stefan Institute, Ljubljana, Slovenia.
e-mail: podgornr@mail.nih.gov

Even before Watson and Crick famously solved the structure of DNA in 1953, scientists were interested in its elastic properties[1]. Until recently, these studies were limited to the bulk, where large numbers of molecules were sampled simultaneously without any possibility of resolving local stresses and strains in a single molecule. However, a number of new methods now exist, such as magnetic beads, glass needles, optical traps and atomic force microscopy (AFM)[2], that are capable of holding, bending and twisting these molecules, one at a time. On page 137 of this issue, Philip Nelson of the University of Pennsylvania and colleagues[3] use high-resolution AFM to critically assess the mechanics of DNA bending on short length scales. Their results suggest that DNA is a lot more bendable than was previously thought.

The way in which DNA can bend, stretch and twist determines just what shapes it can contort itself into (see, for example, Fig. 1). The morphology of DNA has implications for how it recognizes and binds to other molecules, such as proteins, and also for the way it packs into cellular components or viral capsids. For this reason, a number of models try to predict the elastic properties of DNA.

One model that has worked well to explain the elastic properties of DNA molecules is the worm-like chain (WLC) model[4]. This picture treats the DNA molecule like a flexible rubber hose — the stiffer the molecule, the straighter it will be. Microscopically, the model assumes that each segment in the molecule obeys Hooke's law: in other words, the elastic restoring force is proportional to how much the molecule is bent. Elastic stiffness will tend to keep the molecule straight, but it has to compete with the 'wiggles' induced by thermal energy.



D. S. GOODSELL, PDB

Figure 1 DNA molecules can adopt a tightly wound shape, shown here, when packaged inside the nucleus of a cell[11].

A convenient measure of a molecule's stiffness is the persistence length, ξ, which is roughly the distance separating bends along the length of the molecule (Fig. 2). More precisely, the probability that two segments separated by a distance L along the molecule are aligned is given by $\exp(-L/\xi)$. Molecules such as DNA are considered to be 'soft' because their elastic energy is usually comparable to the background thermal energy and they have a relatively short persistence length. Under physiological conditions, the persistence length[5] of double-stranded DNA is between 45 and 50 nm, whereas its overall length varies from several hundreds of nm (as in the experiment by Nelson *et al.*) to micrometres. As a result, chains of DNA molecules tend to be quite tangled.

To test the WLC model and the form of elastic energy it assumes, we have to measure quantities directly predicted by the model. One approach is to measure how the molecule as a whole stretches under an applied force: the WLC model predicts that the molecule should follow Hooke's law for small forces[4]. Another way is to image the conformations of a large number of molecules and compare the frequency of bends with that predicted by the model.

Experiments that measure forces on single DNA molecules at length scales much longer than the persistence length are consistent with the WLC model[4]. However, a number of studies show the limits of the WLC model, for example, in predicting how the elastic properties of DNA change with salt concentration[2,6]. Other limitations show up in experiments that probe the elasticity of DNA at biologically relevant length scales, such as those that are important in DNA packaging, transcription and gene regulation. Nelson and co-workers probe these exact lengths — which are significantly smaller than DNA's persistence length — and argue that DNA is a lot more flexible than predicted by the WLC model.

Nelson and colleagues used high-resolution AFM to image the curvature in a large number of double-stranded DNA molecules over distances as short as 5 nm. The molecules in their experiments were gently adsorbed onto a negatively charged mica surface with the help of small concentrations of $MgCl_2$. When they analysed the statistical frequency of various DNA conformations in the AFM images, the number of highly bent segments was much greater than predicted by the WLC model. Their analysis on these length scales suggests that the elasticity does not follow Hooke's law. Moreover, the authors were able to fit their data to a new general model that they have named the sub-elastic chain model.

The fact that the elastic energy at small length scales (that is, much shorter than the persistence length) does not

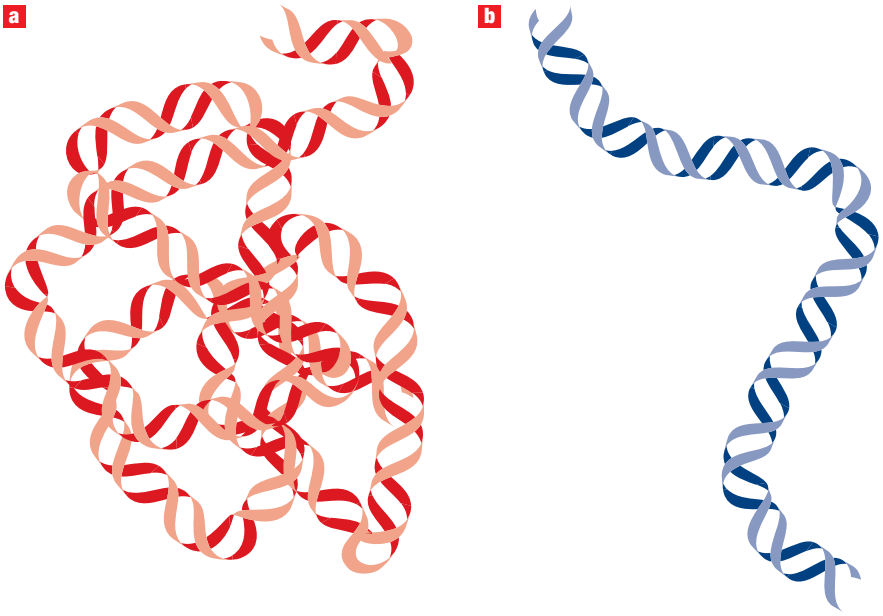


Figure 2 Schematic molecules with different persistence lengths. The more flexible molecule (**a**) changes direction over relatively short distances and so coils up on itself. The stiffer molecule (**b**) has a much longer persistence length, and therefore remains straighter.

obey Hooke's law is by no means at odds with the success of the WLC model in describing force spectroscopy experiments on single DNA molecules[2]. On the length scales probed in those studies, the details of the elastic properties of the segments are washed out by thermal fluctuations, and the molecule as a whole follows Hooke's law. At the scales probed in Nelson's experiments, however, the effect of thermal fluctuations is small and it is possible to observe nonlinear elasticity. In solid and soft matter, there are numerous examples of effective energies depending on the length scale at which they are studied, so it is no surprise to find similar behaviour in semiflexible molecules.

The model proposed by Nelson *et al.*[3] implies that the elastic restoring force is constant when the molecule is bent on small length scales. Similar constant restoring forces are observed when double-stranded DNA is overstretched[7]. This process involves forces in a narrow, almost constant, range around 65 pN, and is followed by complete separation into single strands for even larger forces (hundreds of pN). The constancy of restoring forces in this experiment is a consequence of the thermodynamic equilibrium between two types of differently stretched links. In a similar fashion, the small-length-scale bending of DNA observed and quantified by Nelson *et al.*[3] could be due to an equilibrium between DNA molecules with different values of the local bend angle.

Another possible explanation, to some extent invoked by Nelson *et al.*[3], could be that the DNA elasticity is modified by counterions on adsorption to mica. The divalent cation (Mg^{2+}), used in this work to facilitate DNA adsorption onto mica, prefers to bind to specific sequences along DNA. At these sites, the counterions can promote bending[8] and thus modify the elastic energy. This mechanism would invariably associate the local form of the bending energy to the local sequence of nucleotides, a link that has already been investigated in detail[9].

The results of this study have very broad applications in different areas of biology as well as in bionanoscience in general. Their value is, however, first of all fundamental, providing an insight into the nature and form of DNA elasticity at very small length scales. This has immediate repercussions in many DNA packing problems[10], where DNA is forced to curve at very small length scales, (see Fig. 1). The 'coarse-grained' (long length scale) approach used to extensively model these biological nano-assemblies will undoubtedly be affected by these results.

One wonders whether the breakdown of Hooke's law at very short length scales might also be relevant to other large (bio)molecules with different structures. Indeed, further experiments with other semiflexible molecules are needed to find out if the anomalous elasticity observed by Nelson and co-workers is a general property of all semiflexible molecules, or if it is a consequence of the extraordinary structure of DNA itself.

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