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Review

Serial Crystallography: Preface

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Abstract: The history of serial crystallography (SC) has its origins in the earliest attempts to merge data from several crystals. This preface provides an overview of some recent work, with a survey of the rapid advances made over the past decade in both sample delivery and data analysis.

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The beginnings of Serial Crystallography (SC) are almost as old as crystallography itself. As soon as the early crystallographers in the nineteen twenties attempted to merge the data from more than one crystal, or from different areas of the same large crystal, many of the scaling issues now dealt with in modern SC arose. This merging was done in order to share the damaging radiation dose over a larger volume; generally, these issues were addressed by the standard statistical methods of maximum likelihood least-squared optimization. When using film recording with the oscillation method, the problem later arose of merging partial reflections (those not integrated fully in angle across the rocking curve) from one crystal with those from another [1]. This problem was addressed by defining a "degree of partiality" in a "postrefinement" approach. In addition, the time taken to set up particular Bragg orientations would often result in unacceptable radiation damage before data collection commenced. That problem was solved [2] by determining the crystal orientation, not from goniometer settings, but by computer analysis of "still" diffraction patterns, those not rocked continuously through the Bragg condition during exposure, as is customary in modern crystallography.

New issues of data collection arose with the appearance of the first X-ray Free-Electron Laser (XFEL) MX data sets around 2010, where all the "snapshot" diffraction patterns are stills. Here, the spatial coherence of the beam might exceed the size of the microcrystal, and tens of thousands of micron-sized protein crystal provided data, with 120 diffraction patterns being recorded every second. Some patterns showed clear "shape-transform" effects (interference fringes between Bragg spots due to the finite size of the crystal). It was also quickly understood that an XFEL provided a beam of very poor quality for MX compared to a synchrotron. The major issues were the shot-to-shot variation in beam intensity (about 15%); the large range of crystal sizes, often varying from sub-micron to a few microns; the noisy time-spectrum of each pulse (with perhaps 0.1% bandwidth); and the unknown crystal orientation for each shot. The natural way to deal with this was to adopt a Monte-Carlo integration approach [3] after indexing the Bragg reflections, summing the same reflections from different microcrystals, and thereby averaging over all these large statistical fluctuations from several independent sources. With enough diffraction patterns from different microcrystals in random orientations, sampling would occur at a sufficient number of points across the rocking curve to perform the required angular integration across it, for example. The error (R-split) then can be seen to fall as $k/\sqrt{N}$ for N patterns, with k a constant. We recall that the height of a Bragg peak is proportional to the square of the number of molecules in the crystal, while the angle-integrated intensity is proportional to the number of molecules, and inversely proportional to the size of the unit cell [4,5].

Since that time, many improvements over this global averaging approach have been published with the effect of reducing k through some form of iterative modelling of the rocking curve or spectrum. Many of these improvements are now incorporated as options in the popular "CrystFEL" software

suite [6]. Prior to indexing and merging, useful data must be isolated ("hit-finding") from the total data set, which will include blank shots where the beam misses the crystals, and background treated using programs such as "Cheetah" [7] and its developments. Merging depends on autoindexing methods, which may involve sparse data sets, either in the sense of showing too few Bragg spots to allow indexing by conventional MX software or by showing very weak reflections, or both [8,9]. There have recently been dramatically effective approaches to both of these problems. The use of the Expectation Maximization and Compression (EMC) algorithm uses a similar approach to that used for single-particle data merging: it does not assign crystallographic indices but treats the entire nanocrystal as one molecule. This also adds the parameter of crystal size to the three angular orientation unknown in single-particle analysis, resulting in a powerful approach for data from weaker storage-ring sources for the smallest crystals of small proteins (strongest Bragg spots) but with a large computational cost [10].

Closely linked to the data analysis problem has been the sample delivery problem, and it has been fascinating to see how the methods first developed for the XFEL from 2004 have now been taken over successfully at most synchrotrons. The experimental origins of fast SC can be traced to an early proposal to fire bioparticles in a continuous single-file stream across a beam for diffraction analysis using a Rayleigh jet [11]. The first tests of protein microcrystals in such a system were undertaken at the advanced light source [12]. A system was developed in preparation for the use of this Gas Dynamic Virtual Nozzle (GDVN) at the world's first XFEL, the Linac Coherent Light Source (LCLS) at SLAC, in the first SFX experiments [13,14]. That system was soon after adapted for an optical laser pump-probe mode suitable for studies of photosynthesis. Mixing jets were also developed soon after [15,16] to allow time-resolved X-ray snapshot to be recorded during a chemical reaction. It was understood that these studies are limited to molecules that remain chemically active in crystalline form. Since that time, a plethora of different sample delivery schemes have been developed for the three main modes of SC operation: static structure determination for protein microcrystals, such as the GPCR drug targets [17]; pump-probe studies on light-sensitive protein microcrystals [18]; and mixing jet experiments on microcrystals for chemical dynamics (such as time-resolved imaging of enzyme dynamics [19]. In addition, similar sample-delivery methods may also be required for closely-related serial data collection techniques such as single-particle analysis (in which there may be a single virus per shot), spectroscopy, and time-resolved solution scattering. In each case, one must consider the need for a hydrated sample environment, possibly in vacuum (or helium gas) together with possible laser pumping or solution mixing facilities, while delivering over a thousand samples per second across the beam. These are demanding constraints, with the ultimate goal of minimizing sample waste between shots and obtaining a high hit rate. A review and comparison of methods is given elsewhere [20], including scanned stages ("fixed target" systems), liquid jets, double-focusing jets, viscous jets, conveyor-belt systems, electrospray, acoustic levitation, mix-and-inject systems, and electrokinetic injectors. For some modes (such as pump-probe work on light-sensitive proteins), a completely reliable system that wastes little protein has yet to be developed, while SFX at the European XFEL (and at LCLS II) is complicated by a very high repetition rate of about 1 MHz (in separated pulse-trains at EuXFEL) [21]. For static structure determination, the simplest and most reliable system for protein microcrystals that can be grown in lipid cubic phase (LCP) appears to be the slow-moving LCP jet [22], which does not suffer from clogging and wastes little protein. Scanned stages, with their very high hit rate, can be traced through recent papers [23] and have involved a variety of robotic or flow-based loading systems [24]. A system for mixing droplets onto chip-mounted microcrystals can also be found [25], and a conveyor-belt (tape drive) system is described elsewhere [26], both of which provide high hit-rates. Work toward synchronized droplet jets (triggered by the XFEL photocathode signal) continues, including a system that inserts slugs of oil that travel across the beam between X-ray pulses to conserve protein [27].

As a result of all this, we have seen very rapid progress over the last ten years in the Serial Crystallography (SC) method, both at XFELs (for Serial Femtosecond Crystallography or SFX) (see [28] for a review) and at synchrotrons (for Serial Millisecond Crystallography, or SMX—see, for example [29,30]. For the XFEL, injection of thousands of protein microcrystals into the 10^{12} photons of femtosecond XFEL pulses has allowed for the structural determination of crystals grown in vivo, or of submicron size, and from challenging targets such as membrane proteins and supramolecular assemblies such as ribosomes [31]. For time-resolved XFEL studies, the small crystal size allows for rapid diffusive saturation in mix-and-inject analysis of biochemical reactions (with microsecond diffusion times, shorter than reaction times, for sufficiently small crystals), and full optical saturation of the microcrystal sample by a pump laser in studies of light-driven proteins. The ability to outrun most radiation damage processes avoids the need for sample cooling (which immobilizes proteins) and its artifacts, allowing for time-resolved studies of molecular machines at work in their correct room-temperature thermal bath under near-physiological conditions. For synchrotrons using the new fast detectors and diffraction-limited beamlines, serial millisecond or microsecond crystallography has also proved viable with all the advantages of SFX at XFELs, at lower time resolution, but without the ability to outrun secondary radiation damage due to the photoelectron cascade. In summary, the development of SC at both XFELs and synchrotrons has opened up many new opportunities in structural biology, especially in the exciting new field of atomic-resolution imaging of protein dynamics.

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