Developing Electron Diffraction Methods to Probe Oxidation States in Metalloenzymes

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Understanding oxidation state (OS) variations in enzyme metal-ion cofactors is essential for elucidating enzymatic mechanisms. While traditional spectroscopic techniques can be used to determine oxidation states in materials, they lack spatial resolution. Electron diffraction techniques, such as three-dimensional electron diffraction (3D ED/MicroED) and serial electron diffraction (SerialED), offer unique opportunities for OS determination with structural resolution by probing the electrostatic potential. However, accurately inferring OS from electrostatic potential maps remains challenging due to limitations in data collection and processing protocols, as well as constraints in available atomic scattering models used for refinement.

To study the impact of different atomic scattering models, both independent atom model (IAM) and transferable aspherical atom model (TAAM) based on the MATTS databank[1–3], were evaluated for refinement of an iron complex against 3D ED data. The results demonstrated that IAM significantly overestimates the impact of different OS on the atomic scattering amplitude. In contrast, TAAM significantly improved refinement accuracy and reduced map noise, highlighting the importance of accurate atomic scattering models for interpreting the electrostatic potential map.[4]

Furthermore, using a new serial electron diffraction (SerialED) protocol, we determined the structures of two redox states of the iron-containing protein ribonucleotide reductase R2 subunit (R2a). Isomorphous difference maps computed between the experimental data from the two redox states revealed a signal at the iron sites, which could be attributed to OS changes. Model-derived structure factors supported this interpretation, indicating that OS differences contributed ~12-14% to isomorphous difference peaks, while the remainder resulted from atomic displacement between redox states. These findings suggest that differences in scattering amplitude due to oxidation state changes are already detectable within the current accuracy and precision of the data.

Model-derived structure factors were computed based on TAAM, since this model accurately models partial charges. To compute structure factors using TAAM scattering factors, we developed the Python-based wrapper **pyDiSCaMB**, which served as an interface between the DiSCaMB library[5] and cctbx, the underlying library of Phenix[6]. This development is a crucial step toward implementing TAAM scattering factors in **phenix.refine**, which should enhance phase accuracy and reduce map noise. All in all, this study lays the foundation for oxidation state determination of metal-ion co-factors in metalloenzymes from electron diffraction data.

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