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Correlating folding and signaling in a photoreceptor by single molecule measurements and energy landscape calculations[1]

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Receptor activation is a fundamental process in biological signaling. We study the structural changes during activation of photoactive yellow protein (PYP). This is triggered by photoisomerization of the p-coumaric acid (pCA) chromophore of PYP, which converts the initial pG state into the activated pB state. Mechanical unfolding of Cys-linked PYP multimers probed by atomic force microscopy (AFM) in the presence and absence of illumination reveals that the core of the protein is extended by 3 nm and destabilized by 30 percent in pB. These results establish a generally applicable single molecule approach for mapping functional conformational changes to selected regions of a protein and indicate that stimulus-induced partial protein unfolding can be employed as a signaling mechanism. Comparative measurements, Jarzynski-Hummer-Szabo analysis of the data, and steered MD simulations of two double-Cys PYP mutants reveal strong anisotropy in the unfolding mechanism along the two axes defined by the Cys residues. Unfolding along one axis exhibits a transition-state-like feature where six hydrogen bonds break simultaneously. The other axis displays an unpeaked force profile reflecting a non-cooperative transition, challenging the notion that cooperative unfolding is a universal feature in protein stability. MD simulations with a coarse-grained protein model show that the folding of pG is two-state, consistent with experimental observations. In contrast, the folding free energy surface of a coarse-grained model of pB involves an on-pathway partially unfolded intermediate that closely matches experimental data. The results reveal that interactions between the pCA and its binding pocket can switch the energy landscape for PYP from two- to three-state folding, and show how this can be exploited to trigger large functionally important protein conformational changes.

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