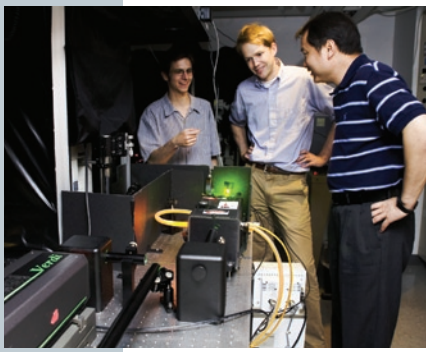
THE AUTHOR FILE

# Xiaoliang Sunney Xie

Single-molecule studies lead to high-throughput sequencing.

In science, logical steps can lead to surprising places. When outlining his research interests, Xiaoliang Sunney Xie splits his laboratory pursuits in three: dynamics of gene expression in living cells, single-molecule enzymology and a label-free microscopy technique that can be used to find biomolecules by detecting vibrations in chemical bonds. Now, a combination of serendipity and hard work has opened up a new direction: high-throughput sequencing.



Udo Loster, Leibinger Foundation

Peter Sims, William Greenleaf and Xiaoliang Sunney Xie (left to right).

Established pyrosequencing techniques work by copying DNA and generating light via a luciferase-based process as new nucleotides are added. The light produced, however, is transient, requiring detection systems that can capture fleeting signals at each DNA strand being interrogated. Fluorescence-based methods, in contrast, produce a stable signal but require many chemical manipulation steps. In this issue of *Nature Methods*, Xie and colleagues describe a sequencing technique that couples the simple workflow of pyrosequencing with fluorescence-based detection. This could allow highly scalable sequencing with rapid turnaround times.

The roots of single-molecule fluorescence enzymology date back more than a decade, to when Xie was a research scientist at Pacific Northwest National Laboratory, working out ways to measure the activity of a single enzyme molecule. For this, he and his colleagues used an enzyme containing a flavin cofactor, which is naturally fluorescent. Shortly afterward, companies such as Helicos and Pacific Biosciences began describing their plans to sequence single molecules of DNA. Xie was interested in applying single-molecule enzymology to DNA sequencing, but that idea lay latent as Xie took on a faculty position at Harvard University. "I had to learn to be a professor," he says.

Xie would occasionally try to interest a graduate student or a postdoc in the idea of fluorogenic sequencing. However, the young scientists usually demurred. "It's easier for me to suggest because I have multiple projects; if one doesn't work, some will work," explains Xie. "For students, it's more high-stakes. Not everyone would want to take the challenge." But Peter Sims, a fourth year graduate student, did, even though he could have graduated with his work on live-cell studies of single molecular motors.

Sims was intrigued by the potential impact of higher-throughput sequencing, but he had not been trained in the chemistry required to make the modified nucleotides that would yield fluorophores, following an approach that had been initially described by GE Healthcare. "He just dove into this and started to learn," says Xie. Xie and Sims worked out a timeline for when Sims would give up this project and fall back to write up his previous work for his Ph.D. thesis.

Just as important as generating the fluorophores was a technique to capture them. William Greenleaf, a postdoc, joined forces with Sims to tackle this problem. "Microreactors, combined with the fluorogenic chemistry, are the essence of the new sequencing technique," says Xie. Greenleaf crafted these resealable vessels out of polydimethylsiloxane, or PDMS. Without this material, says Xie, researchers in his lab would not have made the attempt. "I want to credit George Whitesides [also at Harvard University] who has popularized PDMS," he says. "It makes all sorts of labs on a chip, and it's so useful."

But progress did not come quickly. When produced, the fluorophores would diffuse into the PDMS or would generate an unreliable signal. Another group member, Haifeng Duan, joined the team to make new fluorogenic molecules. However, the deadline Sims and Xie had set was looming, and the project was not working.

Sims and Greenleaf worked out another strategy, but this required sequencing multiple copies of DNA rather than single-molecule sequencing. Xie was traveling in Scotland at the time and recalls a late-night conversation about whether Sims should fall back to a nonsequencing plan for his thesis or continue. "It was a big cell phone bill," Xie says. "I said, 'Peter, please, let's try this very quickly, and if you can do it, your thesis will generate a great deal of interest.'"

They had the data in a few weeks, and Sims described the sequencing technique in his defense, a fact that makes Xie philosophical: "You fight against the wall, and then you change directions a little bit, and that makes all the difference." Sims has another motivation. "I did this because I wanted to graduate," he jokes with Xie.

And although the sequencing technique itself relies on DNA amplification, Xie is hopeful it will provide a pathway to routine genome sequencing of single cells. "Although our technique is not single-molecule detection as we originally expected," Xie says, "it nevertheless allows sequencing a single DNA molecule in an individual cell."

**Monya Baker**

Sims, P.A., Greenleaf, W.J., Duan, H. & Xie, X.S. Fluorogenic DNA sequencing in PDMS microreactors. *Nat. Methods* **8**, 575–580 (2011).