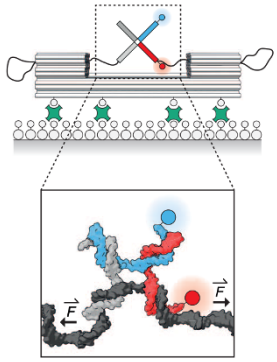
BIOPHYSICS

Single-molecule force analysis, unplugged

A nanoscopic force clamp enables high-throughput single-molecule analysis of DNA under tension without connection to a macroscopic instrument.



A Holliday junction under study in a nanoscopic force clamp immobilized on a surface. From Nickels, P.C. *et al.* *Science* **354**, 305–307 (2016). Reprinted with permission from AAAS.

In recent years, a host of methods have been developed for studying single molecules under tension. Tools such as atomic force microscopy, optical tweezers and magnetic tweezers are now fairly mature; but these techniques suffer from two major limitations. For one, they are generally designed to carry out experiments in a serial rather than a multiplexed manner, and this limits their throughput and the ability to perform appropriate statistical analyses. Secondly, they require that the single molecule under study be physically tethered to a macroscopic instrument, which introduces problems of signal drift and noise that limit sensitivity.

Tim Liedl of Ludwig-Maximilians-Universität, Philip Tinnefeld of Technische Universität Braunschweig and their colleagues aimed to solve both of these issues with a new, miniscule, DNA-based tool. They developed a 'nanoscopic force clamp'—essentially a tiny bracket-shaped device constructed using DNA origami methods.

A single-stranded DNA (ssDNA) entropic spring bridges the gap between the two sides of the nanoscopic force clamp. In the middle of the spring, a target DNA sequence of interest can be inserted using standard cloning methods. The number of bases in the ssDNA spring can be adjusted to apply forces ranging from 0 to 12 piconewtons to the target sequence. A

SYNTHETIC BIOLOGY

REXER HELPS DESIGN BACTERIAL GENOMES

A CRISPR–Cas9-based approach identifies synonymous codons that can be reassigned to encode new chemical building blocks.

The possibilities are endless. A reprogrammed genetic code that goes beyond the natural 20 amino acids and incorporates new chemical building blocks could give rise to newly designed metabolic pathways, new polymers, or even the creation of new organisms. As Jason Chin from the Medical Research Council Laboratory of Molecular Biology in Cambridge points out, a challenge with these aspirational goals is that, in the context of cells, all 64 possible triplets of the genetic code are either assigned the 20 canonical amino acids, or they function as stop codons. Chin has long been interested in engineering the genetic code to encode new building blocks. "If you could reduce the number of codons that encode a particular amino acid," he reasons, "you could then use the freed-up synonymous codons to encode something else."

Kaihang Wang, a senior scientist in the Chin lab, decided to pursue this idea and recode some amino acids. Serine, for example, is encoded by six codons, and Wang planned to replace two of these codons with one of the other four and check the effect on viability. Rather than make changes all over the genome, he focused on a cell-division operon to test the recoding schemes. This operon, rich in the targeted codons, carries 12 essential genes expressed over a large dynamic range. It also contains membrane proteins that need to be folded correctly, a process for which codon choice is known to be important. Any detrimental effect caused by the codon swaps could be read out as a decrease in cell viability.

In order to efficiently replace hundreds of kilobases at once, the team combined the established lambda Red recombination strategy with CRISPR–Cas9 in an approach they termed replicon excision for enhanced genome engineering through programmed

trillion nanoscopic force clamps can be produced in a one-pot reaction. The clamps can then be probed by high-throughput optical analysis using fluorescence resonance energy transfer (FRET).

The researchers put their force clamps to the test in two applications. In the first, they investigated an X-shaped DNA structure called a Holliday junction, which in nature forms during DNA recombination and repair. This structure exhibits switching behavior between two stacking conformers called iso I and iso II, which can be monitored by placing donor and acceptor fluorophores on two different arms of the X structure for FRET analysis. The researchers observed a clear population shift towards the iso II conformation at higher forces, results that were in good agreement with previous optical and magnetic tweezers measurements.

They also studied the interaction of TATA-binding protein with a core promoter DNA sequence. This transcription factor recognizes the minor groove of the TATA box, causing a 90-degree bend in the DNA sequence. However, current single-molecule force analysis tools have not yet been successful in detecting this process. The researchers utilized six different force clamp configurations to cover a range of forces. They found that the bending process was almost completely curbed when the promoter sequence was held under high tensions; their experiments allowed them to calculate the change in binding energy for the interaction.

Liedl is excited about the possibilities offered by the nanoscopic force clamp. Next on the docket is using the device to apply tension to proteins. "By the correct choice of coupling strategy and DNA sequences, it should be possible to connect some interesting proteins to our DNA springs," he says. Further down the road, force clamp experiments in cells may even be possible. "One could inject the structures with a microinjector into the cytoplasm and observe proteins interacting with the force clamp," says Liedl. "After a short while, however, the DNA-based structures or just the spring region surely will be degraded by DNases, so we now will have to focus on making the structures robust towards biodegradation." It will indeed be exciting to watch whether these tiny tools can have a big impact in the single-molecule force analysis field.

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RESEARCH PAPERS

Nickels, P.C. *et al.* Molecular force spectroscopy with a DNA origami-based nanoscopic force clamp. *Science* **354**, 305–307 (2016).

recombination (REXER). "It uncouples the transformation from the recombination step," explains Chin. First, cells are transformed with a bacterial artificial chromosome (BAC), which contains the target with the modified codons together with positive and negative selection markers. After selection for stable expression, the cells are transformed with Cas9 and single-guide RNAs that excise the target fragment from the BAC so it is free to recombine into the *Escherichia coli* genome at the homologous regions.

Wang and the team used REXER to replace two codons each for serine, leucine and alanine in the cell-division operon, and then they sequenced the resulting clones. Some recoding schemes worked very well; for example, the codons TCG and TCA for serine could both be replaced in all instances with AGC. Others failed; for example, TTA and TTG for leucine could not be replaced by CTC. Still others allowed substitutions at most sites but were refractory to them at particular loci. These data yield interesting information about the importance of synonymous codons in certain positions. It will be interesting to further explore why some changes are not tolerated.

This reassignment scheme freed up five codons. In future work, the tRNAs matching these codons will be deleted from the *E. coli* genome, and new tRNAs aminoacylated with unnatural amino acids will be introduced that recognize these codons.

Chin has calculated that REXER could re-engineer the entire *E. coli* genome in 14 iterative steps. "We don't understand how to design recoded genomes *de novo* yet," he says, "so this sectional approach—which provides feedback on what works—makes sense." Every successfully designed section would be used as a template for the next replacement step until the entire genome is swapped.

E. coli is not the only target for REXER, as Chin envisions its applications for eukaryotic systems as well.

Nicole Rusk

RESEARCH PAPERS

Wang, K. *et al.* Defining synonymous codon compression schemes by genome recoding. *Nature* **539**, 59–64 (2016).