Photosynthesis seen in a new light by rapid X-ray pulses

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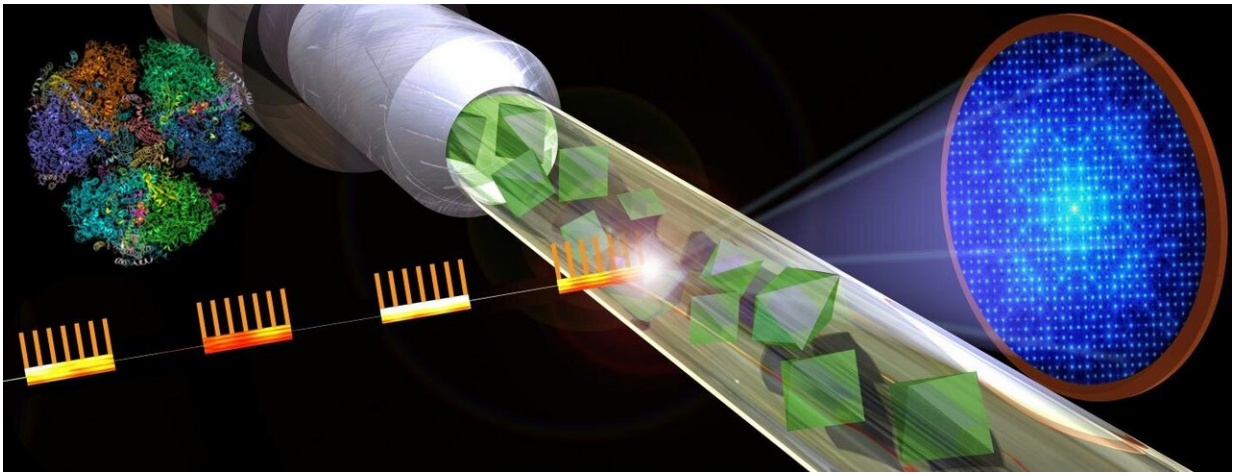

Graphic shows the basic design of an X-ray free electron laser or XFEL, in which brilliant X-ray bursts strike crystallized samples, causing diffraction patterns that can be reassembled into detailed images.X-rays damage biomolecules, a problem that has plagued structure determination efforts for decades. But the X-ray bursts produced by an XFEL are so short--lasting mere femtoseconds--that X-ray scattering from a molecule can be recorded before destruction takes place, akin to using a fast camera shutter. (A femtosecond is a millionth of a billionth of a second, the same ratio as a second is to 32 million years.) Credit: Shireen Dooling for the Biodesign Institute at ASU

The ability to transform sunlight into energy is one of Nature's more remarkable feats. Scientists understand the basic process of photosynthesis, but many crucial details remain elusive, occurring at

dimensions and fleeting time scales long deemed too minuscule to probe.

Now, that is changing.

In a new study, led by Petra Fromme and Nadia Zatsepin at the Biodesign Center for Applied Structural Discovery, the School of Molecular Sciences and the Department of Physics at ASU, researchers investigated the structure of Photosystem I (PSI) with ultrashort X-ray pulses at the European X-ray Free Electron Laser (EuXFEL), located in Hamburg, Germany.

PSI is a large biomolecular system that acts as a solar energy converter transforming solar energy into chemical energy. Photosynthesis provides energy for all complex life on Earth and supplies the oxygen we breathe. Advances in unraveling the secrets of photosynthesis promise to improve agriculture and aid in the development of next-generation solar energy storage systems that combine the efficiency of Nature with the stability of human engineered systems.

"This work is so important, as it shows the first proof of concept of megahertz serial crystallography with one of the largest and most complex membrane proteins in photosynthesis: Photosystem I" says Fromme. "The work paves the way towards time-resolved studies at the EuXFEL to determine molecular movies of the light-driven path of the electrons in photosynthesis or visualize how cancer drugs attack malfunctioning proteins."

The EuXFEL, which recently began operation, is the first to employ a superconducting linear accelerator that yields exciting new capabilities including very fast megahertz repetition rates of its X-ray pulses—over 9000 times faster than any other XFEL—with pulses separated by less than 1 millionth of a second. With these incredibly brief bursts of X-ray light, researchers will be able to much more quickly record molecular

movies of fundamental biological processes and will likely impact diverse fields including medicine and pharmacology, chemistry, physics, materials science, energy research, environmental studies, electronics, nanotechnology, and photonics. Petra Fromme and Nadia Zatsepin are co-corresponding authors of the paper, published in the current issue of the journal *Nature Communications*.

Strength in numbers

Fromme is the director of the Biodesign Center for Applied Structural Discovery (CASD) and leads the experimental team efforts of the project, while Zatsepin led the XFEL data analysis team.

"This is a significant milestone in the development of serial femtosecond crystallography, building on the well-coordinated effort of a large, cross-disciplinary, international team and years of developments in disparate fields" emphasizes Zatsepin, former Research Assistant Professor in the ASU Department of Physics and Biodesign CASD, and now Senior Research Fellow at La Trobe University in Australia.

Christopher Gisriel, the paper's co-first author, worked on the project while a Postdoctoral Researcher in the Fromme laboratory and is excited about the project. "Fast data collection in serial femtosecond crystallography experiments makes this revolutionary technique more accessible to those interested in the structure-function relationship for enzymes. This is exemplified by our new publication in *Nature Communications* showing that even the most difficult and complex protein structures can be solved by serial femtosecond crystallography while collecting data at megahertz repetition rate."

"It is very exciting to see the hard work from the many folks that drove this project to materialize," says Jesse Coe, co-first author who graduated last year with a Ph.D. in Biochemistry from ASU. "This is a

huge step in the right direction toward better understanding Nature's process of electron transfer that has been refined over billions of years. "

Petra Fromme is the director of the Biodesign Center for Applied Structural Discovery (CASD) and leads the experimental team efforts of the project. Credit: Biodesign Institute at ASU

Extreme science

An XFEL (for X-ray free-electron laser) delivers X-ray light that is a billion times brighter than conventional X-ray-sources. The brilliant, laser-like X-ray pulses are produced by electrons accelerated to near light speed and fed through the gap between series of alternating magnets, a device known as an undulator. The undulator forces the electrons to jiggle and bunch up into discrete packages. Each of the perfectly synchronized wiggling electron bunches emits a powerful, brief X-ray pulse along the electron flight path.

In serial femtosecond crystallography, a jet of protein crystals is injected into the path of the pulsed XFEL beam at room temperature, yielding structural information in the form of diffraction patterns. From these patterns, scientists can determine atomic scale images of proteins in close-to-native conditions, paving the way toward accurate molecular movies of molecules at work.

X-rays damage biomolecules, a problem that has plagued structure determination efforts for decades, requiring the biomolecules to be frozen to limit the damage. But the X-ray bursts produced by an XFEL are so short—lasting mere femtoseconds—that X-ray scattering from a molecule can be recorded before destruction takes place, akin to using a fast camera shutter. As a point of reference a femtosecond is a millionth of a billionth of a second, the same ratio as a second is to 32 million years.

Due to the sophistication, size and cost of XFEL facilities, only five are currently available for such experiments worldwide—a severe bottleneck for researchers since each XFEL can typically only host one experiment at a time. Most XFELs generate X-ray pulses between 30 and 120 times per second and it can take several hours to days to collect the data required to determine a single structure, let alone a series of frames in a molecular movie. The EuXFEL is the first to employ a superconducting linear accelerator in its design, enabling the fastest succession of X-ray

pulses of any XFEL, which can significantly reduce the time it takes to determine each structure or frame of the movie.

High risk, high reward

Because the sample is obliterated by the intense X-ray pulses, it must be replenished in time for the next X-ray pulse, which required PSI crystals to be delivered 9000 times faster at the EuXFEL than at earlier XFELs—at a jet speed of about 50 meters per second (160 feet per second), like a microfluidic fire hose. This was challenging as it requires large amounts of the precious protein contained within uniform crystals to reach these high jet speeds and avoid blocking the sample delivery system. Large membrane proteins are so difficult to isolate, crystallize and deliver to the beam, that it wasn't known if this important class of proteins could be studied at the EuXFEL.

The team developed new methods that allowed PSI, which is large complex consisting of 36 proteins and 381 cofactors, that include the 288 chlorophylls (the green pigments that absorb the light) and has over 150,000 atoms and is over 20 times larger than previous proteins studied at the EuXFEL, to have its structure determined at room temperature to a remarkable 2.9 angstrom resolution—a significant milestone.

Billions of microcrystals of the PSI membrane protein, derived from cyanobacteria, had to be grown for the new study. Rapid crystal growth from nanocrystal seeds was required to guarantee the essential uniformity of crystal size and shape. PSI is a membrane protein, which is a class of proteins of high importance that have been notoriously tricky to characterize. Their elaborate structures are embedded in the cell membrane's lipid bilayer. Typically, they must be carefully isolated in fully active form from their native environment and transformed into a crystalline state, where the molecules pack into crystals but maintain all their native function.

In the case of PSI, this is achieved by extracting it with very mild detergents that replace the membrane and surround the protein like a pool inner tube, which mimics the native membrane environment and keeps PSI fully functional once it's packed within the crystals. So when researchers shine light on the green pigments (chlorophylls) that catch the light by the antenna system of PSI, the energy is used it to shoot an electron across the membrane.

Nadia Zatsepin, former Research Assistant Professor in the ASU Department of Physics and Biodesign CASD, is now Senior Research Fellow at La Trobe University in Australia. Credit: Biodesign Institute at ASU

To keep PSI fully functional, the crystals are only weakly packed containing 78% water, which makes them soft like a piece of butter in the sun and makes it difficult handling these fragile crystals . "To isolate, characterize and crystallize one gram of PSI, or one billion billion PSI molecules, for the experiments in their fully active form was a huge effort of the students and researchers in my team" says Fromme." In the future, with even higher repetition rates and novel sample delivery systems the sample consumption will be dramatically reduced."

The recording and analysis of the diffraction data was another challenge. A unique X-ray detector was developed by the EuXFEL and DESY to handle the demands of structural biology studies at the EuXFEL: the adaptive-gain integrating pixel detector, or AGIPD. Each of AGIPD's 1 million pixels are less than a hundredth of an inch across and contain 352 analog memory cells, which enable the AGIPD to collect data at megahertz rates over a large dynamic range. However, to collect accurate crystallographic data from microcrystals of large membrane proteins required a compromise between spatial resolution and sampling of the data.

"Pushing for higher resolution data collection with the current detector size could preclude useful processing of the crystallographic data because the diffraction spots are insufficiently resolved by the X-ray detector pixels" warns Zatsepin, "yet in terms of data rates and dynamic range, what the AGIPD is capable of is incredible."

The novel data reduction and crystallographic analysis software designed specifically to deal with the challenges unique to the massive datasets in XFEL crystallography, whose development was led by collaborators at CFEL, DESY, and ASU, have come a long way since the first high-resolution XFEL experiment in 2011.

"Our software and DESY's high-performance computing capabilities are really being put to the test with the unprecedented data volumes generated at the EuXFEL. It is always exciting to push the limits of state-of-the-art technology," adds Zatsepin.

Membrane proteins: floppy, yet formidable

Membrane proteins like PSI—named because they are embedded into cell membranes—are vital to all life processes including respiration, nerve function, nutrition uptake, and cell-cell signaling. As they are at the surface of each cell they are also the most important pharmaceutical drug targets. More than 60% of all current drugs are targeted to membrane proteins. The design of more effective drugs with fewer side effects is therefore contingent on understanding how particular drugs bind with their target proteins and their highly detailed structural conformations and dynamic activities.

Despite their enormous importance in biology, membrane protein structures make up less than 1% of all protein structures solved to date because they are notoriously tricky to isolate, characterize and crystallize. This is why major advances in crystallographic methods, such as the advent of membrane protein megahertz serial femtosecond crystallography, are undoubtedly going to have a significant impact on the scientific community.

It takes a village

These recent achievements would not be possible without the tireless effort from a dedicated team of nearly 80 researchers from 15 institutions, including ASU, the European XFEL, DESY, the Center for Ultrafast X-ray Science, Hauptman-Woodward Institute, SUNY Buffalo, SLAC, University of Hamburg, University of Goettingen, Hungarian Academy of Sciences, University of Tennessee, Lawrence Livermore National Laboratory, University of Southampton, Hamburg University of Technology, University of Wisconsin. The research group included US collaborators in the NSF BioXFEL Science and Technology Center and a group of international collaborators, including Adrian P. Mancuso and Romain Letrun, lead scientists at the EuXFEL beamline and Oleksandr Yefanov and Anton Barty from CFEL/DESY who worked closely with the ASU team on the complex data analysis.

More information: Chris Gisriel et al, Membrane protein megahertz crystallography at the European XFEL, *Nature Communications* (2019). DOI: 10.1038/s41467-019-12955-3

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