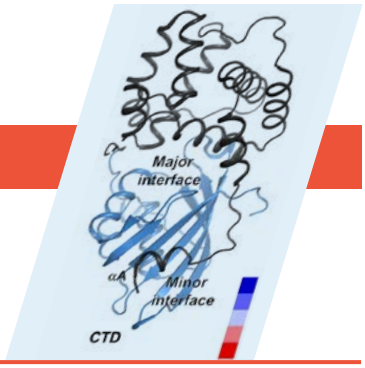
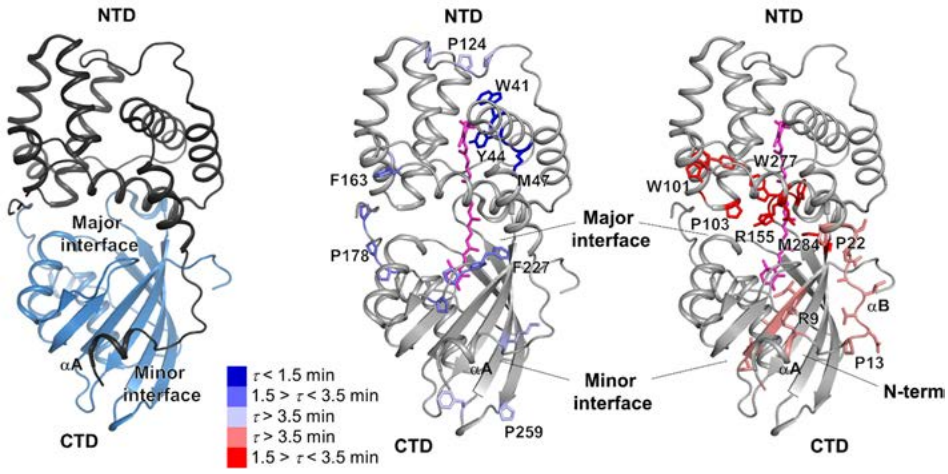
BIOLOGICAL SCIENCES

How Light-Harvesting Bacteria Toggle Off and On





X-ray footprinting provides time-resolved information about where key conformational changes occur. On the left is the overall protein structure. The two structures on the right highlight local areas with increasing protein packing over time (blue shading) and areas with decreasing protein packing over time (red shading). The changes in accessibility are initiated by the movement of the carotenoid molecule (magenta chain).

Scientific Achievement

At the Advanced Light Source (ALS), researchers clarified the atomic-level mechanism that enables light-harvesting bacteria to switch off and on in response to potentially damaging overexposure to light.

Significance and Impact

The results could have long-range implications for artificial photosynthesis and optogenetics—the use of light to selectively activate biological processes.

A prototype of photosynthesis

Cyanobacteria are water-dwelling microbes capable of absorbing sunlight and converting it into chemical energy through photosynthesis. Long ago, ancient versions of these bacteria were incorporated into plant cells, where they eventually evolved into chloroplasts, the organelles responsible for carrying out photosynthesis in green plants. Today, in seeking to develop artificial photosynthesis to harness the sun's abundant energy, scientists look to cyanobacteria to better understand the nuts and bolts of how natural photosynthesis works.

Cyanobacterial "off switch"

One topic of interest is how cyanobacteria respond to too much light. If a sunlight-harvesting system becomes overloaded with absorbed solar energy, it most likely will suffer some form of damage. Nature has solved the problem in cyanobacteria through a protective mechanism—an energy-quenching "off switch" in which excess solar energy is safely dissipated as heat.

In previous work on this topic, researchers focused on orange carotenoid protein (OCP), a light-sensitive protein embedded in cyanobacterial membranes. OCP was found to be triggered by the shifting of a hydrocarbon chain—a carotenoid molecule—between the two main OCP domains—the N-terminal domain (NTD) and the C-terminal domain (CTD). As a result of this translocation, OCP shifts from an "orange" light-absorbing state (OCP^O) to a "red" photoprotective state (OCP^R).

Fluorescence recovery protein

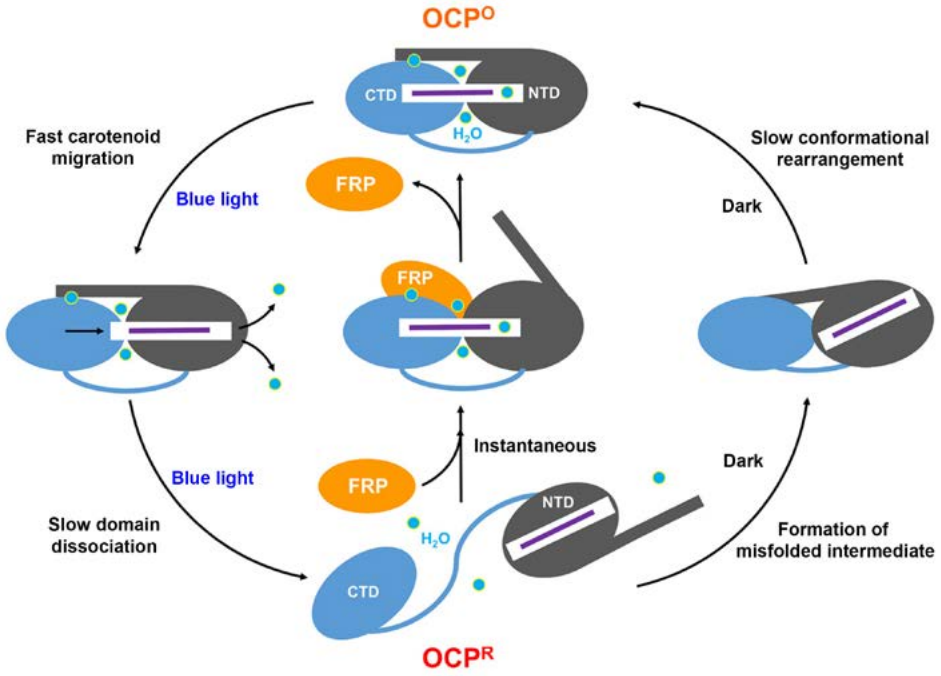
In this work, the researchers studied another protein complex, called fluorescence recovery protein (FRP), to see what role it plays in switching photosynthesis back on. The photoprotective OCP^R state is metastable; it relaxes back to OCP^O slowly in darkness, but instantaneously in the presence of FRP. Thus, determining the exact structural changes that accompany OCP^R formation and its interactions with FRP is critical for a complete mechanistic understanding of the regulation of photosynthesis in cyanobacteria. But although the structure of free FRP has been solved, its detailed interactions with OCP and its precise mechanism of photoactivation has not been well understood.

Following the footprints

To discover how FRP facilitates the resumption of photosynthesis, the researchers performed x-ray footprinting experiments at ALS Beamline 5.3.1. The technique involves exposing FRP–OCP complexes to x-rays while in solution. The high-flux-density x-ray beams ionize water molecules in the solution, generating hydroxyl radicals that label all solvent-accessible areas of the proteins. Timing the x-ray pulses to hit at various intervals after initial excitation of the complexes with blue light provides time-resolved data. The hydroxyl-labeled protein complexes were then fragmented by proteolytic digestion and analyzed by mass spectrometry at the Joint BioEnergy Institute, to produce site-specific maps of the FRP–OCP interaction areas inaccessible to the solvent (the interaction "footprint") at a single-residue resolution.

A working model of photoprotection

Based on the results, the researchers were able to develop a working model of how FRP creates a "shortcut" to OCP relaxation by holding the NTD and CTD together like a scaffolding. They also found that, without FRP, this particular OCP variant forms a misfolded intermediate that may slow down the relaxation process. In general, understanding the mechanisms controlling the thermal dissipation of excess light energy has long-range implications in synthetic biology, for example in the design of selective, light-activated switches for biological processes (optogenetics), or for finer control of photoprotection and consequent enhancement of photosynthetic efficiency.



Working model of photoprotection. Strong blue light induces OCP photoactivation, driven by the fast migration of the carotenoid molecule (purple) into the NTD (gray). Slow domain dissociation leads to a form of OCP^R in which the NTD and CTD (blue) are attached by a flexible linker. FRP (yellow) can bind to the CTD and act as a scaffold for holding the NTD and CTD together, providing a "shortcut" back to the OCP^O state. In the absence of FRP, formation of a misfolded intermediate may slow the relaxation process. Cyan circles show the positions of bound and conserved water molecules, which are critical for the native OCP^O structure.

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