Nonequilibrium steady states can generate apparent ligand-receptor binding heterogeneity

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***Short Abstract* — Ligand-receptor interaction often exhibits heterogeneity in binding affinity, which is usually detected by observation of a curvilinear Scatchard plot. We show that a simple ligand-receptor binding model with nonequilibrium steady states may account for the apparent ligand-receptor heterogeneity. This model can be used to reveal bioenergetic origins of apparent ligand-receptor binding heterogeneity.**

***Keywords* — Ligand-receptor binding. Nonequilibrium steady state. Cooperativity. Cell signaling.**

I. Introduction

Ligand-receptor interaction is essential for initiating cellular signaling and is a focus of much biochemical research and drug discovery efforts. Heterogeneity in ligand-receptor binding affinity is ubiquitous; this heterogeneity manifests as a curvilinear Scatchard plot [1]. Heterogeneity is commonly explained by two or more independent pools of receptor sites or by allostery [2]. Spatial variation in receptor density has also been offered as a potential cause [3]. All of these explanations assume that a ligand-receptor system reaches thermodynamic equilibrium, despite biochemical reactions in live cells being known to operate far from equilibrium. Here, we present a simple analytical model with non-equilibrium steady states; analysis of the model shows that energy-driven reactions affecting ligand-receptor binding can lead to binding heterogeneity.

II. Results

We model receptor dynamics using a four-state Markov chain. In the model, when free, a receptor is allowed to assume one of two interconverting conformations, "inactive" or "active," with different affinities for a ligand. The model also includes two bound receptor forms, which also convert between each other. These two conformations can be considered to represent "native" and "induced" conformations. This model resembles the single-site Monod-Wyman-Changeux model. However, we do not make the assumption of equilibrium. In general, the model reaches a non-equilibrium steady state, which results in a net flux around the reaction loop of the four-state model. Such a net flux at steady state can be sustained by an energy source or a chemical potential difference. For instance, the net flux could be maintained by ligand-induced receptor modification cylces, such as receptor phosphorylation and dephosphorylation, which are driven by constant chemical potentials of ATP, ADP and P_i maintained by the cell. We can obtain an analytical expression for the apparent dissociation constant K_d as a function of the free ligand concentration $[L]$ [4]:

$$K_d = \frac{a + b[L]}{c + [L]}, \qquad (1)$$

where the constants a, b and c are functions of the kinetic parameters of the four-state model. The system relaxes to equilibrium when parameters in the model obey detailed balance, such that $a/c=b$ and K_d is independent of $[L]$ (as in a linear Scatchard plot). Otherwise, K_d ranges from a/c to b as the ligand concentration increases from zero to the saturation level. In other words, the Scatchard plot can become curvilinear, either concave-up or concave-down, depending on parameter values.

III. Conclusion

Ligand recognition by a receptor and downstream information processing by cellular signaling molecules typically involves bioenergetic reactions. Our model illustrates how energy-driven receptor reactions, such as receptor phosphorylation and dephosphorylation reactions mediated by kinases and phosphatases, may influence ligand-receptor interaction and produce apparent binding heterogeneity. This mechanism should not be considered mutually exclusive of other mechanisms that can give rise to a curvilinear Scatchard plot. The potential influence of energy-driven reactions on ligand-receptor binding seems to have been systematically overlooked and re-evaluation of binding data using nonequilibrium models may be prudent.

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