Adrenergic Receptors: Model Systems for Investigation of GPCR Structure and Function

Thesis by

Heather L. Wiencko

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"I may not have gone where I intended to go, but I think I have ended up where I needed to be."

– *Douglas Adams*

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Abstract

Membrane proteins mediate intercellular communication through a wide variety of modes, resulting in changes in the membrane and within the cell itself. One superfamily of integral membrane proteins, G-protein coupled receptors (GPCRs), are responsible for a vast diversity of processes including three of the five senses: sight, smell, and taste. GPCRs comprise 4% of the human genome but are disproportionately represented as pharmaceutical targets: over 50% of the best selling drugs target some member of this superfamily, mitigating the effects of diseases ranging from hypertension to schizophrenia. These receptors exist in equilibrium between their active and inactive states, and either of these states may be stabilized by the binding of an extracellular stimulus that may be either a small molecule or a peptide. The active state of the receptor triggers a response from the associated G-protein, which then controls the release of a second messenger within the cell that initiates other downstream processes. The ubiquity of GPCRs in key biological processes makes them both an attractive target for drug development and a challenge for selective drug design. Their conformational flexibility and membrane environment pose challenges for direct structural characterization, and to date only five of the more than 1,000 known GPCRs have been characterized by

high-resolution crystallography.

The nine adrenergic GPCRs mediate the stress response throughout the body, and are implicated in diseases including hypertension and asthma. While they are among the best studied subtypes of GPCRs, much remains to be learned about selectivity and activation. The first section of this work describes the *ab initio* structure prediction of the turkey β1 receptor and validation using a series of stabilizing mutations. This work preceded the currently available turkey β1 structure, but shows good agreement, especially in the binding site. It validates the latest methods developed for GPCR structure prediction, emphasizes the role of a neutral charge scheme in energy determination, and explores a structure validation strategy based on stabilizing mutations rather than ligand docking. The next section uses the experimental crystal structure as a starting point for nanosecond timescale molecular dynamics, exploring the roles of ligand binding in helix movement that contribute to the transition to an active state. These simulations reveal the early steps in receptor activation, beginning with tilting motions of transmembrane helices 5 and 6 and movement of transmembrane helix 1 closer into the protein core. The last section also uses newly available crystal structures as a starting point, and builds homology models of the human adrenergic receptors for which there are not yet crystal structures. The receptors most closely related to the target structures show the best results, while the less related ones will require further refinement. The best structures provide insight into the binding site of subtype selective antagonists, and can serve as the foundation for future studies.

The central idea of this thesis is that theory and experiment can and must work in concert, with the findings from one propelling advances in the other in the mutual pursuit of knowledge. The methods developed in the course of this work are applied to systems with a great deal of experimental knowledge, but may be applied to those that have been less thoroughly characterized. Over the course of these explorations, new subtleties in adrenergic structure have been illuminated, and may drive further exploration into selective binding and the activation mechanism of these and other receptors.

Contents

List of Tables

List of Figures