Cryo-EM reveals a bi-copper cluster coordinating asymmetric electron transfer in the nitrogenase-like DPOR complex

Rajnandani Kashyap[1], Jaigeeth Deveryshetty[1], Natalie Walsh[1], Monika Tokmina-Lukaszewska[2], Brian Bothner[2], Brian Bennett[3], and Edwin Antony[1,*]

[1]*Department of Biochemistry and Molecular Biology, St. Louis University School of Medicine, St. Louis, MO 63104. Email: rajnandani.kashyap@health.slu.edu*

[2]*Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT*

[3]*Department of Physics, Marquette University, Milwaukee, WI*

Anoxygenic bacteria undergo bacteriochlorophyll biosynthesis in the absence of oxygen and utilize a unique series of enzymes to convert the tetrapyrrole substrate to the chlorophyll. One key enzyme in the biosynthesis pathway is the dark-operative protochlorophyllide oxidoreductase (DPOR). It catalyzes the stereo-specific reduction of the C17=C18 double bond of protochlorophyllide (Pchlide) to chlorophyllide (Chlide). DPOR is widely through to function as a structurally symmetric octameric complex that consists of an electron donor (BchL) and an electron acceptor/substrate binding (BchN-BchB) component protein. Both contain Fe-S clusters and form a transient complex in the presence of ATP. We recently showed asymmetry in electron transfer between the two identical halves of DPOR (Danyal et. al. PNAS 2016).

These findings raise several interesting questions about the structure-function relationships in such electron transfer enzymes that function as higher order complexes. This higher order complex functions with a cascade of events like ATP hydrolysis, protein-protein interactions, and catalysis.

DPOR also has an intricate structure assembly of two identical functional halves featuring homodimer of BchL and heterotetramer of BchN-BchB. Our overall objective is to understand how does this electron transfer protein complex relays information over long range distances and provide exquisite allosteric control over the other half of the complex.

Using Cryo-EM we capture snapshots of the DPOR enzyme during substrate recognition and turnover. The structures reveal that asymmetry is enforced upon substrate binding and leads to an allosteric inhibition of protein-protein interactions and electron transfer in one half. Residues that form a conduit for electron transfer are aligned in one half while misaligned in the other. An ATP-turnover coupled switch is triggered once electron transfer is accomplished in one half and relayed through a bi-copper cluster at the oligomeric interface, leading to activation of enzymatic events in the other. The findings provide a mechanistic blueprint for regulation of asymmetric long-range electron transfer.

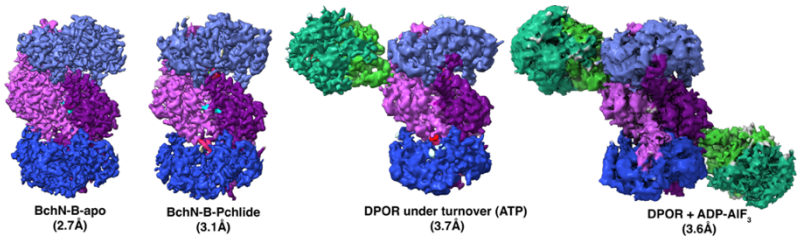

Figure. 1. Cryo-EM structures of DPOR protein component BchN-BchB, BchN-BchB with Pchlide, DPOR complex under turnover conditions (ATP) clearly depicting asymmetrical binding of the electron donor component protein BchL and the DPOR complex in the presence of ADP-AlF3 depicting symmetrical binding just like the X-ray structure (PDB: 2YNM).