IMAGING AND VISUALIZATION

Fluorescence-force spectroscopy: watch your tether

A small change provides large benefits when combining force spectroscopy and fluorescence-based measurements of single molecules.

The tiny forces experienced or transmitted by single biological molecules are integral to their mechanism of action. Researchers have developed extraordinarily sensitive devices to measure or transmit these tiny forces to single molecules and investigate their function. One of these tools is the optical trap.

An optical trap uses infrared light to physically trap a bead or another object at the focal point of a focused laser beam. If one part of a single molecule is linked to the bead using a tether molecule and another part is attached to an immobile substrate, a researcher can use the trap to move the bead and pull on the molecule. Alternatively, the motion of the bead can be tracked to measure forces exerted on it by the molecule. The tether molecule has previously always been relatively short, as this is necessary for measuring forces, but this creates some limitations.

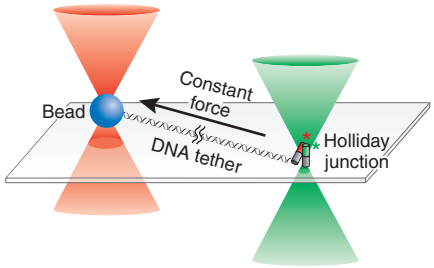

Figure 1 | A very long DNA tether applies a small constant force between an optically trapped bead and a synthetic Holliday junction labeled with red and green fluorophores. The tether also separates the trapping (red) and imaging (green) laser beams during fluorescence force spectroscopy measurements.

Taekjip Ha at the University of Illinois explains these limitations: "If you have a short tether, the intense beam of the trapping laser will also hit the molecule you are studying and photobleach any fluorescent dye tags used to detect dynamic conformational changes in the molecule." This makes it difficult to perform necessary replicate experiments and obtain statistically significant results. "The second problem with very short tethers is if the molecule moves by a few nanometers your bead will move by a similar distance, and the force will change. That is not good if you want to make conformational dynamics measurements as a function of constant force," adds Ha.

To overcome these limitations Ha and colleagues tried using a tether about fifty times longer than normal. They calculated that by using such a long piece of DNA as a tether it would act as a very weak spring and dampen forces transmitted between the trapped bead and the tethered molecule being studied. Using the optical trap and a long tether they found they could apply a constant force of 0.3–10 pN by moving the substrate bound to the tethered molecule while holding the bead in place. Ha adds, "The other advantage of this approach is that the two laser beams

SYNTHETIC BIOLOGY

ENGINEERS MEET SMALL RNA

By studying the quantitative characteristics of small RNA (sRNA)-based gene regulation in bacteria, engineers lay the ground work for using these noncoding RNAs for gene circuits.

Synthetic biology, a field at the crossroads of engineering and biology, depends on input from both disciplines. A good example for a fruitful collaboration between these fields is Terry Hwa from the University of California, San Diego.

A physicist by training with a background in electrical engineering, he is interested in device characteristics, and the synergy between device study and synthetic biology. "By looking at a gadget's characteristics," he explains, "you get some information on how a device might be used by the cell or, alternatively, by us."

One such device that particularly captivated Hwa's attention was sRNAs that contribute to the regulation of protein expression in bacteria. Many of these noncoding RNAs bind specifically to the 5′ untranslated region (UTR) of mRNAs to prevent protein translation and essentially silence the target genes.

Developing tightly regulated gene circuits has been an important task in synthetic biology, and researchers have mainly looked to protein regulators that repress or activate promoters. Hwa was curious to investigate how different sRNAs might act as regulators. Specifically, he was curious to see how the noncatalytic nature of bacterial sRNA regulation, in other words, that sRNAs either co-degrade with or irreversibly bind to their target mRNAs, might affect their role as regulators.

True to their background, he and Erel Levine, a post-doctoral fellow, started by comparing the regulatory characteristics between sRNAs and proteins. They developed theoretical models based on the molecular biology of sRNA-mRNA interaction available in the literature, taking into account the rate constant for an sRNA to find its target, the degradation rate of the target mRNA with and without the sRNA, and the degradation rate of the sRNA with and without the target mRNA. This theoretical work suggested a list of properties inherent to sRNAs: their threshold linear response, that is, they are only active above a certain level of expression; their cross-talk, that is, expression of one sRNA target can relieve the repression of another; and their noise characteristics, that is, they are tightly regulated with very little background.

The next step took Hwa and Levine to the bench to show that these predicted regulations were not only biologically possible, but did actually happen *in vivo*. Hwa's team transformed bacteria with a reporter gene, its 5′ UTR fused to a natural sRNA target and the corresponding sRNA, each transcript under the control

for optical trapping and fluorescence detection are separated by at least 13 μM, so there is no mutual interference."

They used their long tether approach to examine the effect of sub-picoNewton forces on the conformational properties of the Holliday junction, a cross-like DNA structure that forms during recombination of two DNA strands. Such a structure cannot be examined using a conventional optical trap and tether system.

They first labeled two arms of the Holliday junction with different fluorophores that would permit them to detect changes in the distance between the arms by fluorescence resonance energy transfer (FRET). They used the third arm to immobilize the molecule on a glass slide substrate and attached the fourth arm to a long DNA tether (bacteriophage λ DNA) whose other end was attached to an optically trapped bead (**Fig. 1**).

To apply a desired constant sub-picoNewton force to the Holliday junction while performing FRET measurements to look at changes in the structure, Ha and colleagues moved the microscope stage with the substrate toward or away from the stationary trapped bead. The separation of the trapping and imaging beams combined with the use of the vitamin E analog Trolox, to reduce bleaching and blinking, allowed measurements for as long as 250 seconds.

This method will be useful for many other studies that require the application of such small forces. "Our method is readily applicable to any of the nucleic acid structures and their interaction with protein," concludes Ha.

Daniel Evanko

RESEARCH PAPERS
Hohng, S. *et al.* Fluorescence-force spectroscopy maps two-dimensional reaction landscape of the Holliday junction. *Science* **318**, 279–283 (2007).

of its own inducible promoter so that the researchers could independently regulate the level of mRNA and sRNA. Measuring the reporter protein and mRNA levels at various degrees of sRNA expression, they confirmed the key properties of the sRNA-mediated regulation that they had predicted.

Hwa is optimistic that sRNA regulators will find use in developing gene circuits. He says: "Most people who work on bacterial gene circuits use the same small set of protein-based regulators. The feeling is that these tools are quite limited in terms of how you can induce them without causing other unintended changes to the cell. sRNA really gives us a whole new class of gadgets." Hwa also points out that new sRNA-mRNA pairs can be designed based on known templates and they can be made very specific to avoid cross-talk.

Of course, important challenges remain. A number of mRNAs are very short-lived, which does not give the sRNA enough time to find its target and necessitates the stabilization of the mRNA. Another requirement for this regulation to work is the presence of a chaperone, thought to mediate RNA degradation. If an sRNA-based gene circuit were to be established in an 'artificial' cell, this chaperone would have to be supplied.

Despite these challenges, bacterial noncoding RNAs deserve a chance to prove their worth in regulating gene circuits.

Nicole Rusk

RESEARCH PAPERS
Levine, E. *et al.* Quantitative characteristics of gene regulation by small RNA. *PLoS Biol.*, **9**, e229, 2007.

NEWS IN BRIEF

GENOMICS

HapMap version 2.0

Phase II of the human haplotype map (HapMap), characterizing more than 3.1 million single nucleotide polymorphisms (SNPs), has now been released. The data were collected from 270 individuals from diverse backgrounds, and the resulting HapMap has a density of about one SNP per kilobase. This valuable data resource should facilitate studies of human evolution as well as provide insights into the genetic basis of disease.

The International HapMap Consortium *Nature* **449**, 851–861 (2007).

PROTEIN BIOCHEMISTRY

Protein structures from scratch

With an all-atom rebuilding and refinement protocol, Qian and colleagues describe a computational method to improve protein models derived from NMR and X-ray crystallographic data without using any phasing information or homologous structures. Notably, they were also able to successfully predict the structure of a 112-residue protein without using any experimental information other than the protein sequence.

Qian, B. *et al. Nature* **450**, 259–264 (2007).

CHEMICAL BIOLOGY

Click chemistry without copper

The azide-alkyne reactions, the Staudinger ligation and the copper-catalyzed cycloaddition known as click chemistry are useful bioorthogonal labeling reactions. However, the Staudinger ligation is a rather slow process, and click chemistry requires the use of toxic copper. Baskin and colleagues now solve both problems with the development of a copper-free click chemistry reaction.

Baskin, J.M. *et al. Proc. Natl. Acad. Sci. USA* **104**, 16793–16797 (2007).

DRUG DISCOVERY

How drugs inhibit telomerases

Anticancer therapies using drugs that interfere with the maintenance of telomeres by binding to their single-stranded ends have shown good *in vivo* antitumor activity. These drugs were thought to inhibit the telomerase but De Cian and colleagues now show that the drugs inhibit the initial elongation step of the enzyme rather than its activity. This distinction is important when it comes to devising assays to measure the inhibitory potency of these drugs.

De Cian, A. *et. al. Proc. Natl. Acad. Sci. USA* **104**, 17347–17352 (2007).

RNA INTERFERENCE

Controlling pests through RNAi

New methods for controlling agricultural pests are urgently needed. Mao *et al.* and Baum *et al.* describe how to harness the power of RNA interference to control insect pests on crops. With transgenic crops expressing dsRNA specific for knocking down essential genes in insects these two groups independently showed that oral delivery of dsRNA caused lethality.

Mao, Y.B. *et al. Nat. Biotechnol.*, published online 4 November 2007.
Baum, J.A. *et al. Nat. Biotechnol.*, published online 4 November 2007.