Bryan Krantz: From folding to unfolding proteins

Krantz analyzes anthrax toxin to understand the physics of protein unfolding and transport.

As a grad student at the University of Chicago, Bryan Krantz became interested in how proteins fold (1, 2). But as a postdoc at Harvard (with John Collier), Krantz stopped folding proteins, and started unfolding them instead. In particular, he started unfolding anthrax toxin (3).

Most people might think twice before handling anthrax toxin, let alone spending years working with it. But Krantz saw the potential of this toxin for revealing how proteins unfold before they pass through cell membrane channels—a process known as translocation.

Anthrax toxin is secreted from the bacteria *Bacillus anthracis*, and its three protein components—protective antigen, lethal factor, and edema factor—assemble on the surfaces of target cells. The protective antigen inserts itself into a cell membrane and creates a translocase channel, which allows the other two proteins to enter into the cell. Krantz, who now runs his own laboratory at the University of California, Berkeley, is using this toxin system to study the biophysical mechanisms of translocation (4). He believes this method of toxin delivery might also be used by other pathogens.

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HOW AND WHY

What were you like as a kid? Were you interested in science then?

I guess I've been interested in science pretty much since I was a small child. My son Zack is the same way now, just wanting to know why things are the way they are. And I think that naturally leads to asking questions about the world.

Was this something your parents encouraged?

My mom would go to garage sales and pick up books on science; they were called "The How and Why Wonder Books." They were old-school books, probably from the 1960s. It'd be almost any topic you could think of—rocks and volcanoes, weather, biology.

I tended to do better in math and science classes. Though later in college, I managed to take enough English classes to double major in English and Chemistry, but generally I didn't do as well in English.

When did you realize that you wanted to make a career of science?

I had thought about different careers. I was very interested in art and architecture for a while. I was taking drafting classes and things like that in high school. But I eventually decided, maybe due to a little bit of social pressure, "Oh, I should definitely become a physician."

I ended up going to Emory, a liberal arts school, and while I was studying biology there, I realized that I could do research science as a career. I think I became very interested in that during my first or second semester of undergrad, and I began to wonder whether I wanted to be a physician or a research scientist.

I considered doing an MD/PhD program. I applied to medical schools and was accepted at several, but realized that I would rather do just pure research—I felt like I would need that kind of freedom to get the most out of it. So I waited a year, and then applied to grad school.

Are you glad you decided not to go down the medical route?

I think so. People advised me that unless you're really determined to do very clinically based research, the medical degree is probably not going to help you a lot, and it could be a distraction. I would give the same advice to anybody. I think it was definitely a good move for me.

Bryan Krantz

FINDING PROTEIN FOLDING

After Emory, you went to the University of Chicago to study biochemistry.

Yeah, at that point I was interested in biochemistry. I had worked in a laboratory as an undergrad on the small protein ubiquitin. A laboratory at the University of Chicago did work related to what I was doing, so I was interested in joining them.

But ultimately, through laboratory rotations, I came to work in Tobin Sosnick's laboratory, who studied protein folding. We still ended up playing around with ubiquitin and studying how ubiquitin folded. But really I became interested in physical chemistry and biophysics, and it seemed like I'd found the best laboratory for that.

Your thesis was on protein folding?

Yes. The idea of my thesis was to understand how small proteins folded and unfolded. Once you understood the fundamental laws of protein folding, you could use that knowledge to predict how proteins would fold in the cell, even proteins whose structure you did not know.

Text and Interview by Liz Savage
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One area that I worked on was probing the diversity of folding pathways. We thought that there were many possible ways in which a protein can fold, not just one central route. So we developed a methodology for looking at that. And found that indeed some proteins can fold via multiple pathways.

INVESTIGATING ANTHRAX

Your interest in protein folding and protein transport led you to investigate anthrax toxin as a postdoc. Tell me how that happened.

I wanted to study something of cellular relevance, for myself, but also because there was a change in politics at the NIH —there was a renewed emphasis on studying biologically interesting problems. That's how I came to study protein transport.

Ultimately, I worked on anthrax toxin because I felt like it was an area that was understudied for the problem of protein transport. And anthrax toxin's protective antigen component exists in a fully water-soluble form, making it easy to work with. We can treat the toxin with a mildly acidic pH condition so that it inserts into a membrane, forming the translocase channel. So the system seemed self-contained and tractable in terms of developing a model.

Also at that time, around 2001, I thought that this could be a reasonable way to get my work as a postdoc funded, and eventually my first laboratory funded, but I wasn't thinking that far ahead. I thought that the bioterrorism angle would be a way to do that. But really, in my laboratory, we're not as interested in bioterrorism as we are in protein transport.

Aren't there less dangerous proteins to study?

The anthrax toxin is benign relative to tetanus or botulinum toxin, so yes and no. Really the system is attractive because the experiments themselves are tractable biophysically.

Why is anthrax a good model for studying protein translocation?

Toxins in general are quite stable and easy to work with. And so when we started to use the toxin system, we knew that we could readily incorporate these protective antigen protein channels into artificial membranes and use classical electrophysiology to monitor protein translocation. We can essentially watch protein translocation in real time.

In these types of experiments, protective antigen channels are inserted into membranes. The channels conduct cations readily and produce an ionic current once inserted. The lethal factor and edema factor (the substrate proteins) are added to the system, and these proteins block the channel. Raising the transmembrane potential or creating a pH gradient across the membrane then drives the substrates across the membrane, and the ionic current increases. The change in ionic current with time is the translocation kinetics.

So that opens a door to all kinds of biophysical studies that examine the translocation mechanism in detail. For example, we can mutate the channel and determine which residues were critical for transport and do kinetic assays that compare activities.

What about protein transport interests you the most?

The reason I became interested in the question originally stemmed from the fact that most membranes have quite narrow pores, probably as a way to prevent ion leakage and so forth. That means the proteins that translocate through them have to be unfolded. And that dynamic process is so poorly understood. I feel like it's a critical area to study, and there are a lot of possible directions to take it.

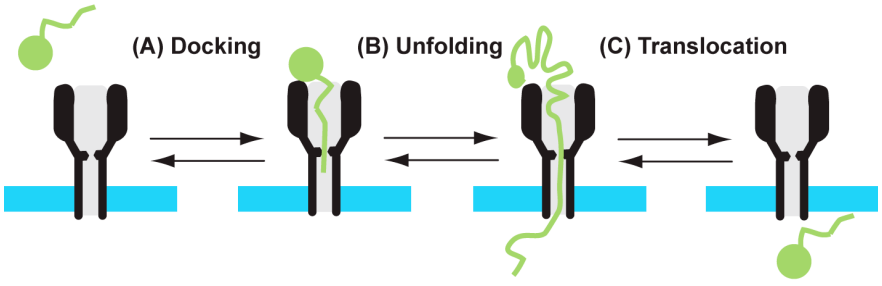
"In my laboratory, we're not as interested in bioterrorism as we are in protein transport."

We've really only scratched the surface, in terms of biophysical studies. We have a good idea of how small proteins unfold and fold in a test tube, but we don't know how proteins unfold in the cell.

In your laboratory at Berkeley, are you still focusing mainly on anthrax?

We're mainly working on anthrax. We're also getting some other projects started involving the outer membranes of bacteria, which is the interface between pathogen and host.

But right now, since the laboratory's only a couple years old, most of the projects still involve anthrax. Because it's a toxin, one might say that its rules and laws for protein transport are going to be different. And I'd agree that some of them probably will be, but I think a lot of them are going to be general, and why not explore the system that is most tractable first? JCB



MICHAEL BARNES, UC BERKELEY

Krantz deciphers the steps of the translocation pathway in anthrax toxin.

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