

Biophysics

A cold break for photoreceptors

Lars-Oliver Essen and Dieter Oesterhelt

Bacteria, plants and animals all perceive light using photoreceptors, each of which carries a bound, organic molecule known as a chromophore. When hit by a photon, the chromophore undergoes an apparently simple reaction — a *cis*-to-*trans* isomerization around a double bond. Light-perception by the chromophore is ultra-fast, taking only a few hundred femtoseconds. But the ensuing structural changes of the chromophore and its protein environment are thermally driven and much slower. For example, it takes more than 10^8 times longer for a photoreceptor to reach its signalling state, generate a cellular signal and, finally, to complete the 'photocycle' by returning to its original state.

How do these photoreceptors translate femtosecond events into millisecond responses so efficiently? In the atomic-resolution study reported by Genick *et al.*¹ on page 206 of this issue, we get a first impression of a biological photoreceptor that has been trapped very shortly after excitation. The authors studied the photoactive yellow protein (PYP), which was discovered in the halophilic bacterium *Ectothiorhodospira halophila*². There, as well as in other phototrophic purple bacteria, PYP serves as a light sensor which, presumably, mediates the negatively phototactic response of these bacteria to blue light.

Originally, PYP was thought to be a small, soluble cousin of the intensively studied bacterial and animal rhodopsins. These proteins are embedded in lipid membranes, and they use the vitamin-A derivative retinal as the chromophore. But X-ray crystallographic work³ revealed that PYP has a completely different protein architecture from its rhodopsin-like ancestors³. It was also found that it carries the simplest photoisomerizable chromophore, *p*-hydroxycinnamic acid (*p*CA). This chromophore contains a single isomerizable double bond, flanked by a bulky phenolic ring on one side and a thioester linkage to PYP on the other side (Fig. 1).

Despite these structural differences, there are several common aspects that make PYP a fascinating model system for the retinal-containing rhodopsin family. First, the protein performs a large redshift for the absorption maximum of the *p*CA chromophore. Second, there are several, spectroscopically well-discriminated intermediates within the photocycle. Third, the triggering is extremely efficient — only one-third of the excited photoreceptors fail to initiate a full photocycle. Last, but not least, PYP is of considerable benefit to the structural biologist. It forms outstanding, high-quality crystals that are amenable to state-of-the-art synchrotron techniques, time-resolved Laue crystallography⁴ and studies on cryo-

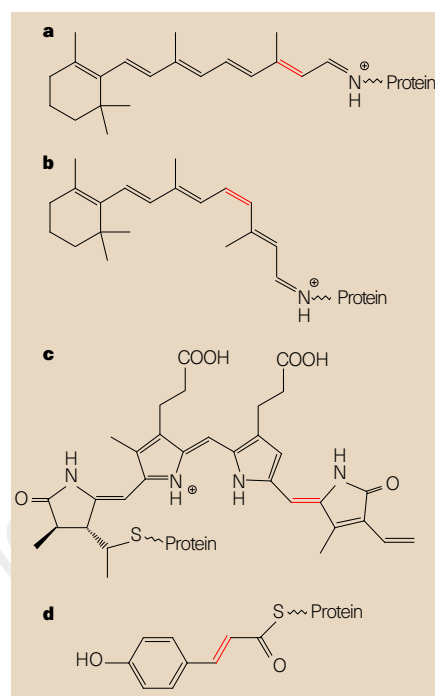


Figure 1 Chromophores from different photoreceptors. a, From archaeal rhodopsins. b, From animal rhodopsins. c, From plant and bacterial phytochromes. d, From bacterial photoactive yellow protein. The isomerizable double bond in each case is highlighted in red.

trapped intermediates at sub-ångstrom resolution, as now reported by Genick *et al.*¹.

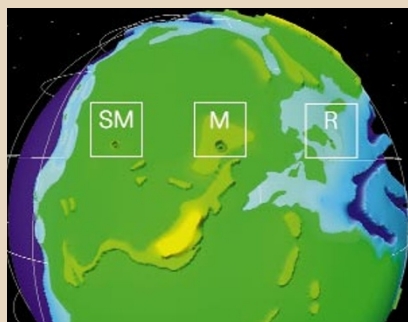
The time-resolved techniques⁵ use a laser flash to initiate the photoreaction in a crystal, followed by a second, 'white' X-ray pulse to record the structural information. Using a

Planetary science

Crater row

A crater chain is an alignment of three or more impact craters, with the same apparent age, thought to be caused by the fragmentation and sequential collision of large bodies with a planet. But finding such chains on the Earth's surface is complicated by the process of plate tectonics which, over geological time, will disperse the craters. Elsewhere in this issue (*Nature* 392, 171–173; 1998), John G. Spray and co-workers describe how they have identified just such a chain. It consists of at least five craters, spread over more than 4,000 km, which were produced about 214 million years ago. The illustration here shows the three main craters in the chain — St Martin, SM; Manicouagan, M; and Rochechouart, R — which line up at constant latitude when the Earth's tectonic plates are reconfigured to their arrangement at the time of the collisions.

In 1994, the break-up of comet Shoemaker–Levy 9 by the tidal forces of



Jupiter, and the subsequent collision of the fragments with the planet, dramatically showed how such crater chains might be formed. The fact that the craters observed by Spray *et al.* lie at a constant latitude indicates that the fragmented body that produced them had likewise been captured into an Earth orbit, with the distance between craters giving the angle through which the Earth rotated between impacts. But the large difference in mass between Jupiter and

the Earth (over 300 to 1), and the Earth's closer proximity to the Sun, mean that it seems unlikely that the Earth would have captured and, through tidal forces, fragmented a comet or asteroid in the same manner as Jupiter.

An alternative view of events is this — a comet or asteroid makes a grazing passage through the Earth's atmosphere; it is fragmented and the pieces are captured into an Earth orbit through the forces involved in 'aerobraking'; the fragments then collide with the planet at their next pass-by (see W. F. Bottke *et al. Icarus* 126, 470–474 (1997); S. G. Love *et al. Lunar Planet. Sci. Conf. XXVIII*, 837–838 (1997)). Such a process would still happen only infrequently. But it is estimated to have affected roughly 1 out of every 1,000 objects that have hit the Earth, and so could have been responsible for the crater chain observed by Spray and colleagues.

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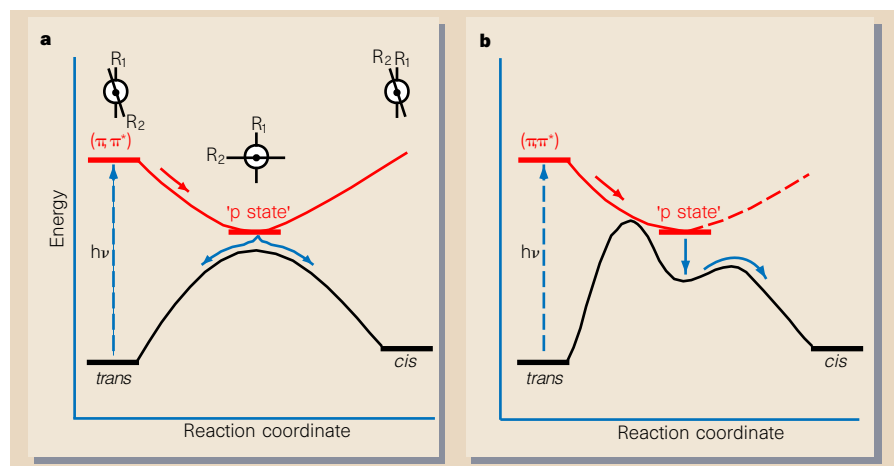


Figure 2 Energy diagrams for the photoisomerization of 1,2-disubstituted ethenes. **a**, In free solution. **b**, In the context of a photoreceptor. The energy profile of the excited (π, π^*) state (shown in red) is different than that for the thermal isomerization. Genick *et al.*¹ have observed that early-state photoreceptor intermediates of the photoactive yellow protein resemble this 'p-state' intermediate.

similar strategy, a 1.9-Å structure of the late, blueshifted I_2 PYP intermediate has been obtained⁴, several milliseconds after excitation. In this intermediate, the *pCA* chromophore flips its double bond to a *cis* configuration by swinging the phenolic ring like a tennis racket. This large movement causes a considerable reorganization of surface-exposed protein residues nearby, and might enable PYP to interact with other signalling molecules. The chromophore is not planar, and the authors supposed this to be the molecular spring for completion of the photocycle.

An absorbed photon supplies more than enough energy to generate such late intermediates. But how is the energy that is initially localized in a 'hot' chromophore converted to the gross structural changes of the protein environment? And how can a photoreceptor catalyse a single reaction pathway, avoiding the unwanted side reactions that are well known to photochemists? The answer might be found in the structures of very early intermediates. However, these are formed too rapidly to be studied by the time-resolved approach, which operates on a nanosecond timescale⁶.

Genick *et al.*¹ offer a simple but elegant solution to these problems. They excited crystals of PYP at liquid-nitrogen temperatures, and assumed that the degrees of freedom that were frozen out were those responsible for the slower thermal relaxations towards the I_2 intermediate. Their 0.85-Å structure of such an early PYP photocycle intermediate shows, for the first time, a chromophore that has started a *trans*-to-*cis* isomerization around its C7–C8 double bond, but has become trapped halfway between.

What does the orthogonal conformation of the double bond tell us about a mechanism for photoreceptors? Consider, as the simplest model, 1,2-disubstituted ethenes in free solu-

tion. When they are excited by light, they form a transient (π, π^*) state in which there is effectively no π bond. Subsequent rotation around the bond gives a staggered, lower-energy conformer in which the adjacent *p*-orbitals are perpendicular to one another (Fig. 2a). This relaxed 'p state' can decay, without emitting radiation, to a *cis* or *trans* isomer with almost equal probability, by crossing to the ground-state energy curve⁷.

Genick *et al.* observe that, in their 0.85-Å structure, the chromophore looks similar to such a *p*-state intermediate. One could speculate that photoreceptors maximize

their quantum efficiencies by selectively stabilizing *p*-state-like intermediates. This is analogous to the way in which enzymes are efficient catalysts — by selective stabilization of the transition state. For PYP, the non-uniform protein environment means that photoisomerization is unidirectional, because the energetic barriers for returning to the original configuration or progressing through the reaction are not equal (Fig. 2b).

To understand the small, structural changes in early photocycle intermediates, we certainly need more sub-ångstrom structures like that presented by Genick *et al.* Unfortunately, it will probably be a long time before we have such an eagle-eyed view of rhodopsins or phytochromes — the quality of these crystals is a long way behind that of PYP. But the new results on PYP mean that crystallographers, quantum chemists and spectroscopists have got good reasons to chat to one another. □

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Meteoritics

Diamonds in the dust

Primitive meteorites are time capsules, containing dust that may have remained virtually unchanged since the earliest days of the Solar System. The dust is often peppered with tiny diamonds, which probably condensed from a stellar outflow before the birth of the Sun. But uncertainty surrounds the types of star involved.

Anja Andersen and colleagues (*Astron. Astrophys.* **330**, 1080–1090; 1998) have investigated the matter by extracting diamonds from the 1969 Allende meteorite, shown here in greatly magnified clusters. They then analysed the diamonds' spectral and absorption characteristics at various wavelengths, and predict the spectrum of a plausible candidate star (in this case, a type of carbon-rich red giant). With a template against which to compare real stellar spectra, the origin of the diamonds could in principle be revealed.

Things are not so simple, however, for diamonds have a comparatively weak influence on stellar spectra. But other lines



of approach may open up because these microscopic gems can act as seeds, and may have become covered with coats of other dust grains. Andersen *et al.* suggest such coatings might be retained and their composition revealed by sensitive extraction techniques — offering further clues to the diamonds' history.

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