NANOPORES

The art of sucking spaghetti

Biomolecules are notorious for their unpredictable flexibility. Some of the smallest nanopores ever created are being used to manipulate individual DNA molecules, with far-from simple results.

ROBERT AUSTIN **is at the Department of Physics, Jadwin Hall, Princeton University, Post Office Box 708, Princeton, New Jersey 08544-0708, USA.**

e-mail: austin@princeton.edu

Bionanotechnology has become a hot area of research as scientists struggle to apply nanotechnology to areas of biological interest. One of the main reasons for this is the realization that nanotechnology can, in principle, be used to examine biomolecules one by one. Achieving single-molecule analysis would have a huge pay-off. For example, single-molecule DNA sequencing means reading the base-pair sequence of a single DNA molecule from a single cell without any of the enormous complications and information distortions created by the existing steps of cloning, polymerase chain reaction amplification, and capillary electrophoresis separation. Unfortunately, analysing single molecules is a fiendishly difficult task, not only because the individual molecules must be moved past a sensor of some sort, but also because extraordinarily sensitive technologies must be developed to respond to their molecular properties.

On page 611 of this issue, Li *et al.*[1] exploit recent advances in the fabrication of synthetic nanopores[2,3] with remarkably small diameters (3–10 nm) to measure the motion of single DNA molecules through the pores. The most surprising result of this work is that the DNA molecules do not thread meekly through these nanopores like a noodle of spaghetti that you suck up, but instead come through the pore in several 'bent hairpin' configurations. The experiments of Li *et al.* use real molecules of biological interest, and are an important step in crossing the gap between the quirky but 'true' nanopores of biology, and the robust, but still too large, nanopores coming from physics.

Single-molecule sequencing is still a distant goal, so before they can address biologically relevant problems, scientists first need to learn how to handle single DNA molecules. DNA can be an extremely long polymer, up to several centimetres in length. But as it has to curl up into the nucleus of a cell (a few micrometres in diameter), it cannot be perfectly rigid. The 'bendability' of a linear polymer can be expressed by a physical length called the persistence length — a concept that comes from statistical physics, and is a measure of how far you can go in a straight line along the backbone of a polymer, on average, before thermal fluctuations move you in another direction. A more accurate statement is that the persistence length is the mean radius of curvature of the molecule at some temperature, T, due

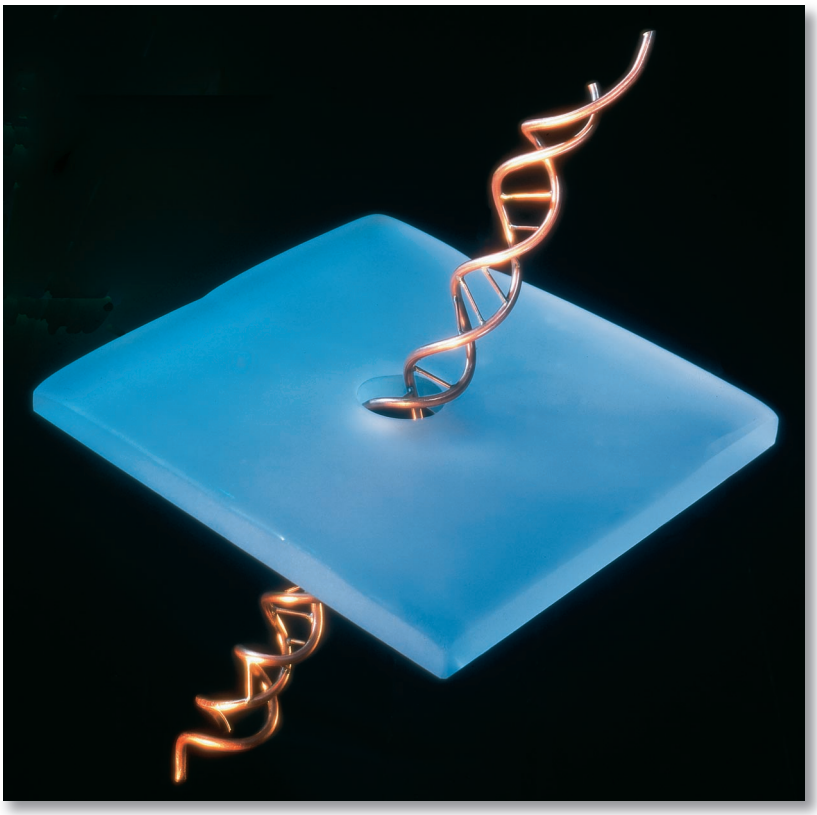

Figure 1 Scientists wishing to straighten out elastic polymers, such as DNA, require very tiny pores a few nanometres in diameter. Li *et al.*[1] use an ion beam to shrink a micropore in a silicon nitride membrane down to nanoscale dimensions. DNA molecules threading through the nanopores don't always behave as expected, but these experiments bring us a step closer to the goal of single-molecule analysis, including DNA sequencing.

Image: Dana Sigall, www.sigall.com.

to thermal fluctuations. To make a molecular 'tape reader' that can straighten out a rapidly bending DNA molecule and examine the base pairs along the polymer, it is necessary to pass the DNA through some opening that has a diameter much less than the persistence length — otherwise you will see only a throbbing tangle, like a vibrating blob of spaghetti, pass by and emerge on the other side.

DNA can exist in two states: as a double-stranded double helix (dsDNA) with a persistence length at physiological salt conditions of about 50 nm, or as a single-stranded molecule lacking the complementary strand (ssDNA) with a far smaller persistence length of about 3 nm. Any nanopore designed to thread a DNA molecule must be no more than 10 nm in diameter for dsDNA and 2 nm in diameter for ssDNA. Of course, biology has always worked at this scale. The protein hemolysin is a classic example of a biological 'true nanopore' that can discriminate ssDNA hairpins at the single base level[4], but it comes at a price. These voltage-biased proteins need to be inserted into a membrane to work properly, and there are the usual issues of stability and reproducibility associated with biological structures.

The solid-state pores created by Li *et al.*[1] using customized ion-beam techniques range in size from 3 to 10 nm in diameter, and so should be able to straighten out dsDNA molecules, and even measure their length to high precision. With the synthesis of even smaller pores, down to 1 nm diameter, by Storm *et al.*[3] using recent developments in electron-beam techniques, studies on ssDNA are also within reach. But whether the nanopore is biological or synthetic, how can you tell when a DNA molecule is moving through it? Quite simply, by assuming that the presence of the molecule will impede the motion of ions that are trying to pass through the hole to carry current from one side to the other. This 'coulomb blockade' can easily be measured by using the same instrumentation previously developed by neurophysiologists to measure channel conductance in membranes.

So, nanotechnology has developed to the point that we can create pores with holes that are much smaller than the DNA persistence length, and electronic techniques exist to detect the presence of a single dsDNA molecule as it threads through a pore. Do we get beautiful electronic signals showing that all dsDNA molecules of the same length pass for the same amount of time through the channels, a big step towards single-molecule sequencing? Alas, the situation is not so simple. Li *et al.* provide evidence that the electronic signals of DNA molecules moving through their nanopores are actually quite complex. There is a wide distribution of passage times and magnitudes of current blockades, so even with reproducible, stable nanopores, one cannot ignore the conformational complexity of biomolecules. The authors ascribe much of the variability they see to thermal-induced bending of the DNA molecule into something like a hairpin, so that a molecule can pass through the pore at half its true length and with twice the blockage of current.

In principle, we understand the statistical mechanics of polymers with a given persistence length quite well[5], so it is surprising that pores with diameters one-fifth of the average bending radius allow such a severely kinked molecule through the pore. However, perhaps DNA when it is jammed into a hole is easier to crumple and bend than we might expect. The persistence length really views DNA as a continuous elastic rod like spaghetti, at some point as we approach the nanoscale the molecular composition of biomolecules becomes important, and we have little to guide us yet on what new phenomena emerge. One way round this problem may be to construct nanochannels rather than nanopores. Such nanochannels would confine the DNA over its entire length rather than at a single point, and if they could be made with a diameter much less than the persistence length, then the energetic cost of a hairpin would be too great and one would expect to see a straight DNA molecule moving by without any kinks. My colleagues and I have tried to build such structures[6], in the hope of wringing out the entropy of these floppy molecules. So far, we have achieved channels of about 10 nm diameter that stretch on for centimetres — a potential genome in a bottle.

Although synthetic nanopores and nanochannels can provide a fruitful laboratory to study the conformational dynamics of worm-like elastic chains, at some point the technology will need to be applied to biologically relevant problems, such as single-molecule sequencing. In this respect, the smaller pores created by Storm *et al.*[3] and more complex types of sensors and probes, which are sensitive to chemical aspects of the polymer, will be useful. The latter is something that nature does better than us. We still have a long way to go before we can construct synthetically, and with great precision, the kinds of complex structures that nature evolved long ago to deal with the physics, chemistry and biology of single-molecule analysis, but the first steps are being made.

References

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