Modular Designed Protein Constructions for Solar Generated H_2 from Water

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Objectives

- *The long-term goal of this grant is to equip synthetic protein designs with light activated electron tunneling-mediated charge-separation and then couple electron transfer to bond-breaking/forming catalysis of water-splitting to form oxygen and hydrogen. We aim to combine the best of natural protein— inexpensive production and self-assembly of structural scaffolds and metal cluster catalytic sites—with robust structures typical of chemical constructs. This goal relies on learning how to abstract and translate sophisticated photocatalysis from natural to synthetic molecular system (maquettes).*

Technical Barriers

- *Bio-inspired designs often needlessly import the complexity that accumulates in proteins during natural selection; such complexity severely limits further engineering. Our synthetic protein maquettes intentionally simplify design, separate the roles played by individual amino acids and purge protein complexity. This allows us to continue to add successive elements of the relatively blunt, basic engineering that underlies natural electron transfer and catalysis. This robustness and simplicity is what is required to integrate light activation, electron transfer and catalytic units.*

Progress Report and Future Directions

Natural design of photocatalytic proteins relies on a few basic engineering principles, such as the use of short distances to speed inherent electron tunneling rates as a means to overcome significant energetic barriers associated with the catalysis. Building this and other basic natural engineering principles into synthetic proteins will succeed if we monitor and control protein complexity during design and testing so that new functions can continue to be added. We ask how many engineering elements are required to achieve a particular biological function; what are the individual biochemical and structural tolerances of these elements and how much of a protein infrastructure is consumed in accommodating the function?

Uncovering the assembly instructions of function in proteins using hydrophilic maquettes:

We have shown that through the stepwise incorporation of engineering elements, it is possible to start from a functionally featureless peptide comprising just three different amino acids with a length and sequence selected to ensure association as a water-soluble, molten globular four-α-helical bundle, and produce a heme-containing protein maquette with native-like tertiary structure that performs the myoglobin or neuroglobin-like function of dioxygen binding to the ferrous heme. We find as schematically presented in Figure 1, that inclusion of two such elements to create a strained bis-histidine-heme ligation is only sufficient to support oxygen

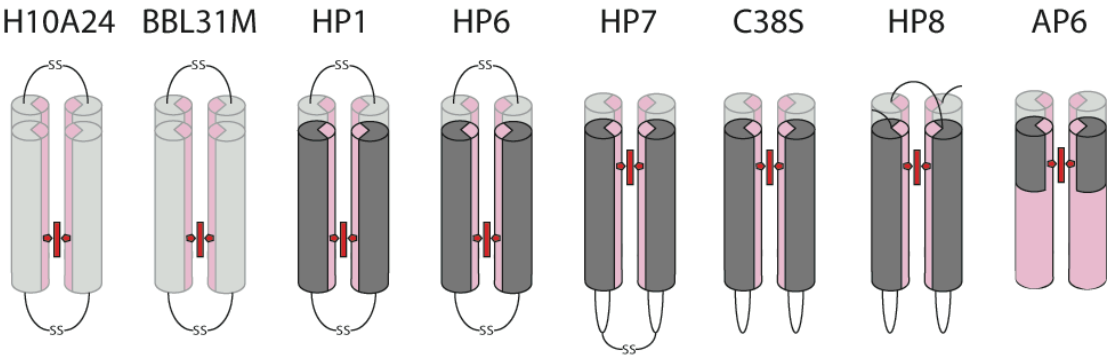


Fig 1. Stable room temperature oxyferrous-heme A or B is formed in maquettes with strained heme Fe histidine ligation *and* constrained helix motion; this is achieved in hydrophilic (HP) maquettes, HP7 and variants. The first four are defined maquette designs altered progressively until HP7. HP8 is a single sequence peptide of the next generation. Early work with amphiphilic (AP) maquette, AP6, also revealed formation of the oxyferrous state and opens the door to functionalizing membrane associated maquettes. Dark grey represents tertiary structures of the helices induced by heme ligation; a second heme added to HP1 structures all four helices (not shown).

binding at low temperature (H10A24-HP6); inclusion of a third element (HP7) to restrict inter-helix motion renders the oxyferrous-heme state stable at 16°C. Restriction of this motion is crucial as water penetration is minimized for stable oxyferrous-heme formation. The demonstration of an uncovering of the 'assembly instructions' for maquettes promoting stable generation of the primary step in respiration and a wide variety of oxidative chemistry represents a major step forward for the development of catalytic maquettes. This sequential, biochemically-informed maquette approach appears viable for the design of synthetic proteins performing catalysis generally. This work was done by Ron L. Koder, J. L. Ross Anderson, K.S. Reddy, Lee A Solomon, Christopher C. Moser and P. Leslie Dutton, is complete and will be submitted to Nature.

Amphiphilic Maquettes:

We have synthesized a new set of amphiphilic maquettes (AP6 series) schematically shown in Figure 2. The tetrameric AP-6 maquettes assemble with up to six ferric hemes B per tetramer. The AP maquettes co-solubilize with diblock copolymers or lipids and on an air-water interface, they compress to specific surface pressure, and can be transferred by Langmuir - Blodgett technique on HOPG (highly ordered pyrolytic graphite) surface. These amphiphillic maquettes show the same ability to control water access and bind oxygen ligands as their water soluble counter parts. In addition, functionalization of the maquettes via self-assembly of hydrophobic porphyrin cofactors to interior histidine ligated positions (including some of the light harvesting porphyrins described below) avoids the solubility problems sometimes found in hydrophillic systems.

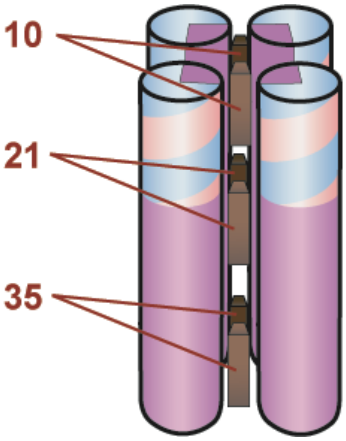


Fig.2: (*a*) Schematic drawing of an AP-6 maquette with 3 heme *b* binding sites. The purple surface residues are hydrophobic and insert into membranes.

Redox centers

a) Naphthoquinone amino acid for one- and two-electron chemistry at potentials reducing enough for H_2 production. In membrane proteins, with few exceptions, quinones are bound weakly and have neither clear binding site sequence nor structural consensus. We have dodged these problems to include a quinone in our maquette designs by synthesizing a novel, Fmoc-

protected naphthoquinone amino acid (**Naq**) functionally similar to the natural menaquinone or vitamin K family. Naq is spatially similar to tryptophan and has been proved to successfully incorporate in peptides along with other natural amino acids using a synthesizer, and without damage from the de-blocking measures required to expose the quinone carbonyls. Naq oxidation-reduction contained in a heptamer (Figure 3, heptaNaq) in aqueous media follows a typical two-electron (n=2.0) oxidation-reduction coupled to two-proton exchange (E_{m7} – 0.05V), while in aprotic solvent Naq potentials for the Qox/SQ and SQ/Qred at –0.60V and –1.33V (vs NHE) are well below the potentials of $2H^+/H_2$ under most conditions. This work is done by Lichtenstein, B.R. Cerda, J.F., Koder, R.L. and Dutton, P.L. and will be submitted as "Reversible Proton Coupled Electron Transfer in a Peptide-incorporated Naphthoquinone Amino Acid." to Chemical Communications.

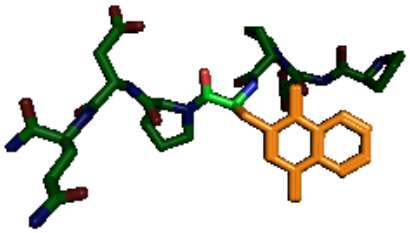


Fig. 3) synthetic redox amino acid Naq in a peptide

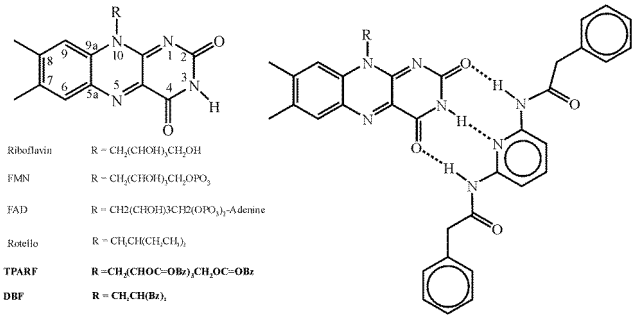


Fig. 4) Controlling H-bonding in flavins

b) Flavins modified for protein association for one and two electron chemistry: The more water-soluble redox analogue of quinones are the flavins. Flavins are ubiquitous in water-soluble oxidoreductase proteins where they can perform one electron chemistry, clear two-step electron/proton oxidation-reductions as well as be a partner for hydride transfer with strict two electron transfers substrates such as the nicotinamides and carbon-carbon bond redox chemistry common in intermediary metabolism. They also are well known to support photo-activated electron transfer. However, the high complexity and reactivity of the flavin has represented a challenge to characterization compared to quinones. We have approached the examination of the flavin moiety as a viable catalytic component by initially synthesizing simplified flavin variants that are highly soluble in organic solvents such as benzene that interact minimally with the several polar groups of flavin and discourage aggregation. Hence molecules such as TPARF and DBF (Fig. 4 left) open the door to analysis by a full range of spectroscopies especially NMR and IR and electrochemistry (see refs 3 and 4) and shown specifically interacting with Rotello H-bonding partners (Fig. 4 right).

c) Iron Cluster module for H_2 generation. We have demonstrated the synthesis of a (m-$SR)_2Fe_2(CO)_6$ complex coordinated to a simple, α-helical peptide via two cysteine residues. This peptide serves as a first generation [FeFe]-hydrogenase maquette and opens the chemical door for the creation of more sophisticated peptides containing second coordination sphere residues designed to modulate the properties of the diiron site. We have also created the first model system for studying the reactions of dithiolato diiron carbonyl compounds in de novo designed peptides. Finally, through redesign, hydrogenase maquettes offer a promising opportunity to develop robust, artificial, water-soluble, base-metal containing, cheap hydrogen production catalysts for use in environmentally friendly energy production strategies (Ref. 5).

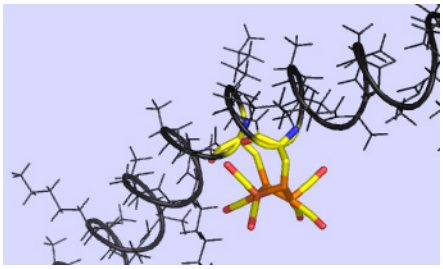
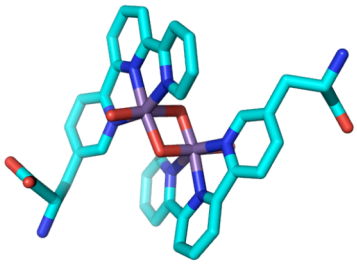
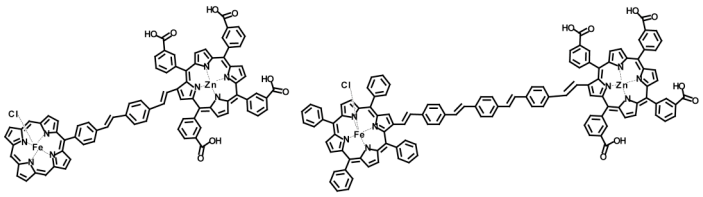


Fig 5) Di-iron hexacarbonyl center akin to H_2ase in a alpha helical synthetic protein.

d) Incorporating Mn clusters. A novel terpyridine amino acid, designed to ligate a Mn_2O_2 cluster in the form that Gary Brudvig shows catalytically splits water in solution, is well on its way to successful synthesis. This material is ready to be Fmoc protected and incorporated into a small Trp-zipper peptide using standard solid phase synthesis to confirm that this peptide geometry is indeed appropriate for Mn_2O_2 ligation (ongoing work by R.L. Koder, B.R. Lichtenstein, and P.L. Dutton).



e) Multimeric Zn- porphyrins. We are incorporating into maquettes a family of photoactive porphyrin dimers that have the potential to be made into dendrimers thereby becoming light harvesting. This is work with David Officer (Wollangong) and Keith Gordon (Dunedin NZ) Binding was achieved to HP-7.



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