Nature Reviews Materials | Published online 7 Sep 2016; doi:10.1038/natrevmats.2016.72

DNA

Working out the knots

NPG/Neil Smith

DNA, like other macromolecules, has the propensity to self-entangle. However, the factors that control the formation of knots and their roles in biological processes are poorly understood, owing to a lack of suitable observation techniques. Now, using a solid-state nanopore system, researchers are able to probe the nature of these elusive features.

Various experimental techniques have previously been used to investigate DNA knots; however, all of them are bulk methods that are restricted to relatively short (<10 kilobase pairs) and/or circular DNA. By contrast, the nanopore technique reported by Cees Dekker and co-workers in *Nature Nanotechnology* allows the observation of individual knots in arbitrarily long, double-stranded linear and circular DNA. "Although such knots are most prevalent at long lengths, there was no method to observe knots in long DNA molecules before our technique," explains Dekker.

Solid-state nanopores are used to study the fundamental properties of biomolecules on a single-molecule level and their interactions (for example, between DNA and proteins), as well as DNA separation and sequencing. The technique involves an electric potential being applied across a membrane that contains a nanopore (typically about 20-nm wide) with an electrolyte-filled reservoir on either side. A charged biomolecule (such as DNA) is driven through the nanopore by an electrophoretic force, where it causes a blockade in the ionic current that depends on the volume of polymer in the pore and, in the case of DNA, on the specific base pairs.

Although DNA translocation through nanopores is the basis of several applications under commercial development (for example, in detection and sequencing) and is suitable for large DNA molecules, the technique has not previously yielded information about the presence and nature of knots. Dekker's team has been studying DNA sensing in nanopores for more than 15 years. "Since I started working on DNA translocation through nanopores, I wondered why DNA would traverse such a tiny pore in a nice head-to-tail fashion without encountering any knots," says Dekker. Now, armed with a much higher temporal resolution compared with their previous nanopore system, they showed that it is possible not only to detect knots, but also to gain unprecedented, detailed information about their properties.

The researchers investigated how the occurrence of knotting depends on the DNA length (up to 166 kilobase pairs), as well as the size and position of individual knots. The occurrence of knotting increased with length, with a constant probability per unit length, which is consistent with the results of previous theoretical simulations. Moreover, under high voltage, they observed that knots in linear DNA slid out as the DNA passed through the nanopore. The most remarkable finding, however, was that the knots were extremely tight (<100 nm).

The implications of this work extend to any application that involves long polymers as well as fundamental studies of knotting in biological systems. "The presence of knots has largely been ignored in many nanopore applications, because the limited resolution of the measurements prevented us from observing them," explains Dekker. "Now that we've shown that the knots are there, it is important to take them into account in applications, such as the detection of DNA-bound protein, and particularly when probing long DNA lengths."

Cristian Micheletti, a theoretician and expert in polymer knots from the International School for Advanced Studies in Trieste, Italy, who was not involved in the study comments: "This vividly confirms the prediction that, for equilibrated DNA chains, the most probable knot length is relatively small. However, the passage through the pore may have also contributed to the tightening of the knot, as we have observed in translocation simulations of knotted, single-stranded DNA." As for a natural (albeit challenging) extension of this work, Micheletti adds: "I hope that this breakthrough can help detect single-stranded DNA entanglement as well in the near future." This would be particularly relevant for sequencing, which requires single-stranded DNA.

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ORIGINAL ARTICLE Plesa, C. *et al.* Direct observation of DNA knots using a solid-state nanopore. *Nat. Nanotechnol.* http://dx.doi.org/10.1038/nnano.2016.153 (2016)