Engineering biogenesis of man-made light- and redox-active protein maquettes

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The translation of modern mechanistic descriptions of natural enzymes into practical engineering guidelines for construction of practical man-made enzymes remains elusive. Our approach is directed toward learning how to emulate the oxidoreductase superfamily, which embraces solar and respiratory energy transduction and the many enzymes of oxidative and reductive metabolism. We are finding that combining empirically determined first-principles design of ?-helical proteins with first-principles photon and electron transfer engineering common to natural oxidoreductases offers a practical path toward reproducing a broad range of oxidoreductase and linked functions in man-made proteins. By banning the import of insufficiently understood sequences and motifs from any one natural oxidoreductase, when we do successfully reproduce function in a stripped-down frame we expose an unrivalled understanding of the fundamentals of natural protein construction and engineering. We have developed a stable of working monomeric proteins – maquettes – comprising 3, 4 and 8 ?-helices of length between 4.5 and 6.2nm, designed for water solubility or membrane association. They have minimalist interiors that offer independent domains for separate functional engineering; the domains are malleable enough to accommodate the range of cofactors familiar in natural light harvesting, reaction centers and oxidoreductases. They are controllably thermostable up to 100C; Apo- and holo-forms crystallize readily down to 1.4Å. They promote diverse natural activities, including light harvesting and energy transfer and charge-separation approximating the core reactions of photosynthesis, cryptochrome and photolyase. They support interactions with dioxygen controllably ranging from stable dioxygen binding to the generation of reactive oxygen species of NADPH oxidase. They promote diffusible inter-protein electron-transfer to natural cytochrome c. They are readily expressible in bacteria: in E coli this extends to cofactor biogenesis (dithiolated heme C) and in cyanobacteria to functional fusion with natural light harvesting proteins. Maquettes will likely find practical applications in vitro but it is their integration with cellular functions where their usefulness to mankind lies.