

*Seth Darst is a professor of Molecular Biophysics at the Rockefeller University in New York City, where his research centers around determining the three-dimensional structure of RNA polymerase, the enzyme at the heart of a cell's ability to make protein from a set of DNA instructions. His work draws on electron microscopy and x-ray crystallography.*



**You've been working with RNA polymerases for years now. What first got you interested in these enzymes and what keeps you coming back for more?**

My background is really in engineering, and when I finally realized I didn't want to be an engineer, I was lucky enough to land a postdoc in Roger Kornberg's lab, where they worked on transcription. So I got interested in RNA polymerase at that point. It's a large macromolecular machine with many functions, it's at the heart of transcription, and it's very heavily regulated, so we're never at a lack of questions to try to answer, and we're nowhere near finishing what we set out to do. That's why I haven't moved on.

**Some of your latest work involves crystallizing RNA polymerase on a lipid bilayer, and if I understand it right, the end result is a long thin tube of lipid wrapped with chains of polymerase. Kind of like a garden hose tightly wrapped in twine. How do you create that structure? What can you tell us about this technique?**

That's actually not so recent – that was my postdoc work in the late 80's, early 90's. The prospect of crystallizing and determining the structure of RNA polymerase by x-ray crystallography seemed kind of far away, pie in the sky. The Kornberg lab had developed ways of getting low-resolution structures using electron microscopy, and this was just a way of developing specimens that were really good for examining by electron microscopy. We still use that technique for things we can't crystallize for x-ray crystallography, but in general we try to use x-ray crystallography.

**With the massive computing power available today, why do we still need to extract, purify, crystallize, and analyze the 3-D structure of polymerases and other enzymes? Can't we build a program that will take into account the amino acid sequence of the enzyme, cofactor information, and spit out a reliable structure?**

No. The field of *de novo* protein structure prediction is advancing a lot for small structures, but the polymerase is 500 kDa and it's very complicated and at the time we did the structure, there were no homologs to compare it with in the database. To predict that kind of complicated structure is nowhere near even feasible.

**Do you think we'll ever get to that level of predicting power or will we always fall back to crystal-making?**

I wouldn't be willing to say, "never," but it's going to be a very long time. I doubt it will happen during my career.

**Some of your recent work explored the changes that a virus, bacteriophage T4, can induce in its host's RNA polymerase.**

Some of the most fundamental insights into transcription regulation and how genes are turned on by activators and turned on by repressors and so forth, came from work in bacteriophages. When bacteriophages infect their host, they often encode small proteins that take over the host

transcription system by altering the regulation and things like that. If we figure out how they do that, not only is that interesting in its own right, but it also sheds light on how the host polymerase is working.

**What's next for your lab?**

We're still plugging away at the polymerase and how it's regulated. There's probably dozens, maybe hundreds of factors that interact with the polymerase and effect the way that it functions at diff steps in the transcription cycle, so we're interested in how these work. We're interested in inhibitors of the polymerase. More of the same, basically.

**What advice would you give students about life as a biophysicist working in academia?**

If I had the time and I told you the story of my career, the striking thing about it is that it's sort of been one random thing after another. I think students should just go with the flow and do what you find interesting at the moment and it will work out. Also, the postdoc process can seem like a place-setter and kind of a waste of time, but I think it's a big mistake not to do a postdoc. I think that's the best time of your career, scientifically speaking. When you're a student you don't really know what you're doing, and when you're a postdoc you know what you're doing and all you have to do is just do it. Then you go on and become a professor, and all of a sudden you have to do all these things that you don't really know what you're doing again. You can't do the things that you know how to do anymore, you have other people doing them.

**What is something about you that most people don't already know?**

I'm a pretty serious piano player.