EFRC ACCOMPLISHMENTS

2014 – 2020

BETCy EFRC

Led by John W. Peters at Washington State University

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SCIENTIFIC ACCOMPLISHMENTS

EFRC MISSION/GOALS: The mission of the BETCy EFRC is to understand how catalysts can more efficiently couple electrochemical potential energy to chemical bond formation at the molecular level. Specifically, the BETCy EFRC will provide the scientific energy community with new and fundamental insights into electron transfer mechanisms in order to provide the necessary guiding principles to design and synthesize next generation catalysts that will reversibly and efficiently store electrochemical energy in the form of chemical bonds. In accomplishing its mission, the BETCy EFRC will unmask, at the atomic level, the molecular mechanisms of complex enzymatic catalysts that already achieve this. We will attack the mechanistic basis for extremely difficult reduction reactions by combining our expertise in state-of-the-art chemistry and biochemistry, cutting-edge theory, and in computational modeling and simulation. Sophisticated and poorly understood mechanisms are at the heart of these biological systems that involve the recently recognized phenomenon of electron bifurcation, together with electron transfer kinetics and proton-coupled electron transfer reactions. Working in combination, these processes can enable the catalysis of challenging reactions at extremely low reduction potentials. The outcome of the collective progress on electron transfer catalysis has established basic principles of how enzymes conserve energy from 2-electron chemical reactions by using selective electron transfer, and how electron flow is chemically controlled to achieve challenging chemical reactions.

1. Mechanism of biological electron bifurcation.
We made groundbreaking discoveries on a bifurcating enzyme known as Nfn (NADH-dependent ferredoxin NADP oxidoreductase) that define the mechanistic principles for enabling flavin-based bifurcation. These discoveries include the detection by transient absorption spectroscopy of a very short-lived (ps) 1-electron intermediate, which had been predicted by our theoretical analyses but not directly observed previously. Our atomic resolution structure of Nfn has detailed the hydrogen bonding and solvation around the bifurcating flavin, implicating dielectric and/or proton-coupled electron transfer as a means of controlling energy levels of flavin oxidation states. We have discovered that Nfn has divergent electron transfer pathways from the bifurcating flavin, each involving a unique site-differentiated cluster of iron and sulfur (one [2Fe-2S] and one [4Fe-4S]) that span a large free-energy gap for controlling electron transfer. The distal ends of these pathways "communicate" with each other via inter-domain interactions that can be observed by H/D exchange mass spectrometry. Thus, Nfn electronically and structurally tunes the communication between redox cofactors and substrate binding sites. We have also discovered a second enzyme, Fix, that catalyzes an electron bifurcation reaction in which high-energy electrons are generated from NADH in the form of the reduced iron-sulfur cluster containing redox protein ferredoxin ([4Fe-4S]), which provides low-potential electrons for N_2 fixation by nitrogenase. Although Fix contains multiple flavin cofactors, we successfully produced a model Fix system and identified individual flavins using circular dichroism to assign reduction potentials and transient absorption spectroscopy to identify a short-lived flavin semiquinone radical. To address the lack of a Fix structural model, we used chemical cross-linking in conjunction with homology modeling to reveal plausible electron transfer pathways within Fix. This work has generated new hypotheses on electron transfer control in this model flavin bifurcation system.

2. New insights into the biological reduction of N_2 to NH_3 and CO_2 to CH_4.
The metal-containing enzyme nitrogenase catalyzes the high activation barrier reduction of N_2 to ammonia (NH_3) and CO2 to CH4. Work in the BETCy EFRC is providing insights into how this enzyme utilizes the chemical energy in ATP hydrolysis to achieve these difficult reduction reactions. An important discovery is that ATP hydrolysis occurs after the transfer of electrons to the substrate, revealing that the energy of ATP hydrolysis is used to reset the system after the electron transfer event. This fundamentally changes our understanding of the energy landscape for coupling chemical energy to reducing potential in this enzyme. It was also discovered that there is communication between the two

symmetric halves of nitrogenase where one half waits for the first half to complete an electron transfer cycle before initiating its own cycle pointing to a complex array of energy couplings in nitrogenase. Studies are ongoing providing new insights into how activities on one side are coupled to activities on the other side. These studies are utilizing calculations to predict regions of the protein that are involved in the energy coupling and then using experiments to test the predictions. In order to better control electron transfer to nitrogenase, we have developed a novel hybrid system consisting of the nitrogenase MoFe protein, adsorbed onto CdS nanoparticles capable of light-driven N_2 reduction to NH_3. We have discovered that the Fe-based nitrogenase system can function in cells to achieve the reduction of both N_2 to NH_3 and the reduction of CO_2 to CH_4, with the evolved CH_4 sufficient to support bacteria that can grow on methane. All of these studies are providing unprecedented insights into how this enzyme achieves the coupling of chemical energy from ATP to achieve the difficult reduction reactions.

3. Determinants of catalytic bias in cofactor based oxidization reduction reactions.
The BETCy EFRC team developed a robust model system for the study of catalytic bias in cofactor-based oxidation-reduction reactions. The analysis of this model system has provided significant insights into understanding potential mechanistic determinants of catalytic bias and resulted in the proposal of a general mechanism of catalytic bias for cofactor-based oxidation reduction reactions. The team has discovered a suite of three [FeFe]-hydrogenases present in a single microorganisms, *Clostridium pasterianum*, that exhibit bias in the direction of catalytic turnover rates that span over a range of six orders of magnitude. The three enzymes, termed CpI, CpII, and CpIII, all harbor the same active site metal cluster, termed the H cluster that consists of a 2Fe unit with carbon monoxide and cyanide ligands bridged to a [4Fe-4S] cubane cluster through cysteinyl ligation. Our work has focused on characterizing the differences in the chemical nature of the amino acid environment and the complement and properties of the accessory [FeS] clusters as determinants of catalytic bias. We have found significant differences in the electrostatics of the H cluster environment and differences in the reduction potentials of the accessory clusters that we have been able to rationalize as determinants of catalytic bias. We have developed a hypothesis that these attributes impose differences in the relative stability of different oxidation states of the hydrogenases. The rate-limiting step for the proton reduction and hydrogen oxidation differ whereby the rate-limiting step for hydrogen oxidation is the binding of hydrogen to an oxidized state and the rate-limiting step for proton reduction is the formation of a reduced state. The relative stabilization or destabilization of these states provides a very simple yet elegant manner in which to promote a catalytic bias and could be applicable for any number of cofactor based oxidation-reduction reactions or metal based synthetic catalysts.

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