

# Structural biology: doors open at the European XFEL

Vivien Marx

X-ray beams at 27,000 pulses per second promise high-resolution views of macromolecules.

As of September 1, 2017, scientists can come to the European X-ray free-electron laser (EuXFEL) for structural biology pursuits. They can collect diffraction data on protein nanocrystals and particles such as viruses, protein complexes and single molecules. They might create dynamic virus ‘movies’ from a series of individual snapshots. After 15 years of development, construction and testing, EuXFEL has ‘lased’: it generated a beam brighter than those produced by all other existing X-ray sources (see **Box 1**, “EuXFEL at a glance”). In more testing, a beam was successfully sent to a ‘hutch’, one of several lead-and-steel-encased rooms at the facility. Some hutches hold gratings, filters and other beam-tuning equipment; others have instruments for measuring samples. From nearby control rooms researchers interact with instruments in the hutches.

The EuXFEL’s big selling point is its high repetition rate, says Henry Chapman, a researcher at the Center for Free-Electron Laser Science in Hamburg, Germany, which is jointly run by the Max Planck Society, the University of Hamburg and DESY, the German Electron Synchrotron. With 27,000 X-ray laser pulses per second, the EuXFEL’s

pulse rate will make it possible to complete an experiment more quickly and with less sample than previously possible. “It could also help for single-particle diffractive imaging, which has been hampered by the fact that the really good shots where the virus or whatever is hit fair

and square in the beam are quite rare,” says Chapman.

Around the world, a number of XFELs are operational or almost there (**Box 2**, “Some X-ray free-electron lasers”), and more are being built, for example, in China. Accelerator-driven FELs and the new EuXFEL offer scientists exposures on a femtosecond ( $10^{-15}$  seconds) or even attosecond ( $10^{-18}$  seconds) timescale so they can measure the structure, variability and dynamics of the experimental objects of their choice<sup>1–5</sup>.

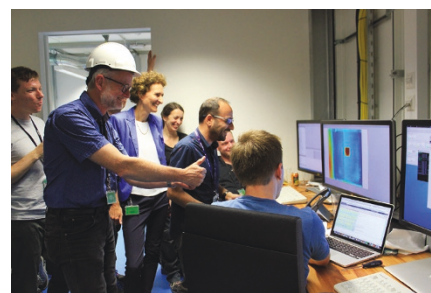
Strictly speaking, biological objects are never identical to one another, says Janos Hajdu, a biophysicist now at Uppsala University who spent much of his career at the University of Oxford. Macromolecular structures adopt various conformations. “Since biology wiggles, it’s all about motion,” he says. This plasticity enables these molecules’ biological functions. Some movements happen in milliseconds, some atomic vibrations last only 10 femtoseconds.

Experiments at the EuXFEL offer the opportunity to capture movements down to the timescale of atomic vibrations. “We are living our dream,” says Hajdu. He is part of a team of early EuXFEL users who will use beamtime for nanocrystallography and studies of single particles such as viruses. One day, he says, single-cell studies might be possible.

Hajdu has long actively made the scientific case for XFELs, including both the Linac Coherent Light Source (LCLS) at Stanford University—the world’s first XFEL, which began lasing in 2009—and the EuXFEL. He and many other researchers can now devise experiments and strategize about looming challenges.

## XFEL possibilities

Using single-particle LCLS–XFEL data, Abbas Ourmazd, a physicist and algorithm



Jessica Mancuso

Earlier this year, the EuXFEL’s first laser beam reached the ‘hutch’.

developer at the University of Wisconsin in Milwaukee, and his colleagues made movies of a virus (published in this issue<sup>6</sup>). Such movies can reveal biologically important information, says Ourmazd. In the future, the EuXFEL could help researchers map energy landscapes of biological molecules, identify functional routes along those landscapes and make movies of those routes, all of which could, he says, “provide a multi-lane highway between experiment and molecular dynamics.” With EuXFEL data it’s possible to get a detailed view of ligand-binding, which underlies all signaling and regulatory processes.

Chapman sees a way to capture play-by-play events, such as those during an enzymatic reaction, on a femtosecond timescale by applying XFEL-based time-resolved crystallography with hundreds, even thousands, of microcrystals. Usually such experiments involve light-triggered reactions, but there are other fast impulses to try, he says. He and colleagues do fast sample mixing: a solution with crystals meets a solution with a ligand or other reactant as the sample courses through microfluidic channels on its way to an injection device. “Fast in that context might be as quick as 0.1 milliseconds,” he says. Separately, he notes, research groups



S. Bejt

The EuXFEL is the place for single-molecule diffraction experiments, says Henry Chapman.

**BOX 1: EUXFEL AT A GLANCE**

The facility is 3.4 kilometers long and generates high-luminosity X-rays at a repetition rate of 27,000 pulses per second. Pulses have been produced at 0.15-nanometer wavelength—atomic dimensions—and the EuXFEL's design could get it to 0.05 nanometers.

Information for users is [here](#). The EuXFEL is set up as a nonprofit company and has involved collaborating researchers and funding agencies from 11 countries: Denmark, France, Germany, Hungary, Italy, Poland, Russia, Slovakia, Spain, Sweden and Switzerland. The UK was part of the project and then dropped out owing to budget constraints, but it is considering rejoining. The EuXFEL has received UK funding—the UK is, for example, part of the SFX user consortium, as are Sweden, Germany and Slovakia.

The facility's accelerator whips electrons to nearly the speed of light. Then, under the sway of magnets with alternating north and south poles, the electrons undulate and emit X-ray flashes. The X-rays and electrons travel together, which pushes the electrons into bunches, with each bunch behaving like a single large charged particle. A micro-bunch of 100,000 electrons can emit ten billion more X-rays than a single electron.

Early users will begin collecting data during their approved beamtime this fall at the research campus in Schenefeld, Germany, near Hamburg. The beam can be tuned as



EuXFEL, H. Müller-Elsner

Electrons reach nearly the speed of light in the superconducting accelerator modules, shown in yellow.

experimenters need and then sent to such experiment instrument 'hutches' as the Femtosecond X-ray Experiments instrument (FXE) or the Single Particles, Cluster and Biomolecules and Serial Femtosecond Crystallography instrument (SPB/SFX).

Sources: Henry Chapman, DESY; Janos Hajdu, Uppsala University.

use pulse electric fields to find flexible parts of a protein.

The EuXFEL is the place for single-molecule diffraction experiments, says Chapman. "And we have a crazy idea on how to use the fluorescence light from the sample to get structure," he says. Hundreds more X-ray photons are absorbed by atoms in the sample than actually scatter to form a diffraction pattern. These photo-excited atoms will fluoresce as X-rays with different wavelengths, depending on the element in question. He and his colleagues want to measure this fluorescence by using pulses to obtain a diffraction pattern.

Chapman and others want to automate sample delivery to the XFEL so that in less than 10 seconds researchers could obtain a single structure or a snapshot of a structure in a series. "So we could have huge throughput," he says. This throughput could support fragment screening in drug development, improve the research-oriented structural biology pipeline and address crystallization and screening bottlenecks. Fragment screening at the XFEL could "vastly increase" the speed of tasks done at synchrotrons, allowing bigger libraries of fragments and reducing the amount of protein needed.

Researchers can begin to transfer techniques from conventional crystallography

to XFELs. Traditionally, they use optical microscopy to find workable crystals. Now that researchers experiment with "invisibly small crystals," it's faster to deliver a sample into a beam to see whether it diffracts, says Chapman. In less than an hour, thousands of crystallization conditions can be tested. Large combinatorial studies can reveal how the structure changes at different temperatures or pH levels.

**Combining techniques**

To date, research at LCLS has pioneered single-molecule imaging and serial crystallography studies, says Charlotte Uetrecht, a scientist at the Heinrich Pette Institute, which is linked to the Leibniz Institute for Experimental Virology in Hamburg. The EuXFEL will be more intense in that it lets researchers look at smaller systems and, she says, drive methods for exploring single protein complexes. XFEL-based

work has been limited to virus particles that are relatively large, she says.

The high pulse rate enables high-throughput explorations of different states in the molecules' energy landscape; researchers can monitor protein complexes in action in previously impossible ways. It's a highly time-resolved view also of energetically disfavored transition states that are of great interest in drug discovery and that help scientists explore function.

Utrecht leads a project called VISAVIX, which combines mass spectrometry and XFEL-based work. The approach lets her deepen research into something mass spec has revealed: no protein complex, as she says, "comes in a single flavor." Each one has a variety of modifications or flexible regions, which can lead to broad conformational ensembles and different binding equilibria. With VISAVIX, structural analysis is

**BOX 2: SOME X-RAY FREE-ELECTRON LASERS**

[EuXFEL](#)

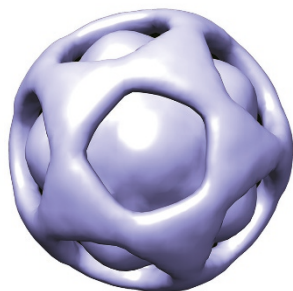
[FERMI facility](#)

[Linac Coherent Light Source at Stanford \(LCLS\)](#)

[Pohang Accelerator Laboratory \(PAL\) X-ray Free-Electron Laser](#)

[SPring-8 Angstrom Compact Free Electron Laser \(SACLA\)](#)

[Swiss Free-Electron Laser \(SwissFEL\)](#)



The X-ray diffraction volume of a virus.

A. Hosseiniadeh et al., Univ. of Wisconsin

accelerated because data images can be captured for just selected samples.

Both mass spec and XFEL work under vacuum conditions, which simplifies the coupling of these two approaches. Last year Utrecht and her team ran a test at FLASH, a smaller version of the EuXFEL. “We learned a lot about the system,” she says, most importantly that high-intensity radiation did not damage the mass spec’s electronics. The group is analyzing their fragment data now, and she hopes to run her first experiments at the EuXFEL in 2018 or 2019.

For XFEL-based studies of bovine virus BEV2, biophysicist Alke Meents developed a ‘fixed-target approach’ to ease sample loading and reduce the number of crystals needed<sup>7</sup>. It uses beamtime more effectively, he says, and can attract more users to serial femtosecond crystallography (SFX), a technique in which many microcrystals are delivered into the beam to generate a set of diffraction data. Meents joined Chapman’s group last year. He needed crystals to get a detectable signal above noise in the BEV2 experiment, but XFELs will make it feasible to ‘image’ non-crystallized particles.

Meents is a collaborator on a few EuXFEL proposals—for example, he is involved with the SFX user consortium at the Single Particles, Cluster and Biomolecules instrument—but he has not submitted his own proposal. He and his colleagues are currently advancing their fixed-target sample-delivery method in which thousands of crystals are loaded onto a chip and raster-scanned. He is exploring how to get the beam to scan ‘over’ the sample, akin to techniques in laser-based micromachining.

The team is taking the idea to nearby PETRA III, a synchrotron where more beamtime is available and it’s possible to make the major instrument modifications this experiment requires. The scientists have experimented at LCLS with large chips

that hold more than 50,000 crystals. If they can scan the chip at 1 kHz, data for a solved structure could be collected in less than a minute, says Meents. That 1-kHz data acquisition rate is far from the EuXFEL’s 5-MHz repetition rate with pulses delivered in bunches.

These ‘bunch trains’ are challenging for users, especially for fixed-target experiments. For those experiments, Meents would need to move the sample 10  $\mu\text{m}$  during the 200-nanosecond pause between the pulses in order to shoot the next sample position. One way to address this is by scanning the beam over the sample. To do so, one could set up a reflective X-ray mirror a few meters upstream of the sample. Between pulses, the lever arm would move the mirror according to calculated angular values. It’s challenging and still in development, he says, but it’s more realistic than trying to scan the sample one hundred times faster.

With optical imaging, most labs take multimodal approaches, but with XFELs researchers must get as much information as possible from a single shot exposure. They want all the photons: both the elastically and the inelastically scattered and fluorescence photons. Detectors need energy resolution that lets experimenters distinguish between photons from different interaction processes. In this vein, Meents and his colleagues are currently exploring how to use Compton scattering to image larger biological samples using synchrotron radiation. Combining all of these efforts along with new data analysis methods, he says, should help them catch up to the electron microscopy community.

With XFELs, scientists can potentially approach the scale reached with electron microscopy for single-particle imaging, says Meents, but X-rays damage the sample with radiation. Researchers in electron microscopy obtain around 1,000 times more information out of a sample than researchers working with X-rays before the sample sustains too much damage.

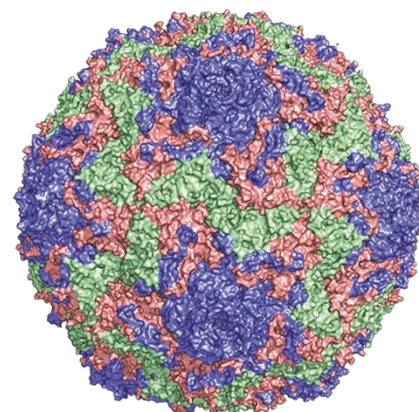
When using XFELs, experimenters can ‘outrun’ radiation damage because they collect data right after X-ray diffraction but before the sample is destroyed. “Despite all the software methods, which need to be further developed for this, I see a great need and potential in having the optimal instrumentation for such kind of experiments,” says Meents. The brightest X-ray beams can now be nanofocused, but experimenters often rely on sample holders and instrumentation designed 20 years ago, he says.

Another issue is background scattering, which should be lower than it is in most XFEL-based experiments, says Meents. Just reducing scatter will deliver a big boost in resolution.

### Handling pulse trains

A day of beamtime at the EuXFEL is worth 225 days at LCLS, says Chapman, but it’s a day with some challenges. The Adaptive Gain Pixel Detectors must keep up with the data deluge delivered by the X-ray ‘trains’, which are 2,700-pulse bunches at 4.7 MHz that repeat ten times a second with 220 ns between pulses. The detector can store 3,500 frames per second, which is enormous capacity, he says, but it’s only around 10% of a full beam. Even though experimenters can’t leverage all generated data, the pulse enables many types of experiments. A researcher could set up a reaction and probe it at this rate and capture many biological processes. For higher resolution, experimenters can interleave data from different trains and initiate a reaction at slightly different times.

The EuXFEL beam’s high pulse rate is beneficial in terms of quick turnaround and sample consumption, but, says Hajdu, one needs to leave time between shots for the “smoke to clear.” Debris from the first shot may still be in the interaction region that researchers are studying. “Ideally one would need evenly distributed sets of pulses,” he says. The pulse pattern has some history: it was meant to enable certain types of physics experiments and then became “ingrained” in the project, and it’s not ideal for structural studies. “We have to live with it for the moment,” he says. Changing it will cost money. Some remedies experimenters can try include beam-tuning to drop pulses



Structure of a bovine virus determined with a ‘fixed-target’ approach.

A. Meents, CFEL



from the pulse train or spacing pulses farther from one another.

Overall, the EuXFEL holds tremendous potential for structural biology, says Uetrecht. “However, it is not a true single-molecule technique yet,” she says. A myriad of images of a single species must still be combined to obtain a structure. “It would be awesome to record 3D structures at high resolution in a single shot, thus really for each particle,” she says. Holographic approaches show great potential, especially for large objects such as cells.

### Applied research

Besides the EuXFEL, Europe is home to SwissFEL, part of the Paul Scherrer Institute (PSI) in Villigen, Switzerland. Pilot experiments start later this year and it will open to the research community in 2018. In contrast to the EuXFEL’s 27,000 pulses per second, SwissFEL delivers 100 pulses per second, says Michael Hennig, CEO of leadXpro, a PSI spin-out. He spent two decades at Roche directing structure-based research. This pulse rate difference involves different experiments and measurement strategies.

SwissFEL is set up for academic and commercial research, says Hennig. His company focuses on structural biology and biophysics methods for drug discovery, most notably for membrane protein targets such as ion channels, transporters and G-protein-coupled receptors. Working with solubilized membrane proteins, he and his team use cryo-EM or X-ray crystallography to study binding properties of potential drug molecules and to design future drugs. Hennig wants to find lead molecules with “novel chemotypes” with pronounced specificity and efficacy. With structures in hand, drug developers can take on challenging systems as they, for example, search for potential small molecules that target protein–protein interactions of transporters. He has performed experiments at XFELs such as LCLS in the United States and SACLA in Japan, and he and his team have applied for beamtime at the EuXFEL.

Interaction with beamline scientists is critical from sample prep to data analysis, he says. His team might, for example, request adjustments to the beamline settings. “One of the most thrilling areas of research with XFELs are investigations of protein dynamics,” says Hennig. Compared to what is observed with traditional cryo-crystallography, he and his team see evidence for different conformations and B-factors when structural analysis is performed at room temperature such as



Charlotte Uetrecht combines mass spectrometry and XFEL-based research.

with serial crystallography. “Together with investigation of time-resolved ligand-binding,” he says, “I am convinced this will have an impact on computational analysis and design of future drug molecules.”

### Data torrent

Experimenters at the EuXFEL will

harvest a torrent of data about their biological molecules. Data analysis tools and data portals are emerging<sup>8</sup> (see “XFEL projects, tools, data portals” on [Methagora](#)). With its repetition rate, the EuXFEL is at the extreme end of the big data challenges now common in many fields, says Uetrecht. “Automatic data-sorting to discard empty or background-only images will be essential,” she says. The detectors at the EuXFEL “feature a veto system to do exactly that.”

In VISAVIX experiments, there will be fewer big data challenges given that the researchers are likely to see many “empty shots,” says Uetrecht. Because of the low background provided by the mass spectrometer, it will be comparatively easy to distinguish empty frames and real data. What could be difficult are the potentially long data analysis times for single-particle imaging compared to the data-acquisition rate. “In the long run, I really hope that the colleagues at EuXFEL will provide an integrated data analysis pipeline,” she says. Given the amount of data generated in experiments, just the transfer of these data to home institutions will be time-consuming.

One measure of XFEL success, says Ourmazd, is the degree to which others, including pharmaceutical industry researchers, are convinced that the use of these machines and the existing and emerging data analysis algorithms is beneficial. He wishes data analysis tools were integrated into the planning for the facilities. “We have learned to build magnificent machines, but too often neglect to give them brains,” he says.

Especially given the complexity and size of the data sets from XFEL-based experiments, it’s critical to interact with facility staff, both before and during experiments, says Hennig. SFX data analysis is one key area where advances will make electron density maps

more telling about the binding of a drug candidate and a protein target.

### More XFELs

The EuXFEL is operational, the Pohang Accelerator Laboratory XFEL in South Korea has lased, and SwissFEL will soon be operational. Tabletop XFELs are under development. LCLS has built a new beamline called Macromolecular Femtosecond Crystallography, and LCLS-II, a facility upgrade, is in the works. As Chapman explains, LCLS-II involves the same accelerator technology used for the EuXFEL with its high repetition rates. “The competition between facilities is absolutely fantastic for users like us,” he says. There are not yet as many XFELs as there are synchrotron sources, but XFELs “have proven to be much more useful and versatile than was ever imagined.”

Structural biology was once a scientific frontier. That changed as the field grew after



The XFEL community shows a frontier spirit, says Janos Hajdu.

the first diffraction patterns were generated in 1934, says Hajdu. Unlike electron microscopy, which was developed during World War II, or protein crystallography, which dates back to the early 20th century, XFELs are a young technology: the first lasing at the world’s first XFEL

was in 2009. “Everything has to be developed from scratch,” he says. XFEL-based research is exciting, and one day soon it will have industrial applications, too. International collaboration in the XFEL community works, and he senses “a frontier spirit” that is sparking new science.

**Vivien Marx is technology editor for *Nature Methods* ([v.marx@us.nature.com](mailto:v.marx@us.nature.com)).**

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