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# Small-Angle Neutron Scattering Reveals Extended Conformations in Electron-Bifurcating ETF

Electron bifurcation enables energy conservation in biological systems by coupling exergonic and endergonic redox reactions. In bifurcating electron transfer flavoproteins (Bf-ETFs), a large-scale conformational rearrangement is thought to gate electron flow by positioning flavins to enable or prevent inter-flavin transfer. However, the conformational ensemble sampled in solution has remained poorly understood. Here, we combine small-angle neutron scattering (SANS), small-angle X-ray scattering (SAXS), and advanced molecular modeling to probe the solution conformations of the bifurcating ETF from Acidaminococcus fermentans (AfeETF). Our SANS data reveal a dominant population of extended conformations in oxidized ETF, with radii of gyration ~4 Å larger than any known crystal structures, comprising over 50% of the ensemble. Reduction of flavins shifts the equilibrium toward more compact states, as validated by both NADH and dithionite-treated samples. Using ensemble optimization via a genetic algorithm informed by over 1,600 conformations generated from Bilbo-MD and metadynamics simulations, we achieve excellent agreement with SANS data. Contrast variation SANS experiments with selectively deuterated partner protein (BCD) further support conformational remodeling upon complex formation. These results challenge the static two-state model of ETF dynamics, proposing instead a spectrum of accessible conformations where extended forms act as structural intermediates linking the canonical “open”and “closed”states during catalytic turnover. This work establishes solution-phase structural dynamics as a critical component of electron bifurcation and highlights SANS-guided ensemble modeling as a powerful strategy for elucidating functionally important protein motions.

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## Topical Area

Biology and life sciences

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