STRUCTURAL BIOLOGY

XFELs probe protein dynamics

A new study shows that time-resolved serial femtosecond crystallography can reveal high-resolution intermediates of photoactive yellow protein.

Unlike conventional X-ray crystallography, time-resolved X-ray crystallography (TRX) can image proteins in action. TRX is generally done using the Laue method, in which a protein crystal is illuminated to induce a chemical reaction. Structural changes are then probed using short X-ray pulses at several time delays. The Laue method has proven powerful, and it has been used to study light-sensitive proteins such as the photoactive yellow protein (PYP) in great detail. However, the Laue method, as presently practiced with synchrotron radiation, has limitations. For example, it can be used only with large crystals, which can hinder uniform reaction initiation. In addition, the time resolution is limited by the length of the X-ray pulse used to probe the structure, which is around 100 ps.

Marius Schmidt at the University of Wisconsin–Milwaukee and a team of collaborators wanted to see whether they could bypass these limitations to ultimately achieve TRX with higher time resolution. The majority of the scientists involved are members of a US National Science Foundation Science and Technology Center called BioXFEL. According to the BioXFEL website, one of the major missions of this center is "to watch biomolecular machines at work, using X-ray lasers to better understand how life works at the molecular levels." With this goal in mind, Schmidt and his colleagues set out to test whether X-ray free-electron lasers (XFELs) could be used to carry out time-resolved serial femtosecond crystallography (TR-SFX).

XFELs are suitable for observing ultrafast events because they emit femtosecond pulses of X-rays whose peak brilliance is dramatically higher than that of X-ray beams available at synchrotrons. XFELs are used in serial femtosecond crystallography, during which thousands of microcrystals are delivered by a liquid jet injector and then illuminated with a single X-ray pulse. A diffraction pattern is then collected and analyzed for each illuminated crystal, yielding structural information at near-atomic-level resolution.

Schmidt and his colleagues used PYP as a model protein for TR-SFX and compared their results to data obtained using the Laue method. According to Schmidt, they were "on a quest to open the fast picosecond and femtosecond time range for biomolecules and observe what is going on there." He added that for PYP, "there is evidence for a sort of a fast shock wave that travels through the molecules once they absorb a photon. We want to explore whether we can quantify this in terms of structural change." To begin the experiment, they initiated the PYP chromophore reaction with a nanosecond blue laser pulse. They then probed the illuminated crystals by serial femtosecond crystallography after a time delay. By using two different time delays—10 ns and 1 μs—after reaction initiation and then analyzing data from over 100,000 microcrystals, the team was able to capture different populations of intermediates in the PYP photocycle. They found that their 1-μs-delay TR-SFX data set was more easily interpretable than the corresponding 1-μs-delay Laue-method data and that it allowed clear visualization of structural intermediates.

In addition to increasing the time resolution of TRX, TR-SFX has other major advantages that should greatly facilitate studying protein dynamics. According to Schmidt, "the biggest advantage is probably that reversible (cyclic) and nonreversible (noncyclic) reactions are on the same footing with TR-SFX because the crystals are discarded after only one X-ray shot." In addition, he says, TR-SFX "opens the door to structure-based enzymology" because TR-SFX uses microcrystals that have rapid diffusion times, allowing enzymatic reactions to be triggered quickly by mixing substrate and crystals.

Schmidt plans to focus future studies on increasing the time resolution of TR-SFX and on studying other proteins that are not intrinsically photoactivatable, either using photocaged compounds or by treating crystals with substrate immediately before analysis.

Rita Strack

RESEARCH PAPERS

Tenboer, J. *et al*. Time-resolved serial crystallography captures high-resolution intermediates of photoactive yellow protein. *Science* **346**, 1242–1246 (2014).

© 2015 Nature America, Inc. All rights reserved.
npg