**4ba Systematic Approaches to the Protein Engineering of Highly Specific Receptor-Ligand Pairs**

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The ability to manipulate naturally occurring proteins to bind and respond to synthetic ligands in a manner independent, or orthogonal, from the influence of natural proteins and ligands, constitutes a significant challenge in protein engineering. Such a tool has important utility in the creation of gene switches for the control of heterologous gene expression in numerous applications such as gene therapy, metabolic engineering and tissue engineering. To date, there are only a handful of examples of engineering proteins with significantly altered ligand specificity. Many of these examples do not involve a systematic engineering approach, and therefore cannot be readily generalized to the engineering of new specific receptor-ligand pairs. Here, we report the development and application of two strategies for engineering proteins with significantly altered ligand selectivity. The first approach involves systematic, stepwise site saturation mutagenesis of individual ligand-contacting protein residues. The second utilizes a novel library creation method for the directed evolution of proteins, involving the simultaneous randomization of multiple key protein sites. It is envisioned that the described technologies could provide powerful, broadly applicable tools for engineering receptors/enzymes with improved or novel ligand/substrate specificity.