Engineering origins of natural photo-electrochemical enzymes.

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Mechanistic understanding of modern natural photosynthetic reaction centers and allied light-activated redox enzymes has advanced in recent years, but the origins of their light, electronic and chemical engineering remain elusive. To address this, we use proven empirical expressions of electron tunneling [1] to determine what component parts of elementary chemistry and physics principles underlying light-driven electronic charge-separation are adopted, and to what degree adapted, to establish functional fitness in the engineering of a range of different natural photosystems. Of matching importance is the establishment of what component parts are not adopted and the evaluation of the generic parameters they contribute to the engineering of natural photosystems.

The minimal charge-separating construct is a cofactor dyad comprising one light-activatable chromophore and one redox-active electron donor to it or an electron acceptor from it. Because the singlet excited state (usual in biology) that drives the initial photochemical charge-separation decays in ~10ns, the tunneling-mediated charge-sparation must occur in the picosecond time range to avoid energy loss. Near van der Waals distance between the dyad components can facilitate tunneling in this time range even at low driving force ($-\Delta G$), while increasing $-\Delta G$ affords greater distance between the dyad pair and suppresses energy loss by thermal repopulation of the singlet excited state and nanosecond return to the ground state (Boltzmann: e.g. 0.2eV slows this decay route to >10 μs). However, limitation of loss through direct charge-recombination back to the ground state in dyads requires that the value of the $-\Delta G$ remaining after charge-separation is significantly larger than the reorganization energy (Marcus: λ, typically about 0.9 eV in protein) in order to access the rate-suppressing inverted region. Successful suppression of this direct route to achieve high-efficiency charge-separation stable until the biochemically practical millisecond time is achievable in principle by significant lowering of λ, say to 0.2- 0.4 eV, or by selecting incident activating photon energy ($h\nu$) toward the blue end of the solar spectrum (near-IR to blue; 1-3 eV). However, there is no evidence that modern dyads exist as viable electronic charge separating devices in nature. The closest relatives are represented by the one-electron flavoenzyme photolyase and two-electron protochlorophyllide reductase, in which a captured substrate acts as a close dyad partner directly reduced/oxidized coupled to chemistry in picoseconds and dodges the challenges of tunneling engineering. Other relatives, represented by the flavin cryptochrome-like proteins that are engaged in blue-light activated charge-separation in the 10 μs time for signalling and navigation, have recruited other cofactors (tryptophans) and are formally tetrads.

Indeed, extension of a dyad by one or more donors and/or acceptors alters the engineering landscape substantially and offers greater engineering tolerances to directed alteration and a broader creative platform for selected variations and redesign. The transition from dyad to triad, most straight-forwardly a light activatable pigment flanked by an acceptor and a donor, relaxes the engineering constraints on the key parameters, in particular the engineering of a low λ-value. Our results reveal that a donor-pigment-acceptor triad engages in the engineering of photo-electro-chemical enzymes capable of drawing solely on electron tunneling parameters to convert light energy (extending to the near-IR) into that stored as an oxidant and a reductant expressed as widely separated redox potentials in a charge separated state selectably stable from the nanosecond to well beyond the millisecond timescale typical for catalysis. Expanding triads to tetrads, pentads and beyond can further diminish engineering constraints and increase tolerances for choice of design parameters of the early steps, but adds little to the overall efficiency of the process. The most obvious contribution is in the added distance between oxidant and reductant and escape of the time constraints of charge-coupling to provide freely diffusing electron tranporter proteins, of nanometers length cofactor chains and cofactor clusters for multielectron catalysis.

Quantum and engineering efficiency of natural photochemical processes has long been a point of attention. Our calculated efficiencies of photochemical charge separations stable into seconds times in optimally engineered dyads, triads and beyond, are significantly higher than those measured in natural anoxygenic and oxygenic photosystems. The increase in efficiency at a millisecond in optimally engineered triads using an unadapted λ of 0.9 eV can be 25% while an engineered λ of 0.3 eV can be > 30%.

This work has revealed that there are considerable areas in the energy and structural landscapes that are unadopted for design and engineering of terrestial photosystems. Our findings provide impetus to develop higher efficiency photosystems for new products in manmade solar constructions *in vitro* or in modified living cells *in vivo*. The results also provide an engineering basis for considering the generation of photosystems in extra-terrestial settings.

References: [1] Page C. C. *et al* (1999) *Nature, 402,* 47-52. [2] Moser C. C. *et al* (1992) *Biochem Biophys. Acta 1797(9),* 1573-86.