Cryo-trapping Crystal Studies of Photoreceptor PixJ Yield New Insights into its Photoconversion Mechanism

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Proteins are not the static models we create out of x-ray diffraction or cryo-EM data, but are dynamic, moving molecules that inhabit an ensemble of states and can change conformation to drive signaling cascades and catalyze reactions. To understand how these signaling cascades work at the atomic level, we are performing cryo-trapping experiments with crystals of the photosensing GAF domain from the blue/green-light photoreversible cyanobacterial photoreceptor PixJ. To observe intermediate structures along the photoconversion pathway, we are using two cryo-trapping techniques, temperature-scan cryo-crystallography and pump-quenching. The temperature-scan data shows small changes permitted at very low temperatures via F_O-F_O, which correspond to early intermediates during the photoconversion reaction, as PixJ transitions from its dark-adapted Pb state to its activated Pg state. These smaller changes include the chromophore isomerization and reorientation in the binding pocket. Pump quench experiments allow us to observe larger changes and later intermediates that occur along the photoconversion pathway with time resolution, including binding pocket rearrangements. Together, these two techniques enable a more complete understanding of the photoconversion pathway as compared to studying the static Pb and Pg end point structures alone.

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