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Extended Abstract

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Time-resolved diffraction experiments at an X-ray free electron laser reveals structural changes in bacteriorhodopsin

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X-ray free-electron lasers (XFEL) supply a billion-fold jump in the peak X-ray brilliance when in contrast with synchrotron radiation. One place the place XFEL radiation has an impact is time-resolved structural studies of protein conformational changes. This presentation will describe how we used time-resolved serial femtosecond crystallography at an XFEL to probe light-driven structural modifications in bacteriorhodopsin. Bacteriorhodopsin is a light-driven proton pump that has long been used as a model system in biophysics. The mechanism by which light-driven isomerization of a retinal chromophore is coupled to the transport of protons up-hill against a transmembrane proton concentration gradient involves protein structural changes. Collaborative research performed at SACLA (the Japanese XFEL) has probed structural modifications in microcrystals on a time-scale from nanoseconds to milliseconds. Structural effects from this research enabled a whole image of structural changes happening during proton pumping through bacteriorhodopsin to be recovered. Structural statistics of the unique conformational states of the two prototypical light-sensitive membrane proteins, bacteriorhodopsin, and rhodopsin, have been acquired in the past by X-ray cryo-crystallography and cryo-electron microscopy. However, these methods do not allow for the shape dedication of most intermediate conformations. Recently, the plausible of X-Ray Free Electron Lasers (X-FELs) for tracking the dynamics of light-triggered processes through pump-probe serial femtosecond crystallography has been verified using 3D-micron-sized crystals. In addition, X-FELs supply new possibilities for protein 2D-crystal diffraction, which would allow to examine the route of conformational changes of membrane proteins in a close-to-physiological lipid bilayer environment. Here, we describe the strategies in the direction of structural dynamic research of retinal proteins at room temperature, the use of injector or fixed-target based serial femtosecond crystallography at X-FELs. Thanks to current development specifically in sample delivery methods, serial crystallography is now also feasible at synchrotron X-ray sources, accordingly expanding the possibilities for time-resolved structure determination. The function of a protein is determined by its shape which defines unique properties in an organic context and its dynamic interactions with diverse companions such as ions, lipids, hormones, different proteins, and nucleic acids. Detailed structural information on protein complexes has had a gorgeous influence on our appreciation of organic systems and is integral for rational drug discovery, rationally designed protein engineering, and biocatalysis. However, static structural statistics also has its obstacles on account that the biological function of most proteins is based on conformational adjustments in response to stimuli or protein-protein interactions. Dynamic records of protein motions protecting their conformational landscape together with structural statistics would, therefore, lead to a more unique perception of their organic function and appreciably enhance our capability for rational protein engineering. About 90% of all structures in the Protein Data Bank have been bought via X-ray crystallography. This approach depends on crystals to make bigger the structural records and reach enough sign to noise ratios. Unfortunately, this method delivers fine outcomes with large, well-ordered crystals that are often hard to obtain, in particular for membrane proteins. Radiation harm poses every other crucial barrier that limits the viable facts fine and may additionally alter the shape of proteins in maybe misleading ways. X-ray free-electron lasers (X-FELs) overcome obstacles of classical crystallography by outrunning radiation damage with distinctly wonderful femtosecond X-ray pulses briefer than the timescale of most crystal harm processes. The excessive brilliance of the X-ray pulses additionally allows for collecting data from crystals smaller than few micrometers, which is not possible at synchrotron sources. As character crystals are destroyed immediately after data collection, a stream of new crystals is injected into the X-ray course for each X-FEL pulse to ensure continuous data collection.

Bottom Note: *This work is partly presented at 10th Edition of International Conference on Structural Biology* March 15-16 2018 Barcelona, Spain