Lei Wang: Expanding the language of life

From sticking unnatural amino acids into proteins to using somatic hypermutation to jump-start protein evolution, Lei Wang has pioneered new ways to probe cellular processes and develop new drugs and industrial enzymes.

Organisms generally have only 20 amino acids to work with. True, they've managed to accomplish quite a bit with those ingredients, but imagine the possibilities if more choices were available. While a graduate student in Peter Schultz's lab at UC Berkeley, Lei Wang figured out a way to stick unnatural amino acids into *E. coli* proteins at rare stop codons. He did this by borrowing protein synthesis components, called orthogonal tRNA/aminoacyl synthetase-tRNA pairs, from other organisms such as the rugged archaebacteria (1). The resulting protein chimeras have extraordinary properties. For example, Wang has made a growth hormone that sticks around longer than its natural counterpart and a mutant receptor that glows when meeting its formerly orphaned ligand.

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From 2002 to 2005, Wang worked as a postdoc with Roger Tsien (cowinner of the 2008 Nobel Prize in chemistry) at the Salk Institute. There, Wang attempted to hasten protein evolution by using somatic hypermutation—a process used by B cells to diversify immunoglobulins—to induce mutations in selected target genes. Using this strategy, Wang developed a new and improved red fluorescent protein with increased far-red emissions and photostability (2, 3), surpassing the best efforts of structure-based protein engineers. With high-throughput screening systems, this technology could generate a variety of therapeutically and industrially useful proteins. Think better detergents, both in the lab and at the laundromat.

Since 2005, Wang has headed up his own lab at the Salk Institute, where he continues to push the envelope of protein evolution. He is also using unnatural amino acids to explore biological processes in mammalian cells and model organisms (4, 5).

STARTING OUT

Where were you born, and did you know what you wanted to be when you grew up?

I was born in southeast China. I think I always wanted to be a scientist. It's not because I knew that being a scientist would be exciting. It was simply because at that time, when I was young, every teacher told us in the classroom, "You want to be a scientist." It's a strong message once you get it stuck in your mind. In the end, I was good at scientific courses, so I thought it would be a good idea to become a scientist.

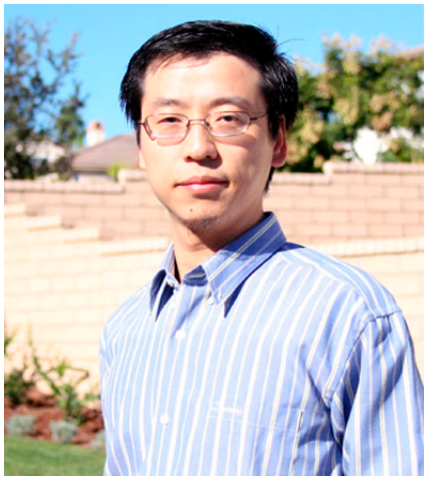
How did you begin working on unnatural amino acids?

Pete Schultz had been putting unnatural amino acids into proteins in vitro for a long time. I think I joined the lab at just the right moment. When I was a first-year graduate student, Pete had been wanting to expand this technology in vivo. I talked with him about what project to work on, and he actually wanted me to work on a different project—single molecule imaging—because I had a physical chemistry background. But I wanted to work on the unnatural amino acid project because I thought it was a really exciting idea and a very crazy idea as well!

At the time, I was very naïve. I told Pete, "If you don't want me to work on the unnatural amino acid project, I'm not going to join your lab." I was glad he wasn't offended. Afterwards, when I talked to other students, I got really scared that I'd said that to him [laughs].

What exactly is an orthogonal tRNA/aminoacyl-tRNA synthetase pair, and how do you generate it?

Orthogonal just means we don't want the introduced tRNA/aminoacyl-tRNA synthetase pair to cross-talk with the endogenous tRNA/synthetase pairs inside of the cell. If cross-talk happens, then you mess up the genetic code and mess up the whole message. When I was a student in Pete's lab, we tried many, many different methods, and it turns out the most efficient way is just to borrow a tRNA/synthetase pair from a different organism. For example, if you want to do this in mammalian cells, you just borrow a tRNA/synthetase pair from *E. coli*.



Lei Wang

HARNESSING HYPERMUTATION

In the Tsien lab, you used somatic hypermutation to introduce mutations into target proteins. How is this superior to conventional mutagenesis methods?

We developed this methodology basically trying to take advantage of the immune system, because our immune system can generate a variety of different antibodies, and these antibodies can be specific for different antigens,

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Text and Interview by Kira O'Day
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which is really a powerful system. But our immune system can only mutate immunoglobulin genes, not any other proteins. We thought, "Can we take advantage of this ability to evolve other proteins?" Then we could simply grow the cells and look for proteins with the desired property. Using in vitro mutagenesis methods, the main limitation is the transfection efficiency. But if you do everything inside of living cells, then you just need to grow a lot of cells without doing all the labor-intensive work.

Once you've removed transfection efficiency as a limiting factor, what limitations remain?

Now the limitation is how you design a screening method to look for the desired property. When we evolve fluorescent proteins, you can see them using FACS or using a microscope, which is very convenient and can be high-throughput as well.

What sorts of properties might you want to evolve in proteins?

There's a lot of potential in terms of protein engineering. For example, we want to engineer better protein drugs, and we also want to engineer enzymes that can tolerate high temperature or harsh conditions, because they have a lot of industrial applications. For example, enzymes are added to laundry detergent to help clean the clothes. Those enzymes need to tolerate high temperatures when you use warm water, and they need to be stable. You can greatly increase those properties using engineering. You can also evolve proteins to degrade toxic chemicals in the environment.

SYNTHESIZING SUPER-PROTEINS

How is your lab using unnatural amino acid technology?

Once the unnatural amino acid technology was set up, we wanted to apply it to interesting biological questions. The main idea is that if you can genetically incorporate unnatural amino acids in live cells, then you have the ability to introduce all kinds of novel chemical and physical properties into proteins in a site-specific way. And then you can take advantage of the new properties you introduce to do all sorts of interesting experiments in a more precise way. One way of looking at it is that we are enabling biochemistry in vivo, which is more accurate because all the biochemical reactions are happening inside of the cell, in the native environment.

The first experiment we did was to try to understand how ion channels self-inactivate. We started with conventional site-directed mutagenesis, but when we replaced one natural amino acid with another one, we found no difference at all. Then we switched to the unnatural amino acid and, surprisingly, by just extending a side chain by about one angstrom, you could see a significant and dramatic change in the ability of the channel to inactivate itself. This actually tells us that the size of a certain part of the protein is important for its function. It's not because of charge, or because of hydrogen bonding, but simply because of the bulkiness.

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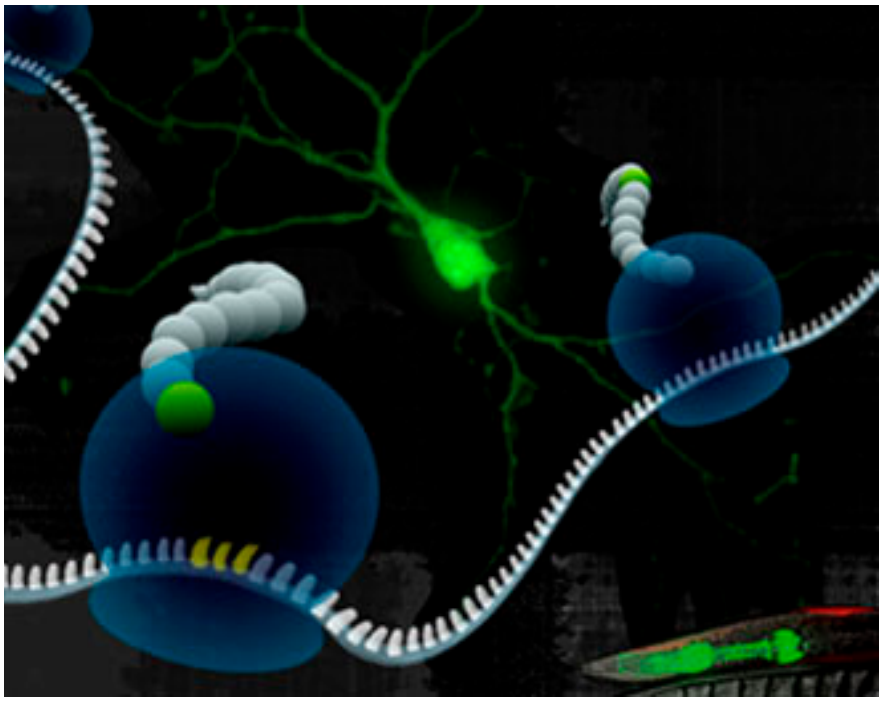
A second, more demanding direction we're taking is to try to engineer new protein properties using unnatural amino acids, so that we can control or modulate protein functions in a non-invasive way. If you can control protein function, then you can control cellular processes, either to better understand the process itself or to engineer new cellular properties.



Representation of an unnatural amino acid being incorporated during protein translation.

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