78f Cell-Specific and Ligand-Specific Parameters Affect Ligand Efficacy in a Kinetic Model of G Protein Coupled Receptor Signaling

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G protein coupled receptors (GPCRs) are transmembrane proteins that are involved in a variety of physiological functions (e.g. immune responses, pain, emotion and memory). Upon ligand binding, receptors change conformation to allow for G protein binding and activation. The cubic ternary complex model (cTCM) is a thermodynamically complete equilibrium model that has been widely used to describe how GPCRs in different conformations induce signaling (Weiss et. al., J. Theor. Biol. 178:169, 1996). However, kinetic models are necessary to understand many short-term GPCR-induced responses such as intracellular cAMP and Ca^{2+} activation as well as rapid cellular behaviors such as migration and phagocytosis.

Starting with the cTCM we develop a kinetic model that additionally incorporates other receptor phenomena such as G protein activation and receptor desensitization. As is common in large signal transduction networks, there are significant uncertainties in model parameter values and it is difficult to intuit model behavior. We use Latin Hypercube Sampling (LHS) (Blower and Dowlatabadi, Int. Stat. Rev. 62: 229, 1994) to efficiently sample the parameter space. Partial rank correlation coefficients are used to quantify model sensitivity to these variations in inputs and to identify parameters that are most likely to influence model behavior. Response generation in this system is quantified as positive, neutral, or negative changes in G protein activation upon ligand binding. In this way we directly relate our results to pharmacological classifications of ligand efficacy, positive, neutral, and inverse agonism.

In this work we focus on identifying particular regimes of model behavior, i.e. positive, neutral, and inverse agonism, and which parameters are critical to determining such behavior. Although drug development focuses on altering ligand-specific parameters, we find that both ligand-specific and cell-specific parameters play critical roles in determining the character of the response. Additionally, the time at which the response is measured (i.e. prior to or after steady state is established) greatly determines the response characteristics and thus the characterization of ligand efficacy in our model. Not surprisingly, we find that the ligand-specific parameter most important for response generation is the effectiveness to which the ligand induces an active receptor conformation. The efficacy of the ligand, however, is influenced by several cell-specific parameters including receptor and G protein expression, the receptor-G protein coupling efficiency, and the relative expression of active and inactive receptor species. For example, increasing G-protein concentration over the physiological range while receptor number remains constant can change a ligand from a negative to a positive agonist or vice versa depending on ligand characteristics. This latter finding is significant in that receptor and G protein expression levels are routinely varied using molecular biology techniques and can be experimentally measured.

In summary, by quantifying model sensitivity to parameter variation we demonstrate that not only ligand-specific but also cell-specific parameters may determine the character of ligand efficacy. Over or under expression of receptor and G protein or expression of different isoforms of G proteins may give very different results than those expected in endogenous systems. As large signaling databases (SigPath, Alliance for Cell Signaling, NCGR's PathDB) become populated with data and as more complex models of signaling pathways become possible it will become increasingly important to systematically assess parameter uncertainty and quantify regimes of model behavior.