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Harder and faster: electrochemical solutions to problems in metalloprotein biophysics

Content

Redox properties of metal-containing active sites are critically important to many biocatalytic processes: one third of all proteins contain a redox-active metal, and ca 22% of submissions to the Protein Data Bank contain a transition metal. It is often difficult or impossible to prepare well-defined samples of all catalytically-relevant intermediates, in particular high-valent catalytic intermediates (often the most critical and elusive), in a suitable form for biophysical or structural studies. This is particularly problematic for short-lived intermediates that are only formed in response to metalloprotein function or redox catalysis. To understand fully why metalloproteins are so efficient we need to thoroughly characterise the kinetic, structural, and coordination changes associated with these intermediates. In turn, this requires methods for reliable preparation of specific oxidation states, and for triggering subsequent redox reactivity.

We have developed electrochemical strategies that remove chance from sample preparation by allowing redox state to be precisely controlled, and catalysis selectively turned 'on' or 'off'. These approaches ultimately enable in situ characterisation of metalloproteins using a range of biophysical methods. Here I describe applications of electrochemistry that enable photodamage-free X-ray absorption spectroscopy of metalloproteins at room temperature (including advanced methods such as HERFD-XAS), even on high flux beamlines, and provide a platform for triggered, time-resolved structural biology of redox catalysis by metalloenzymes on nanosecond-millisecond timescales.

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