Interprotein electron transfer in single crystals

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Although interprotein electron transfer (ET) depends on factors inherent to long-range electron tunneling, numerous studies have shown that interprotein ET is exquisitely sensitive to protein association modes and conformational changes at protein-protein interfaces. To correlate structure with reactivity for inter-protein ET reactions, we have characterized photochemically-induced ET reactions in single crystals of Zn-porphyrin-substituted cytochrome *c* peroxidase (ZnCcP) and cytochrome *c* (Cc) and metal-substituted azurins. Through use of homologs, site-directed mutagenesis, and solvent isotope substitution we have crystallized five different complexes of ZnCcP with Cc, and measured corresponding forward ET rates between CcP ^{3}Zn-porphyrin and Cc Fe(III)-heme, and back ET rates between Cc Fe(II)-heme and Zn-porphyrin$^+$. Conservative point mutations at the protein interface drastically change how CcP and Cc associate. . Reflecting donor-acceptor distances that differ by over 10 Å, back ET rates change over three orders of magnitude in these complexes; however, forward ET rates are surprisingly similar in each case. Forward ET in the high-affinity WT complex greatly diminishes below 0 °C, indicating gating. D_2O substitution slows ET rates between Cc and both ZnCcP and a SAM-modified electrode. Crystals or electrode films prepared in H_2O, but then soaked in D_2O recapitulate the slower ET properties over a period of hours. Comparison of inter- vs. intra- protein ET in crystals of metal-substituted azurins show how ET rates can be differentially affected by pH-dependent structural alterations in the crystal lattice. Taken together these studies define structures and interfaces over which electrons travel over long distances, and match them to rates. Relative ET rates are sensitive to association modes, which themselves are easily perturbed by small structural changes. Due to the constraints of the crystal lattice, any conformational gating processes must be limited to relatively small motions.