**Cooperativity transitions driven by higher-order oligomer formations in ligand-induced receptor dimerization**

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While cooperativity in ligand-induced receptor dimerization has been linked with receptor-receptor couplings via minimal representations of physical observables, effects arising from higher-order oligomer (e.g., trimer and tetramer) formations of unobserved receptors have received less attention. Here, we propose a dimerization model of ligand-induced receptors in multivalent form representing physical observables under basis vectors of various aggregated receptor-states. Our simulations of multivalent models not only reject Wofsy-Goldstein parameter conditions for cooperativity, but show higher-order oligomer formations can shift cooperativity from positive to negative.

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**Introduction.** Collective behavior is a phenomenon common in human, animal, cellular, and biomolecular systems. Despite varying significantly in terms of the type and composition of biological components, fundamental dynamical properties allow collectives to exhibit rapid or gradual responses in complex environments. For example, group behaviors of wild baboons have been precisely linked to characteristic “S”-shaped (or sigmoid) response curves via pairwise interactions of subgroups [1]. The dynamics of animal groups also exhibit parallels with collective behaviors among biomolecules in living cells, such as the process by which hemoglobin binds to oxygen [2–5]. Thus, by abstracting structural details of biomolecules and relating biochemical interactions directly to mathematical networks, we can consider key insight regarding collective behavior and likely demonstrate various aspects of biomolecular binding systems.

Collective biomolecular behavior is generally referred to as cooperativity, its main functions being to allow biomolecular binding systems to exhibit either positive or negative sigmoid responses [2–5]. For example, a conformational change in proximal and distal regions of hemoglobin complex enables efficient transport of oxygen between the lungs and tissue, exhibiting positive cooperativity: steeper sigmoid (or switch-like) responses with a threshold in a concentration range of stimuli [2–5]. Receptor systems coupling to G-proteins can, however, display more gradual sigmoid curvature, achieving less decisive but also less restricted with respect to a wide concentration range of signaling molecules [2–4]. Such gradual cooperative responses are known as negative cooperativity.

In standard systems biology approaches, the fundamental rules governing cooperativity in living cells can be investigated by mapping and analyzing biomolecular networks and their parameter conditions. A key challenge of analyzing network models is finding meaningful and nonintuitive effects. Using data-driven (or inductive) modeling approaches, network models are generally constructed with various biochemical parameters (e.g., equilibrium binding constants) but restricted to observable components (or states) imposed by experimental techniques (e.g., live-cell imaging via biomolecules tagged with fluorescence emitters). The network models can lead to the parameter conditions exhibiting either positive or negative cooperativity. It is often overlooked, however, that these conditions can vary greatly by incorporating realistic but unobserved components (or states) into the network models. For example, the Monod-Wyman-Changeux (MWC) model that describes allosteric regulations of proteins always exhibits positive cooperativity; nevertheless, modifications in the scale of protein’s conformational changes proposed by Koshland, Nemethy and Filmer, offer parameter regions that allow negative cooperativity [2, 6–8]. By introducing hidden components (or states) in the network models, cooperativity can shift from positive to negative (or negative to positive). Such cooperativity transitions thus hinder physical interpretations of the cooperativity extracted from the data-driven modeling approaches.

Collective behavior of cell-surface receptors is a key function for enabling the efficient transduction of biochemical signals to cellular interiors. Prior experimental studies have explored the origin of negative cooperativity in dimer formations for equilibrium binding of ligands to cell-surface receptors, mainly using the simplest dimerization model formulated by Wofsy and Goldstein [9–18]. While this dimerization model predicts the parameter conditions that give rise to negative cooperativity, effects arising from higher-order oligomer (e.g., trimer and tetramer) formations of unobserved receptors have received less attention. In this article, we consider dimen-
ligands with receptors, the rates of association ($k_i$ in the dimerization model. In first-order interactions of observed states. There are $N$ vectors in the formation of nulls ($Φ \times Φ'$, $M / M'$ and $D'$ are the observed receptor-state vectors in the formation of nulls, monomers and dimers, respectively. Each observed receptor-states are represented under basis vectors of various aggregated receptor-states. $k_i$ and $d_i$ are the association and dissociation rates of the $i$-th index, respectively. $L$ is the ligand concentration.

Matrices can be written in the form of

$$k_{x0} = k_{x0} F_{x0}, \quad k_{x1} = k_{x1} F_{x1}, \quad k_{x2} = k_{x2} F_{x2} \quad (2)$$

where $k_{xi}$ and $F_{xi}$ are the receptor-receptor association rate and $N \times N$ scaling matrices of the $i$-th index, respectively.

For convenience, we redefine the dimensionless lumped parameter that constrains the fraction of dimer formations in the absence of ligands. The lumped parameter can be rewritten in the matrix form of

$$k_x = k_x F_{x0} \quad (3)$$

where $k_x$ is the dimensionless lumped parameter originally defined in the WG formulations [9].

**Multivalent models.** We construct monovalent ($N = 1$) and bivalent ($N = 2$) cell-models of ligand-induced receptor dimerization. We then use the E-Cell platform [19] [20] to simulate the cell-models of biological fluctuation that arise from stochastic changes in the cell surface geometry, number of receptors, ligand binding, molecular states, and diffusion constants. These cell-models assume that the non-diffusive receptors are uniformly distributed on a flat cell-surface measuring 100 $\mu$m and 100 $\mu$m in the horizontal and vertical axes. We also assume that the total receptor concentration, binding affinity and dissociation rates for each interaction are given by $T = 4.977$ #receptors/$\mu$m$^2$, $K_0 = 1.00$ nM, $d_0 = 0.01$ sec$^{-1}$, $d_1 = 10^{-5}$ sec$^{-1}$, $d_2 = 1.00$ sec$^{-1}$, and $d_{x0} = d_{x1} = d_{x2} = 1.00$ sec$^{-1}$. The relation of the local equilibrium constants to the association and dissociation rates is also given by $K_i = d_i / k_i$ where $i = 0, 1, 2, x0, x1, x2$. In a concentration range of ligand stimuli from $10^{-4}$ to 100 nM, we run model-simulations for a period of 100,000 sec to verify the complete convergence of receptor response to full equilibrium.
The scaling factor and matrices in the monovalent model are given by \( k_x = T/K_{x0} \) and \( F_{x0} = F_{x1} = F_{x2} = \alpha \). In the bivalent model, we assume that the symmetric scaling matrices can be written in the form of

\[
F_{x0} = F_{x1} = \left( \begin{array}{c} \alpha \\ \gamma \sqrt{\alpha \beta} \end{array} \right), \quad F_{x2} = \left( \begin{array}{c} 0 \\ 0 \end{array} \right)
\]

where \( \alpha, \beta \) and \( \gamma \) are matrix elements. \( \gamma \) must be less than unity to satisfy the positive definite condition. The second-order interactions forming oligomers (e.g., dimers and trimers) in the null and monomeric observables are given by

\[
\Phi^\dagger \mathbf{k}_{x0} \Phi \quad \text{(5)}
\]

\[
\mathbf{M}^\dagger \mathbf{k}_{x1} \Phi \quad \text{(6)}
\]

where \( \Phi^\dagger \) and \( \mathbf{M}^\dagger \) represent dual vectors of the null and monomeric observables, respectively. In these formulations, the second-order interaction of the null observables can exhibit null dimers, trimers and tetramers: \( r + r \rightarrow rr, \ r + rr \rightarrow rrr \) and \( rr + rr \rightarrow rrrr \). Monomeric dimers, trimers and tetramers can be also formed through the second-order interactions between the null and monomeric observables: \( R + r \rightarrow Rr, \ rR + r \rightarrow rRr \), \( R + rr \rightarrow Rrr \) and \( rR + rr \rightarrow rRrr \). There is no dimeric trimers and tetramers defined in the bivalent model.

The WG formulation \[9\]. The network diagram of the WG dimerization model is equivalent to that of the monovalent model. The dimerization process was, however, formulated under a “special” assumption that the local equilibrium constant of direct receptor-receptor (or second-order) interaction \( (K_{x0}) \) is independent of first-order interactions of receptors associated with ligands \( (K_0, K_1, K_2) \). The total cell-surface receptor concentrations and the number of ligand-induced oligomers per a unit surface-area are given by

\[
T = (1 + LK_0)X + K_{x0}(1 + 2LK_1 + L^2K_1K_2)X^2 \quad \text{(7)}
\]

\[
B = LK_0X + K_{x0} \left( LK_1 + \frac{1}{2}L^2K_1K_2 \right)X^2 \quad \text{(8)}
\]

where \( X \) and \( L \) are the concentration of unbound receptors and ligand concentration input in nM, respectively. The 1/2-factor in the third term of Eq. \[8\] is meant to count the dimers as single molecules. Unit representations of local equilibrium constants in this formulation are not consistent with the units in our multival lent formulation, thereby requiring a unit transformation: \( K_i \rightarrow K_i^{-1} \) where \( i = 0, 1, 2, x0 \).

This WG formulation leads to a parameter condition that approximately exhibits negative cooperativity. The condition can be written in the form of

\[
\frac{K_1(K_1 - K_2)}{(K_1 - K_0)^2} \geq \sqrt{1 + 4k_x - 1} \quad \text{(9)}
\]

where \( k_x = TK_{x0} \) is the dimensionless lumped parameter. This relation implies that the model always exhibits positive cooperativity if \( K_1 = K_2 \).

**FIG. 2. Model comparison.** We compare the binding curves and Scatchard plots among the three models assuming \( K_1 = K_2 = 100K_0 \): WG formulation, monovalent model \( (\alpha = 1) \), and bivalent model \( (\alpha = \beta = 1, \gamma = 0) \), for \( k_x = 0.01 \) (a,b), \( k_x = 0.10 \) (c,d), \( k_x = 0.30 \) (e,f). The solid and dashed black lines represent the response curves for the monovalent model and the WG formulation, respectively. Black crosses represent the bivalent model.

**Model comparison.** Cooperative effects can be generally seen in the concavity of the Scatchard plots. We compare the Scatchard plots among the three cell-models configured to have same parameter values. Model differences can be clearly seen in Figure 2a,c,e. In standard systems biology approaches, the Hill function can be fitted to the binding curves to quantify the cooperative characteristics in the cell-models. The Hill function

\[
\text{Hill function: } \frac{n[L]}{n[L] + K} \quad \text{(10)}
\]
can be generally written in the form of
\[ B(L) = \frac{B_0 L^n}{K_A^n + L^n} \]  
(10)
where \( L \), \( B_0 \), \( K_A \) and \( n \) represent ligand concentration, maximum area-density of the ligand-induced oligomers, ligands occupying half of the oligomers and the Hill coefficient, respectively. If the Hill coefficient is less than unity \((n < 1)\), then the receptor system exhibits negative cooperativity. If \( n > 1 \), then cooperativity is positive. There is no cooperativity if \( n = 1 \).

For a fixed \( k_x = 0.10 \), the best fit Hill coefficients of the monovalent model are mapped as a function of \( K_1 \) and \( K_2 \). In Figure 3(a), the cooperativity mapping result is shown and compared with the WG condition given by Eq. (9). This comparison result clearly shows suppression of the negative cooperative region in the monovalent model, implying inconsistent cooperative responses between the monovalent model and the WG formulation.

**Diagonalization.** To see the cooperative effects that arise from the second-order interactions forming the higher-order oligomers in the bivalent model [see Eq. (5) and (6)], we diagonalize the lumped parameter matrix \( \mathbf{k}_x \). Eigenvalues are given by
\[
\lambda_{\pm} = \frac{k_x}{2} \left( \alpha + \beta \pm \sqrt{(\alpha - \beta)^2 + 4\gamma^2 \alpha \beta} \right) = \lambda_0 \pm \Delta 
\]  
(11)
where the dynamic range is \( \gamma \lambda_0 \leq \Delta \leq \lambda_0 \).

We compare cooperative responses between the monovalent and bivalent models when the eigenvalues of the lumped parameter matrix in the bivalent model are identical \( \lambda_+ = \lambda_- \). Figure 3(b) shows cooperativity transition of the bivalent model as a function of the \( \lambda_0 \) component in Eq. (11). While cooperativity is always positive in the monovalent model (black line), cooperativity in the bivalent model is shifted from positive to negative through the increase of \( \lambda_0 \) (red line). In the lower \( \lambda_0 \)-range, the second-order couplings are weakly linked with cooperative responses, displaying identical cooperativity between the monovalent and bivalent models. The higher \( \lambda_0 \)-values in the bivalent model can, however, increase the number of trimers \((rrR)\) and tetramers \((rrrr)\) in the monomeric observable \( \mathbf{M}' \). Also, these higher-order oligomers are weakly linked with the first-order couplings that represent ligand-dissociation \((rrR \rightarrow rrr, rrrR \rightarrow rrrrr)\) and -association \((rrR \rightarrow rrR, rrrR \rightarrow rrrRR)\). Because of these model parameter relations, the bivalent model exhibits the transition of cooperativity in the higher \( \lambda_0 \)-range.

We also evaluate cooperative responses in the bivalent model in the case of differing eigenvalues \( \lambda_+ \neq \lambda_- \). Figure 3(c) shows cooperativity of the bivalent model as a function of the \( \lambda_0 \) and \( \Delta \) components in Eq. (11). As the ratio of these components converges to unity \( \Delta/\lambda_0 \rightarrow 1 \) \( (\alpha = 0) \), the bivalent model becomes equivalent to the monovalent model, exhibiting positive cooperativity in the full \( \lambda_0 \)-range. While cooperativity is always positive at \( \Delta/\lambda_0 = 1 \) \( (\beta = 0) \), the trimer and tetramer formations in the monomeric observable can change cooperativity from positive (red region) to negative (blue region) for \( \Delta/\lambda_0 < 1 \) \( (\beta > 0) \).

Our results may have biophysical implications in data-driven modeling approaches. Many biomolecular binding processes have been modeled under the scenario that the second-order couplings are weakly linked with cooperative effects. For example, the dimerization model that describes heregulin (HRG) binding with ErbB receptors in living cells was constructed using fluorescence microscopy images via HRG tagged with tetramethylrhodamine [13]. In this model, the first-order coupling representing a conformational change of receptor complex in the monomeric...
observable ($rR \leftrightarrow r'R$) plays a key role that gives rise to negative cooperativity. Nevertheless, there exist various aggregated receptor-states hidden in the physical observables. These states are realistic but unobserved through the fluorescent imaging techniques. By incorporating the second-order interactions forming higher-order oligomers that can include unobserved receptors into the dimerization model, cooperativity may shift from positive to negative, and vice versa. Because of these transitions, the data-driven modeling approach is not a straightforward method to identify physical sources giving rise to negative cooperative effects in the receptor systems.

**Conclusion.** In this article, we explored the origin of negative cooperativity in dimer formations for equilibrium binding of ligands to cell-surface receptors, in terms of biochemical parameters for associations and dissociations. While receptor-receptor couplings in the ligand-induced receptor dimerization have been previously linked with cooperativity via minimal representations of physical observables, cooperative effects that arise from the mixture of various aggregated receptor-states hidden in each observed receptor-states have received less attention. In particular, we examined the cooperativity of monovalent and bivalent models. Our results from model simulations showed the suppression of negative cooperative regions in the monovalent model, thereby implying violation of parameter conditions expected from the WG formulation. We also demonstrated that the presence of higher-order oligomer formations in the bivalent model leads to the transition of cooperativity from positive to negative, thus affecting physical interpretations of the cooperativity extracted from data-driven modeling approaches. Furthermore, it is interesting to extend our state-vector representations to more general model frameworks by incorporating heterodimer formations of receptor-family members (e.g., dimerization of ErbB3 and EGF receptors [21, 24]).

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