PNAS PROFILE

Profile of Nieng Yan

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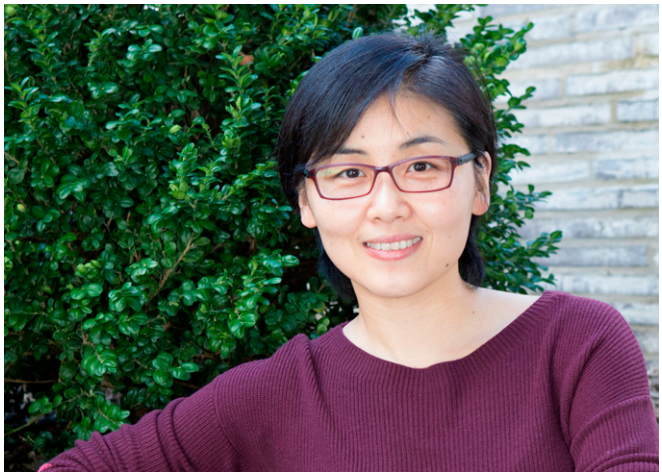
As a child, Nieng Yan became fascinated with the microscopic world. Growing up in Beijing, China, she was a fan of the Chinese novel *Journey to the West* and was particularly captivated by one of its major characters, Monkey King, and his ability to transform into anything. "Even in elementary school, I thought: If this Monkey King shrinks to the size of a cell or even smaller, what will he see at such scales?" says Yan. "I think that's the root of my interest in structural biology, because structural biology is all about revealing the detailed structures of our molecules at this very fine scale." Yan pursued this interest in structural biology throughout her scientific career, and her research has helped elucidate the structure and function of membrane proteins using X-ray crystallography and cryoelectron microscopy (cryo-EM). She helped characterize several important membrane transport proteins, including human glucose transporters and voltage-gated sodium and calcium channels. These structural studies have helped uncover the mechanisms of many of these proteins and could also help with discovering potential drug targets. In her Inaugural Article (1), Yan describes her latest studies of one of these sodium channels, Nav1.7. Now a professor of molecular biology at Princeton University, Yan was elected to the National Academy of Sciences as an International Member in 2019.

Interest in Membrane Proteins

Yan received a Bachelor's degree from Tsinghua University in Beijing in 2000 and traveled west to pursue a doctorate at Princeton University. "I think my real passion for science and structural biology probably started in graduate school when I joined professor Yigong Shi's laboratory at Princeton University," she says. "I realized how powerful biochemistry and structural biology [are] in revealing mechanisms, and how the conformational change of one amino acid can affect a cascade of signaling events."

By 2004, Yan had finished her doctorate and was eager for new challenges for her postdoctoral research. "As a graduate student, I completed two extremely challenging projects in apoptosis, so when I came to the end of my PhD training, I was super confident and thought I needed to challenge myself," she says. "At that time, membrane proteins were the most challenging area for structural biology," says Yan.

Yan liked living in Princeton and was keen to stay, and Shi suggested that she start a new branch of his laboratory focused on membrane proteins. Yan worked on the sterol regulatory element binding protein (SREBP) pathway, which is a central pathway that controls cellular homeostasis of cholesterol and involves membrane proteins. "I realized I was very naïve, and at that time the techniques were not ready for membrane proteins, so instead of solving the structures of the real players in this pathway, we took a detour," says Yan.



Nieng Yan. Image credit: Denise Applewhite, Princeton University, Princeton, New Jersey.

Among other things, Yan solved the structure of an intermembrane protease from bacteria. "It took me and my lab mates 1.5 years to solve this structure, and I accumulated both expertise and confidence," she says. Both came in handy during the next phase of her career.

Introduction to Cryo-EM

Around 2007, Yan took a vacation to Beijing and visited her former professors at Tsinghua University. One of them, Nanming Zhao, was now the senior vice dean of the medical school and asked her if she had ever published in high-profile journals. Fortuitously, Yan had just recently had a first-author paper in *Nature* (2) and, to her surprise and delight, she was soon offered a professorship at the medical school at Tsinghua University.

"I love both Tsinghua University and Princeton University, and it's funny, when I left Tsinghua for Princeton, I was already thinking that it would be great if one day I could come back to work for Tsinghua," she says. "And then my dream was realized much earlier than I had expected." Similarly, when Yan left Princeton in 2007, she thought it would be great to come back there eventually. Indeed, she returned a decade later as a professor of molecular biology, a position she holds to this day. "That dream was also realized much earlier than I had expected," she says.

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While at Tsinghua University, Yan started solving crystal structures, initially on bacterial homologs of eukaryotic proteins. "This was during the period when X-ray crystallography was the mainstream approach, and crystallization of membrane proteins was a pain," she says. "The entire structural biology of membrane proteins remained in its early phase, and we knew so little about them," says Yan. That would change with advances in cryo-EM over the next few years.

Yan says she owes her quick transition from X-ray crystallography to cryo-EM to Shi, who also moved to Tsinghua University in 2008 and encouraged the university leadership to invest in an EM facility. "We were structural biologists mainly using X-ray crystallography, and not many of us were aware of how promising EM was," says Yan, adding, "Yigong Shi really was the one with the vision."

Tsinghua University invested millions of dollars on a new EM facility and also recruited several professors with expertise in cryo-EM. "By 2011, we already had this core in cryo-EM, so when the breakthrough in cryo-EM resolution happened we could immediately adopt this new technology into our own research," says Yan.

According to Yan, a landmark advance in cryo-EM occurred with the publication of the structure of the protein TRPV1 in 2013 (3). "Just 1 year later, we resolved our first cryo-EM structure for the voltage gated calcium channel Cav1.1," she says (4). "I think the key is we were already ready with the infrastructure and with the expertise," says Yan. These proved crucial to the structural breakthroughs Yan made over the next several years.

Structural Characterization of Membrane Transporters

At Tsinghua University, one of Yan's research directions entailed solving the structure of glucose transporters. "The highlight of that period was the structural determination of the human glucose transporters GLUT1 and GLUT3," she says. "We solved the structures of human glucose transporters in three different conformations—outward open, outward occluded, inward open—and basically these structures together revealed the so-called alternating access working cycle of glucose transport," says Yan.

Yan is particularly proud of using rational design to decipher the structure of GLUT3 based on the GLUT1 structure (5, 6). "That was truly satisfying," she says. Her work on glucose transporters has important implications for drug discovery. "These transporters are overexpressed in cancer cells and represent potential drug targets for the treatment of cancer," says Yan (7).

Yan also set her sights on characterizing voltage-gated sodium (Nav) and calcium (Cav) channels, which are involved in initiating electrical signaling through action potentials. "I'm pretty happy that we were able to solve the first structures of eukaryotic sodium and calcium channels and then revealed their architecture, their folding principle, and more importantly, we discovered the mechanism underlying the so-called fast inactivation of sodium channels" she says. "That's something I'm very proud of. Because sodium and calcium channels are also important targets for drug discovery, I am hoping that we can eventually contribute to the fight against various diseases," she says.

Sodium channels are involved in a range of physiological processes, from heart beats to muscle contractions, and mutations in the channels are associated with a variety of pathologies, including arrhythmia and epilepsy. Deciphering the structures could help identify potential druggable sites. Some of these sodium channels—Nav1.7, Nav1.8, and Nav1.9—are also involved in pain sensation, and blocking these channels could help develop nonaddictive painkillers. "Because of the opioid crisis in the United States there is an urgent need for a new generation of painkillers, and these are very promising targets," says Yan. Her Inaugural Article (1) describes her recent work on one of these sodium channels, Nav1.7.

Capturing Different Conformations

Sodium channels undergo conformational changes to fulfill their function, and in 2019 Yan described the inactivated conformation of Nav1.7 (8). "We still needed at least two more conformations: the activated one with its pore open, and another one called the resting state," she says.

Capturing the resting state is particularly challenging; under physiological conditions, a strong electric field is needed to hold this conformation. However, solving the structure of membrane proteins involves isolating them from the membrane, which results in a loss of the membrane potential and of this resting-state conformation. "Our strategy in this paper (1) is to introduce mutations that may shift Nav1.7's voltage dependence, so we were hoping to generate a variant of Nav 1.7 that remained at the resting state even when there's no membrane potential," explains Yan.

Yan introduced mutations by rational design and discovered a different conformation of Nav1.7. "Instead of making a protein that favors resting state in the absence of membrane potential, this variant structure may represent a so-called closed-state inactivation and exhibits a totally different conformation from the previous ones," she says. It wasn't quite the resting-state conformation, but it did provide useful information about it. "The first voltage-sensing domain is in a completely down conformation and the pore domain is tightly closed, so this probably is reminiscent of what a resting state should be, but this channel has four repeats, and we've basically only engineered one quarter of it," says Yan. "So there's still a long way to go for us to capture the resting state, but I think this is kind of an important first step."

The new conformation provides insights into several disease mutations identified from patients suffering from pain. "The mechanism for these mutations was not immediately clear when we solved the previous structure, which is in a different state, but now the answer was very clear," says Yan. "This highlights the importance of solving the sodium channel structures in different conformations."

Yan plans to continue characterizing Nav1.7 using mutagenesis to try to capture the authentic resting state. Meanwhile, they are exploring methods to solve structures of membrane proteins in lipid vesicles (9) and hoping to introduce a controllable ion gradient to mimic the membrane potential. She also plans to harness new techniques, such as

combining structure prediction with advanced computational approaches to facilitate drug discovery. "One of my goals is really to find drugs for pain syndrome," says Yan. She is also excited at the promise of cryoelectron tomography for in situ structural biology. "I think that's kind of the future, and that would probably reveal information about these proteins that I cannot imagine of now," says Yan.

Yan appreciates the transformative effects of new technology. "I have benefited tremendously from the resolution revolution of cryo-EM," says Yan. "Merely 10 years ago, I thought it was kind of [a] mission impossible to solve these sodium and calcium channels, but with this technological breakthrough membrane proteins became quite tractable for structural analysis," she says. "I feel pretty lucky at having made such great progress in solving these structures, and constantly receiving this positive feedback, that only made me be more motivated."

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