Role of Fluctuations in Ligand Binding Cooperativity of Membrane Receptors

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(Received 20 August 2010; published 21 April 2011)

Signal transduction upon binding of a ligand to a membrane protein can occur not only via allosteric conformational changes but also through fluctuations. We report a numerical study on the influence of conformational fluctuations on the cooperativity of a binding reaction in a simple model of an integral membrane receptor consisting of transmembrane helices. We find that small fluctuations lateral as well as perpendicular to the membrane can increase the cooperativity, with the former more dominant. Moreover, too much fluctuation induces negative cooperativity. Proteins with fewer than four helices do not show positive cooperativity under any circumstances. This behavior is rather robust, and independent of the receptor topology or ligand size. Fluctuations measured in all-atom molecular dynamics simulations of a $G$-protein-coupled receptor fall within the predicted region of maximum cooperativity.

DOI: 10.1103/PhysRevLett.106.168103 PACS numbers: 87.16.Xa, 87.14.ep, 87.15.ak, 87.15.Ya

The functioning of transmembrane signaling proteins such as those belonging to the $G$-protein-coupled receptors (GPCRs) is typically explained by an allosteric conformational change upon binding of a signaling molecule (e.g., a hormone), which conveys environmental information to the inside of the cell, setting off signaling cascades further downstream that ultimately lead to a cellular response [1]. Usually the allosteric conformational change is envisioned as a switch between two (meta)stable structures of the receptor protein. However, while recently detailed structures of several GPCRs have become available [2], the molecular allosteric mechanism of GPCRs still remains elusive. As proteins are flexible molecules, the allosteric signaling process might also depend on a change in conformational fluctuations [3]. Such a possibility has been explored for DNA binding proteins [4–6]. In addition to experimental evidence that supports a more dynamical mechanism of GPCRs [7], molecular dynamics (MD) simulations have ruled out previously proposed static mechanisms that consider only conformational changes [8].

Any model of the working mechanism of GPCRs will contain both a specific and a generic part. The specific part depends on the details of the molecular interactions between protein and substrate, the generic part focuses on common features of all GPCRs, for instance, that they have 7 transmembrane helices (TMs). The present Letter focuses on such generic questions. Indeed, the fact that protein receptors are almost always built from several TMs connected by flexible linkers suggests that fluctuations might be important for this generic part. The flexibility of the receptor protein would then result in fluctuations in the position of these TMs with respect to membrane and to each other. The presence or absence of signal molecules may have a pronounced effect on the amplitude of these fluctuations. The hypothesis of this work is thus that a GPCR protein’s TM helices exhibit relative motion, and that it is this flexibility, rather than a conformational change, that causes signal transduction. We explore the consequences of this hypothesis by focusing on the cooperativity of the binding of the extracellular signal molecule and the intracellular guanosine diphosphate liganded $G$ protein. Note that we focus on fluctuations within a single membrane receptor, not on those due to relative motions of rigid proteins in a multireceptor complex. As we are interested in generic, rather than specific, aspects of GPCR-mediated signal transduction, we consider a highly simplified model, that only takes into account ligand binding and receptor flexibility but leaves out all detail on the atomistic scale. Such simple models, while disregarding atomistic details, can still provide essential physical insights in the underlying phenomena. Moreover, these models allow tuning the different types of fluctuations explicitly and separately, which is not straightforward to implement with MD simulations. Here, we consider two types of fluctuations: (1) the lateral flexibility of TMs within the membrane; (2) the flexibility of TMs normal to the membrane.

The model consists of $N_L = 2$ ligands, one extra and one intracellular, that can both bind to an integral receptor. The membrane is modeled as a surface of thickness $h_M$ impenetrable to ligands, positioned in the $x, y$ plane, at the center ($z = 0$) of a cubic box with box length $L$. The ligands are hard spheres of radius $r_L$, and thus are excluded from a region $|z| < r_L + h_M/2$. To restrict the ligands to their respective volume, the simulation box is periodic only in the $x, y$ direction. The receptor is a chain of TMs represented as $N_R$ rigid rods connected by springs that represent the linker sequences between the TMs and other TM-TM interactions. Each rigid rod consists of $n_R = 8$ adjacent hard spheres of radius $r_R = \sigma$, and thus has a length $h_R = 2n_R\sigma$. The TMs are kept perpendicular to the
membrane but are allowed to diffuse laterally and fluctuate perpendicularly. (In principle, rotational freedom could also be allowed, but here we keep the model as simple as possible.) Both symmetric and asymmetric geometry of intracellular or extracellular domains are considered by setting the size of ligands $r_L^{in} = r_L^{ex}$ and $r_R^{in} = 2r_L^{ex}$, respectively. The geometrical parameters of the model are chosen approximately proportional to their typical values in nature. Setting the unit of length $\sigma = 2.5 \text{ Å}$, the TM radius $r_R = 2.5 \text{ Å}$ corresponds roughly to the radius of a real $\alpha$ helix. A small ligand such as a hormone is modeled by a compact ring structure due to the constraints of membrane lipids [7]. We mimic this ring structure by adding an extra link between the receptor’s first and last TMs.

To investigate the influence of this topological constraint, both ring and string topology are studied. The number of TMs and string topology are studied.

For each of these systems we considered 5 values for the lateral fluctuation parameter $k_BT$, and 15 values for the perpendicular fluctuation parameter $k_BT$, ranging from 0.04$k_BT$ to $\infty$. The total number of different system settings is thus $32 \times 5 \times 15 = 2400$. For each of these settings we performed parallel tempering [10] runs consisting of 13 replicas, with $1 < \beta < 6$. A single replica ran $\sim 10^8$ cycles. Each cycle consisted on average of a trial move for every particle. A replica swap was attempted every 250 cycles. The acceptance ratio of the particle move and replica swap was $\sim 0.2$ and $\sim 0.8$, respectively.

Essentially, the current system can be regarded as a four state chemical reaction $s_0 \leftrightarrow s_1 \leftrightarrow s_2$, where the labels 0, 1, 2 denote the number of ligands bound to the receptor, $s_0$ and in denote extra and intracellular ligand, respectively. Cooperativity in binding is defined as the difference between the sum of the standard free energy differences of the substeps (binding to one ligand only) and the standard free energy difference between reactions and products [11,12] (also known as the allosteric free energy $\Delta G$ [3,4,6])

$\Delta G = \Delta G_{01a} + \Delta G_{01s} - \Delta G_{02a} \approx \Delta G_{01s}$. The standard is set by the volume (or pressure) of the simulation box. Using the relation between the free energy and the equilibrium constant $\beta\Delta G^0 = -\ln K$ it immediately follows that $K_{ij} = k_BT \ln (\frac{p_{ij} \rho_{ij}}{p_{ij} \rho_{ij}})$. Where $K_{ij} = p_{ij} / p_i$ is the equilibrium constant of subreactions $s_i \leftrightarrow s_j$, and $p_i$ denotes the population of $s_i$. $c > 0$ and $c < 0$ indicate positive and negative cooperativity, respectively. The populations $\rho_{ij}$, $P_{ij}$, $P_1$, $P_2$ can be extracted directly from the MC simulations. Since the ligand-TM attraction is modeled by a square well, we can simplify the analysis by combining the number of ligand-TM bonds $n$ for each ligand into a “binding pattern” of 2 numbers $n_{ex}, n_{in} \in [0...N_R]$. The probability to find a certain pattern can then be summarized in a $(N_R + 1) \times (N_R + 1)$ matrix $Z$. Here entry $Z_{ij}$ for index $
For all cases, as for asymmetric ligands and three types of lateral fluctuations, the topology receptor with asymmetric ligands as a function of

\[ \frac{1}{n} \]

on average to moderate perpendicular fluctuations. Cooperativity over inverse temperature depends on the fluctuation parameters. Averaging the first result populations and corresponding free energy for a specific parameter setting.

The cooperativity \( c(\beta) \), while a function of \( \beta \), is relatively constant over the range of \( \beta \), but much more dependent on the fluctuation parameters. Averaging the cooperativity over inverse temperature \( \langle c \rangle_\beta \) allows compressing the simulation results into two-dimensional cooperativity landscapes as a function of the number of TM \( N_R \) and the perpendicular fluctuation harmonic constant \( k_{RM} \).

In Fig. 3 we plot these landscapes for both symmetric and asymmetric ligands and three types of lateral fluctuations. For all cases, \( c(\beta) \) is small if \( N_R < 4 \), suggesting that a low TM number does not lead to strong positive cooperative binding of the two ligands. In the case without lateral fluctuation (left column) \( \langle c \rangle \) is positive for \( N_R \geq 4 \) and \( 0.4k_BT < k_{RM} \sigma^2 < 40k_BT \). These \( k_{RM} \) values correspond on average to moderate perpendicular fluctuations 0.1 \(-1 \sigma \) (0.25–2.5 Å). For higher values of \( k_{RM} \sigma^2 \gg 40k_BT \), no positive cooperativity is observed. Note that for \( N_R \geq 7 \) the equilibrium ring is too large for a small ligand to bind to a sufficient number leading to \( p_2 = p_{11} = 0 \) and a undefined \( c \). Allowing lateral fluctuation (middle column) shifts and increases the maximum of \( \langle c \rangle \) to values of \( k_{RM} \sigma^2 > 40k_BT \), but does not alter the position of the maximum in terms of \( N_R \). The effect of lateral fluctuations is more dominant than that of normal fluctuations; i.e., reducing lateral fluctuation decreases cooperativity, while reducing normal fluctuations increases it. Changing from the ring to the string topology (right column) allows further flexibility in the lateral movement but does not change the qualitative picture. This implies that neighbor TM distance fluctuation is much less dominant than the shape deformation of receptor from the equilibrium ring structure. Introducing asymmetry between ligands (lower row) only slightly reduces \( \langle c \rangle_\beta \) with respect to the symmetric case, but induces a negative \( \langle c \rangle_\beta \) for small \( k_{RM} \). For all \( N_R \).

Our results are in agreement with Ref. [3] in which cooperativity is proposed to originate from a shift in vibrational density of states that accompanies the ligand binding. Such a shift is also observed in our model, as a suppression of fluctuation upon binding. We can compare our numerical results for \( N_R = 2 \) and no lateral fluctuation with an analytical model along the lines given in Ref. [6]. Approximating the influence of (symmetric) ligand binding by an additional harmonic coupling \( k_c \), the system is effectively a system of coupled harmonic oscillators. The cooperativity in this model is \( c = \frac{k_BT}{2} \ln \left( \frac{k_{RM} + k_c}{k_{RM} - k_c} \right) \). Since \( k_c \) is by definition non-negative, the cooperativity \( c \geq 0 \). Our results indeed show that the cooperativity is \( c = 0 \) for \( k_{RM} \to \infty \), and is increasing for small \( k_{RM} < 1 \), as predicted, but is slightly negative for intermediate values of \( k_{RM} \). This is caused by \( N_R = 2 \) configurations with ligands sideways bound to both receptors, which are partly excluded in the \( s_0 \) state. While this result is clearly due to the simplicity of our model, we believe that the overall features of the cooperativity landscapes are robust.

To evaluate the existence and scale of the flexibility of membrane receptors and to justify our model, we performed a 240 ns MD simulation of a \( \beta 2AR \) receptor. We first deleted the T4L residues of \( \beta 2AR \) (PDB entry 2RH1), capped the exposed termini of Leu-210 and Lys-263, mutated it to wild type sequences [8], and then embedded this clipped \( \beta 2AR \) into a phosphatidyl ethanolamine (POPE) bilayer normal to the \( xy \) plane. Using the GROMOS87 force field, simple point charge water and Berger lipid model [13], the resulting system consists of 308 lipids, 73 Na\(^+\), 77 Cl\(^-\), and 12 153 water molecules and totally 55 546 atoms in a box \( 9.7 \times 9.7 \times 8.6 \) Å (see Fig. 1 for a snapshot of the system). The production run was performed at 298 K with the \( v \)-rescale thermostat and at 1 bar with the Parrinello-Rahman barostat. In this 240 ns trajectory, the standard deviation (SD) of approximated Gaussians for \( x, y \) components of the distances between the center of mass (COM) of each linked TM-TM pair are \( \langle x_{\text{MD}}^2 \rangle_{1/2} = 0.310 \) Å, \( \langle y_{\text{MD}}^2 \rangle_{1/2} = 0.225 \) Å, respectively. The SD of the \( z \) component of COM distance between the TMs and the whole protein is chosen to quantify the normal fluctuation and reads \( \langle z_{\text{MD}}^2 \rangle_{1/2} = 0.203 \) Å. These values are then transformed into our model units and compared with corresponding \( k_{RM} \) and \( k_{RR} \) values (or equivalently the histogram of fluctuations in state \( s_0 \)), since the MD simulation only represents \( s_0 \) in our simple model: \( \beta k_{RR} \sigma^2 = \sigma^2/\langle x_{MD}^2 \rangle_{1/2} = 40 \) and \( \beta k_{RM} \sigma^2 = \sigma^2/\langle z_{MD}^2 \rangle_{1/2} = 150 \). These values fall within the region of optimal positive cooperativity in Fig. 3. While the comparison is indirect, as we cannot measure the cooperativity directly in the MD simulation.
simulations, these results agree with our hypothesis that naturally occurring receptors have evolved to a region of high cooperativity for optimal signal transduction.

In summary, our results suggest that cooperative binding of ligands to multiple TMs, and thus shape selectivity, might be a dominant factor underlying the working mechanism of integral membrane receptors such as GPCRs. Cooperativity is determined by the interplay between the geometry of the ligands with the modes of fluctuations (lateral and normal) of the receptor. Communication through fluctuations between extra and intracellular domains is only optimal when the shape of ligands influence the allowed modes of fluctuation. In addition, we predict that all membrane receptors in nature should have at least four TM helices to gain positive cooperativity. These predictions could be further explored via extensive all-atom MD simulations that measure TM fluctuations of both liganded and unliganded forms of GPCRs, promising NMR techniques [14,15], scattering experiments [16], or mutation studies that suppress fluctuations by, e.g., introducing chemical bonds.

We acknowledge financial support from the Marie Curie EuroSim project. D. F. acknowledges support from ERC Advanced Grant No. 227758, EPSRC Grant No. RG58958 and Royal Society No. RG50412.