

Serial Crystallography of Proteins

U Weierstall¹, R B Doak¹, J C H Spence¹, D Starodub¹, D Shapiro², P Kennedy¹, J Warner¹, G G Hembree¹, P Fromme³ and H Chapman⁴

¹ Department of Physics, Arizona State University, Tempe, AZ 85287, USA

² Advanced Light Source, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720, USA

³ Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287, USA

⁴ University of California, Lawrence Livermore National Laboratory, 7000 East Ave., Livermore, CA 94550, USA

Electron or X-ray diffraction from a beam of laser aligned molecules has recently been proposed as an approach to structure determination of proteins, particularly those that are difficult to crystallize and are too large for structure determination by NMR. In our method, a beam of hydrated proteins (or possibly microscopic protein crystals) is injected into an interaction region at the intersection of two crossed beams: a diffracting probe beam (continuous electron or X-ray beam) and an alignment beam (continuous polarized IR laser). The interaction region will usually be in a high vacuum environment to allow for evaporative cooling of the droplet beam and to minimize scattering of the diffracting beam by residual gas molecules. In the interaction region the proteins are aligned via torque exerted on the molecule by the electric field \mathbf{E} of the laser beam, acting on an \mathbf{E} -field induced dipole moment of the molecule. If N identical aligned molecules are in the interaction region at any instant and if the coherence patch of the probe beam is not appreciably larger than the target molecule, then the probe beam produces a diffraction pattern having N times the intensity of that from a single molecule. Since the molecules are constantly replenished (transit time across the interaction region ~ 200 ns), a serial diffraction scheme of this sort allows much longer exposure times than with a static N -molecule crystal. Moreover each molecule receives a dose far below the critical dose that would cause damage at atomic resolution. A transmission diffraction pattern for one orientation builds up at the detector as scattered intensity from successive identical single proteins is recorded. After a useable diffraction pattern has been accumulated, the detector is read out, the alignment laser polarization rotated, and a new diffraction pattern is recorded for a different molecular orientation. In this way a 3-D diffraction dataset can be recorded. From this dataset the charge density of the molecule can be reconstructed with an iterative Gerchberg-Saxton-Fienup algorithm. Serial diffraction requires an injection method to deliver protein molecules or microcrystals - preferably uncharged, fully hydrated (in detergent micelles if membrane proteins), spatially oriented, and with high flux - into a focused probe beam of electrons or x-rays of typically only a few tens of microns diameter. We have examined several potential droplet sources, i.e. electrospray, Rayleigh droplet sources, nebulizers and airojet focusing sources, as to their suitability for this task. Experimental results indicate, that the airojet focused source is the most suitable, since clogging problems are avoided and submicron size droplets can be generated from much larger nozzles. Recently we theoretically evaluate the incident x-ray fluence required to obtain a given resolution from (1) the calculation of the count rate at the maximum scattering angle for a globular object, (2) the explicit simulation of diffraction pattern for a GroEL-GroES protein complex, and (3) the frequency cut off of the transfer function for a phase reconstruction algorithm in the projection approximation. These calculations show that at the available flux at the ALS, preliminary experiments with 2nm resolution should be possible. Supported by NSF award IDBR 0555845 and ARO award DAAD190010500.

Reference: <http://arxiv.org/abs/physics/0701129>