Redesigning photosynthetic charge separation

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The x-ray structure of bacterial photosynthetic reaction centers transformed our view of the relationship between structure, spectroscopy and function and has been the basis of nearly every physical and biological measurement on photosynthetic assemblies for the last 20 years. The most striking finding was the presence of two reasonable electron transfer pathways, the L and M (or A and B) branches, yet under ordinary conditions, electron transfer occurs only along the L branch of chromophores. Many labs have engineered local changes to probe the origin(s) of this unidirectional electron transfer. Two groups pursued larger-scale symmetrization in *Rb. capsulatus:* the D_{LL} mutant (Robles *et al.*, 1990), in which D helix residues M192-M217 that make contact with most of the reactive components were replaced with those at L165-L190; and the *sym1* mutant (Taguchi *et al.*, 1992) in which residues M187-M203 were replaced with L160-L176. Neither led to electron transfer to the M-branch; however, both produced novel phenotypes. Remarkably, D_{LL} RCs assembled, but with the primary electron acceptor H_L missing, so L-side electron transfer is blocked. The i>sym1 mutant led to the surprising discovery that P is much more difficult to oxidize to P^+ than in wild type, and this was traced largely to the replacement of Phe at position M195 with His, which can hydrogen bond to the ring I acetyl group of the M macrocycle of P, P_M (Stocker *et al.*, 1992). Subsequently, the role of residue M195, the symmetry related residue at position L168, and those at positions L131 and M160 in *Rb. sphaeroides*, were all shown to systematically affect the oxidation potential of P, primarily by adding or removing hydrogen bonds to the acetyl or keto carbonyl groups of the two halves of P (Lin *et al.*, 1994). This strategy has been extensively developed by many groups, but the D_{LL} mutation has been much less studied because, as originally described, pure reaction centers could not be isolated.

We have returned to the D_{LL} system in the context of contemporary knowledge about mutations that affect co-factor redox properties and mild methods for isolation of reaction centers. Since electron transfer to H_L cannot occur, this system is ideal for probing the role of B_L, since B_L^- should still be formed, and for observing M-side electron transfer. We have recently reported that systematic mutations in the vicinity of P, BL, BM and HM can produce a very large yield of M-side electron transfer (Chuang *et al.*, 2006). To date, no evidence has been found for the presence of appreciable yields of $P^+B_L^-$ at room temperature. The results suggest that reaction energetics dominate native unidirectional electron transfer.

While significant progress has been made re-engineering the reaction center, nearly every step was based on empirical findings, rather than predictions. Electrostatic calculations can provide a basis for the analysis of reaction energetics, however, the level of structural information that is needed is rarely available. We have developed vibrational Stark shifts in proteins as a precise local and directional measure of electrostatic energy changes upon mutations.

Robles SJ, Breton J, & Youvan D. (1990) *Science*, **248:** 1402-5.

Taguchi AA, Stocker JW, Alden RG, Causgrove TP, Peloquin JM, Boxer SG & Woodbury NW. (1992) *Biochemistry* **31:** 10345-55.

Stocker JW, Taguchi AK, Murchison HA, Woodbury NW & Boxer SG. (1992) *Biochemistry***31:** 10356-62.

Lin X, Murchison HA, Nagarajan V, Parson WW, Allen JP & Williams JC. (1994) *Proceedings of the National Academy of Science USA*, **91:** 10265-9.

Chuang JI, Boxer SG, Holten D & Kirmaier C. (2006) *Biochemistry* **45:** 3845-51.