MS08-1-8 Towards time-resolved structural studies of tryptophan 2,3-dioxygenase
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Abstract
Structural studies of heme proteins have historically been complicated by radiation damage manifesting around the heme active site. As a result, an outstanding challenge in the field is the determination of time-resolved structural intermediates of oxygen activation at room temperature and pressure. Tryptophan 2,3-dioxygenase (TDO) is a tetrameric heme protein containing one heme cofactor per monomer. The active site binds divalent oxygen, filling the vacant distal ligand of the heme iron, and catalyses the oxygenation of L-tryptophan via the activation of oxygen. The study of TDO has clinical relevance – the product of this reaction, n-formylkynurenine, is upregulated in certain cancer cells and contributes to the suppression of anti-tumour immunity [1]. Recently, using cryo-trapped X-ray and neutron methods, a consensus on the precise chemical nature of the heme protein Fe(IV)=O reactive intermediates have been developed [2]. However, these methods are technically difficult to achieve and interpret while avoiding photon-induced radiation damage to the vulnerable heme active site. X-ray free electron lasers (XFELs) provide a new method to probe reaction mechanisms through serial femtosecond crystallography: the resulting structures are pristine, and when combined with simultaneous X-ray emission spectroscopy, the redox state of the heme iron can be validated. Here we present rotation and serial crystal X-ray structures of TDO from Diamond Light Source and PAL-XFEL, and an optimisation protocol to produce microcrystalline slurries suitable for serial diffraction experiments. We also present spectra from cryo-cooled single crystal microspectrophotometry of TDO macrocrystals, this data represents progress towards understanding oxygen diffusion through TDO crystals relevant for time-resolved XFEL studies.

References
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[2] Moody PCE et al. Acc Chem Res. 2018;51(2):427-435