People & Ideas

Liz Rhoades: Single molecules tell many stories

Rhoades studies protein folding and binding using single-molecule fluorescence techniques.

Recent advances in lasers and fluorescent dyes have made it possible to observe individual proteins as they fold into their mature conformation or bind to other proteins. Liz Rhoades is harnessing these techniques to answer questions about the biology of amyloid family proteins—whose aggregation is associated with neurodegenerative syndromes such as Parkinson's and Alzheimer's disease (1).

With an undergraduate degree in physics, followed by graduate and postdoctoral work in biophysics (2, 3), Rhoades approaches her work with a different perspective than would a cell biologist, but her efforts are helping unravel some enduring mysteries surrounding amyloid proteins, including what structural conformations these proteins normally assume (4). They're also providing insights into what induces these proteins to form the aggregates that are the hallmarks of neurodegenerative diseases (5). We called Dr. Rhoades in her lab at Yale University to find out more about her work and the technologies that drive it.

MAJOR CHANGE

What was your first exposure to science?

It was really when I went to public school, where I always had really good math and science teachers. But I didn't think about being a scientist until I was already at college; I was pretty sure I would be an English major when I went to University. Then, when I got to Duke, I had to take the distribution requirements—classes that weren't in my main area of study. I had started thinking about going into environmental engineering, so I decided to take some basic science classes. At the beginning of my sophomore year I needed to take organic chemistry, but I didn't register for the class in time to get a lab section that I liked, so I decided I would take physics instead, to get it out of the way. As it turned out, I really liked my physics class. That was largely due to the professor, Alec Schramm, who was fantastic, even though he wasn't on the faculty—I think he was either a postdoc or a graduate student at the time. I decided to become a physics major.

What did you plan to do after your degree?

Graduate school seemed like a nice option, as I think it does to many people finishing up an undergraduate degree in an area where it's not immediately obvious what kind of job you'll do. I'd been doing research every summer, but in the summer before my senior year, I ended up in a biophysics lab at the University of Tennessee. I really liked what I was doing there, so in my senior year I took a lot of biology courses, and I decided I really wanted to be in a biophysics graduate program.

You were in graduate school when you first encountered proteins involved in amyloid disease…

I did my graduate studies with Ari Gafni at the University of Michigan. I was studying a peptide called Islet Amyloid Polypeptide, which is implicated in the pathology of type II diabetes. It's found aggregated and deposited in extracellular plaques, and we were trying to understand why it aggregates and what pathway leads to the formation of aggregates. Although I didn't work on amyloid proteins during my first postdoc, I ended up coming back to the subject for my second postdoc, and that's primarily what we're working on in my group now.

> "FCS is a powerful approach to look at fluctuations that happen on [very fast] time scales."

IMAGE COURTESY OF JERRY DOMIAN

Liz Rhoades

NOTHING TO FRET ABOUT

In your first postdoc you used Förster resonance electron transfer (FRET) to look at protein folding…

Yes. In FRET, the excitation state of an acceptor fluorophore depends on its proximity to a donor fluorophore. This means that it can be used to detect interactions between two proteins, and also as a direct conformational probe of a protein. For conformational studies, both the donor and the acceptor fluorophore are attached to the same protein, and the relative amount of fluorescence emission from the donor and the acceptor is dependent on the conformation of the protein. You can get both static information about the states that are populated, and information about the dynamic interchange between those states—in other words, both kinetic and equilibrium data. Because we were doing FRET measurements on molecules that were inside immobilized lipid vesicles, we could also follow a single protein for an extended period of time and watch it switching back and forth between the conformational states.

Text and Interview by Caitlin Sedwick
csedwick@gmail.com

Why did you do two postdocs?

I had done my first postdoc in Israel at the Weizmann Institute with Gilad Haran. I chose to go there because I wanted to work abroad. The Institute was a fantastic place to work. I really enjoyed my time there. It's a really interesting environment full of very talented people from all over the world.

After a couple of years, I was ready to wrap up my time in Israel, but I wasn't certain I was ready to take up a professorship right away. I applied for some faculty positions anyway, and I was actually offered a job at Stockholm University, but I decided instead to take another postdoc in Watt Webb's lab at Cornell. I was very excited to get the opportunity to work with him.

Why was that?

I'd read so much of his work, and he had tools that I really wanted to learn, particularly fluorescence correlation spectroscopy (FCS). I proposed to him that we study an amyloid system using the tools that he already had in his lab. The project that I initially envisioned—which involved using FCS to understand how an amyloid protein called α-synuclein localizes to specific types of model membranes—never actually got done. Instead, we used FCS to make quantitative measurements of α-synuclein binding to different types of model membranes, which allowed us to investigate the role of specific lipids and of electrostatics on binding.

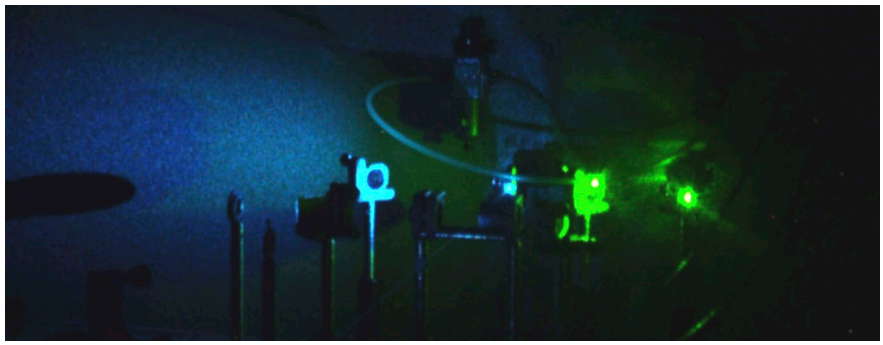

IMAGE COURTESY OF ADAM TREXLER

Strong, tightly focused laser light illuminates single-molecule fluorescence.

"How do the physiological failures of the aging brain lead to the aggregation of what is normally a very soluble protein?"

CONCENTRATE ON DETAIL

How does FCS work?

Molecules undergo Brownian motion in solution. If you use a focused laser as your illumination source, the focal volume defines your observation volume. As fluorescent molecules diffuse by Brownian motion, they move in and out of the focal volume, which results in fluctuations in the fluorescence intensity measured from this volume. From these intensity fluctuations, you can calculate something called a temporal autocorrelation curve. Fitting data to this curve yields information about the diffusion of the molecule and its concentration in solution. For example, a compact, folded protein diffuses more quickly than when it's unfolded, aggregated, or bound to another protein.

FCS is also a powerful approach to look at fluctuations that happen on time scales much faster than diffusion. When combined with single-molecule FRET, which is useful for longer time scale dynamics, you can get very detailed information about the conformational fluctuations of your protein. For example, if you're using FRET to monitor the state of a folded protein, then as long as the protein stays folded, it's going to have constant donor and acceptor fluorophore intensities—at least on a time scale of tens of milliseconds, which is what you normally use for these types of measurements. But combining this with the information from FCS allows you to observe the dynamic behavior—for example, fluctuations in packing state or slight conformational changes of the protein in the folded state.

Can this technology be used in intact cells, as well?

That's an advantage of this technique and something that has been demonstrated elegantly both in cells and, very recently, in intact living embryos. The ability to do FCS and FRET in a cell or living organism means that you have the potential to look at interactions with native binding partners and make quantitative measurements of these interactions in physiological systems. One of the projects in my lab is to make a structural map of what α-synuclein looks like in various parts of the cell—for example, does it have the same structure when it's associated with different cellular substructures?

What else are you working on now that you have your own lab at Yale?

α-Synuclein is strongly implicated in the pathology of Parkinson's disease, and while there are a number of genetic and environmental factors that lead to the disease, the primary risk factor is aging. A question we are very interested in answering is, how do the physiological failures of the aging brain lead to the aggregation of what is normally a very soluble protein? One aspect of this that we've been thinking a lot about is the role of oxidative stress. Oxidative stress can lead to modifications of either lipids or proteins. Native interactions, and thus the native function, of α-synuclein could be perturbed by modifications that arise from an oxidative environment. We would like to understand whether direct oxidative modifications to α-synuclein either affect its ability to function normally or its dysfunctional role in Parkinson's disease.

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