Neutralising nature’s smallest arrows

With a background in biochemistry and biophysics, Dr Bryan Krantz is passionate about gaining further insight into the molecular basis of cellular protein unfolding and translocation. Here, he discusses how he is using the anthrax toxin as a model system for dissecting translocation-coupled unfolding and polypeptide delivery across membranes.

Can you outline how you first developed an interest in the research of bacterial toxin translocation?

My earliest exposure to biomedical research was in Dr Keith Wilkinson’s laboratory at Emory University, USA. There, I researched ubiquitin-dependent protein degradation — a process that ultimately tags proteins for their eventual unfolding and degradation by a large adenosine triphosphate (ATP)-powered molecular machine. Interestingly, by some strange twist of fate, I ran into Dr John Collier — whom I later worked with at Harvard Medical School — and watched his research presentation on diphtheria toxin translocation. We had a great conversation about adapting the toxin as a means of delivering heterologous proteins into cells for new therapies. Coupled with my undergraduate research experiences, it was this conversation that switched my ambitions from medical school to graduate school to examine the biophysics of protein unfolding.

I subsequently joined Professor Tobin Sosnick’s laboratory as a graduate student at the University of Chicago, and used engineered metal-ion binding sites and isotope effects to observe the folding transition states of small globular model proteins. My long-term vision began to develop a few years into the work, as I ultimately wanted to apply these physical principles and methods to understanding the molecular basis of cellular protein unfolding and translocation — a problem central to cellular physiology.

What are your current research objectives?

Toxins have always been nature’s smallest arrows. New knowledge of how they work, where they hide and how to neutralise them will continue to be critical to all aspects of human civilisation. Hence, my goal is to translate our research into applications that are not only critical to biodefence and biosecurity but also central to human and environmental health.

Moving forwards, our goal is to use the anthrax toxin to gain insights into protein translocation; influence the development of new protein drugs to treat cancers, inflammation and other chronic diseases; probe the in vivo structure of the toxin to improve anthrax vaccines and immunotherapies; and adapt the toxin into a multiplexed nanosensing system to detect toxicants critical to food security, biodefence, and environmental and human health. Last but not least, we are also interested in pore-forming bacterial toxins associated with periodontitis, a condition now linked to chronic diseases such as dementia and atherosclerosis.

Could you explain what translocase channels and polypeptide clamps are in reference to translocation?

A translocase channel is a protein or protein complex that inserts into membranes and forms a water-filled nanopore through which proteins translocate to the other side of the membrane. Within a given channel, there is a series of nonspecific binding sites that engages the translocating chain during translocation. These sites are called polypeptide clamps, implying that they do not bind the translocating polypeptide in the traditional static sense. In the case of anthrax toxin, we are working on the hypothesis that clamp sites are dynamic and can bind and release the chain during translocation in a manner that efficiently couples the driving force.

What methods do you employ in your research?

Our laboratory is adept at using molecular techniques to specifically engineer proteins. We use biophysical methods like electrophysiology to characterise protein translocation, even at the single-channel level. To gain insight into the structures of these toxins, we use electron microscopy and X-ray crystallography. Unfortunately, any of these given methods suffers in its ability to obtain a molecular movie of protein translocation, but like many fields these structure/function approaches must be taken together in order to gain mechanistic insights.
Toxins and translation

Researchers working in the Krantz Laboratory in the Department of Microbial Pathogenesis at the University of Maryland, USA, are conducting basic scientific research to shed new light on the physical principles of bacterial toxin translocation across membranes.

**AS A LETHAL disease caused by the bacterium Bacillus anthracis**, anthrax can be traced back to the beginnings of the Old Testament and early Mesopotamian writings. For instance, it is widely thought that the Book of Genesis' outline of the fifth plague in Egypt – namely, a sickness that affected livestock – describes typical symptoms of anthrax, while other references to the disease can be found in early Hindu and Greek literature. Many scholars have proposed that the most serious anthrax outbreak in history was Black Bane, a deadly epidemic that swept through Europe in the 1600s and claimed at least 60,000 human lives.

More recently, anthrax has re-emerged in the guise of biological warfare. While state-sponsored bioweapons programmes are no longer in operation, this deadly toxin continues to be exploited by terrorist groups, rogue nations and disturbed individuals. For example, the infamous Amerithrax incident in 2001 – which occurred just weeks after the terrorist attack on the World Trade Center in New York City and involved the mailing of *B. anthracis* spores to high-profile US Senators and media outlets – culminated in 22 infections, five of which proved fatal.

**A MODEL SYSTEM**

It was the Amerithrax incident that prompted Dr Bryan Krantz to devote his work to investigating the protein unfolding and translocation mechanism of the anthrax toxin. By this point, he had built a solid foundation of knowledge about the biophysics of protein folding and developed a passion for applying physical principles and methods to central issues in cellular physiology. Krantz therefore went to work as a postdoctoral fellow in the laboratory of Dr John Collier at Harvard Medical School, USA, where the anthrax toxin was used as a model system to dissect translocation-coupled unfolding and polypeptide transport.

This initial research paved the way for Krantz’s subsequent position as Assistant Professor in the Department of Molecular and Cell Biology at the University of California and his current role at the University of Maryland, where he is Associate Professor in the Department of Microbial Pathogenesis. Today, research in his laboratory primarily centres on the basic science and biophysical chemistry of membrane proteins involved in cellular transport and trafficking. “Nature has evolved proteins to be inherently stable, since their folded state is almost always their functional state,” Krantz outlines. “Stability is, however, an impediment when a protein is no longer needed or when it is localised in the wrong place in the cell. Hence, soluble proteins tagged for degradation can be unfolded using a molecular machine powered by the chemical potential of adenosine triphosphate (ATP); and those requiring delivery across membranes can be similarly unfolded by translocases in the membrane via ATP or electrochemical potential gradients.”

**PROTEIN TRANSLOCATION**

By elucidating complex protein unfolding mechanisms, Krantz and his team aim to increase their understanding of protein translocation across membranes, and explore various strategies for adapting the anthrax toxin for use as a targeted protein and small molecule drug delivery vehicle. To date, for instance, the researchers have shown that a transmembrane proton gradient is necessary for driving the unfolding and translocation of the anthrax toxin’s lethal and oedema factors. Additionally, they are investigating how peptide-binding sites known as clamps enable translocation while simultaneously avoiding tightly bound kinetic traps. “Ultimately, this question is one of force transduction or how the proton gradient is converted into a productive mechanical force that drives unidirectional transport,” states Krantz. The team is interrogating these mechanisms with a range of cutting-edge tools and techniques: synthetic polypeptide substrates, protein engineering of the translocase channel, single-channel electrophysiology, and biophysical and structural studies.

**ARCHITECTURE AND DETECTION**

In addition to exploring transmembrane protein translocation, Krantz’s laboratory...
also focuses on the in vivo architecture of the anthrax toxin and nanopore toxicant sensing. Importantly, existing therapeutic approaches for anthrax have inconsistent success rates and are likely to be based on incomplete scientific knowledge; for example, immunohistochemical studies from the 1960s imply that the in vivo toxin derived from anthrax animal models has alternative configurations – and emerging research findings suggest that there are two parallel toxin assembly pathways. In view of this, Krantz’s team is studying the macromolecular structure of anthrax toxin complexes, thus preparing the groundwork for a more complete knowledge of the molecular basis of immunity. Ultimately, this will contribute to the development of vaccines and passive immunotherapeutics, as well as a deeper understanding of the pathogenesis of anthrax.

The researchers are also attempting to forge deeper insights into the structure and function of the anthrax toxin nanopore and its related systems. In this context, they aim to develop a multiplexed bioelectronics sensing array with the capacity to communicate raw data remotely for the purposes of signal processing and the identification of toxic analytes. A powerful sensing method like this would have significant implications in a number of areas, including food storage: “The increased surveillance of grain stores – which are highly vulnerable to fungal contamination by pathogens – will be economically advantageous to tomorrow’s food challenges,” Krantz points out. “Developing nanopore sensors that more cheaply and rapidly monitor a more distributed food supply will aid food security.”

**DRIVING DRUG DELIVERY**

A further area of research in Krantz’s laboratory is centred on the possibility of manipulating toxic proteins to deliver drugs to cells. Excitingly, this is something that could be used in the treatment of cancer; for example, the delivery of engineered bacterial toxins with specific growth factor domains to cancer cells could prove to be an effective means of chemotherapy. Related to this, the researchers have discovered that the anthrax toxin associates closely with a large molecular-weight polyglutamate capsule polypeptide – and they are currently exploring the potential of using this polypeptide as a nanoparticle sponge that has the ability to carry and deliver cationic drug molecules to cancer cells. Further possibilities in this realm include using the toxin as a carrier for anti-inflammatory protein factors to specific targets.

Ultimately, Krantz’s innovative research endeavours have applications in a range of areas. Going forwards, the researchers in his laboratory are eager to continue investigating the molecular mechanisms that underpin anthrax toxin assembly and translocation. By applying their ingenuity and expertise to new basic science discoveries, the team hopes to unearth real-world benefits that range from human and environmental health to biosafety and biodefence.

**THE ROOT OF PERIODONTAL DISEASE**

Ranging from gum inflammation to serious damage of the soft tissue and bone that support the teeth, periodontal disease is common among adults worldwide. However, with over 500 species of bacteria inhabiting the oral cavity, its aetiology is highly complex – and some periodontal pathogens have been implicated in systemic inflammation leading to atherosclerosis, and neuroinflammation leading to dementia. Crucially, a deeper understanding of pore-forming toxins is essential for achieving a fuller understanding of the pathogenesis of these diseases. To this end, Krantz and his colleagues are using microbiome, proteome and RNA sequencing expression patterns to probe complex infectious communities, as well as electrophysiological methods to ascertain pore-forming functional activities.

**PHYSICAL PRINCIPLES OF BACTERIAL TOXIN TRANSLOCATION ACROSS MEMBRANES**

**OBJECTIVES**

- To increase understanding of protein delivery across membranes
- To explore the potential adaptation of the anthrax toxin for use as a targeted protein and drug delivery vehicle for diseases such as cancer
- To improve anthrax vaccines and immunotherapies by probing the in vivo structure of the toxin
- To develop the toxin into a multiplexed nanosensing system to identify toxicants critical to food security, biodefence, and environmental and human health

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BRYAN KRANTZ completed his PhD in the Department of Biochemistry and Molecular Biology at the University of Chicago in 2002. From 2006-14, he worked as Assistant Professor in the Department of Molecular and Cell Biology at the University of California. He has received many honours in his career and published numerous articles in international journals.