

Cysteine-mediated mechanism disrupts energy transfer to prevent photooxidation

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Unlike higher plants, which are often awash in sunlight, green sulfur bacteria survive in some of the darkest and most inhospitable environments for photosynthetic organisms. From the bottom of the Black Sea to the underside of thick microbial mats growing atop hot springs, these anaerobic archaea subsist on the rare photons that penetrate into their dark environments. For this reason, it is surprising that they would evolve a photoprotective mechanism. Indeed, until the work by Orf et al. (1), no photoprotection mechanisms have been well established in green sulfur bacteria; photoprotection was primarily the domain of higher plants and photosynthetic organisms that had the luxury (curse?) to exist in light so bright that it could damage their photosynthetic apparatus. Although green sulfur bacteria need not face bright sunlight, they still combat photo-induced oxidative damage when the redox potential of their environment rises. Orf et al. (1) demonstrate that an operative photoprotection mechanism exists in green sulfur

bacteria and that this mechanism is activated by oxidation of two cysteine residues.

This new photoprotection mechanism identified by Orf et al. (1) differs from more familiar motifs; the new mechanism employs amino acid residues instead of isomerization of dedicated photoprotective chromophores, such as carotenoids. It also seems to protect against damage from a single excitation (rather than multiple excitations). That is, the mechanism depends on redox potential, not light intensity. In contrast, photoprotective mechanisms are normally used by plants, algae, and bacteria to withstand conditions of excess sunlight (2). Typical strategies include nonradiative relaxation, adjustments of the proton gradient in the system to control the rate of redox reactivity, and physical detachment of the involved photosynthetic machinery (2–4). These approaches limit the risk of multiple simultaneous excitations inducing triplet formation and leading to generation of reactive oxygen species. In this context, the process described by Orf et al. (1) is a new nonphotochemical quenching mechanism.

The photosynthetic light-harvesting antenna in green sulfur bacteria consists of a large chlorosome built primarily of bacteriochlorophyll *c*; this chlorosome absorbs light and funnels energy to the reaction center. Between the chlorosome and the reaction center is a baseplate assembly and, in many cases, a spacer that allows reducing equivalents to reach the reaction center; this spacer, a bacteriochlorophyll *a* (BChl *a*)-containing pigment–protein complex, is known as the Fenna–Matthews–Olson complex (FMO). FMO functions as an excitonic wire conducting energy to the reaction center while allowing room for reducing agents to replenish electrons in the reaction center, thereby facilitating charge separation (5). This trimeric FMO protein contains eight BChl *a* molecular chromophores per monomer, which are bound to the protein scaffold (6). Seven of the BChl monomers are arranged in an internal hydrophobic pocket, forming an energy conduction channel for downhill energy transfer, whereas the eighth BChl binds near the baseplate

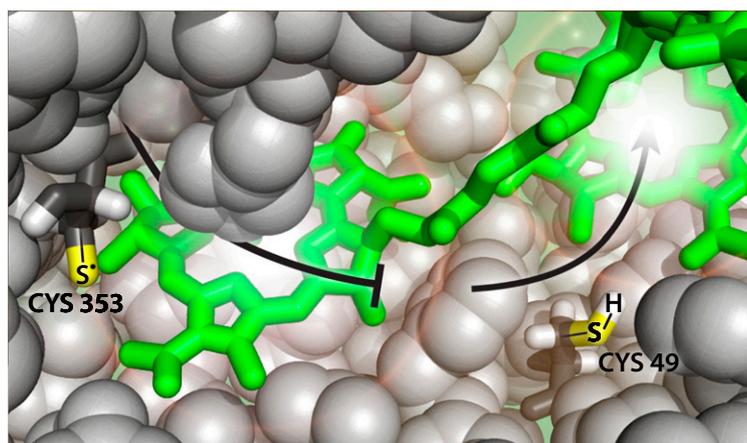


Fig. 1. The oxidation states of the cysteine residues control the excitonic energy transfer through FMO to the reaction center. Energy flows when these residues are reduced, but nonradiative relaxation quenches the excitation when they are oxidized. In this regard, the redox potential of the environment effectively gates energy flow through FMO, in analogy to an excitonic transistor. This image was created in PyMol using file 3ENI from the Protein Data Bank.

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interface. Although the monomers are identical, variations in their local environments shift their electronic energy gaps with respect to each other, and their spatial arrangement determines the coupling among the BChls.

FMO is among the most well-studied photosynthetic complexes for several reasons: It has only eight chromophores, is both water-soluble and robust, has yielded crystal structures at 1.3 Å resolution (6), and has relatively well-resolved spectral features at convenient energies for transient optical spectroscopy. This spectral resolution and the asymmetric chromophore arrangement lead to the straightforward assignment of the BChls. One challenge of working with FMO is that it must be expressed in its native organism (an obligate phototroph) to assemble properly and, until very recently, we lacked robust genetic control over the green sulfur bacterial photosynthetic apparatus. Using a suite of genetic tools only recently developed for *Chlorobaculum tepidum* (7), Orf et al. (1) examined an unusual fluorescent signature, originally observed decades earlier, in the FMO light-harvesting complex. They discovered that the oxidation states of two cysteine residues (Cys49 and Cys353), located near the lowest-energy BChl a pigment (Fig. 1), are responsible for the change in fluorescence yield. The authors confirm this hypothesis through a series of redox, ligand, and mutation experiments, and they further characterize the process using a variety of spectroscopies. Additionally, they conclude that these cysteine residues protect the green sulfur bacteria from photodamage when they experience simultaneous oxidizing conditions and illumination. Oxidation of the cysteine residues affects the photophysical behavior of the BChl a at site 3. This site corresponds spatially to the lowest-lying exciton, from which transfer to the reaction center proceeds (8). Orf et al. (1) found that, when oxidized, these cysteine residues facilitate nonradiative quenching. This observation reveals that in addition to its role as a highly efficient energy conduit and a physical spacer, FMO also functions as an “excitonic transistor,” where the redox potentials of the neighboring cysteine residues gate the energy transport toward the reaction center.

One possible advantage for FMO’s photoprotection mechanism, in contrast to carotenoid-based ones, is the low metabolic cost of reversing the process. In photosystem II of higher plants, photoprotection involves the enzyme-induced de-epoxidation to convert violoxanthin to zeaxanthin (4, 9). This process is called the xanthophyll cycle, and it occurs in light-harvesting complex II in response to pH. In cyanobacteria, nonphotochemical quenching occurs when carotenoids isomerize in response to intense green or blue light. These conformational changes induce reorganization of the light-harvesting antenna, causing thermal dissipation with an 80% quenching efficiency (10, 11). In contrast, Orf et al. (1) report structural invariance for FMO upon activation of its protoprotective mechanism. Because FMO exists in an aqueous periplasmic niche, carotenoids cannot simply diffuse away and recycle, and enzymatic reduction would represent a serious metabolic cost for this light-starved organism. The elegant mechanism identified by Orf et al. (1) can be quite metabolically inexpensive.

The discovery of the new photoprotection mechanism also suggests opportunities to explore what other roles local electrostatic effects might play in the electrostatics of FMO and other biological systems. It is striking that two humble hydrogen atoms in a massive protein are empowered to govern the photosynthetic energy transfer process (1). Because the cysteine residues are so rare but highly conserved in this complex, we speculate that their role carries a strong selective advantage. However, the impact of highly localized features such as these is regularly outside the scope of many contemporary analyses of FMO. Aside from its natural role in photoprotection, this mechanism could be used to probe how photosynthetic complexes avoid concentration quenching, how local electrostatic environments facilitate energy

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transfer, or even novel signaling in photobiology. One could even imagine engineering cysteine residues into light-harvesting systems to use the proton-specific chemistry to probe local electrostatic effects on electronic dynamics of a variety of systems, or even using unnatural amino acids tailored to realize other electrostatic roles not found in nature.

Both experimental and theoretical investigations of the dynamics in FMO have often used a more coarse-grained approach that cannot reveal the explicit role of local structure on the scale of individual protons. Until now, it was not clear that building such detailed models would be necessary to understand energy transfer; this microscopic functional modeling represents a grand challenge to the community. Due to its size and complexity, FMO has been treated by approximate methods, such as open quantum system and mixed molecular mechanical/quantum mechanical approaches (12–17). These methods have contributed significantly to the understanding of energy transfer dynamics in FMO, but they may miss the impact of highly localized effects such as those raised by Orf et al. (1).

In a broader context, the findings of Orf et al. (1) also raise many new questions. Is this photoprotection mechanism highly conserved in green sulfur bacteria? Oxidative stress is present in many photosynthetic environments—especially those that evolve oxygen, such as plants. We are aware of very specialized strategies to isolate the photosynthetic apparatus from reactive oxygen species ranging from crassulacean acid metabolism to leaf curling to cyclic electron transport (18). Do any of these species also use this amino acid-based photoprotection approach, as opposed to the carotenoid-based approach? Have they only escaped notice because we did not know to look for cysteines as a photoprotective agent? Will this approach become a new laboratory tool allowing a redox-based dial for probing coupling and dynamics? These intricate photoprotection motifs demonstrate once again that it is not easy being green.

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