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X-ray lasers for structural and dynamic biology

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Abstract
Research opportunities and techniques are reviewed for the application of hard x-ray pulsed free-electron lasers (XFEL) to structural biology. These include the imaging of protein nanocrystals, single particles such as viruses, pump–probe experiments for time-resolved nanocrystallography, and snapshot wide-angle x-ray scattering (WAXS) from molecules in solution. The use of femtosecond exposure times, rather than freezing of samples, as a means of minimizing radiation damage is shown to open up new opportunities for the molecular imaging of biochemical reactions at room temperature in solution. This is possible using a 'diffract-and-destroy' mode in which the incident pulse terminates before radiation damage begins. Methods for delivering hundreds of hydrated bioparticles per second (in random orientations) to a pulsed x-ray beam are described. New data analysis approaches are outlined for the correlated fluctuations in fast WAXS, for protein nanocrystals just a few molecules on a side, and for the continuous x-ray scattering from a single virus. Methods for determining the orientation of a molecule from its diffraction pattern are reviewed. Methods for the preparation of protein nanocrystals are also reviewed. New opportunities for solving the phase problem for XFEL data are outlined. A summary of the latest results is given, which now extend to atomic resolution for nanocrystals. Possibilities for time-resolved chemistry using fast WAXS (solution scattering) from mixtures is reviewed, toward the general goal of making molecular movies of biochemical processes.

(Some figures may appear in colour only in the online journal)

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Contents

1. Introduction

The recent invention and development of the hard x-ray free-electron laser (XFEL) has opened up new opportunities for structural biology. Before the turn of the century, it was believed that true single-molecule imaging using scattered radiation would never be possible, because the radiation dose needed to achieve sufficient high-angle elastic scattering would, as a result of inelastic processes, destroy the molecule (Breedlove and Trammel 1970). Theoretical work had suggested that short pulses might outrun radiation damage (Solem 1986), but no experimental results existed. If we consider a small x-ray beam which forms a near-delta function in time, into which we may pack as many photons as possible, it is clear that damage-free elastic scattering could be obtained regardless of dose, resolution and sample size, down to the single-molecule level.

As a result of recent experiments, we now know that if the dose is delivered quickly enough, it is indeed possible to outrun radiation damage (Chapman *et al* 2006a). In this way we can obtain sufficient image-forming elastic scattering before radiation damage dominates or even begins, thus allowing the possibility of molecular movies by a snapshot 'diffract-and-destroy' method (Neutze *et al* 2000). Since the damage, which occurs after termination of the incident pulse, may destroy the sample, this method requires a constantly refreshed supply of identical particles, such as molecules or perhaps viruses. So far atomic resolution by this method could only be attained by taking advantage of the coherent amplification of Bragg scattering from nanocrystals. But it is now clear that only the need for engineering advances in XFEL and sample injector technology (brightness, beam diameter, repetition rate, hit rate, water background) prevents single-molecule imaging, not the more fundamental problem of radiation damage. If molecular snapshots are recorded in many random orientations and the molecules assume a limited number of conformations, then the snapshots might be sorted according to their orientation and conformation (Frank 2006) and merged to form a three-dimensional molecular movie (Huldt *et al* 2003). This sorting process is only possible if the conformational and orientational changes can be distinguished, as demonstrated in simulations (Fung *et al* 2009).

Emma *et al* (2010) provides a report on the capabilities of the first hard-x-ray laser, the Linac Coherent Light Source (LCLS) at SLAC near Stanford, USA, while others are now under construction or commissioning around the world. These spatially coherent light sources operate in a pulsed mode which provides time-resolved 'snapshot' x-ray images of proteins, both in nanocrystalline and single-particle form, in their native environment and at room temperature (RT). In addition it is now clear that these sources can indeed generate femtosecond x-ray pulses brief enough to terminate before radiation damage (which ultimately destroys the sample) sets in. In this way we may break the nexus between damage, sample size, dose and resolution (Howells *et al* 2009), thus avoiding the need to freeze samples for damage protection. (The effects of electronic damage, which occurs during a pulse, are discussed later in this review.) When applied to protein nanocrystals, we will refer to this serial, destructive-readout, 'diffract-before-destroy' method as serial femtosecond nanocrystallography (SFX), to distinguish it from single-particle methods, where samples such as viruses are used.

Macromolecular crystallography (MX) at synchrotrons, the most successful technique for protein structure determination, provides charge-density maps of proteins limited in resolution by both crystal quality and radiation damage. The process of finding the correct conditions for growing the large, well-diffracting protein crystals required for MX can take years. MX samples are usually frozen to reduce radiation damage, and the crystallization process usually (but not always) allows only a single protein conformation to be studied. The results from other techniques, such as cryo-electron microscopy and atomic force microscopy, make it increasingly clear that this shortcoming of MX is limiting our view of protein interactions. Recent MX at RT has shown how flash cooling to reduce radiation damage can bias hidden structural ensembles in protein crystals and remodel the conformational distribution of 35% of side-chains, while eliminating the packing defects necessary for functional motions. Thus MX at RT can reveal motions crucial for catalysis, ligand binding and allosteric regulation (Fraser *et al* 2010). Despite valuable progress in time-resolved protein crystallography discussed in section 7, what is urgently needed is a time-resolved technique, which can image individual proteins at subnanometer resolution in three dimensions, in their native environment, unaffected by damage from the imaging radiation.

Both the serial crystallography SFX method recently demonstrated at the LCLS (Chapman *et al* 2011) and single-particle (virus) imaging experiments at this XFEL (Seibert *et al* 2011) address the limitations of MX in structural biology caused by crystal quality and radiation damage.

The idea that the early coherent elastic scattering might provide a high-resolution x-ray hologram of organic material, before it is destroyed, was first analyzed in detail by Solem (1986), who predicted that 10 nm resolution might be possible using 1 ps pulses, and who described a 'self-shuttering' mechanism. Doniach (1996) discussed time-resolved holographic crystallography using XFELs, while detailed simulations by Neutze *et al* (2000) provided estimates of resolution for various pulse durations and intensities by tracking the atomic motion following the photoelectron cascade, which vaporizes a sample. Experiments using the FLASH soft x-ray XFEL at the Deutsches Elektronen-Synchrotron (DESY) in 2006 (Chapman *et al* 2006a) demonstrated this 'diffract-before-destroy' principle. In these experiments radiation damage is reduced or eliminated by using an x-ray pulse so intense and brief that it terminates before damage processes affect the length scale of interest, yet contains sufficient photons to produce a useful diffraction pattern from the initial burst of elastically scattered photons (Barty *et al* 2011). Using hard x-rays at the LCLS, single x-ray pulses of 30–70 fs duration containing about 7×10^{11} photons of 9 keV have been found to produce diffraction patterns from micrometer-sized crystals of Lysozyme (Boutet *et al* 2012) at RT extending beyond 2 Å. The corresponding radiation dose in that case was 33 MGy/pulse, similar to the Henderson 'safe