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De novo phasing of protein crystallography data from a free-electron laser

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Free-electron lasers (FELs) are pushing back the limits of possibility in protein crystallography. Using the high-intensity, femtosecond duration pulses afforded by FELs allow data collection from micrometer-sized crystals while outrunning radiation damage. Moreover, FELs may be used for pump-probe experiments with unprecedented time resolution. However, the intricacies of FEL data collection pose specific challenges: as every FEL pulse destroys the sample, data are mostly collected from a stream of microcrystals and averaged to remove the variations in crystal size and quality as well as shot-to-shot variations in beam parameters. This technique is called serial femtosecond crystallography (SFX). In SFX, several tens of thousands of images typically need to be averaged to obtain reasonably accurate structure factor amplitudes. We previously showed that SFX yields structure factor amplitudes accurate enough to detect the weak anomalous signal of endogenous sulfur atoms. Now we show that SFX can be used to collect data accurate enough for de-novo phasing of a protein structure[1]. Using a model system (gadolinium-derivatized lysozyme) we collected ~60,000 diffraction images and obtained structure factor amplitudes that allowed phasing by single-wavelength anomalous diffraction. This first demonstration of de novo phasing from FEL data leads us to anticipate that FEL-based crystallography will become an important tool for the structure determination of proteins that are extremely radiation sensitive or that are difficult to crystallize, such as membrane proteins.

[1] *Barends et al., Nature 505:244-247 (2014)*

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