Protein Cell 2010, 1(11): 965–966
DOI 10.1007/s13238-010-0135-6

Protein & Cell

NEWS AND VIEWS

Solving X-ray protein structures without a crystal: using X-ray Free Electron Laser, the fourth generation synchrotron light sources

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A synchrotron light source is a source of electromagnetic radiation artificially produced by specialized electron accelerators. Compared to the commonly used in-house X-ray sources, it is wavelength adjustable, much stronger and more focused. In the last two decades, synchrotron usage has become the mainstream for X-ray protein structure determination. Taking the advantage of micro-focus light beams of the third generation synchrotron, the size of a usable protein crystal for data collection decreases to micron level, which increases the rate of macromolecular structure determination to about 10 new protein data bank entries per day. The fourth generation synchrotron light sources not only inherit the above advantages of traditional synchrotron but also develop them to a higher level: you do not even need a protein crystal for atomic resolution X-ray structure determination by using the nano-focus beam line at the fourth generation synchrotron! This is obviously exciting news for those structural biologists working on membrane proteins and large macromolecular complexes which are notorious for crystallization.

X-ray Free Electron Laser (XFEL), provided by the fourth generation synchrotron light sources, has femtosecond pulse length and both peak and average brightness largely exceeding the beam of 3rd generation sources. XFEL is a laser that shares the same optical properties as conventional lasers such as emitting a beam consisting of coherent electromagnetic radiation which can reach high power, but it uses a relativistic electron beam as the lasing medium, which moves freely through a magnetic structure. In other words, it is coherent in phase and has very high brightness. The world's first XFEL, called Linac Coherent Light Source (LCLS), was completed at Stanford's Linear Accelerator Center in 2009 (Hand, 2009). Theoretically, a beam emitted by this machine will have "a brightness that is 10 billion times greater than that of any existing X-ray source on earth," reported by the press office at the Argonne National Laboratory.

At an XFEL, a nano-focus beam line is available. Together with the incredible intensity, a crystal with 1 μm^3 might be large enough to yield the necessary scattering data. Of course the radiation damage will cause serious problems here. So people need to change their strategy for collecting a complete data set from rotating one crystal during exposure while collecting and merging the images from maybe hundreds of different crystals. It is unlikely that a nano-crystal could survive from one shot of XFEL. The very short pulse of XFEL also provides a way out of the damage problem. Compared to the picosecond pulse of a conventional synchrotron, XFEL could provide femtosecond pulse length. A computer simulation showed that one can rapidly image a sample with such a short pulse before the atoms in the molecule may be destroyed (Neutze et al., 2004).

The most revolutionary impact of an XFEL on structural biology is the collection of X-ray scattering data without crystals. The basic idea of processing X-ray scattering data of single molecule is developed from electron microscopy (EM) studies (Huldt et al., 2003). In EM research, the images are sorted and averaged by the cross correlation between them. Then the mutual orientation of the averaged images can be determined based upon the idea that any two projections through a three-dimensional object have at least one same line. It is very similar in X-ray studies that any two scattering images share at least one curve in common where the Ewald spheres intersect in reciprocal space. By sorting and averaging the X-ray scattering images collected from

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molecules with random orientation, it will be possible to reconstruct the X-ray scattering intensity throughout the Ewald sphere, which means the reciprocal space could be reconstructed continuously. In contrast with sampling the reciprocal space discretely by collecting data from a crystal lattice, a continuous reciprocal space will provide enough data for directly phasing (Neutze et al., 2004). In general, the whole process includes three steps: (1) Classification of the similar patterns; (2) Orientation of the class averaged patterns; and (3) Reconstruction of the structure with a variant of dual-space iteration method. A demonstration of classification of a simulated data set with 10^6 patterns from a 528 kDa protein has been reported (Bortel et al., 2009). In this circumstance, the resolution that the final model could reach would be limited by how well those images were sorted and averaged. Actually, the related theory is not recent, but it had been trapped in books until the XFEL showed up with its huge X-ray flux.

What more we can expect from XFEL is the improvement in time resolved structure studies. The atoms in macromolecular system vibrate in a period of 100–1000 fs typically (Neutze et al., 2004). Due to this, it is impossible to study basic photo-chemical phenomena with the traditional synchrotron beam line with picosecond pulse. XFEL is just like a camera with extraordinarily fast shutter speed (femtosecond), by which every single movement of a molecule could be recorded clearly in principle. In addition, because samples with submicron scale are large enough for XFEL, the difficulties of time resolved structure studies with crystalline proteins will no longer exist. For example, it would be unnecessary to diffuse the substrates to a protein crystal and be afraid of that the conformational changes of the active site will destroy crystal packing. In addition to the current XFEL at LCLS, the European X-Ray Free-Electron Laser (XFEL) in Germany is scheduled to be completed in 2014, and Japan hopes to have a free-electron laser built in 2010 next to its SPring-8 synchrotron in Harima (Hand, 2009). Of course, nothing is certain except that the 2010s will see a world of “dynamic” structural biology brought by XFEL.

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