STRUCTURAL BIOLOGY

Making protein crystals fly

A new device for injecting membrane-protein microcrystals into the path of an X-ray free-electron laser beam improves the efficiency of serial femtosecond crystallography.

The structural biology community is abuzz with excitement over X-ray free-electron lasers (XFELs). This technology has been available for only a few years, but rapid methodological developments are already having a strong impact on the protein crystallography field. These lasers are a billion times brighter than the best synchrotron radiation sources and produce ultrashort, ~50-femtosecond X-ray pulses, ideal for collecting high-quality single diffraction patterns from single, micrometer-sized protein crystals before radiation damage destroys the crystal. Single-crystal diffraction snapshots are then computationally merged to solve a protein structure. This powerful approach is known as serial femtosecond crystallography (SFX).

Most SFX studies to date have utilized a liquid injector to stream a steady supply of millions of microcrystals into the path of the XFEL beam. "The problem with these injectors is that the flow is too fast for the repetition rate of the [XFEL], which is 120 hertz," explains Vadim Cherezov of The Scripps Research Institute. "Only one crystal out of tens of thousands of crystals gets hit by the beam, and all others are wasted between the pulses. It's not possible to slow it down because the flow becomes unstable."

Recent work from a large international team of researchers to develop a new injector may greatly improve the efficiency of SFX. Cherezov's group has been using an established method of crystallizing G protein–coupled receptors (GPCRs) in lipidic cubic phase (LCP) medium. This approach often produces high-quality but small crystals, perfectly sized for SFX. The problem is that LCP is a gel and therefore incompatible with a liquid-based injector. The solution, developed by Uwe Weierstall and coworkers at Arizona State University, is a new device called an LCP microextrusion injector. With this tool, a gel-like crystal stream can be injected into the XFEL beam at lower speeds than the liquid injector allows, thereby minimizing waste

SENSORS AND PROBES

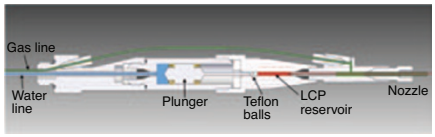
FISHING FOR FASTER FINDINGS

Quick-hybridizing probes help scientists image the high-speed events leading up to gene transcription.

The elegant plots and heat maps commonly used to illustrate gene expression patterns in research articles mask the considerable variability that can exist between cells in an otherwise identical population. This 'noise', which arises through differences in the timing of various steps leading from initiation to completion of transcription, can have a meaningful effect on cell behavior but is all but impossible to quantify with standard analytical techniques.

Researchers in Robert Tjian's lab at the Janelia Farm Research Campus have now devised a valuable imaging tool that enables real-time analysis of the activity of individual RNA polymerase molecules at subsecond timescales. Zhengjian Zhang and Andrey Revyakin had initially set out to monitor transcription with labeled oligonucleotide probes that hybridize with target sequences on nascent RNA chains, but they continually met with failure. "We just couldn't detect any signal," says Revyakin. "We got to thinking about why, and the idea [that] came to mind was that RNA secondary structure was probably the culprit." Indeed, many RNAs self-hybridize at physiological temperatures, potentially slowing down the rate of probe hybridization to a degree that it becomes nearly impossible to catch high-speed events.

The researchers used a computational strategy to identify sequences for which such interactions are unlikely to occur and found that the most promising probe sequences were those that entirely lack guanine—most likely because this nucleotide is capable of forming both strong base pairs with cytosine and weaker pairings with uracil. This probe design approach did not meaningfully reduce the sequence complexity that can be achieved, and Zhang and Revyakin found that they could readily design target-specific 19-mer oligonucleotides featuring only adenine, cytosine and uracil. And as predicted, the resulting absence of secondary structure led to greatly improved



An LCP injector enables serial femtosecond crystallography of membrane proteins crystallized in gel-like LCP medium. Figure from Weierstall *et al.*, Nature Publishing Group.

of precious sample (Weierstall *et al.*, 2014).

Using the LCP-based approach, Cherezov's team recently solved two GPCR structures at high resolution: the human serotonin 5-HT_{2B} receptor, bound to its agonist ergotamine at 2.8-angstrom resolution (Liu *et al.*, 2013), and the human smoothened receptor, bound to its antagonist cyclopamine at 3.2-angstrom resolution (Weierstall *et al.*, 2014). Their success is particularly notable given that GPCRs are notoriously difficult either to produce in quantity for SFX using liquid injectors or to generate as large crystals for traditional synchrotron diffraction.

Unlike synchrotron diffraction, which requires cooling crystals to cryogenic temperatures to minimize radiation damage, with SFX, diffraction snapshots can be taken at room temperature before radiation damage has a chance to set in. "Cryo-cooling introduces sample artifacts into the structure," explains Cherezov. "The room-temperature data give us a much better idea of the structure in the native environment." Because the data processing steps for traditional crystallography and SFX approaches are completely different, his team was heartened to see that a traditional cryo-cooled structure and a room-temperature structure for the serotonin receptor were largely similar, though loop regions in the room-temperature structure showed greater dynamic flexibility, as one might expect.

For crystallographers wanting to try their own hand at LCP crystallization, the Arizona State engineering team is happy to provide construction and use details.

Allison Doerr

RESEARCH PAPERS

Liu, W. *et al.* Serial femtosecond crystallography of G protein–coupled receptors. *Science* **342**, 1521–1524 (2013).

Weierstall, U. *et al.* Lipidic cubic phase injector facilitates membrane protein serial femtosecond crystallography. *Nat. Commun.* **5**, 3309 (2014).

performance, with hybridization rates 100-fold faster than those typically observed for standard RNA probes.

As an initial demonstration, the researchers used their fast fluorescence *in situ* hybridization (fastFISH) method to characterize a relatively simple model system: the bacteriophage T7 promoter. By measuring the binding of fluorescently labeled T7 RNA polymerase to an immobilized target DNA and the subsequent hybridization of a fastFISH probe to the nascent RNA, they could reproducibly quantify the timing of the various transcriptional stages with resolution at the scale of hundreds of milliseconds. "One of the surprises was that this worked so well," says Zhang. "We achieved an ultimate efficiency of detection of about 80%, which was limited primarily by issues in the chemical labeling of the probe." Interestingly, the rates determined for events such as polymerase binding to the promoter and subsequent initiation of transcription (or 'promoter escape') were consistently faster than those obtained in previous single-molecule studies. As fastFISH is performed at physiological temperatures with minimal experimental interference, Zhang believes these measurements may better reflect the reality within the cell. "Other experiments have involved stretching or other manipulations of the promoter that are likely to affect some of the kinetics," he says.

The next step will be to apply this method to deconvolute the timing of transcriptional stages by human RNA polymerase, a far more complex system that depends on the participation of dozens of additional proteins. "The jury is still out as to which step of initiation is the rate-limiting one," says Revyakin. "We want to ask how much time each factor takes to bind to the promoter and how much time passes between the binding of the polymerase and promoter escape." However, fastFISH has also drawn interest from researchers in other fields, and the authors note that the rapid kinetics of fastFISH could also potentially facilitate similar investigations of splicing or translation, or even lead to faster folding of nanostructures based on RNA or DNA 'origami'.

Michael Eisenstein

RESEARCH PAPERS

Zhang, Z. *et al.* Single-molecule tracking of the transcription cycle by sub-second RNA detection. *eLife* **3**, e01775 (2014).