Serial Data Collection Approaches and Dose Slicing in Protein Crystallography

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Significant technical developments are revolutionising structural biology, including noise-free X-ray detection and pulsed X-ray free electron laser sources (XFEL). Serial crystallography is now becoming firmly established at high-brilliance synchrotron sources. Thousands of nanocrystals are needed, each contributing a single X-ray exposure, thereby changing the way in which a crystallographic experiment is approached. Experiments can be carried out at cryogenic temperatures, but they may also be performed at ambient temperatures, then giving access to more physiological environments and additionally making structural transitions directly observable. This will add dynamic information on structural changes or transitions, observed over time. Prominent targets are enzymes or higher order molecular complexes.

We studied the dodecameric Pdx1 enzyme, capable of forming PLP, an active form of vitamin B6, through a series of covalent intermediates [1]. Using online UV-Vis spectroscopy we follow not only formation of catalytic intermediates *in crystallo* but also detect specific radiation damage through spectral changes upon X-ray irradiation. Our analysis extended to buffer components contributing through solvent radiolysis.

Serial crystallography will allow studying systems like PLP synthase and probing the structural changes in the enzyme complex at different timepoints. Micro- or nanocrystalline samples are required to unlock this capability for which we developed a workflow optimising crystal growth for size and homogeneity [2]. Our approach to serial data collection uses a chip-based fixed target capable of dose slicing [3]. The nano-fabricated chip has 25,600 positions for nanocrystals, and data from 1-3 chips typically yield the 10,000 hits required for a high-quality dataset. The system is capable of delivering one structure per hour either at the Diamond Light Source (I24 microfocus beamline) or at an XFEL source (SACLA, Japan) where chip motion is synchronous to the XFEL repetition rate of 30 Hz.

There may be additional gains with using nanocrystalline samples, as shown from theoretical calculations by Nave and Hill [4]. Photoelectrons that are primarily cause of damage are predicted to escape the crystal volume which should increase crystal lifetimes, an effect that would be pronounced at higher photon energies [5, 6]. Our measurements of radiation damage at cryogenic temperatures on lysozyme crystals deliver experimental support for prolonged lifetimes using smaller crystals at higher energies.

1. Rodrigues MJ, Windeisen V, Zhang Y, Guédez G, Weber S, Strohmeier M, Hanes JW, Royant A, Evans G, Sinning I, Ealick SE, Begley TP, Tews I. *Lysine relay mechanism coordinates intermediate transfer in vitamin B6 biosynthesis.* Nat Chem Biol. 13 (2017), 290-294.
2. Beale JH, Bolton R, Marshall SA, Beale E, Carr SB, Ebrahim A, Moreno-Chicano T, Hough MA, Worrall JAR, Tews I, Owen RL. *Successful sample preparation for serial crystallography experiments.* J Appl Cryst. 52 (2019), 1385-1396.
3. Ebrahim A, Moreno-Chicano T, Appleby MV, Chaplin AK, Beale JH, Sherrell DA, Duyvesteyn HME, Owada S, Tono K, Sugimoto H, Strange RW, Worrall JAR, Axford D, Owen RL, Hough MA. *Dose-resolved serial synchrotron and XFEL structures of radiation-sensitive metalloproteins.* IUCrJ. 3 (2019), 543-551.
4. Nave C, Hill MA. *Will reduced radiation damage occur with very small crystals?* J Synchrotron Radiat. 12 (2005), 299-303.
5. Sanishvili R, Yoder DW, Pothineni SB, Rosenbaum G, Xu S, Vogt S, Stepanov S, Makarov OA, Corcoran S, Benn R, Nagarajan V, Smith JL, Fischetti RF. *Radiation damage in protein crystals is reduced with a micron-sized X-ray beam.* PNAS. 108 (2011), 6127-32Sanishvili et al. (2011), PNAS 108(15), 6127–6132.
6. *Ueno G, Shimada A, Yamashita E, Hasegawa K, Kumasaka T, Shinzawa-Itoh K, Yoshikawa S, Tsukihara T, Yamamoto M. Low-dose X-ray structure analysis of cytochrome c oxidase utilizing high-energy X-rays. J Synchrotron Radiat. 26 (2019), 912-921.*