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# Modular Designed Protein Constructions for Solar Generated H<sub>2</sub> from Water

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## Progress Report and Future Directions

**Generic tunneling barriers in natural proteins:** The theoretical basis for our electron tunneling expressions and their utility for calculating relevant tunneling parameters in natural and man-made photosynthetic systems is empirical. The generality of the expressions so far developed for analyzing parameters of electron tunneling in protein is therefore subject to reconsideration and refinement when new electron-transfer data from well-characterized proteins is received. The addition of over 20 new non-physiological electron tunneling reactions from semi-synthetic proteins continues to support our view that tunneling-barrier height has not been naturally selected to help or hinder electron transfer in natural photosynthesis. The barrier height does dictate the spatial essential required for light activated charge separating elements to generate high yield charge separated states stable into the millisecond times. Thus, natural reaction center proteins are scaled to approx 2.5 nm (25Å) while covalently bridged biomimetics require 5 nm (50 Å) and hence must be over twice as large to achieve the same performance.

**Coupling electron tunneling to redox catalysis:** We have extended our expressions to guide the engineering and construction of maquettes that catalyze oxidative and reductive chemistries. We are moving from pure electron tunneling, now beginning to be well described, to simple catalysis involving two-electron hydride transfer that occurs in the large class of dehydrogenases and to two-or-more electron transfers linked to bond forming/breaking common in oxidative and reductive catalysis. There is an important interplay between distance and driving force in the design and engineering of natural oxidoreductases when an overall exergonic series of electron transfers includes one or more endergonic (uphill) steps. To some extent the increased height of an uphill step can be compensated for by shrinking the distance between donor and acceptor while still maintaining overall sequential electron-tunneling rates within the approximately millisecond working threshold. We find that the balance between tunneling distance and uphill driving force sketches an unexpectedly broad engineering choice in distances and driving forces to achieve acceptable nanosecond to milliseconds sequential electron-transfer rates. The distance/energetic boundaries where sequential electron-transfer mechanisms become unworkably slow are clear.

**Synthetic protein backbone design:** Our aims to reproduce photochemical energy conversion and chemical oxidation-reduction catalysis require the development of maquette interiors that collect the characteristics fundamental to catalytic fitness in nature. Maquette interior design and the incorporation of cofactors go hand in hand. Despite the apparent low dielectric interior of the unlinked homodimeric maquettes, H/D exchange measurements indicate they offer comparatively little restriction for

## Objectives

Our long-term objective is to harness the energy of the sun for the production of inexpensive chemicals and fuels useful to mankind. We aim to achieve this with simple, design-flexible artificial proteins called maquettes. Maquette protein designs are informed by engineering and structural principles we learn from impressive natural photosynthesis. Our aim is to turn these principles into guidelines for the practical assembly of maquettes equipped for tailored photochemistry.

## Technical Barriers

Creation of very long-lived, light-activated charge-separated states will allow the accumulation of reductants and oxidants in the vicinity of catalytic centers within the protected environment of the artificial protein. With these lifetimes, even slow catalytic centers with significant activation energy barriers for making hydrogen or other fuels can operate, and then be iteratively redesigned to improved speed and function.

## Abstract

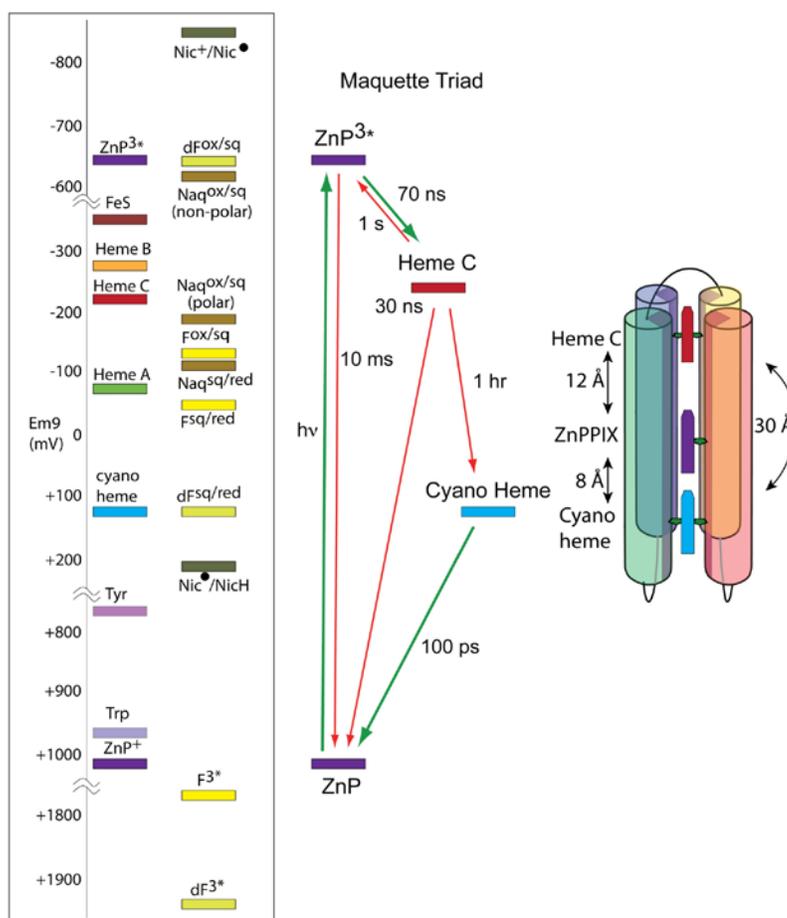
There is an urgent need to meet global demands for clean, inexpensive and renewable sources of energy. Sunlight is an abundant energy source tapped by biological photosynthesis. Our project is inspired by the efficiency and speed of biological photosynthesis that draws on water as its source of electrons for reduction of CO<sub>2</sub> to produce its cellular materials and fuels. Our project is directed to the abstraction of key engineering and construction principles from natural photosynthetic proteins and their application to the creation of a family of artificial photosynthetic proteins tailored to harnessing solar energy to drive production of useful chemicals and fuels including hydrogen. It is also our intention that any artificial proteins proving valuable to mankind as planned, will be designed ready for expression in bacteria for minimized costs of scaled production and assembly.

the entry of water. Regulation of water access is vital to the design of sites engaged in initial charge separation (low reorganization energies) and catalysis (chemical transformation sharply promoted or suppressed by water). We disulfide-linked maquette helices in a candelabra-like geometry, restricting helical motions, to dramatically slow H/D exchange and restrict water access to the interior of our simple maquettes well beyond the millisecond time scales of typical catalysis. We note that the interior of an analogous single-chain maquette excluded water despite substantial protein motion during rapid heme ligand exchange.

**Tetrapyrrole designs:** The lower symmetry of the single-chain maquette allows new design and assembly opportunities including covalent anchoring at specific positions of tetrapyrroles such as heme C as well as binding of single light activated centers such as Zn protoporphyrin IX and Zn-pheophorbide and bacteriochlorophyllide in the presence of companion redox centers and tetrapyrroles. We have found the new single chain maquette family avidly binds tetrapyrroles with nanomolar dissociation constants provided they are polar on one side and nonpolar on one or other sides. We are finding that the maquettes that support the fastest and simplest binding time courses are those that in the apo form are known to have multi-structured interiors that become structured upon porphyrin/chlorin coordination as indicated by NMR. We can secure porphyrin cofactors at 9.4 and 15.9 Å edge-to-edge distances by constructing histidine ligation sites along a helix 3 or 4 turns apart. We avoid 1 or 2 turns because of steric collision between adjacent porphyrins. However, there is no such limit to histidine placement in an adjacent helix. Presently, we secure porphyrins in adjacent helices at the level of 0, 2 or 4 helical turns which leads to edge-to-edge distances between different tetrapyrroles of 3.4, 7.2 and 17.9 Å. The demonstrated flexibility in controlling which tetrapyrroles bind where (bis-histidine sites bind Fe tetrapyrroles, mono-histidine sites Zn, covalent sites Fe or Zn) and the spacing between tetrapyrroles provide a good palette for positioning these cofactors at optimal locations, orientations and distances apart in the design of reaction center maquettes and for catalysis.

**Multi-electron redox cofactors:** We have designed non-tetrapyrrole cofactors to serve as other candidates for light-activated-triad electron donors and acceptors, for cofactor relays to add stability to the initial charge-separated states and for multi electron function at catalytic sites. These include a naphthoquinone amino acid (Naq), which is functionally similar to the natural

menaquinone or vitamin K family and structurally can substitute for tryptophan. We use an intein strategy to splice synthetic Naq peptides with expressed sequences. We have also developed a nicotinamide amino acid and secured various flavins to the maquette frameworks. Commonly, we react brominated flavins with Cys sulfur. We are increasing the flexibility of the approach by modifying flavins for attachment through the 8-a-methyl position, which tends to raise the flavin midpoint potential, in addition to our traditional 6-H position. We have also demonstrated the synthesis and characterization of a  $(m\text{-SR})_2\text{Fe}_2(\text{CO})_6$  complex coordinated to a simple,  $\alpha$ -helical peptide via two cysteine residues. Although this prototype showed no  $\text{H}^+/\text{H}_2$  exchange catalysis, the peptide serves as a first generation [FeFe]-hydrogenase maquette and opens the chemical door through redesign for the creation of more sophisticated peptides containing second coordination sphere residues designed to modulate the properties of the di-iron site. We have also reengineered the four-helix bundles of a natural bacterioferritin to include light-activated chlorins that serve to photo-oxidize Mn, in a manner analogous to the photo-



Average energy levels of redox centers secured inside maquettes. The first column on the left lists one-electron donors/acceptors and the second column two-electron cofactors. The middle schematic shows energy levels and lifetime selected in a triad presently under construction depicted on the right.

assembly of the Mn cluster of the oxygen evolving center in natural photosynthetic photosystem II.

**Light-activated maquettes:** We have explored the nanosecond to milliseconds spectral signatures of light activated electron transfer along the length of maquettes in chromophore dyads, using Zn PPIX or flavins as light-activated centers and various hemes as donors and acceptors. Longer charge separated lifetimes will be available in the light-activated triads we are currently constructing and testing in the single-chain four-helix bundle maquette designs (see figure). On the basis of our electron tunneling analysis, our current triad design should allow stable charge separation on the many minutes timescale, longer than any present chemical or biological construct, making it easier to achieve successful oxidative or reductive electron transfer at catalytic sites within the maquette. After successful construction of very long-lived charge-separated states, we will add a terminal cluster of redox centers to accumulate reductants within the maquette for catalysis at promising chemically constructed hydrogen producing centers secured within the maquettes.

#### Publication list (including patents) acknowledging the DOE grant or contract

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