Multinary complex formations in GPCR signaling activations

Masaki Watabe,\textsuperscript{1} Hideaki Yoshimura,\textsuperscript{2} Satya N. V. Arjunan,\textsuperscript{1,3} Kazunari Kaizu,\textsuperscript{3} and Koichi Takahashi\textsuperscript{1,4}

\textsuperscript{1}Laboratory for Biologically Inspired Computing, RIKEN Center for Biosystems Dynamics Research, Saita, Osaka 565-0874, Japan
\textsuperscript{2}School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
\textsuperscript{3}Lowy Cancer Research Centre, The University of New South Wales, Sydney, Australia
\textsuperscript{4}Institute for Advanced Biosciences, Kanto University, Fujisawa, Kanagawa 252-8520, Japan

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Eukaryotic cells transmit extracellular signal information to cellular interiors through the formation of a ternary complex made up of a ligand (or agonist), G-protein, and G-protein coupled receptor (GPCR). Previously formalized theories of ternary complex formation have mainly assumed that receptors can only take the form of monomers. Here, we propose a multinary complex model of GPCR signaling activations via the formations of various aggregated receptor states. Our results from model simulations imply that the receptor aggregation processes can govern the signaling activity in a regime inaccessible by previous theories. In particular, we show how the affinity of ligand-receptor binding can be largely varied by various oligomer formations in the low concentration range of G-protein stimulus. More broadly, our work provides concrete modeling principles to explore general relations between receptor aggregation and various signaling properties.

Introduction.— G-protein coupled receptors (GPCR) in eukaryotic cells form a remarkable modular system over the cell membrane, its main function being to provide cells with a wide means of signal communications between extracellular molecules (e.g., hormones and neurotransmitters) and intracellular signaling G-proteins. GPCR signal communication can be achieved through conformational changes in receptors, as well as the complex formation with three different components: a ligand (or agonist), G-protein, and GPCR. This complex formation serves as an activated signaling component, accordingly changing the affinity of ligand-receptor binding as a function of G-protein stimulus. These ideas have been formalized into mechanistic theories of G-protein stimulus, termed ternary complex models \cite{1,2}, following in particular, the assumption that receptors can only take the form of monomers (see Figure 1).

Thousand of GPCRs diffuse on the cell membrane, randomly interacting with each other and spontaneously forming oligomers such as dimers and trimers. Such receptor aggregation may, for example, extend the colocalization period of the ternary complex, amplifying signaling activity \cite{3}. Likewise, in a wide concentration range of ligand stimulus, group behavior such as cooperativity induced by receptor dimerization may be constrained by the affinity of higher-order oligomer formations \cite{4,5}. Recent studies analyzing receptor aggregation have shown various novel aspects of receptor systems, implying modifications to ternary complex models \cite{3,4,5}. This lies in contrast to previous theories which have mostly focused on scenarios where receptor-receptor couplings (i.e., direct interaction of two receptors) are weakly linked with GPCR signaling activations.

In this letter, we propose a multinary complex model of GPCR signaling activations via dimer formations of ligand-bound receptors, represented by a multivalent form of physical observables under basis vectors of various aggregated receptor states (see Figure 2). We perform model simulations to explore the functional role of receptor aggregation in GPCR signaling activations. Crucially, we show how a mixture of various aggregated receptor states can lead to the transition of ligand-receptor binding affinity in a regime which cannot be predicted by ternary complex models. We finally discuss that such receptor aggregation is a phenomenon common in various biological cells, and also of relevance more broadly beyond GPCR signaling activation. Our work suggests new avenues for extracting general relations between receptor aggregation and various signaling properties.
**Model framework.** — In the receptor state vector representation of physical observables, the multiary complex model is described by a function containing the probabilities of biochemical interactions that form various aggregated receptor states. All possible aggregated receptor states via dimer formation of ligand-bound receptors can be treated mathematically as basis vectors in a multidimensional real vector space.

First, we assume that the observed state vectors of receptors are represented in terms of ligand-bound and unbound receptors. Figure 2 shows network diagrams of the multiary complex model. Null ($\Phi$, $\Phi'$), monomeric ($M$, $M'$) and dimeric ($D'$) observed state vectors of receptors are given by

$$\Phi = \begin{pmatrix} r \\ r r \\ \vdots \\ r N \end{pmatrix}, \quad M = \begin{pmatrix} R \\ R r \\ \vdots \\ R r^{N-1} \end{pmatrix}, \quad \Phi' = \begin{pmatrix} r \cdot r \\ r \cdot r r \\ \vdots \\ r \cdot r^{N} \end{pmatrix},$$

$$M' = \begin{pmatrix} R \cdot R \\ R \cdot R r \\ \vdots \\ R \cdot R r^{N-1} \end{pmatrix}, \quad D' = \begin{pmatrix} R \cdot R \cdot R \\ R \cdot R \cdot R r \\ \vdots \\ R \cdot R \cdot R r^{N-1} \end{pmatrix}$$

where $r$, $R$ and $G$ represent the receptors, the ligand-bound receptors and G-proteins, correspondingly. $N$ refers to the number of receptors that can be aggregated in the $\Phi$ and $M$ observed states. There are $N^2$ elements in the $\Phi'$, $M'$, and $D'$ observed states.

Null ($G \cdot \Phi$, $G \cdot \Phi'$), monomeric ($G \cdot M$, $G \cdot M'$) and dimeric ($G \cdot D'$) observed state vectors of the G-protein-bound receptors are also given by

$$G \cdot \Phi = \begin{pmatrix} G \cdot r \\ G \cdot r r \\ \vdots \\ G \cdot r^{N} \end{pmatrix}, \quad G \cdot M = \begin{pmatrix} G \cdot R \\ G \cdot R r \\ \vdots \\ G \cdot R r^{N-1} \end{pmatrix},$$

$$G \cdot \Phi' = \begin{pmatrix} G \cdot r \cdot r \\ G \cdot r \cdot r r \\ \vdots \\ G \cdot r \cdot r^{N} \end{pmatrix}, \quad G \cdot M' = \begin{pmatrix} G \cdot R \cdot R \\ G \cdot R \cdot R r \\ \vdots \\ G \cdot R \cdot R r^{N-1} \end{pmatrix},$$

$$G \cdot D' = \begin{pmatrix} G \cdot R \cdot R \cdot R \\ G \cdot R \cdot R \cdot R r \\ \vdots \\ G \cdot R \cdot R \cdot R r^{N-1} \end{pmatrix}$$

where $G \cdot r$ and $G \cdot R$ represent G-protein-bound receptors. $N$ refers to the number of receptors that can be aggregated in the $G \cdot \Phi'$, $G \cdot M'$, and $G \cdot D'$ observed states.

In first-order interactions of a ligand, G-protein, and receptor, the rates of association ($k_{bi}$, $k_{ai}$, $k_{gi}$, $k_{bi}$) and dissociation ($d_{bi}$, $d_{ai}$, $d_{gi}$, $d_{bi}$) of the $i$-th index are represented by $N \times N$ diagonal matrices acting upon the

![FIG. 2. Network diagrams of multiary complex formation via ligand-bound receptor dimerization.](image)
The Hill function can generally be written in the form of

generalized functions of receptors:

\[ M \text{ curves of the monomeric and dimeric observed state vec-} \]

\[ \text{torial receptor concentration,} \]

\[ \alpha \]

\[ \text{where} \]

\[ K \]

\[ \text{is the number of receptors, ligand binding, molecular} \]

\[ \text{states, and diffusion constants. These cell-models assume} \]

\[ \text{that non-diffusive receptors are uniformly distributed on} \]

\[ \text{the cell membrane. The source code of the multivalent} \]

\[ \text{cell-models is provided in the Supplemental Material} \]

\[ \text{17.} \]

\[ \text{In a concentration range of ligand stimulus from} \]

\[ 10^{-3} K_{00} \text{ to } 10^{3} K_{00}, \text{ we ran model simulations for a pe-} \]

\[ \text{riod of 100,000 sec to verify the complete convergence of} \]

\[ \text{receptor response to full equilibrium. To quantify cooper-} \]

\[ \text{ative characteristics in the multivalent cell-models, the} \]

\[ \text{Hill function can be fitted to the ligand-receptor binding} \]

\[ \text{curves of the monomeric and dimeric observed state vec-} \]

\[ \text{tors of receptors:} \]

\[ \text{M, G \cdot M, M', G \cdot M', D' and G \cdot D'.} \]

\[ \text{The Hill function can generally be written in the form of} \]

\[ B(L) = \frac{B_0 L^n}{L^n + K^m} \]

\[ \text{where} \]

\[ L, B_0, \text{ and } n \text{ represent ligand concentration, max-} \]

\[ \text{imum area-density of the ligand-bound receptor, and the} \]

\[ \text{Hill-coefficient, respectively;} \]

\[ K' \text{ denotes overall affinity of} \]

\[ \text{ligand-receptor binding as a function of G-protein stim-} \]

\[ \text{ulus from} \]

\[ 10^{-3} K_{00} \text{ to } 10^{3} K_{00}. \]

\[ \text{The model parameter values are given as follows: total} \]

\[ \text{receptor concentration,} \]

\[ T = 4.977 \text{ receptors/μm}^2; \]

\[ \text{cooperativity factor,} \]

\[ \alpha = K_{00}/K_{00} = K_{00}/K_{00}; \]

\[ \text{binding} \]

\[ \text{and dissociation rates for each first-order} \]

\[ \text{interactions,} \]

\[ K_{\ell_1} = K_{\ell_2} = 100 K_{00}, \]

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\[ K_{\ell_1} = K_{\ell_2} = d_\ell_1 = d_\ell_2 = d_{g0} = d_{g1} = d_{a0} = d_{a1} = d_{a2} = \]

\[ d_{g0} = d_{g1} = d_{g2} = d_{a3} = 1.00 \text{ s}^{-1}; \]

\[ \text{and binding affinity and dissociation rates for each second-order} \]

\[ \text{interactions,} \]

\[ K_{\ell_0} = K_{\ell_1} K_{\ell_0} / K_{00}, \]

\[ K_{\ell_2} = K_{\ell_2} K_{\ell_2} K_{\ell_3} / K_{00}^2, \]

\[ d_{x0} = d_{x1} = d_{x2} = d_{y0} = d_{y1} = d_{y2} = d_{y3} = 1.00 \text{ s}^{-1}. \]

\[ \text{The local equilibrium} \]

\[ \text{constants of the association and dissociation rates of} \]

\[ \text{the} \]

\[ \text{index satisfy the relation} \]

\[ K_i = d_i/K_i. \]

\[ \text{We assume that the scaling factors of diagonal ele-} \]

\[ \text{ments in the first-order association} \]

\[ (k_{\ell i}, k_{a i}, k_{g i}, k_{b i}) \]

\[ \text{can be written in the matrix form of} \]

\[ k_{x i} = k_{x i} F_{x i}, \]

\[ k_{y j} = k_{y j} F_{y j} \]

\[ \text{where} \]

\[ i = 0, 1, 2 \text{ and } j = 0, 1, 2, 3; \]

\[ k_{x i, y j} \text{ and } F_{x i, y j} \text{ are} \]

\[ \text{the second-order association rates and} \]

\[ N \times N \text{ scaling} \]

\[ \text{matrices, respectively.} \]

\[ \text{For convenience, we define a dimensionless lumped pa-} \]

\[ \text{rameter that constrains the fraction of oligomer forma-} \]

\[ \text{tions in the absence of ligands. The lumped parameter} \]

\[ \text{that constrains the fraction of oligomer forma-} \]

\[ \text{tions in the first-order association} \]

\[ (k_{\ell i}, k_{a i}, k_{g i}, k_{b i}) \]

\[ \text{can be written in the form of} \]

\[ k_{x} = k_{x} F_{x0} \]

\[ \text{where} \]

\[ k_{x} = T/K_{x0}. \]

\[ \text{Multivalent cell-models.— To begin, we constructed} \]

\[ \text{multivalent} \]

\[ (N = 1 \sim 5) \text{ cell-models of multinary com-} \]

\[ \text{plex formations. We then used the E-cell system ver-} \]

\[ \text{sion 4 [10] to simulate cell-models of biological fluctua-} \]

\[ \text{tion that arise from stochastic changes in the cell surface} \]

\[ \text{geometry, number of receptors, ligand binding, molecular} \]

\[ \text{states, and diffusion constants. These cell-models assume} \]

\[ \text{that non-diffusive receptors are uniformly distributed on} \]

\[ \text{the cell membrane. The source code of the multivalent} \]

\[ \text{cell-models is provided in the Supplemental Material} \]

\[ \text{17.} \]

\[ \text{FIG. 3. Model comparison between the simplest ternary} \]

\[ \text{complex model (left column) and the monovalent cell-model} \]

\[ \text{(right column). (a) Ligand-receptor binding curve as a func-} \]

\[ \text{tion of ligand stimulus} \]

\[ L/K_{00}, \text{ is given by Eq. (7) where} \]

\[ G/K_{00} = 0.1, \alpha = 0.03 \text{ (blue), 0.3 (light-blue), } 1.0 \text{ (black} \]

\[ \text{dots), 3.0 (light-violet), and 30 (violet). (b) Overall affinity} \]

\[ \text{of ligand-receptor binding} \]

\[ (K' / K_{00}) \text{ as a function of G-protein stimulus} \]

\[ G/K_{00}, \text{ is given by the Eq. (8), for} \]

\[ G/K_{00} = 0.1 \text{ and} \]

\[ \alpha = 0.03, 0.3, 1.0, 3.0, 30. \]

\[ \text{(c) Colors represent the binding} \]

\[ \text{affinity of the ternary complex model and the monovalent cell-model,} \]

\[ \text{respectively.} \]
and dissociation rates \((d_{ri}, d_{si}, d_{bi}, d_{xi}, d_{xy})\) of the \(i\)-th and \(j\)-th indices are all unity. The scaling matrices of the second-order association rates \((k_{xi}, k_{yj})\) are also given by

\[
F_{xi} = F_{yj} = \frac{\mathcal{J}_{NN}}{N}
\]

where \(i = 0, 1, 2\) and \(j = 0, 1, 2, 3\); \(\mathcal{J}_{NN}\) represents all-ones matrix where every element is equal to one. In our model framework, the second-order interactions of receptors exhibit various oligomers (see Figure 2d): \(\Phi + \Phi \rightarrow \Phi', M + \Phi \rightarrow M'\) and \(M + M \rightarrow D'\). G-protein-bound oligomers are also formed through the second-order interactions between G-proteins and receptors (see Figure 2d): \(G \cdot \Phi + \Phi \rightarrow G \cdot \Phi', G \cdot M + \Phi \rightarrow G \cdot M'\) and \(G \cdot M + M \rightarrow G \cdot D'\). In the monovalent cell-model \((N = 1)\), there are various dimers via the second-order interactions: \(r + r \rightarrow rr\), \(R + r \rightarrow rR\), \(R + R \rightarrow RR\), \(Gr + r \rightarrow GrR\), \(Gr + r \rightarrow GrR\), \(Gr + r \rightarrow GrR\), \(Gr + r \rightarrow GrR\) and \(Gr + r \rightarrow GrR\). Moreover, the higher-order multivalent cell-models \((N = 2 \sim 5)\) also exhibit dimers, as well as higher-order oligomers; for example, trimers \((rrr, rRr, rRR, GrRr, GrrR\) and \(GrRr\)), tetrers \((rrrr, rrrR, rRrr, Grrrr, GrrrR\) and \(GrrRr\)) and pentamers \((rrrrr, rrrrr, rrrRR, Grrrrr, GrrrrR, GrrrrR\) and \(GrRrrR\)).

**The simplest ternary complex model** [1][2].— The network diagram of the multivalent complex model (see Figure 3) converges to that of the simplest ternary complex model (see Figure 1) as \(N = 1\) and \(k_x \rightarrow 0\). Overall ligand-receptor binding states \((R, GR)\) in the ternary complex model can be written in the form of

\[
B(L) = \frac{B_0 L}{L + K'}
\]

where \(L\) and \(B_0\) represent ligand concentration and maximum area-density of the ligand-bound receptor, respectively. Overall affinity of ligand-receptor binding as a function of G-protein stimulus is given by

\[
K' = K_{00} \left( \frac{1 + G/K_{g0}}{1 + G/(\alpha K_{g0})} \right)
\]

where \(\alpha\) is the cooperativity factor that satisfies the relation \(\alpha = K_{g0}/K_{00} = K_{00}/K_{g0}\). If \(\alpha = 1\), there is no affinity transition.

**Model comparison.**— To clearly see the effects arising from the second-order interactions of receptors, we compare the ligand-receptor binding curves between the simplest ternary complex model \((k_x = 0)\) and the monovalent cell-model \((k_x > 0\) and \(N = 1)\). Figure 3 clearly shows differences in the binding curves. In the simplest ternary complex model, the cooperativity factor \(\alpha\) in Eq. (8) plays a key role in largely varying the overall affinities of ligand-receptor binding in the high concentration range of G-protein stimulus (see Figures 3a, b, and c). The affinities of the binding curves shown in Figures 3a and b, can be decreased \((K' < K_{00})\) or increased \((K' > K_{00})\) as a function of the cooperativity factor. Such affinity transitions are also shown in Figure 3, varying from blue to red regions in the high \(G/K_{g0}\) range.

While there is no direct interaction of receptors in the simplest ternary complex model, the second-order association rate \((k_{x0})\) in the monovalent cell-model plays a key role to largely change the overall affinity in the low concentration range of G-protein stimulus (see Figure 3b, e and f). The affinity of the binding curves shown in Figure 3b and c, can be increased \((K' > K_{00})\) as a function of the lumped parameter \(k_x\). Such affinity shifts are also shown in Figure 3, represented by red colored region in the low \(G/K_{g0}\) range. In the absence of G-protein stimulus \((G \rightarrow 0)\), the overall network diagram of the multivalent complex model converges to the dimer formations of ligand-bound receptors (see Figure 2b). In particular, the dimer formations are modeled under a specific parameter condition that can exhibit positive cooperativity \((K_{00} = K_{00})\), increasing the affinity of ligand-receptor binding [4][5]. Because of these model parameter relations, the monovalent cell-model displays an affinity transition in the low \(G/K_{g0}\) range.

The affinity transitions can be also seen in the higher-order multivalent cell-models: bivalent \((N = 2)\), trivalent \((N = 3)\), tetravalent \((N = 4)\), and pentavalent \((N = 5)\). For \(\alpha = 1\) and \(G/K_{g0} = 10^{-3}\), Figure 4 shows the affinity transitions in multivalent cell-models as a function of the lumped parameter \(k_x\). While the affinity in the multivalent cell-models is always unity if \(k_x = 0\) \((K' = K_{00})\); black dashed line, the affinity can be increased through the increase of \(k_x\) \((K' > K_{00})\); colored lines). Also, affinity-splitting between the monovalent cell-model (red line) and higher-order multivalent

![FIG. 4. Transition of the overall affinity in the multivalent cell-models is shown as a function of the lumped parameter \(k_x\), assuming \(\alpha = 1\) and \(G/K_{g0} = 10^{-3}\). Each colored line represents the monovalent (red), bivalent (blue), trivalent (green), tetravalent (pink), and pentavalent (violet) models; the dashed black line denotes no receptor-receptor interactions of receptors.](image-url)
cell-models (blue, green, pink and violet lines) becomes apparent in the high $k_r$ range.

**Conclusion.**— Many diffusive GPCRs in the cell membranes randomly collide with each other, spontaneously taking the form of various oligomers such as dimers, trimers and tetramers. The functional role of receptor oligomerization (or aggregation) in GPCR signaling activations, however, has been elusive to date. In this letter, we constructed a multiary complex model to investigate biophysical effects arising from various aggregated receptor states in GPCR signaling activations. Our results from model simulations revealed that receptor oligomerization functions to largely vary the overall affinity of ligand-receptor binding in a regime which cannot be ruled by cooperativity factor in the simplest ternary complex model.

Furthermore, such receptor aggregation in the cell membranes is ubiquitous across various biological cells and of relevance more generally beyond GPCR signaling activations presented here. The function of oligomer formation through receptor-receptor interactions leads to the general questions: what is the relation between receptor aggregation and various signaling properties, e.g., the amplification and propagation of noisy signals [18, 19], and the physical limit and sensitivity to chemical concentration sensing in ligand-receptor binding [20,24]? Our work sheds light on these interesting questions from the perspective of theoretical biophysics, and suggests concrete modeling principles to explore general rules of receptor aggregation governing signaling activities and properties in various signal transduction systems.

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* Corresponding author; masaki@riken.jp
† Corresponding author; ktakahashi@riken.jp

[1] A. De Lean, J. M. Stadel, and R. J. Lefkowitz, A ternary complex model explains the agonist-specific binding properties of the adenyate cyclase-coupled β-adrenergic receptor., The Journal of biological chemistry 255, 7108–17 (1980).
[2] T. Kenakin, Theoretical aspects of GPCR-ligand complex pharmacology. [Chemical Reviews 117, 4-20 (2017)]
[3] T. Nishiguchi, H. Yoshimura, R. S. Kasai, T. K. Fujiwara, and T. Ozawa, Synergetic roles of Formyl Peptide Receptor 1 oligomerization in ligand-induced signal transduction, Private communication (2019).
[4] M. Watabe, S. N. V. Arjunan, W. X. Chew, K. Kaizu, and K. Takahashi, Cooperativity transitions driven by higher-order oligomer formations in ligand-induced receptor dimerization, Phys. Rev. E 100, 062407 (2019).
[5] C. Wofsy, B. Goldstein, K. Lund, and H. S. Wiley, Implications of epidermal growth factor (EGF) induced egr receptor aggregation., Biophys. J. 63, 98–110 (1992)
[6] C. Wofsy and B. Goldstein, Interpretation of Scatchard plots for aggregating receptor systems., Math. Biosci. 112, 115–54 (1992)
[7] M. Hiroshima, C.-g. Pack, K. Kaizu, K. Takahashi, M. Ueda, and Y. Sako, Transient Acceleration of Epidermal Growth Factor Receptor Dynamics Produces Higher-Order Signaling Clusters, Journal of Molecular Biology 430, 1386–1401 (2018)
[8] H. Guo, S. An, R. Ward, Y. Yang, Y. Liu, X.-x. Guo, Q. Hao, and T.-r. Xu, Methods used to study the oligomeric structure of G-protein-coupled receptors, Biosci. Rep. 37, BSR20160547 (2017)
[9] M. Hiroshima, Y. Sasaki, M. Okada-Hatakeyama, and Y. Sako, Dynamically varying interactions between heregulin and ErbB proteins detected by single-molecule analysis in living cells., Proc. Natl. Acad. Sci. U.S.A. 109, 13984–9 (2012)
[10] M. Hiroshima and Y. Sako, Regulation Mechanism of ErbB-Heregulin Interaction Shown by Single-molecule Kinetic Analysis in Living Cells., Biophys. Physicobiol. 53, 317–318 (2013)
[11] M. Yanagawa, T. Yamashita, and Y. Shichida, Comparative fluorescence resonance energy transfer analysis of metabotropic glutamate receptors: Implications about the dimeric arrangement and rearrangement upon ligand bindings. Journal of Biological Chemistry 286, 22971–22981 (2011)
[12] M. Yanagawa and Y. Shichida, Dimerization of G-protein-coupled receptors: Molecular mechanism of the interprotomer communication, Seikagaku 83, 949–956 (2011).
[13] G. Milligan, G protein-coupled receptor dimerisation: Molecular basis and relevance to function, Biochimica et Biophysica Acta - Biomembranes 1768, 825–835 (2007).
[14] Y. Teramura, J. Ichinose, H. Takagi, K. Nishida, T. Yanagida, and Y. Sako, Single-molecule analysis of epidermal growth factor binding on the surface of living cells, EMBO J. 25, 4215–4222 (2006).
[15] T. Uyemura, H. Takagi, T. Yanagida, and Y. Sako, Single-molecule analysis of epidermal growth factor signaling that leads to ultrasensitive calcium response., Biophys. J. 88, 3720–30 (2005)
[16] G. E. Breitwieser, G Protein-Coupled Receptor Oligomerization: Implications for G Protein Activation and Cell Signaling. Circulation Research 94, 17–27 (2004)
[17] M. Bai, Dimerization of G-protein-coupled receptors: Roles in signal transduction, Cellar Signalling 16, 175–186 (2004)
[18] K. Kaizu, K. Nishida, Y. Sakamoto, S. Kato, T. Niina, N. Nishida, N. Aota, M. Koizumi, and K. Takahashi, Ecell system version 4 (2019); W. X. Chew, K. Kaizu, M. Watabe, S. V. Munimady, K. Takahashi, and S. N. V. Arjunan, Surface reaction-diffusion kinetics on lattice at the microscopic scale. Phys. Rev. E 99, 1–16 (2019) W.-x. Chew, K. Kaizu, M. Watabe, S. V. Munimady, K. Takahashi, and S. N. V. Arjunan, Reaction-diffusion kinetics
on lattice at the microscopic scale, Phys. Rev. E 98, 1–24 (2018).

[17] See Supplemental Material at https://github.com/ecell/ecell4_docs/blob/master/en/examples/example13.ipynb for the source code of the multiary complex model.

[18] M. Ueda and T. Shibata, Stochastic signal processing and transduction in chemotactic response of eukaryotic cells, Biophysical Journal 93, 11–20 (2007).

[19] T. Shibata and K. Fujimoto, Noisy signal amplification in ultrasensitive signal transduction, Proc. Natl. Acad. Sci. U.S.A 102, 331–6 (2005).

[20] T. Mora and I. Nemenman, Physical Limit to Concentration Sensing in a Changing Environment, Physical Review Letters 123, 198101 (2019).

[21] K. Kaizu, W. de Ronde, J. Pajmans, K. Takahashi, F. Tostevin, and P. R. Ten Wolde, The berg-purcell limit revisited, Biophysical journal 106, 976–85 (2014).

[22] B. Hu, W. Chen, W. J. Rappel, and H. Levine, Physical limits on cellular sensing of spatial gradients, Physical Review Letters 105, 1–4 (2010).

[23] W. Bialek and S. Setayeshgar, Physical limits to biochemical signaling, Proc. Natl. Acad. Sci. U.S.A 102, 10040–5 (2005) arXiv:0301001 [physics].

[24] H. C. Berg and E. M. Purcell, Physics of chemoreception, Biophysical journal 20, 193–219 (1977).