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Applications of Room Temperature Serial Crystallography to Metalloproteins using Silicon Fixed Targets at Synchrotron and XFEL beamlines

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Metalloproteins, particularly those containing transition metals in high valent states are highly sensitive to redox state changes as a consequence of X-ray exposure during data collection. If not understood and interpreted correctly, this site-specific damage may lead to misinterpretation of structural data and mis-assignment of structures to e.g. enzyme mechanistic states. Increasing attention has been paid to room temperature crystallography to allow for protein dynamic freedom and in order to facilitate time-resolved crystallography. The binding of ligands to proteins may be affected both by temperature and redox state. The faster rate of radiation damage with dose in room temperature crystals poses challenges for data collection methodologies. I will describe room temperature fixed target 'chip' serial synchrotron crystallography (SSX) measurements at Diamond beamline I24 and serial femtosecond crystallography (SFX) experiments at the SACLA X-ray Free Electron Laser (XFEL), Japan. This system may be implemented with few changes at either synchrotron micro-focus or XFEL beamlines allowing us to directly compare the effects of radiation damage in SSX structures to XFEL structures collected using a near identical strategy i.e. where the nature of the X-ray beam is the primary experimental variable. I will describe applications of this approach to several metalloprotein systems [1-3].

References

- [1] Resolving polymorphs and radiation-driven effects in microcrystals using fixed-target serial synchrotron crystallography. Ebrahim, A. *et al.*, (2018) *Acta Cryst.* D75, 151-159.
- [2] Dose-resolved serial synchrotron and XFEL structures of radiation-sensitive metalloproteins. Ebrahim, A. *et al.*, (2018) *IUCr* 6, 543-551.
- [3] High-throughput structures of protein-ligand complexes at room temperature using serial femtosecond crystallography. Moreno-Chicano, T., *et al.* (2019) *IUCr* 6, 1074-1085.

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