Profile of Kevan M. Shokat

Protein kinases are the workhorses of the cell, orchestrating complex cellular activities by carrying out a relatively simple chemical modification: the transfer of a phosphate molecule from ATP to a protein or lipid substrate via a process called phosphorylation. Kinases are crucial to the function of all living organisms, and deregulated kinase activity lies at the heart of humanity's most pernicious diseases, including cancer, cardiovascular disease, neurodegeneration, and diabetes. But deciphering the role of each of the more than 500 kinases encoded in the human genome has proven remarkably challenging.

A kinase's function—and its role in disease—can only become clear once researchers know which proteins it phosphorylates. Kevan Shokat, Chairman of the Department of Cellular and Molecular Pharmacology at the University of California, San Francisco (UCSF), and recently elected member of the National Academy of Sciences, uses the tools of chemistry and biology to better understand what each kinase does. He pioneered a technique to identify the substrates of individual kinases and has developed a method to precisely control a particular kinase's activity using small-molecule inhibitors. He uses these tools to figure out which kinases could be good drug targets. Recently he has translated his findings into the development of drugs for the treatment of cancer and immune dysfunction, which are currently being tested in human clinical trials.

Out of Print

Raised in Berkeley, California, Shokat credits his early interest in chemistry to his parents' Bay area printing business. There, he learned how to operate printing presses and bindery equipment and to mix inks to develop various colors. "I think inadvertently, working for my parents' business, I was practicing a lot of chemistry but not learning about it," he says. When he entered high school, a teacher stirred his interest in biology. "The other classes I had were not the most challenging, and I hadn't really been exposed to the most formal chemistry classes in high school, so when I got to college, I thought I was going to be a biology major," Shokat recalls.

He went to Reed College in Portland, Oregon, and began taking the standard coursework for biology majors interested in medical school, which included chemistry. As he learned about hydrophobicity and hydrophilicity in an organic chemistry course, he thought back to the offset printing process, which is based on the repulsion of oil and water. "It's all about mixing the ink and using the balance between the ink and water to deliver a very fine pattern of ink onto paper," he says.

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Soon he officially switched his major to chemistry and began spending his free time doing independent research. As a junior, he carried out computer modeling analyses of enzyme kinetics, which led to the publication of his first paper with Reed professor Ron McClard (1). For his senior thesis, Shokat synthesized and characterized enzyme inhibitors. "When I got into the lab work of chemistry, it reminded me of the satisfaction of printing and making something, working with your hands; the craft of it," he says.

Uniting Chemistry and Biology

Shokat enjoyed independent research so much that he decided to pursue graduate studies. Still unsure of what direction to take, he applied to several MD/PhD programs, and—as a back-up plan—a few PhD programs in chemistry. As he traveled the country interviewing for MD/PhD programs, he stopped off to interview for the chemistry PhD program at the University of California, Berkeley (UC Berkeley). Nick Galakatos—one of Shokat's professors at Reed—urged him to meet with Peter Schultz, who had recently started his laboratory at UC Berkeley. Shokat describes his meeting with Schultz as "mind-blowing." "I had never heard about projects that allowed you to expand the genetic code, or make enzymes out of antibodies at will, or redirect nucleases to cut selective stretches of DNA or RNA," he recalls. "These projects seemed amazing." By the end of their talk, Shokat had decided to do a PhD with Schultz at UC Berkeley.

Through his work in Schultz's laboratory, Shokat found opportunities to bridge his interests in chemistry and biology. Schultz had recently shown how the molecular recognition capabilities of antibodies—immune proteins that bind with high affinity to specific biological targets—could be exploited to catalyze chemical reactions. Shokat expanded this line of research by developing strategies to tailor the specificity of these so-called "catalytic antibodies," opening the door for a wider range of chemical reactions that promised to be of great value to chemistry, biology, and medicine (2, 3).

As he neared the completion of his PhD in 1991, Shokat yearned to diversify. "I knew enzymology and chemistry, but didn't know much about cells," he says. His wife was doing a residency at UCSF, so he narrowed his search for postdoctoral research opportunities to the Bay area. After visiting several immunology and cell biology laboratories, Shokat felt excited by all of them. "After having joined Pete's lab while his lab was so young, I kind of got the spark for joining a very new lab and getting it going," Shokat says. The laboratory of Stanford University immunologist Chris Goodnow fit the bill.

From Catalytic Antibodies to Kinases

Goodnow studied how the B cells of the immune system learn to distinguish an organism's own "self" molecules from those of an invading microbe. Shokat was already familiar with B cells, some of which produce antibodies, thanks to his doctoral research. "I thought there was some consistency between catalytic antibodies and B cell immunology, but it was a rude awakening to see how little I knew about immunology when I started my postdoc," he acknowledges. "It's an amazingly complex area."

More lessons were yet to come. "I thought I was just there to learn techniques, to be able to handle B cells. . .and learn how to do mouse experiments," he recalls of his early days in Goodnow's laboratory. During his first 6 months, Shokat indeed learned the techniques necessary for his exploration of how the immune system avoids attacking certain molecules (4–6). "But I think it took me the next 2 years to even begin to

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 15046–15052 in issue 37 of volume 108.

understand how to design experiments, to think about the right question, and to know when you have sufficient evidence."

At the same time, the experiences of two of Shokat's friends helped to illustrate some of the limitations of the contemporary tools used to answer biological questions, and ultimately helped to shape his contemporary research interests. The first was Shokat's laboratory mate, Mike Cooke, who was trying to identify which kinases were dysregulated in B cells during an autoimmune state. Cooke explained to Shokat how he would disrupt the gene encoding each kinase in the mouse, then study the resulting phenotype. However, Shokat was bothered by shortcomings in the design. "I kept asking him, 'How does this experiment tell you which kinase is involved?'" He recalls thinking, "There's some missing tool here. We need a better way of telling which precise kinase was working."

Meanwhile, a friend working in a neighboring laboratory had just discovered that his knockout mice lacking the β1 adrenergic receptor, which regulates heart rate and contractility, displayed no apparent phenotype. "It was the moment where I could see that if you took a pure molecular biology approach, you could get at the systems of proteins that were important, but you couldn't often go to the exact protein. Even if you had the most powerful genetic system that was available then—gene knockouts in mice—biology could essentially adapt faster than the tool you were using to perturb it," Shokat says. "Right then, it got me thinking that this is where chemistry could really be the missing piece," he says. He started his own laboratory at Princeton University in 1994 with the goal of identifying the direct substrates of protein kinases—something that had proven intractable with traditional genetic, biochemical, and chemical approaches, owing in part to the high degree of overlap in substrate specificity among kinases. Shokat developed a molecular tagging system to identify kinase substrates by exploiting the highly conserved nature of the ATP-binding sites of kinases. He synthesized an unnatural, radioactively labeled ATP analog and engineered an enlarged ATP-binding site in the kinase Src so that it would be the only protein capable of using the bulky ATP analog to transfer radioactive phosphate groups to its substrates (7, 8). Under these conditions, all radioactively labeled proteins are necessarily substrates of modified Src because no other kinase can efficiently use the ATP analog.

Bag of Tricks

Using this chemical–genetic strategy as a blueprint, Shokat began many collaborative projects to identify the substrates of other kinases and to map signaling networks. However, he quickly recognized the need to hone the technique to identify low-abundance substrates. Over the next decade, Shokat developed additional ATP analogs to label substrates with what he calls different types of chemical "handles" that could be used to capture and purify the tagged substrates from protein mixtures, increasing the likelihood that low-abundance substrates could be identified (9–11). Shokat ultimately aims to use this technique to identify all of the direct substrates of each of the kinases in the human genome and to map kinase signaling networks.

In 1999 Shokat returned to the West Coast as an associate professor at USCF. As his kinase substrate identification work was gaining momentum, he realized that he might also be able to use a similar chemical–genetic approach to develop highly selective chemical inhibitors of kinases and use such a tool to better understand each kinase's role in the cell. He chemically modified nonspecific kinase inhibitors so that they were complementarily shaped to fit the modified form of Src or other kinases with similarly mutated ATP-binding sites (12). The technique, which enables rapid, reversible inhibition of a desired kinase, has since been used to probe the functions of more than 70 kinases.

One drawback to the method is that the enlargement of the ATP-binding site severely impaired the activity of some kinases. Shokat's Inaugural Article (13) describes a method to achieve the same specific pharmacological control over an engineered kinase without enlarging the ATP-binding site. Instead, Shokat introduced a reactive cysteine residue in the ATP pocket and synthesized inhibitors that bind that site via covalent complementarity rather than shape complementarity, thereby improving the specificity of the approach.

Unexpected Effects of Kinase Inhibitors

At times the results from Shokat's iterative approach to the study of kinases have been particularly eye-opening. "It's amazing," says Shokat, "because you get different phenotypes when you perturb kinases with genetics than you get with small molecules. Inhibiting a kinase is not just turning it off—it does other things to it." For example, Shokat collaborated with Peter Walter, a biochemist at UCSF, and applied the chemical–genetic strategy to study the bifunctional kinase Ire1, which phosphorylates itself to activate its second function as an endoribonuclease (RNase) (14). "We made the mutation in the Ire1 kinase domain, and it wasn't as active as the wild-type enzyme. So we were a little distressed that the mutant wouldn't behave, and that would ultimately make it not a very useful model," Shokat says. However, a postdoctoral fellow in Walter's lab added the inhibitor of the mutant enzyme to his assay anyway, and the results were shocking: the kinase inhibitor enhanced the activity of the RNase domain. "We always think a kinase's job is to add a phosphate, and if you block that, its function should be blocked," Shokat says. "That [result] really led us to start thinking that the conformation of the kinase domain is also important, and small molecules can perturb that. It took the drug regulation of kinases to another level, because now it wasn't just an off switch, it was an on switch, and kinases weren't just catalysts, but they were conformational switches."

Most recently this phenomenon played out in a collaborative project with Neal Rosen, an oncologist at Memorial Sloan-Kettering Cancer Center (15). "We were trying to understand the curious result of a clinical trial of a Raf inhibitor, where the drug was curing one cancer but actually causing another cancer in patients," Shokat says. Rosen had found that the Raf inhibitor blocked Raf activity in cells with a mutant, constitutively active form of Raf but unexpectedly enhanced Raf activity in cells with wild-type Raf. "It got us thinking that basically the Raf drug could bind to monomers of Raf, and then promotes Raf dimerization, which was where the kinase was more active," Shokat recalls. The team used Shokat's chemical–genetic tricks to test this idea. They made an enzymatically "dead" version of Raf kinase and added the inhibitor, expecting that inhibiting a dead enzyme would have no effect, but if Raf's conformation was important, then adding the inhibitor should still activate Raf. It worked. "It was fun to see [our technique] uncover completely new mechanisms of kinase regulation," Shokat says.

Shokat also adapted his chemical–genetic tool to help identify which kinases were targeted by small-molecule inhibitors. "I came up with the idea of basically taking 'perfect' inhibitors, like our chemical genetic ones, and figuring out the pattern produced by each of those at a genome-wide level, and then comparing the subsets of those to an unknown inhibitor," Shokat says. "We originally thought that if a kinase inhibitor targets two kinases, then the pattern of genes it changes will just be the sum of inhibiting kinase A perfectly or kinase B perfectly." However, what he found was that the pattern was actually A plus B, plus a subset that neither A or B inhibited (16). "That gave me the eye-opening realization that inhibitors that inhibit multiple targets can achieve things that

single-target inhibitors can't achieve on their own."

This discovery led to Shokat's recent interest in polypharmacology—an emerging paradigm in drug discovery that embraces promiscuous compounds that inhibit more than one target because such compounds may be more potent therapeutically. In his quest to design selective molecules to inhibit only certain members in the family of lipid kinases known as PI3-Ks, Shokat uncovered a dual inhibitor that blocks one PI3-K family member as well as the kinase mTOR—both of which are promising targets for the treatment of inflammation and cancer (17). Furthermore, this molecule inhibited the growth of glioma cell lines more potently than an inhibitor of PI3-Ks that does not target mTOR (18). More recently, Shokat was part of a team that designed a series of molecules capable of simultaneously inhibiting two different classes of kinases that are frequently dysregulated in human cancers (19). Through this work the team inadvertently discovered a potent inhibitor of mTOR, which is now in clinical trials to treat cancers.

After nearly 2 decades of research at the interface of chemistry and biology, Shokat continues to be captivated by his research. "I'm just blown away by the simple observation that small organic molecules can rewire entire signaling networks, causing cells to die, allowing others to live," he says. "It's something that as I get older, I get even more amazed by. It's just great to see a compound progress up the path towards impacting someone's tumor."

Nicholette Zeliadt, *Science Writer*

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