Conformational equilibria underlying electron bifurcation in *Thermotoga maritima* Fix/EtfABCX

Daniel T. Murray[1], Xiaoxuan Ge[2], Gerrit J. Schut[2], Michal Hammel[1], Michael W. W. Adams[2], Greg L. Hura[1]

[1]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory

[2]Department of Biochemistry and Molecular Biology, University of Georgia, Athens

Electron bifurcation (EB) is an evolutionarily ancient means of energy conservation used by anaerobes and, despite progress from recent studies, its mechanism remains incompletely understood. Flavin-based EB (FBEB) is a flavoprotein-mediated process in which a high-potential electron is generated in a thermodynamically favorable fashion by simultaneously dropping the potential of a second electron before their respective donation to acceptors. The mechanism of FBEB is enigmatic as the transfer of energy from the electron that drops potential to the electron that gains potential must be carefully orchestrated to avoid equilibration and the associated conformational changes are unknown.

The cryo-EM structure of one such FBEB enzyme – the *Thermotoga maritima* (*T. maritima*) Fix/EtfABCX ("EtfABCX" hereafter) – suggests that, upon NADH reduction of its EB centers, a low-potential electron transfers to ferredoxin and high-potential electrons reduces a menaquinone (**Fig. 1**). EtfABCX is a membrane-associated dimer of ABCX heterotetramers – termed "superdimer" – with each heterotetramer – referred to as "supermonomers" – in possession of distinct EB centers and electron transfer (ET) pathways that converge at a shared, central active site cavity. The EB subcomplex of EtfABCX – EtfAB – houses the flavins behind EB and serves as a proxy for examining the superdimer.

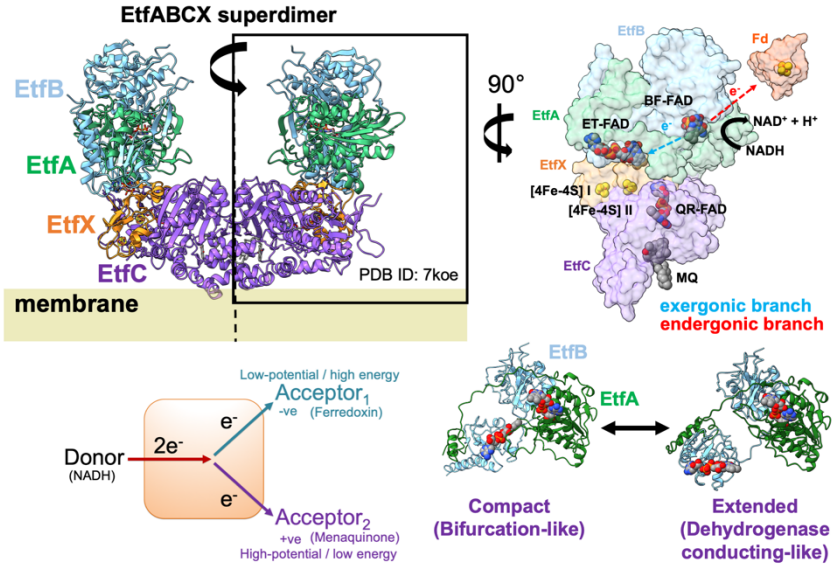

Fig. 1. *Top left*: Structure of EtfABCX. *Top right:* Supermonomer of EtfABCX with cofactors and EB scheme labeled. *Bottom left*: EB by EtfABCX. *Bottom right:* Conformational states of EtfAB.

Presented here, anaerobic size exclusion-coupled small-angle X-ray scattering (SEC-SAXS) analyses show that EtfABCX exists in a conformational equilibrium with respect to its EtfAB subcomplex and is characterized by compacted and extended states, the abundance of which are impacted by flavin reduction and coenzyme binding (**Fig. 1**). The conformations identified in EtfAB's conformational equilibrium provide snapshots of novel states for the *T. maritima* enzyme and recapitulate some states identified in homologous FBEB systems. Similarly, modeling of EtfABCX superdimer shows it exists in a combination of states with respect to its EB subcomplexes, suggesting a cooperative mechanism reliant on both supermonomers for optimal efficiency. Correlation of EtfABCX's conformational equilibrium with steps in its ET pathway suggests a means with which EtfABCX may progress through its catalytic cycle. Further, flavin reduction alone is insufficient to elicit domain movements, but requires a structural "trigger" induced by NAD binding. Collectively, these observations provide a dynamic structural framework for tracing EtfABCX's catalytic cycle and provide a foundation for future studies of oxygen-sensitive enzymes involved in EB using small-angle scattering.