MS25-2-1 Charge refinement of metal ion cofactors in protein crystals using MicroED
#MS25-2-1

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Abstract
Microcrystal electron diffraction (MicroED) is an attractive alternative to X-ray diffraction for studying micro and sub-micron sized protein crystals. In contrast to X-ray diffraction, electron diffraction probes electrostatic potential distribution within the specimen and not electron density. Because of this, MicroED can be used to determine charge states in residues and metal cofactors within metalloenzymes. In the broader perspective, determining charge state of metal cofactors in different states of an enzymatic reaction could provide insight into the charge transfer at the enzyme active site. However, more insight is needed in how different charge states of a metal cofactor are reflected in the electron diffraction data and if this difference is within the accuracy of the experiment.
In this study, the R2 protein of class I ribonucleotide reductase (R2a) containing a diiron centre (FeIIIFeIII) has been used as a model protein to simulate electron diffraction data. Data was simulated by calculating the structure factors from an available R2a structure model. The simulated data was further used for structural refinement in SHELXL, using different oxidation states of the iron ions in the refinement model. The refinement outcome could successfully detect the correct charge of the iron in the metal centre. Furthermore, MicroED data has been collected on R2a crystals. Two different strategies, namely cryo-FIB milling and optimization of microcrystals, were explored for preparing crystals. The later method produced ideal plate-like crystals for MicroED data collection up to 2 Å resolution. Future work will focus on optimizing sample preparation for MicroED, collecting high quality data and refining the charge states of diiron centre against experimental data.