The structural basis for redox-dependent conformational switching in the InaD PDZ5 domain

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***Short Abstract* — An important goal in biology is to understand the structural principles underlying complex functional properties of proteins. An excellent model system to address this problem is the InaD multi-PDZ domain scaffolding protein required for vision in *Drosophila*. Light activation of the visual signaling cascade triggers a disulfide redox-based allosteric conformational switch in the fifth PDZ domain ($InaD^{PDZ5}$) that regulates its affinity for its target ligand – the PLC-β effector molecule. This switch is necessary for fast vision, a conserved property of daytime-active flying insects. The thermodynamics of coupling between disulfide bond formation, conformational switching, and substrate binding have been described, and provides the necessary foundation to deduce the mechanism(s) linking these three equilibria. Here, we attempt to define the structural basis for the $InaD^{PDZ5}$ conformational switch through statistical modeling of PDZ evolution, targeted mutagenesis, and transplantation of the mechanism into an unrelated, non-switching PDZ domain.**

***Keywords* — Proteins, biophysics, allostery, signaling, redox**

I. Background

PROTEIN allostery plays a critical role in mediating and regulating the flow of information within cells. However, the structural basis and origins of allosteric phenomena remain difficult to understand. What approach can tell us how distant sites on proteins communicate? How can such communication be established in a naïve domain through a process of random variation and selection? Here, we address these questions in context of a complex allosteric switch critical for vision in the *Drosophila* compound eye. In the photoreceptor neurons, light stimulation leads to transient conformational switching of $InaD^{PDZ5}$ due to reversible formation of an intramolecular disulfide bond. This activity regulates binding to the PLC-β effector molecule, and consequently, the sensitivity of visual signaling—a process termed "dynamic scaffolding" [1, 2].

How can we work out the underlying mechanism of redox-based conformational switching? One systematic approach is statistical analysis of PDZ domain evolution [3], which reveals a sparse but physically contiguous network of coevolving amino acids that both includes and is built around the disulfide-bonded positions in $InaD^{PDZ5}$. To study the role of these positions in redox-dependent conformational switching, we take two approaches: (1) targeted mutagenesis, following effects on disulfide redox potential and on PLC-β binding, and (2) directed transplantation of the coevolving network into another member of the PDZ family ($PSD95^{pdz3}$) that shows no conformational switching. To date, we have primarily pursued the latter experiment, focusing on the strength and dynamics of disulfide bond formation and substrate binding in chimeric forms of $PSD95^{pdz3}$. Together, these experiments are designed to test the necessity and sufficiency of the coevolving network with PDZ5 for specifying redox-dependent switching in PDZ domains.

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II. Results

A crystal structure of $PDZ3^{I336C/A375C}$ reveals the presence of a partially-occupied disulfide bond within the core of the protein, but biochemical assays show very little equilibration with the environmental redox potential of the bulk solution. $InaD^{PDZ5}$-like disulfide equilibrium potentials and sensitivity to environmental redox do, however, begin to emerge as co-evolving positions are introduced systematically into $PSD95^{pdz3}$. In total, a chimeric $PSD95^{pdz3}$ with the top 25 coevolving positions swapped displays a redox titration well fit by the Nernst expression and with a standard redox potential within several millivolts of that for wild-type $InaD^{PDZ5}$.

III. Conclusion

Our work to date begins the process of identifying the mechanism of redox switching in $InaD^{PDZ5}$. Future work will focus on the targeted mutational analysis of $InaD^{PDZ5}$ and on further biophysical characterization of the disulfide redox reaction in chimeric $PSD95^{pdz3}$ proteins. Ultimately, we hope to have an understanding of how $InaD^{PDZ5}$ works and how the seemingly complex allosteric mechanism could emerge through sequence variation in other PDZ domains.

References

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