BIOPHYSICS

Unwinding to measure tension

Two independent groups develop tension probes based on molecular beacons to measure mechanical stimuli in live cells.

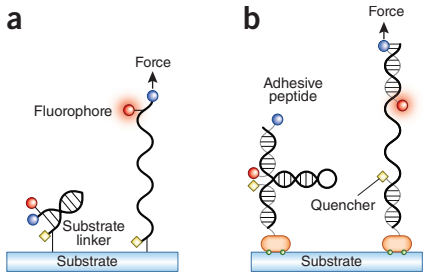
Organisms sense and interact with surfaces around them and with other cells largely via integrins, and advanced tools are needed to measure tension at these cell-surface receptors. To improve on currently available tools, two independent groups recently took advantage of molecular beacon technology to develop tension probes to measure mechanotransduction.

Christopher Chen, who is affiliated with Boston University and Harvard University, and his colleagues developed a single-oligonucleotide probe (Blakely *et al.*, 2014). Their system comprises a fluorophore and a quencher on opposite ends of an oligo that forms a hairpin. One end of this probe is linked to a substrate, whereas the other has an adhesive peptide that binds integrins. When cells bind and pull against their integrins, the applied force unzips the hairpin, separating the fluorophore from the quencher, which results in fluorescence.

Khalid Salaita's group at Emory University independently created a modular probe (Zhang *et al.*, 2014). Instead of a single oligo with four modifications, they use three oligos: one has a quencher and links to the substrate, the second has a fluorophore and an adhesive peptide, and the unmodified third oligo hybridizes to each of the first two and contains the hairpin that unzips with application of force. Salaita explains the advantage of this modular design: "You'll spend time doing synthesis on the two arms, the anchoring arm and the ligand arm, but the hairpin oligo is unmodified, so you can generate a library… of different hairpin oligos to dial in the force required to open the hairpin."

To measure forces (in the low piconewton range), both groups immobilized their probes on a surface and subsequently plated cells on that surface. For both probe designs, the observed fluorescence is a measure of unfolded hairpins and therefore of aggregate forces at a given location in the cell, which can be tracked over time. Thus, with readily accessible fluorescence microscopes, these probes enable localization studies for a receptor of interest along with its mechanics—simultaneously and in living cells.

In proof-of-concept studies of integrins, both groups showed that in focal adhesions, the forces were localized at the cell edge and the magnitude of forces varied across adhesions over time.



Schematics of tension probes developed by the Chen (left) and Salaita (right) groups. Adapted from Blakely *et al.* and Zhang *et al.*, Nature Publishing Group.

The two tension probes have advantages for different applications. The reliance on hybridization to hold the tension probe together makes Salaita group's modular version less robust: the DNA duplexes can shear if additional force is applied after the hairpin unfolds or over time. "Our probes are better suited for receptors that don't apply high forces, such as growth factor receptors," notes Salaita. In their study of integrins, the probes were stable for only about an hour. And therein lies the advantage of the single-oligo probe, as Chen notes: "we can observe the adhesion maturation process and other slower processes that might take hours or days."

These tension probes can be used to address hypotheses about the role of mechanical transduction in signaling. "Many pathways involve recognition of cell-surface receptors, and mechanics may be involved in improving the fidelity of information transfer," says Salaita. "These pathways can and will be studied using these tension probes." And combined with high-resolution microscopy, the tension probes can provide even richer information. Chen adds that they "are hoping to use these for high-resolution measurements and for substrates where it is difficult to measure forces, such as topographic environments."

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RESEARCH PAPERS

Blakely, B.L. *et al.* A DNA-based molecular probe for optically reporting cellular traction forces. *Nat. Methods* **11**, 1229–1232 (2014).

Zhang, Y. *et al.* DNA-based digital tension probes reveal integrin forces during early cell adhesion. *Nat. Commun.* **5**, 5167 (2014).