

# Towards Understanding The Conformational Changes Behind Electron Bifurcation In Thermophilic Metalloenzymes Using Small-Angle X-Ray Scattering

Dan Murray<sup>1</sup>, Gerrit Schut<sup>2</sup>, Xiaoxuan Ge<sup>3</sup>, Michal Hammel<sup>4</sup>, Michael Adams<sup>5</sup>, Greg Hura<sup>6</sup>

<sup>1</sup>Lawrence Berkeley National Lab <sup>2</sup>Department of Biochemistry and Molecular Biology, University of Georgia, <sup>3</sup>Department of Biochemistry and Molecular Biology, University of Georgia, <sup>4</sup>LBNL, <sup>5</sup>Department of Biochemistry and Molecular Biology, University of Georgia, <sup>6</sup>Molecular Biophysics and Integrated Bioimaging Division (MBIB), Lawrence Berkeley National Laboratory  
murraydt@lbl.gov

Electron bifurcation (EB) is a recently discovered avenue of biological energy conservation that enables an unfavorable oxidation-reduction (redox) reaction by coupling it with a favorable one, so that the net reaction is favorable (Peters, et al, Curr Opin Chem Biol, 2016). The multi-subunit electron-transferring flavoprotein-menaquinone oxidoreductase ABCX (EtfABCX) is one enzyme that catalyzes EB through the exergonic reduction of menaquinone (high potential/favorable) coupled to the endergonic reduction of ferredoxin (low potential/unfavorable). Reduced ferredoxin drives many global microbial processes, including production of hydrogen gas (H<sub>2</sub>), methane (CH<sub>4</sub>), and nitrogen (N<sub>2</sub>) fixation. EtfABCX is a membrane-bound dimer (EtfABCX<sub>2</sub>) found in the thermophilic bacterium, *Thermotoga maritima* (Adams, FEMS Microbiol Rev, 1994). For EB to occur, the two-electron donor, enzyme cofactors, and electron acceptors must be orchestrated for efficient conformational gating so that the created higher potential electron does not lose its energy to the lower potential electron. The static cryo-EM structure of EtfABCX (Feng, et al, PNAS, 2021) greatly enhances our knowledge, but further investigation is needed to fully understand the EB mechanism as an uncharacterized conformational change is expected to decouple the electrons in the bifurcation from one another, the existence of which will likely occur in solution as observed with small-angle X-ray scattering (SAXS).

A truncation of EtfABCX, EtfAB, has been studied by both High-Throughput-SAXS (HT-SAXS) and Size-Exclusion Chromatography and Multi-Angle Light Scattering-coupled SAXS (SEC-MALS-SAXS) on the SIBYLS beamline at the Advanced Light Source. Data showed domain disassociation of the EtfAB subcomplex upon reduction by NADH, supporting the hypothesis that conformational changes are involved in EB. Results from EM and SEC-MALS-SAXS of EtfABCX indicate the enzyme forms a transient mixture of dimers and tetramers (EtfABCX<sub>2</sub>-EtfABCX<sub>2</sub>). The tetramer exists as an in vitro artifact resulting from hydrophobic patches integral to membrane insertion of the dimer (Feng, et al, PNAS, 2021). The data associated with the tetrameric fraction required software-assisted deconvolution of the SEC-MALS-SAXS intensity peaks (Meisburger, et al, JACS, 2016), but the resulting analysis proved inconclusive. To obtain the dimer's SAXS curve, SEC-MALS-SAXS was measured of EtfABCX in the presence of nonionic detergents to eliminate the tetramer population. Now, EtfABCX's conformational changes through varying redox states are being characterized using anaerobic SAXS. The resulting data from these experiments will be analyzed to elucidate allosteric interactions of EtfABCX that enable EB and the subsequent generation of H<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub> fixation.

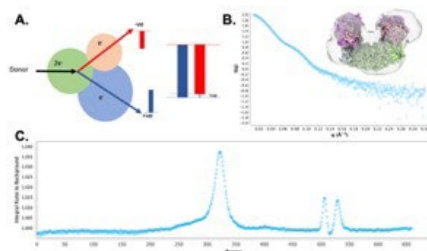


Figure 1.