Untying a nanoscale knot

Mechanical unfolding of a single DNA G-quadruplex structure with and without a stabilizing ligand can be used to calculate the binding strength of the ligand and could help to identify drugs to target these important biological assemblies.

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Experiments that apply piconewton forces to individual molecules have provided great insight into the folding of proteins and nucleic acids, and into the interactions between these molecules and various ligands. In such experiments, optical tweezers are used to trap individual molecules while exerting a known force. Creatively manipulating this force gives dynamic and energetic information on the function of nucleic acids and the associated ligands found in cells. For example, these experiments have been able to track the progress of an individual RNA helicase as it translocates along, and unwinds double-stranded RNA, and have been used to probe RNA folding, from simple hairpins to complex tertiary structures. Observing dynamic intermediates, including transition states and even misfolded states, allows a complete reconstruction of the energy landscape. Mao and co-workers now report in Nature Chemistry that such experiments can be used to measure the interactions between DNA G-quadruplex structures and stabilizing ligands, to obtain equilibrium interaction energies. This information could provide insight into the activity of drugs that target G-quadruplexes.

G-quadruplexes are tetrameric structures formed by guanine-rich DNA sequences. Short segments of DNA that contain such sequences, regularly interspersed with other bases, are bound to each other through Hoogsteen interactions — alternative base-pairing interactions to the more familiar Watson–Crick pairing seen in the DNA duplex. Individual G-quadruplexes may stack in a variety of conformations, stabilized by monovalent ions in solution. Such G-quadruplex structures are unusually stable and may cause polymerase enzymes to stall. Although G-quadruplexes may exist throughout the human genome, particular attention has focused on those formed in the single-stranded ends of telomeric DNA.

Telomeric DNA is important for normal cell function and is usually shortened with each copying cycle, eventually resulting in cell death. Cancerous cells often produce more of the enzyme telomerase — which acts to extend the telomere — and this can result in uncontrolled cell growth. However, the human telomere, which is rich in 5'-TTAGGG-3' repeats, forms G-quadruplexes that are believed to maintain its length in opposition to the action of telomerase. Because various ligands may stabilize the G-quadruplex, thereby opposing the action of telomerase, it is hoped that they could be used to discourage further extension of the telomere and thus have potential as cancer treatments.

Unravelling a complex knot-like structure such as the DNA G-quadruplex poses intricate experimental challenges. In addition to the obvious difficulty of grasping and pulling apart a nanometre-scale object, disrupting these small structures is typically a non-equilibrium process, which makes it difficult to determine quantitatively the interactions holding the G-quadruplex together. Moreover, these notably stable chromosomal structures can be stabilized by binding ligands that further inhibit disruption of the complex nanoscale object.

Building on their previous work using optical tweezers to unfold G-quadruplexes, Mao and co-workers use a single-molecule assay to force the unfolding of individual G-quadruplexes (Fig. 1). Cycles of unfolding and relaxation reveal a distribution of unfolding forces in the range of 10–30 pN. Incubating these G-quadruplexes with a binding ligand leads to a relative decrease in the population of this distribution and the growth of another, at a distinctly higher range of forces, typically 30–50 pN — although the exact values vary with the ligand. These higher forces reflect the increased stability of the ligand-bound complex. The relative sizes of the two distributions vary with ligand concentration, and this allows for the calculation of an equilibrium binding constant. Monitoring rapid changes in the applied force allows the growth of the ligand-bound population to be charted in time.

The clear utility of this assay is that it can probe the binding strengths of ligands over many orders of magnitude, even for ligands with low solubility in standard experimental buffers. Furthermore, single-molecule experiments may now benefit from relatively recent advances in theoretical physics, with the Jarzynski equality and Crooks’s fluctuation theorem being among the techniques that allow equilibrium free-energies to be deduced.
even during non-equilibrium experiments\(^1\). These are crucial aids for experiments that may not be able to probe the longer timescales required to achieve equilibrium. Mao and co-workers\(^2\) use Jarzynski’s equality to determine the free energy of the G-quadruplex with and without binding ligands and their results directly quantify the greater stability of the G-quadruplex in the presence of these ligands.

The only limitation of the method is the serial nature of data collection and the large number of events that must be observed to establish clearly interpretable distributions. Other single-molecule studies use multiple binding sites along a single DNA molecule\(^3\), and serial arrangements of G-quadruplexes could speed the throughput of collection. Mao and co-workers\(^4\) have partly remedied this drawback by establishing that the equilibrium dissociation constant may be calculated using data for only one ligand concentration, rather than requiring experiments over a range. Overall, these exciting results highlight a novel way to characterize the kinetics and thermodynamics of ligand binding to G-quadruplexes, which could be used in the rational design of drugs that target these structures.

References