People & Ideas

Dan Fletcher: A recipe for cooking up cellular machines

Fletcher specializes in reconstituting subcellular structures to test their behavior.

Growing up on a big piece of land in Texas, Dan Fletcher explored how things in both the natural and mechanical realms were put together. But in his early school days a particularly traumatic dissection of a bloated fetal pig sent him in the direction of "hard, clean" sciences. By the end of his doctoral work developing new instruments that combined optical and atomic force microscopy (AFM), he realized that such technology could help in the study of cells, in all their messiness.

As a postdoc, he studied the physics of cell motility with Julie Theriot at Stanford University, including measuring the nanonewton-scale forces generated by actin comet tails propelling lipid vesicles (1). Now a professor of bioengineering and biophyics at the University of California, Berkeley, Fletcher investigates how cells transduce mechanical signals into biochemical signals and vice versa. His group's advances in AFM have allowed biophysical studies to jump from single molecules to cells and subcellular assemblies (2), letting them twist, compress, or pull on membranes, vesicles, and cytoskeletal networks to see how they respond. In 2007, his lab showed that branched actin networks exhibit a unique mechanical property—reversible stress softening—that arises from their branched architecture and allows the network to be both stiff and soft under different forces (3).

A big fan of recreating simple cellular structures in the lab, Fletcher and collaborators have used reconstitution to show how protein crowding can curve membranes (4), how spindle assembly depends on compartment volume (5), and how membranes can bundle actin (6). We recently chatted with Fletcher about his feats in the lab and forays into public service.

USING THE FORCE

Why do we care how much force organelles or cytoskeletal components generate?

That's a question I have gotten not too infrequently from reviewers. If we can understand where and when forces are generated by cells, and how individual molecules respond to those forces, then we can pull the covers back on what I would argue is a hidden set of signals. In cells, forces are simply another way to communicate.

We know that if you pull on a motor protein, such as myosin, its catalytic activity slows down. What's less clear is how that happens on the collective, multimolecular scale where cellular behavior emerges. The challenge of understanding that mechanical language got me interested in cell motility, membranes, and actin networks—the transmission lines for mechanical signals.

How did your technical improvements to AFM allow for cell-level studies?

We made improvements in a few ways. First, we integrated AFM with fluorescence imaging in ways that harness the power of both techniques to address basic cell biological questions. Second, we engineered ways to do longer timescale measurements by adding a second cantilever to control for drift in the microscope system. Third, we used cantilevers not just as probes for force measurement but also as substrates for adhesion of cells and organization of proteins.

> "We think of reconstitutions as physical proofs of behavior."

A goal of our work combining AFM with both cells and reconstituted systems is to reveal the importance of force and mechanical properties. They aren't just relevant to bones but also to membranes, actin filaments, and even how cells respond to drugs.

ADD MEMBRANES, STIR

Your group investigates the behaviors of reconstituted giant lipid vesicles and other minimalist cell-like compartments. Do you worry you might miss a key ingredient of a process?

I think it comes down to how you interpret reconstitution experiments. When we reconstitute something, we discover what this combination of molecules, membranes, and physics *will* do. And the cell can either take advantage of that or it has to suppress it in some way.

PHOTO COURTESY OF PEG SKORPINSKI

Dan Fletcher

I don't see these experiments as telling us, "This is what the cell must be doing with this set of molecules." That's where the danger of over-interpreting or missing a critical element of a process lies.

One example is the work we did showing that filopodia-like structures form when we grow actin networks on giant vesicles. The idea is that the membrane *can* play a role in bundling actin filaments, something that had previously been ascribed only to "bundling" proteins.

This approach just changes the question a little bit. Instead of asking what protein I must add to make an activity happen, we think of reconstitutions as physical proofs of behavior.

In thinking about those physical proofs, you also use the term "boundary conditions." What do you mean by that?

Maybe a good analogy is the ingredients versus the recipe. So much attention has been paid to identifying components that the context of those components is sometimes not appreciated.

A good example of boundary conditions is in our recent paper with Rebecca Heald's

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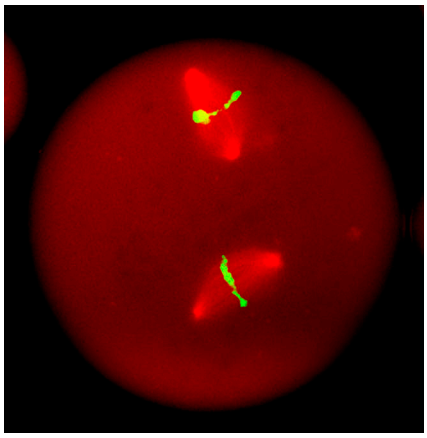

IMAGE COURTESY OF MATT GOOD AND MIKE VAHEY, UC BERKELEY

Chromosomes (green) align along spindles (red) whose lengths are determined by the volume of reconstituted cytoplasmic droplet compartments.

group on spindle scaling. We assembled this structure in compartments with defined sizes. And even though we put exactly the same material—*Xenopus* egg extract—into each one, the compartment size ends up affecting the size of the spindle. So volume would be a boundary condition.

I think that's the recipe part of making a dish that is missing from the molecular view of cell biology in many cases. We know the parts, but we don't always know how to put them together. There's so much to be learned about the role these boundary conditions play—forces, volumes, even gravity. With reconstitution we can isolate and explore them.

I should probably admit at this point that I'm not a very good cook!

You and your colleagues recently showed that amphipathic helix insertion into one side of the lipid bilayer by the protein epsin1 is not necessary for creating highly curved membrane tubules. How did you know something else was going on?

If you ask a physicist how crowding of molecules on one side of a membrane might affect the shape of that membrane, the answer is straightforward: you are going to generate a pressure that induces bending. From a biology point of view, there is the observed connection between helix insertion and membrane curvature. Both of those perspectives are right. But mechanism can be a tricky thing to track down.

This work with colleagues at Sandia National Laboratories is a great example of how reconstitution can provide insight into a collective phenomenon. It was only by directly quantifying the physical consequences of either having high densities of proteins on a membrane or not, and changing how they are attached to that membrane (replacing the helix with a direct lipid attachment), that we could learn how protein density contributes to curving membranes.

Reconstitution gives you that level of control that lets you ask a very specific question. It's the happy medium between the really messy pig dissections and the really clean physics.

POLITICS AND HIGH-SPEED FUN

In 2008-2009, as a White House Fellow in the Office of Science and Technology Policy, you worked on projects ranging from biodefense to a health initiative for expectant mothers. What did you learn?

Whenever a politician gives a speech about how the energy problem is going to be solved or how cancer rates will be decreased, they are talking about work they expect university researchers to do. Research can feel like a very personal endeavor. But it's one with many, many stakeholders—people who very much need the solutions we are aiming for. And there is no back up. There isn't a secret set of scientists to solve the energy crisis or healthcare issues. There's nobody else. It really is up to researchers in universities, national labs, and industry.

> "Mechanism can be a tricky thing to track down."

I try to keep in mind that, for my basic research to be useful, it's got to be passed on, and I can play a role in handing it off to the next person on the road to solving the big problems.

One such hand-off to the real world is the CellScope, a smartphone- or tablet-mounted microscope for imaging biological samples. How did that idea come about?

I thought it would be a good project for my optics class back in 2006 to figure out how to retrofit a mobile phone camera to convert it into a microscope. I assumed I would show an example at the end of the class, but then I couldn't find anything.

So the prize for the best project was that I'd sponsor students to work over the summer to help build a prototype. We built it, and, indeed, you could see blood cells. You could even do fluorescence. And that got us really excited about using these for diagnostic applications. Over the past several years, we've built devices for tuberculosis, malaria, filariasis, and ophthalmic indications like diabetic retinopathy, as well as an otoscope device for the ear. Many more diseases to go.

Your website features quirky slow-motion videos of, among other things, students being water-ballooned. What's going on?

We bring a high-speed camera for research purposes to the Physiology Course at the Marine Biological Laboratory in Woods Hole, Massachusetts, where I have been teaching for the last several years. But late at night we pull the camera off the microscope and just have fun.

There's a freedom to being at Woods Hole where you can try things out you'd never try (and probably shouldn't try) in your lab back home. The same is very much true with experiments. You try new and risky experiments there and are oftentimes amazed by the results. Being there brings back the joy of discovery. Plus, it gives us a good excuse to throw water on people.

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3. Chaudhuri, O., et al. 2007. *Nature*. 445:295–298.
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6. Liu, A.P., et al. 2008. *Nat. Phys.* 4:789–793.

PHOTO COURTESY OF KAHI KAI, HAWAII

A CellScope being used to image marine algae, plankton, and crustacean larvae right after their collection from the ocean.