# Macromolecular Imaging with X-ray free-electron lasers

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Free-electron lasers produce X-ray pulses with a peak brightness a billion times that of beams at a modern synchrotron radiation facility. This has provided a disruptive new technology for imaging and structural biology. A single focused X-ray FEL pulse completely destroys a small protein crystal placed in the beam, but not before that pulse has passed through the sample and given rise to a diffraction pattern. This principle of diffraction before destruction has given the methodology of serial femtosecond crystallography for the determination of macromolecular structures from tiny crystals without the need for cryogenic cooling [1,2]. Consequently, it is possible to carry out high-resolution diffraction studies of dynamic protein systems with time resolutions ranging from below 1 ps to milliseconds, from samples under physiological temperatures and other conditions.

The ability to record diffraction of biological materials using extremely intense and spatially coherent X-ray pulses has also been of interest for imaging non-crystalline samples, such as virus particles and single molecules [3]. Such single-particle imaging is being developed but is challenging due to the very low signal levels (compared to background sources) of tiny non-crystalline particles. There is a very significant advantage of measuring continuous diffraction from non-crystalline objects since it contains vastly more information than is encoded by the Bragg peaks in diffraction patterns of crystals. The increase in information makes it possible to directly determine the diffraction phases, overcoming the well-known phase problem in crystallography [4].

A driving goal of our work is to reduce the size of crystals down to their ultimate limit, which is the single molecule or virus particle. Much progress has been made on reaching this challenging goal, and most of the steps (such as sample preparation, recording low-background diffraction, and merging of data) have been demonstrated. We are exploring ways to increase signal to noise by holographic approaches, such as by high-magnification projection imaging, using multilayer Laue lenses of high numerical aperture [5].

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[4] K. Ayyer *et al.*, *Nature*, **2016**, *530*, 202–206.
[5] S. Bajt *et al.*, Light Sci. Appl. **2018**, *7,* 17162.