

Structural and electrostatic determinants of interaction specificity in G-protein mediated signaling

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For signaling cascades to function correctly, their components must recognize their appropriate partners accurately. This requirement presents a challenge for living cells, as *related* components are used repeatedly in both parallel and intersecting cascades within the same cell. Signaling therefore requires that the interactions of *particular* protein-family members be tailored to each signaling cascade via interaction specificity. Understanding the structural basis for such selectivity is a major goal in both experimental and computational biology. Yet, beyond single representative examples, little is known of how specificity is determined among members of large protein families, including those involved in signal transduction.

We developed a “bottom-up” approach to decipher interaction specificity - combining an experimental activity benchmark with a structure-based computational mapping of function/specificity determinants at the protein family level. Our approach uses a variant of *in silico* mutagenesis – perturbing the *charges* of each residue and differentiating between side-chain and main-chain energetic contributions. Electrostatic energies are calculated using the Finite Difference Poisson-Boltzmann method (as implemented in the DelPhi program). Using a consensus approach across multiple crystal structures, we established a structure-to-sequence map predicting which residues are essential for function and which residues can modulate specific interactions with the cognate protein partner. We validated our residue-level map using experimental mutagenesis and redesign of gain-of-function mutants. We also applied this approach to additional proteins families, showing the generality of our structure-based method and highlighting the inherent advantages of a structure-based approach to decipher specificity determinants at the protein-family level.