Title: Bringing to Light the Mechanism of Energy Conservation by Flavin-Based Electron Bifurcation

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Abstract:
An organism's ability to extract energy from its surroundings—and to do it better than its competitors—is a key requirement of survival. Until recently it was thought that in all of biology, from microbes to humans, there were only two methods to generate and conserve the energy required for cellular metabolism and survival, substrate-level phosphorylation and electron transport-linked phosphorylation. In the last few years, flavin-based electron bifurcation has gained acceptance as a third, fundamental mechanism of biological energy conservation. Bifurcation is fundamental to the biochemistry that drives microbial life at the thermodynamic limits observed for global anaerobic processes, including methanogenesis, acetogenesis and hydrogen metabolism.

A key feature common to all bifurcating enzymes is the ability to use the free energy generated by an exergonic oxidation-reduction reaction to drive a coupled endergonic reaction, although how this is achieved remains a topic of intense controversy. This study demonstrates how the flavin-based bifurcating enzyme, NADH-dependent ferredoxin-NADP⁺ oxidoreductase I (Nfn), catalyzes the formation of an energy-rich product, reduced ferredoxin (Fd), from the less energetic donor, NADPH, by coupling this reaction to the thermodynamically favorable reduction of NAD⁺ by NADPH.

Our results indicate that the high barrier reaction is driven by the formation of an unstable flavin anionic semiquinone intermediate that promotes one of the electrons from the two-electron donor, NADPH, to a highly reduced state sufficient to reduce ferredoxin. This flavin intermediate has not been previously observed due to its high reactivity. Its characterization, along with the energetic landscape of the additional redox cofactors in Nfn, allows us to construct a mechanistic understanding of how electrons are bifurcated over more than one volt of electrochemical potential, a range typically associated with photosynthetic biology. Notably, the electron produced at the bifurcating flavin site has sufficient energy to drive a broad range of difficult reactions with products that are of industrial relevance, such as alcohols and alkanes. An understanding of how this reaction is achieved in Nfn will not only guide the design of novel catalysts with large driving forces and high efficiencies, but may also lead to advances in our understanding of metabolic pathways and the design of new bioconversion processes through synthetic biology.