Communicating helices: molecular simulation of allosteric receptor proteins
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Citation for published version (APA):

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Role of fluctuations in binding cooperativity of membrane receptors

Signal transduction upon binding of a ligand to a membrane protein can occur not only via 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. The 2-dimensional version of the model with moderate lateral fluctuations reveals zero cooperativity for a protein with less than three helices. For a protein with 3-7 helices, positive cooperativity is gained and increase with the number of helices. In the 3-dimensional version of the model, we find that small fluctuations lateral as well as perpendicular to the membrane can increase the cooperativity, with the former more dominant. Too much fluctuation induces negative cooperativity. Tilting and rotation of TM helices always reduce 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 predicted in our 3-dimensional model.
6.1 Introduction

A living cell needs to be able to sense the change in its surroundings to survive in a constantly varying environment. This complex sensing process is typically envisioned as an elaborate signaling cascade, initiated by a capture of the environmental signals at the cell membrane. The received signals are then amplified, transformed and passed on and ultimately lead to a change in the gene expression pattern of the cell causing an adaptation in the cell behavior. It is of broad interest to understand the molecular mechanism of the initial step of the signaling process at the cell membrane and to possibly control and adapt these mechanisms.

Diverse approaches are adopted by a cell to sense different types of signals. Some signals such as ions, can pass through ion-channels whose opening and closing are controlled by the difference in the ion concentration between the two sides of the membrane. Other signals, e.g. light-sensitive compounds, odors, pheromones, hormones, and neurotransmitters, are molecules that are either too big to penetrate the lipid membrane, or are toxic, and thus should be prevented from doing so. Typically such signals are sensed by the extracellular part of a trans-membrane (TM) protein receptor and introduce a change in the flexible receptor machine. This change is passed through the membrane receptor towards its intracellular side, stimulating further downstream signaling pathways. This latter category of signaling is of special interests to the pharmaceutical industry mainly for three reasons: (I) penetration of the potential therapeutic drugs (the signal molecules) through the cell membrane is not a requirement; (II) integral membrane receptors, such as G-protein-coupled-receptors (GPCRs) are expressed virtually in every cell type; (III) these receptors typically possess a highly specific binding pocket for the signal. In fact, 30%-50% of the currently marketed drugs are directly or indirectly targeting the GPCRs. Therefore it is not surprising that this protein family is the subject of intense study.

6.1.1 G-Protein-Coupled-Receptors

G-protein-coupled receptors (GPCRs) are cell surface receptors that can be activated by a plethora of stimuli, including peptide hormones, odors, small molecules, ions, and light. GPCR-mediated signaling plays important physiological and pathophysiological roles in almost all cell types. As one of the most successful protein families in the sense of evolution, GPCRs and their signal transduction can be traced back to probably ~1.2 billion years ago, before the plant/fungi/animal split. Interestingly, although the GPCR superfamily comprises at least five distinct families/subfamilies, among which very little sequence homology exists, all GPCRs possess a common topology: the seven transmembrane (7TM) α-helices linked by three extracellular loops and three intracellular loops.

6.1.1.1 A static view of GPCR signaling

Our current understanding of GPCR signaling is fairly limited. Although it has been shown that signaling across the membrane can happen via a single GPCR or oligomeric forms of GPCRs, the detailed mechanism of these processes remains largely unknown. Here we consider only the signaling via a single receptor. Signaling of a single GPCR is typically interpreted as a simple step wise cyclic process, as illustrated in FIG. 6.1. Initially the 7TM receptor is pre-coupled with a GDP-liganded G-protein; Step (I) a signal (photon or molecule) binds to the extracellular binding pocket of the receptor, changes the receptor
and correspondingly catalyzes the replacement of GDP by GTP on the G-protein; Step (II) the GTP-liganded G-protein dissociates into two parts (G\(_{\alpha}\) and G\(_{\beta\gamma}\)) that catalyze distinct downstream reactions; Step (III) the extracellular ligand stays bound to the receptor while the GTP-hydrolysis (GTP to GDP) happening on G\(_{\alpha}\); Step (IV) another diffusive GDP-liganded G-protein close by or the GDP-liganded G-protein reassembled by the original G\(_{\beta\gamma}\) and the GDP-liganded G\(_{\alpha}\) after GTP-hydrolysis assemble with the receptor, the signal escapes the receptor; the system returns to the initial state. However, the dramatic increase of complexity of the GPCR-regulated intracellular communication is still hindering most experimental efforts to reveal the kinetics of each step of this classic cycle with significant accuracy. Thus the validity of this classic picture is still on debate.

In this static explanation, the crucial mechanism that conveys information from the outside to the inside of the cell is the conformational change of the receptor after ligand binding, usually envisioned as a switch between two (meta)stable states of the receptor. The current dominance of the concept of a necessary change in the average conformations in interpreting...
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the signaling mechanism of a protein is understandable, as most protein structures are resolved via crystallography, a method naturally limits the intrinsic motions of a highly flexible protein in confinement so that its structure becomes accessible. However, as protein crystallography provides little dynamical insight, interpreting the signaling mechanism in terms of conformational changes is far from complete.

Dynamics of proteins can be accessed by NMR spectroscopy\[20\]. Although applicable only to relatively small proteins in solution at the moment, the progress of the NMR techniques seems highly promising. Popovych et al studied a signaling protein CAP that binds to the DNA via NMR and found no conformational change(s) of this dimer protein before and after ligand binding to any of the monomers\[19\], suggesting a dynamically driven communication between the two monomers.

The concept of a dynamical mechanism of GPCRs is also supported by new evidences from both experimental studies\[10\] and molecular simulations. So far a large number of Molecular Dynamics (MD) simulations\[7, 5, 15, 8, 21, 9\] have been applied to different types of GPCRs. Notably, two MD studies have ruled out a previously proposed “static” mechanism - the ionic lock hypothesis - that considers only conformational changes\[7, 21\]. Through mutational studies on residues Glu268 and Asp130 of the $\beta_2$AR receptor, Ballesteros et al suggested that the ionic interaction between the two residues is a key interaction to lock the receptor in its inactive state\[2\]. Furthermore, as $\beta_2$AR belongs to Class A - the largest family of GPCRs, disruption of the ionic lock was proposed as a general step towards the activation of GPCRs. However, in the 2$\mu$s MD simulations by Dror et al, breakage of this ionic lock was observed even in the inactive state of $\beta_2$AR\[7\]. Romo et al further showed via MD that in an inactive apo-$\beta_2$AR\[21\] the lock fluctuates rapidly among three states: closed (locked), semi-open with a bridging water, and fully open.

6.1.2 Fluctuations in GPCRs

Another apparent problem with the perspective of conformational changes is its specificity to the sequence of a protein. All GPCRs have diverse sequences but share a conserved structure - the seven transmembrane $\alpha$-helices linked by flexible linker sequences. Why is it seven? Why for example not three instead? There must be certain common parts of the mechanism of all GPCR signaling processes that are consistent with this conspicuous structural similarity. To discuss this general part of GPCR signaling, we have to abandon the notion of any specific conformational change and attempt to find other concept(s) underlying the mechanism. One possible alternative is that the signaling is conveyed through a change in the scale of internal fluctuations of the transmembrane helices (TMs) of the receptor.

As proteins are flexible molecules, it is in fact natural to consider the internal fluctuations of the membrane receptors as part of the signaling mechanism. The idea of such entropic effect can at least be traced back to 1984\[6\]. Cooper et al introduced a theoretical model about allostery (SEC. 6.3) purely from fluctuations without conformational change. They interpreted the effective interactions between the two bodies in allostery in terms of thermodynamic free energy (SEC. 6.3.2). Recently, this concept of communication between distant binding sites purely via fluctuations has been explored for DNA binding proteins via NMR techniques\[19\] and coarse grained modeling\[10, 25\].
6.2 Fluctuations in an apo-β2AR via MD

In order to verify the existence of the internal fluctuations of the transmembrane helices of GPCRs and quantify the scale of such fluctuations, we performed a 240ns MD simulation of a β2AR receptor without any ligands, as illustrated in FIG. 6.2.

We first deleted the T4L residues of β2AR (PDB entry 2RH1), capped the exposed termini of Leu210 and Lys263, mutated it to wild type sequences[7] and then embedded this clipped β2AR into a phosphatidyl ethanolamine (POPE) bilayer normal to the xy plane. Using the GROMOS87 force field, SPC water and berger lipid model[4], the resulting system consists of 308 lipids, 73 Na+, 77 Cl− and 12153 water molecules and totally 55546 atoms in a box ≈ 9.7 × 9.7 × 8.6Å . The production run was performed in the NPT ensemble at 298K with the v-rescale thermostat and at 1bar with the Parrinello-Rahman barostat.

In this 240ns 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

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6.1.3 Outline of the chapter

In this chapter we investigate the question whether fluctuations in the membrane receptor can play a role in the signaling mechanism of GPCR by means of molecular simulations. In SEC. 6.2 we first establish the fluctuations present in a fully atomistic model of the β2AR receptor. These results can act as a reference point. Then we investigate the effect of fluctuations by employing a simple coarse grained model, in which we can specifically change the number of helices as well as the interactions. As a signal is transmitted from one side of the membrane to the other, the receptor needs to be highly cooperative (see SEC. 6.3). We quantify this cooperativity by means of the allostERIC free energy, which we compute for many different system settings using Monte Carlo. The simplified model is introduced in SEC. 6.4. In SEC. 6.5 the results for a two dimensional 2D implementation of the model are discussed, followed by the results for the three dimensional 3D version in SEC. 6.6. We end with conclusions.

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Figure 6.2: Visualization of setup of the 240ns Molecular Dynamics simulation of a bold β2AR receptor. (a) sideview. (b) topview. Color codes: blue cell membrane of lipid bilayer; red β2AR receptor; orange water molecules.
are $\langle x_{MD}^2 \rangle^{1/2} \approx 0.310 \text{Å}$ and $\langle y_{MD}^2 \rangle^{1/2} \approx 0.225 \text{Å}$ respectively. The SD of $z$ component of COM distance between the TMs and the whole protein is chosen to quantify the normal fluctuation and reads $\langle z_{MD}^2 \rangle^{1/2} \approx 0.203 \text{Å}$ . Tilting of the seven TMs with respect to the normal direction of the membrane are in the range $8.5^\circ$-$20^\circ$, each with an average SD of $\sim 1.5^\circ$.

6.3 Allosteric mechanisms require cooperativity

GPCR signaling is an allosteric mechanism since the signal molecule and the G-protein bind to different sites on the receptor that are rather distant from each other ($\sim 30 \text{Å}$). As they are separated by the membrane, any direct interaction between the two, such as electrostatics is impossible. The only way the two ligands can communicate with each other is through the mediation of the receptor. To study the effects of fluctuations on the communication between the extra and intracellular domains via an integral membrane receptor, we must quantify the effective interaction between the two ligands by the receptor. This effective interaction is often referred to as ligand binding cooperativity. This section reviews the concept of cooperativity and one widely used approach to quantify it - the allosteric free energy (AFE). Another widely used quantity for cooperativity - the Hill coefficient - is not useful in our case, as is discussed in Appendix SEC. 6.8.

6.3.1 What is cooperativity?

Cooperativity is a central concept for understanding supra-molecular self-assembly. Cooperativity arises from the interplay of two or more interactions. If the two component interactions favor each other, i.e. they have an extra indirect attractive interaction, the system they form will be more than the sum of the two, yielding a positive cooperativity. When the extra effective interaction is repulsive, the two are not compatible to assemble, leading to a negative cooperativity.

Whether the cooperativity is positive or negative can also be reflected by the population of the intermediates between the molecular components and the complete assembled structures (partially bound or assembled), because the relative population of the intermediates with respect to the reactants reveals the strength of the component interaction. Systems of very positive cooperativity exhibit very low concentration of the intermediates; only the initial and final states are significantly populated. For such systems, it is either "all" or "nothing": (I) at the microscale, the individual molecules are likely to be either all bound or all unbound; the system spends little time in intermediate states; (II) at the macroscale, the behavior of the ensemble is characterized by a very sharp population switch from mainly free to mainly bound over a small change in the concentration of the ligands.

Cooperativity occurs widely in biology. A signaling process is often a process of assembly or disassembly of an upstream signal, a receptor (or a translator) and a downstream signal. In the case where the binding of the downstream protein is crucial for passing on the information, a sharp transition in the concentration from the unbound to the fully bound regime will be observed. The opposite is true for negative cooperativity. If the disassembly of the downstream molecule is required to pass on the information, binding of the upstream molecule should disfavor the binding of the downstream one to the receptor, leading to a negative cooperativity: the fully bound state (upstream + receptor + downstream) is much less populated than the intermediates (upstream + receptor or downstream + receptor).
6.3 Allosteric mechanisms require cooperativity

6.3.1 Three body assembly

Here we formulate cooperativity for the simplest case - a three body assembly reaction scheme. Let $R$ be a receptor, $A$ and $B$ be two ligands of different species. The complete assembly of the three bodies can be summarized as:

$$A + B + R \overset{K}{\rightleftharpoons} ABR$$ (6.1)

The reference reaction scheme is the sum of the two sub-reactions representing the two component interactions (A-R and B-R), assuming that they are statistically independent:

$$A + R \overset{K_A}{\rightleftharpoons} AR$$ (6.2)

$$B + R \overset{K_B}{\rightleftharpoons} BR$$ (6.3)

Here $K = \frac{[ABR]}{[R][A][B]}$, $K_A = \frac{[AR]}{[R][A]}$, $K_B = \frac{[BR]}{[R][B]}$, denote the equilibrium constants for the corresponding reactions.

We now address the question: is the total complete assembly reaction easier or more difficult to occur than the reference scheme? This can be measured by comparing the ratio between $K$ and $K_AK_B$:

$$\alpha = \frac{K}{K_AK_B}$$ (6.4)

$$= \frac{[ABR]/[R]}{([AR]/[R])([BR]/[R])}$$ (6.5)

$$= \frac{[ABR][R]}{[AR][BR]}$$ (6.6)

The meaning of $\alpha$ is clear from Eqn. 6.5 taking the population of the unbound receptor $[R]$ as a reference to populations of all other states of the receptor, how does population of the fully bound state of the receptor $[ABR]/[R]$ compare with the population of the two intermediates $[AR]/[R]$ and $[BR]/[R]$, assuming they are statistically independent. $\alpha > 1$ denotes a positive cooperativity while $\alpha < 1$ means a negative cooperativity. Cooperativity is the measure of the effective coupling between the binding events[13], do they favor ($\alpha > 1$), disfavor ($\alpha < 1$) or are independent of ($\alpha = 1$) each other.

6.3.2 Allosteric free energy (AFE)

The allosteric free energy (AFE) is a widely accepted measure of cooperativity. It can simply be deduced from Eqn. 6.5 by taking the logarithm of $\alpha$. Let the concentration of a substance mean its population (probability) of the states times a standard concentration $[R]$, we obtain a relation between three free energy differences for the three reactions: from the initial state
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R to the intermediates AR, BR and final state ABR respectively:

\[ c = \frac{1}{\beta} \ln(\alpha) \]
\[ = \frac{1}{\beta} \ln \left( \frac{[ABR]/[R]}{([AR]/[R])([BR]/[R])} \right) \]
\[ = -\frac{1}{\beta} \ln \left( \frac{[AR]}{[R]} \right) - \frac{1}{\beta} \ln \left( \frac{[BR]}{[R]} \right) + \frac{1}{\beta} \ln \left( \frac{[ABR]}{[R]} \right) \]
\[ = \Delta G_{R|AR} + \Delta G_{R|BR} - \Delta G_{R|ABR} \quad (6.7) \]

Here \( \Delta G \) in the last line is the difference of Gibbs free energy between the corresponding states. Note that the cooperativity \( c \) we define is simply the opposite of the definition of AFE found in most literature\[10, 25, 13\]. \( c > 0 \) indicates positive cooperativity while \( c < 0 \) and \( c = 0 \) means negative or no cooperativity respectively. In this chapter we consider the term cooperativity and the allosteric free energy as being one and the same quantity \( c \).

6.4 A simple model of membrane receptor signaling

We are interested in the effects of a change in the receptor fluctuations. An ideal (and naive) solution is tuning the fluctuations directly and studying the consequences of such tuning. However, this is not straightforward to implement within the framework of all-atom Molecular Dynamics simulations. Moreover, as we aim to capture the general part of GPCR signaling, a simplified model that captures the general structural features of all GPCRs is more practical. All-atom MD simulations are expensive and too specific as they must involve a single representative type of GPCR. Finally, simple models not only are easier to sample but also enable an extensive study of the relations between cooperativity and the number of TM helices.

In this section, we first define the basic components of our simple model of membrane receptor signaling and then discuss two approaches to define cooperativity for this model, namely (I) using the radial distribution function of the distance between the extra and intracellular signal ligands and (II) using the binding matrix. Detailed analysis of the variants of our model with increasing level of complexity are discussed in later sections. In SEC. 6.5 and SEC. 6.6, we present the numerical studies via Monte Carlo sampling of the 2D and 3D model.

6.4.1 Model components

As shown in FIG. 6.3, we consider a very simple setup of membrane receptor signaling. The system consists of two signal ligands, one extracellular \( L_1 \) and, one intracellular \( L_2 \), both can bind to an integral receptor \( R \). While the ligands are modeled as hard spheres of radius \( r_{ex} \) and \( r_{in} \), the receptor \( R \) is a chain of \( N_R \) transmembrane helices (TMs) represented as rigid rods connected by springs of strength \( k_{RR} \) that represent the linker sequences between the TMs and other TM-TM interactions. Each TM rod is of length \( h_R \) and consist of \( n_R \) spheres of radius \( r_R \).

We let \( r_1, r_2, r_i^R \) be the vector position of extracellular ligand, the intracellular ligand and the TM \( i \) respectively. Each ligand can bind to the corresponding terminal sphere of each
6.4 A simple model of membrane receptor signaling

Figure 6.3: Communication between extracellular signal (blue) and G-protein (green) is mediated only by one integral receptor (red) composed of a number of transmembrane helices (TMs). In this simple model both signal molecules cannot penetrate the membrane, and bind to all TMs with the same strength.

TM. All linker sequence couplings between neighbor TMs are kept identical as \( k_{RR}((x^R_i - x^R_{i+1})^2 + (y^R_i - y^R_{i+1})^2) \), where \( x^R_i \) and \( y^R_i \) are respectively the x and y components of \( r^R_i \). For a 3D representation, we need to consider the fluctuations of TMs perpendicular to the membrane. We model such fluctuations with a harmonic potential \( k_{RM}(z^R_i)^2 \), where \( z^R_i \) is the z component of \( r^R_i \). Here the membrane is considered as a rigid cuboid spanning the xy plane and is of thickness \( h_M \) on the z direction, impenetrable to the ligands - the membrane interacts with the ligands via only hardcore repulsion. The harmonic spring with force constant \( k_{RR} \) and equilibrium lateral distance \( r_{RR}^0 \) represents the fact that neighboring TMs are linked through extracellular and cytoplasmic loops, and nonbonded interactions, and constrained by membrane lipids. For simplicity all ligand-TM pair interactions are assumed identical. All other interactions are described in the sections for the 2D and 3D simulations.

6.4.2 Evaluation of cooperativity

6.4.2.1 Radial distribution function (RDF)

Since the essence of cooperativity is the effective interaction between two bodies, a natural choice to define cooperativity for ligand binding in membrane receptors is the radial distribution function (RDF). In statistical mechanics, a radial distribution function, also known as pair correlation function, \( g(r) \), gives the probability to find an atom at a distance \( r \) from one particular atom at the origin, normalized with respect to the average density. The simple spherical geometry of ligands allows using the RDF as a quantity for analyzing the population of bound states.
integration over the relevant regions of represent these populations.

through the binding matrix. The partition function for the system can be written as

A more generally applicable approach to analyze cooperativity of membrane receptors is

6.4.2.2 Binding matrix

membrane, i.e. if the two ligands are identical.

RDF approach is valid, but only with the system that is symmetric on both sides of the

Nevertheless, for a symmetric system we can still get \( p^{ub} = p^{bu} = (p^{ub} + p^{bu})/2 \). Thus this

The RDF is formulated in Eqn. [6.8] Here \( \beta = 1/k_B T \) is the inverse thermodynamic

temperature. \( r_{12} \) represents the distance between two ligands. \( \delta(x) \) is the Dirac delta

function. \( Z = \iiint dr_1 dr_2 dr_{RE} e^{-\beta (E(r_{12}) + E_1(r_{1R}) + E_2(r_{2R}))} \delta(r_{12} - |r_1 - r_2|) \)

is the partition function of the system. \( \zeta = \zeta(r_{12}) \) is a geometrical correction term: (I) for a 2D system \( \zeta = 2\pi r_{12} \); (II) for a 3D system, \( \zeta = 4\pi r_{12}^2 \). For simplicity, we have written the configuration position of the center of mass of all receptor TMs as a position vector \( r_R \).

We have split the potential \( U(r_1, r_2, r_R) \) into two contributions: a direct interactions of ligands \( E(r_{12}) \) and a direct interaction between one of the ligand and the receptor \( E_1(r_{1R}) + E_2(r_{2R}) \). As \( L_1 \) and \( L_2 \) are located at two separate volumes divided by the membrane, it is natural to assume \( E(r_{12}) = 0 \).

Computing the cooperativity \( c \) requires populations of all four states of the system: no ligands bound, only extracellular ligand bound, only intracellular ligand bound, both ligands bound. With \( u \) meaning "unbound" and \( b \) denoting "bound", we use \( p^{uu} \), \( p^{ub} \), \( p^{bu} \), \( p^{bb} \) to represent these populations. \( p^{bb} \) and \( p^{uu} \) can be conveniently calculated from the RDF by integration over the relevant regions of \( g(r) \):

\[
p^{bb} = \int_0^{r_{box}^L+2r_R} dr_{12} \zeta(r_{12}) g(r_{12}) \quad \quad (6.9)
\]

\[
p^{uu} = -\int_0^{r_{box}^L+2r_R} dr_{12} \zeta(r_{12}) g(r_{12}) \quad \quad (6.10)
\]

Here \( r_{box} \) is the maximal distance possible between the two ligands in the simulation box.

A similar integration approach can be applied to \( g(r) \) to evaluate the population of the intermediates, but only the total population of both intermediates \( p^{ub} + p^{bu} \) together, not \( p^{ub} \) and \( p^{bu} \) separately:

\[
p^{bu} + p^{ub} = \int_0^{r_{box}^L+2r_R} dr_{12} \zeta(r_{12}) g(r_{12}) \quad \quad (6.11)
\]

Nevertheless, for a symmetric system we can still get \( p^{ub} = p^{bu} = (p^{ub} + p^{bu})/2 \). Thus this RDF approach is valid, but only with the system that is symmetric on both sides of the membrane, i.e. if the two ligands are identical.

6.4.2.2 Binding matrix

A more generally applicable approach to analyze cooperativity of membrane receptors is

through the binding matrix. The partition function for the system can be written as

\[
Z = \int dr_1 \int dr_2 \int dr_{RE} e^{-\beta (E_0(r_R) + E_1(r_1R) + E_2(r_2R))} \quad (6.12)
\]
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where the notation $r_1, r_2$ and $r_R$ has the same meaning as in Eqn. 6.8. Let $E$ be a potential energy. $E_0$ is the internal energy of the receptor, $E_1, E_2$ the interaction energy between the ligands and the receptor. The integrals run over the volume $V$ of the box.

As all binding strengths are assumed identical as $\epsilon$, one can write a series of conditional average of this partition function as in Eqn. 6.13.

$$z_{ij} = \frac{Z_{ij}}{Z} = \frac{\int dr_1 \int dr_2 \int dr_R e^{-\beta(E_0(r_R) + E_1(r_1, r_R) + E_2(r_2, r_R))} \delta(E_1 - i\epsilon) \delta(E_2 - j\epsilon)}{\int dr_1 \int dr_2 \int dr_R e^{-\beta(E_0(r_R) + E_1(r_1, r_R) + E_2(r_2, r_R))}} \; (6.13)$$

$$= \langle \delta(E_1 - i\epsilon) \delta(E_2 - j\epsilon) \rangle \; (6.14)$$

With $i, j \in [0, N_R]$ being the number of bonds established respectively between the extracellular ligand and the TMs and the intracellular ligand and the TMs, this matrix $z_{ij}$ is the binding matrix of dimension $N_R + 1$, giving the probability to find a particular pair $i, j$. In principle, the two extreme states, i.e. the unbound and fully bound state, should correspond to the probability peaks at $z_{00}$ and $z_{N_R N_R}$ respectively, whilst other peaks correspond to the intermediates. However, as we show below, due to possible geometrical constraints, the bound state may not be located at $z_{N_R N_R}$, but is found at some $z_{nn}$ with the integer $n \in (N_R/2, N_R]$. Taking the division of states at $n > N_R/2$ ensures that the bound state is defined by at least half of the TMs in contact with the ligand. We define the four states of our system based on the binding matrix as follows:

The populations of the four states, introduced in the previous section, are given by sums over the binding matrix $z_{ij}$.

$$p^{uu} = \sum_{i,j=0}^{n-1} z_{ij} \equiv \frac{Z^{uu}}{Z} \; (6.15)$$

$$p^{ub} = \sum_{i=0}^{n-1} \sum_{j=n}^{N_R} z_{ij} \equiv \frac{Z^{ub}}{Z} \; (6.16)$$

$$p^{bu} = \sum_{i=n}^{N_R} \sum_{j=0}^{n-1} z_{ij} \equiv \frac{Z^{bu}}{Z} \; (6.17)$$

$$p^{bb} = \sum_{i,j=n}^{N_R} z_{ij} \equiv \frac{Z^{bb}}{Z} \; (6.18)$$
The cooperativity (allosteric free energy) \( c \) is easily defined from the binding matrix via

\[
[R] = p_{uu} = \sum_{i,j=0}^{n-1} z_{ij} \equiv \frac{Z_{uu}}{Z}
\]

(6.19)

\[
[L_2R] = p_{ub} = \sum_{i=0}^{n-1} \sum_{j=n}^{N} z_{ij} \equiv \frac{Z_{ub}}{Z}
\]

(6.20)

\[
[L_1R] = p_{bu} = \sum_{i=n}^{N} \sum_{j=0}^{n-1} z_{ij} \equiv \frac{Z_{bu}}{Z}
\]

(6.21)

\[
[L_1L_2R] = p_{bb} = \sum_{i,j=n}^{N} z_{ij} \equiv \frac{Z_{bb}}{Z}
\]

(6.22)

\[
c = \frac{1}{\beta} \ln \alpha = \frac{1}{\beta} \ln \frac{p_{uu} p_{bb}}{p_{ub} p_{bu}} = \frac{1}{\beta} \ln \frac{Z_{uu} Z_{bb}}{Z_{ub} Z_{bu}}
\]

(6.23)

### 6.5 Numerical study of 2D model

For simplicity, we first consider the simple model in 2 dimensions, ignoring the dimension vertical to the membrane. The system consists of a receptor in the form of a chain of hard disks connected by springs, which interact via the ligands with a short range attractive potentials. Both ligands can bind to the receptor, but do not interact with each other directly as they are supposed to be on different sides of the membrane. The membrane is assumed to be a plane holding the receptor TMs.

#### 6.5.1 Model setup

The Hamiltonian of the system is:

\[
H(r^N) = \sum_{i<j}^{N_R} U_{HS}(r_{ij}) + \sum_{j}^{N_R} \sum_{i}^{N_R} U_{RL}(r_{ij}) + \sum_{i}^{N_R-1} \frac{1}{2} k_{RR}(r_{x,y,i,i+1}^2 - r_{RR}^0)^2
\]

(6.24)

where \( U_{HS} \) represents the hard-core interaction among all receptor particles (hard disks representing the TM helices), with \( r_{ij} \) denoting the distance between particles \( i \) and \( j \) (except between the two ligands that are separated by the membrane) \( U_{RL} \) is the binding attraction between a ligand and a TM, modeled by a square well potential with width \( \Delta \) and depth \( \epsilon \). In the third term, \( r_{x,y,i,i+1} \) denotes the lateral distance between \( TM_i \) and \( TM_{i+1} \). The spring coupling neighbor TMs is represented by \( k_{RR} \) and equilibrium lateral distance \( r_{RR}^0 \). Note that there is no direct interaction between the ligands.

Regarding the radius of receptor disk \( r_R = \sigma \) as the unit of length, we start with a model as simple as possible: (I) ligands are relatively big and symmetric \( r_1^{(1)} = r_1^{(2)} = 2.5 \sigma \); (II) The TM-ligand binding well has a width \( \Delta = 1.5 \sigma \) and a depth \( \beta \epsilon = \beta \epsilon U_{tot}/N_R = 12/N_R \) to ensure a change in \( N_R \) does not introduce a trivial energetic preference of the fully bound state \( p_{bb} \); (III) the neighbor TM coupling spring is relatively loose with \( k_{RR} = 4k_B T \), \( r_{RR}^0 = 3 \sigma \). As a real receptor in the membrane is constrained by the lipids into a compact
ring-like object, we consider both the string topology as given in Eqn. 6.24 and the ring topology in which an extra \(k_{RR}\) spring couples the head TM, and tail of TM_{NR}. The periodic 2D box is \(60\sigma \times 60\sigma\) to accommodate the most extended configuration of the receptor.

### 6.5.2 Simulations details

We sample this model via Monte Carlo and replica exchange techniques. The easiest way to observe the transition from \(p^{uu}\) dominant regime to the \(p^{bb}\) dominant regime under the NVT ensemble is to run simulations a range of inverse temperature \(\beta_i \sim \beta_j\), which is equivalent to changing the well depth \(\epsilon\). Replica exchange is then a natural choice to enhance the sampling at large \(\beta\), since multiple Monte Carlo simulations at these \(\beta\) values are necessary anyway.

We consider \(N_R = 1 \sim 7\) systems with ring and string receptor topology, resulting in totally \(7 \times 2 = 14\) distinct systems. Each run of each system consists of 16 replicas \(\beta_i \in [1/4, 4]\) where \(\beta_i - \beta_j = 1/4\). A single replica ran \(\sim 10^9\) cycles. Each cycle consisted on average of a trial move for every particle. A replica swap was attempted every 25 cycles. The acceptance ratio of the particle move and replica swap was \(0.3 \sim 0.6\) and \(0.4 \sim 0.8\) respectively.

### 6.5.3 RDF analysis

As the binding matrix turned out to be more suitable to compute the cooperativity for our system, we discuss results in terms of RDF only for the systems with string topology.

#### 6.5.3.1 Two minima in the effective ligand-ligand potential

We convert the RDF to an effective ligand-ligand potential via \(\beta \psi(r_{12}) = -\ln(g(r_{12}))\). FIG. 6.4 illustrates \(\beta \psi\) for the string topology with \(N_R = 3\). As expected the global shape of \(\beta \psi(r_{12})\) is independent of the temperature. The two minima observed correspond to the bound state \(p^{bb}\) and the intermediates \(p^{ub} + p^{bu}\) respectively. The \(p^{bb}\) and \(p^{ub} + p^{bu}\) follow directly from Eqn. 6.9 and Eqn. 6.11. The two states can therefore be distinguished and split apart at \(r_{12} = 2r_L = 5\sigma\), as the major structural difference between them is whether or not the two ligands on each side of the membrane are separated by the TMs in the x-y plane. The remaining flat part of the effective potential can be integrated to represent the unbound state \(p^{uu}\).

#### 6.5.3.2 Entropic attraction between ligands

The \(\psi(r_{12})\) is not purely determined by the attractive part of the interact potential but also has an entropic component. To see this, we run simulations setting \(\beta = 0\) to observe entropic effects due to the hardcore repulsive interactions among the particles.

FIG. 6.5 illustrates such entropic attraction via \(-\ln(g(r_{12}))\) for all seven cases \(N_R = 1 \sim 7\) at \(\beta = 0\). When \(\beta = 0\), all potentials are turned off. The free energy minima we observe can therefore only be explained in terms of entropy. In FIG. 6.5, we consider the simplest case \(N_R = 1\): due to the existence of the ligands the excluded volume between a TM particle and an isolated ligand is \(V_0 = \pi((r_L + r_R)^2 - r_R^2)\). Three possible relevant arrangements are shown: (a) when \(r_{12} > 2(r_R + r_L)\), the total excluded volume is \(V_{excl} = 2V_0\); (b) \(V_{excl}\) decreases from \(2V_0\) as \(r_{12}\) decreases from \(2(r_R + r_L)\); (c) when \(r_{12} \to 0\), \(V_{excl}\) approaches...
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6.5.3.3 Bound and intermediate states indistinguishable for \( N_R < 3 \)

Following Eqn. [6.9] the bound population \( p^{bb} \) can be defined via

\[
p_{RDF}^{bb} = \int_0^{2r_L} dr_{12} 2\pi r_{12} g(r_{12}) \tag{6.25}
\]

FIG. 6.6A shows the transition of \( p_{RDF}^{bb} \) from 0 to 1 as a function of \( \beta U_{tot} \) for systems with \( N_R = 1 \sim 7 \). For \( N_R < 3 \), \( p_{RDF}^{bb} \) can not increase all the way to unity but is limited to \( p_{bb} \sim 0.4 \). This is due to the fact that for a receptor holding \( N_R = 1,2 \) TMs, the bound state \( (L_1L_2R) \) and the intermediates \( ([L_1R], [L_2R]) \) can not be distinguished energetically, as illustrated in FIG. 6.6B. When \( N_R = 1 \), maximally two bonds can be established between the two ligands and the single TM disk, in both scenarios \( r_{12} > 2(r_R + r_L) \) and \( r_{12} < 2(r_R + r_L) \). Similarly for \( N_R = 2 \), in both scenarios, 4 bonds can be established, leading to the same potential of bindings and thus almost the same populations in both scenarios. As a result, \( p_{RDF}^{bb} \), as defined by cutting the RDF at \( r_{12} = 2(r_R + r_L) \) will never exceed 0.5. For \( N_R = 3 \), when the TM disks are in between the two ligands \( (r_{12} > 2(r_R + r_L)) \), maximally only 5 bonds can be established, one bond less than in the fully bound state \( L_1L_2R \) \( (r_{12} < 2(r_R + r_L)) \).

As pointed out in SEC. 6.4.2.1, RDF analysis is only convenient for symmetrical ligands. To quantify the cooperativity in the binding of two asymmetrical ligands, we have to use

**Figure 6.4:** Examples of \( \beta \psi(r_{12}) = -\ln(g(r_{12})) \) at \( N_R=3 \). Two minima are identified. When \( r_{12} < 2r_L \), both ligands are on the same side and embraced by all TMs, which corresponds to \( p^{bb} \). The other minimum is at \( r_{12} > 2r_L \) with two ligands separated by the TMs, corresponding to the intermediates \( p^{bu} + p^{ub} \).
6.5 Numerical study of 2D model

Figure 6.5: Pure entropic attractions between ligands due to TM-ligand hardcore repulsion. (A) $-\ln(g(r_{12}))$ at $\beta = 0$; (B) schematics showing this excluded volume effect for the case $N_R = 1$. Let $V_0 = \pi (r_L + r_R)^2$ be the excluded volume between the ligand and a single TM disk, we observe three scenarios: (a) $r_{12} > 2(r_R + r_L)$, the total excluded volume is $V_{\text{excl}} = 2V_0$; (b) $V_{\text{excl}}$ decreases from $2V_0$ as $r_{12}$ decreases from $2(r_R + r_L)$; (c) when $r_{12} \to 0$, $V_{\text{excl}}$ approaches the minimal value $V_0$. The fact that less possible microstates are excluded in regime (c) than in regime (a) creates an entropic free energy minimum at $r_{12} = 0$.

Figure 6.6: Transition of $p_{RDF}^{bb}$ as function of (A) $\beta U_{\text{tot}}$ and (B) $1/V$ at $\beta U_{\text{tot}} = 30$. 

(A) $r_{12} > 2(r_R + r_L)$

(B) $r_{12} < 2(r_R + r_L)$
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Figure 6.7: Examples of binding matrix for $N_R = 5$. (A) String receptor $\beta U_{tot} = 21$; Ring receptor: (B) $\beta U_{tot} = 21$; (C) $\beta U_{tot} = 30$; (D) $\beta U_{tot} = 48$.

the binding matrix description. Moreover the binding matrix provides a cleaner separations of the bound, and unbound states.

6.5.4 Binding matrix analysis

6.5.4.1 Ring versus string topology

FIG. 6.7 exemplifies the binding matrix at different binding regimes for the ring receptor topology for $N_R = 5$. While two major peaks representing $p^{bb}$ and $p^{uu}$ can be observed at $\beta = 21$ and $\beta = 48$ respectively, $p^{bu}$ and $p^{ub}$ are populated at intermediate value of $\beta U_{tot} = 30$ close to the half saturation point.

Note that the peak representing $p^{bb}$ is not entirely located at $z_{N_R N_R}$. This is because a ring receptor of 5 TMs at the equilibrium configuration ($r_{i,j} = 3\sigma$) cannot accommodate the ligands of diameter $5\sigma$ inside. This specific geometrical constraint prevents the system to establish all the TM-ligand bonds and thus to find the global energetic minimum. As shown in FIG. 6.7A, for a string receptor, where such constraint is removed, not only the half saturation point shifts to a lower $\beta U_{tot} = 21$, which corresponds still to a unbound regime for the ring receptor, but also the $p^{bb}$ peak shifts back to $z_{N_R N_R}$.

6.5.4.2 Allosteric free energy

Following Eqn. 6.23, we can plot populations of all four binding states, $p^{uu}$, $p^{bu}$, $p^{ub}$ and $p^{bb}$, explicitly as a function of $\beta$ in FIG. 6.8A,B. We also computed the corresponding free energy $F^{uu}$, $F^{bu}$, $F^{ub}$ and $F^{bb}$ according to $F = -\ln(p)/\beta$. We plot the free energies with respect to $F^{uu}(\beta)$ in FIG. 6.8C,D. Here we observe again the shift of location of the unbound-bound transition defined as $p^{uu} \simeq p^{bb}$ to higher $\beta U_{tot}$ when changing the string topology to a ring topology. This observation is explained by the fact that ring like topology cannot easily accommodate the ligands, thus lowering the temperature of the transition. The cooperativity following from Eqn. 6.7 is shown in FIG. 6.9. The cooperativity, $c(\beta U_{tot})$ is not a trivial function of $N_R$ or $\beta U_{tot}$. For $N_R = 1, 2$ $c$ is zero, agreeing with the RDF analysis where the bound state and the intermediates are indistinguishable. While a ring receptor has a generally higher $c$ than a string receptor, both topologies of the receptor shares a common trend of $c$ with respect to $N_R$. At low energies $c$ becomes positive at $N_R = 3$, reaches the maximum at $N_R = 4$, remains positive but decreases when $N_R > 4$. However, for high $\beta U_{tot}$
6.5 Numerical study of 2D model

Figure 6.8: $N_R = 5$ Binding populations: (A) string receptor; (B) ring receptor. Free energy of states: (C) string receptor (D) ring receptor.

Figure 6.9: Allosteric free energy of 2D model $c(\beta U_{tot})$. (A) string receptor; (B) ring receptor.

This trend reverses, and $c$ becomes actually most cooperative at $N_R = 7$. We are interested in the $c$ values close to the transition region where $p^{bb}$ is close to 0.5. As the transition regions are located at high $\beta U_{tot}$ (20-50$k_BT$, see FIG. 6.8), these results indicate that the cooperativity $c$ increases as $N_R$ increases and reaches the maximum at $N_R = 7$.

6.5.5 Conclusions from 2D model

From the numerical study of our 2D model, we conclude the following: (I) for $N_R < 3$, as the bound state and intermediate states can not be energetically distinguished, cooperativity is zero; (II) The cooperativity $c$ tends to increase with increasing $N_R$ and reaches its maximum at $N_R = 7$; (III) As shown by the difference between the ring and string topology of the receptor, the lateral geometrical constraints play important roles in the binding pattern of the system and changes quantitatively but not qualitatively the pattern of $c$. 
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6.6 Numerical study of 3D model

6.6.1 Model setup

The 3D 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 periodic box with box length \( L \). The receptor is a chain of TMs represented as 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 allowed to diffuse laterally and fluctuate in the perpendicular direction to the membrane. The orientation of the TMs is either fixed in direction perpendicular to the membrane (in SEC. 6.6.3.1) or is allowed to tilt slightly and rotate (in SEC. 6.6.3.3). The two ligands move in the extracellular and the intracellular volume respectively. To avoid that a ligand enters the volume of the other, the simulation box is made periodic only in the \( x,y \) direction. The ligands are modeled as hard spheres of radius \( r_L \). FIG. 6.3 illustrates the model setup.

The Hamiltonian for the model reads

\[
H(r^N) = \sum_{i<j}^{n_R \times N_R} U_{HS}(r_{ij}) + \sum_j^{N_L} \sum_i^{N_R} U_{RL}(r_{ij}) + \sum_i^{N_R-1} \frac{1}{2} k_{RR}(r_{i,i+1} - r_{0,RR})^2 + \sum_i^{N_R} \frac{1}{2} k_{RM} z_i^2
\]

(6.26)

This Hamiltonian contains an additional term with respect to Eqn. 6.24 that denotes the internal fluctuation of each TM in the \( z \)-direction which is restrained by \( k_{RM} \) towards the center of the membrane \( z_i = 0 \).

Two scenarios are studied: (I) TMs are not allowed to tilt or rotate with respect to their center of mass but can move and fluctuate in the normal and lateral directions. (II) TMs can move rotate, tilt and fluctuate laterally and perpendicularly. For Scenario (II), tilting is limited by an upper bound angle \( \theta_{cut}(\varphi) \), which enters the Monte Carlo acceptance criterion.

The geometrical parameters of the 3D model are chosen approximately proportional to their typical values in nature. Setting the unit of length \( \sigma = 2.5 \)Å, the TM radius \( r_R = 2.5 \)Å corresponds roughly to the radius of a real \( \alpha \)-helix. A small ligand such as a hormone, is modeled by \( r_{ex}^{L} = 1.1\sigma = 2.75 \)Å. The membrane thickness \( h_M = 12\sigma \) and the TM length \( h_R = 16\sigma \) correspond to approximately 30Å and 40Å respectively. Asymmetry between the ligands is also considered, by setting a larger intracellular ligand \( r_{in}^{L} = 4.4\sigma = 11 \)Å to represent a larger ligand (e.g. part of a G-protein). The box size is set to \( L = 35\sigma \) to accommodate the most extended configurations. Ligands cannot enter the membrane, and hence are excluded from a region \(|z| < r_L + h_M/2\). No particles are allowed to move out of the box in \( z \)-direction and all satisfies \(|z| < z_{box}/2 = 12.5\sigma \).

The square well ligand-TM potential is set with a shorter width \( \Delta = 0.1\sigma \) and again a depth \( \beta \epsilon = \beta U_{lat}/N_R = 12/N_R \). The equilibrium lateral distance is set \( r_{0,RR} = 2\sigma \) to yield a compact receptor in the membrane plane.

6.6.2 Simulation details

Monte Carlo simulations of the model were performed with the following settings. The number of TMs of the receptor varied from \( N_R = 1 \rightarrow 8 \). For scenario (I), both symmetric
and asymmetric ligands, ring and string receptor topology, are considered giving in total $2 \times 2 \times 8 = 32$ different systems. For each of these systems we considered 5 values for the lateral fluctuation parameter $k_{RR}\sigma^2 = [4, 40, 400, 4000, \infty]k_BT$, and 15 values for the perpendicular fluctuation parameter $k_{RM}\sigma^2$ ranging from 0.04$k_BT$ to $\infty$. For Scenario (II), we consider only the receptor of ring topology with symmetric ligands and a moderate degree of lateral fluctuation $k_{RR}\sigma^2 = 150k_BT$. Ten values of the maximum tilting angle $\theta_{cut} = 1 - 10^9$ are simulated for $N_R = 1 - 8$. The total number of settings of the simulations is $32 \times 5 \times 15 + 8 \times 10 = 2480$.

For each of these settings we performed parallel tempering \cite{24} runs consisting of 13 replicas, with $1 < \beta < 8$. 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.

### 6.6.3 Results

For the 3D model, we only analyze cooperativity from the binding matrix and not from the RDF. As the 3D box is only periodic in the lateral membrane plane, not on z-direction, one has to define $g(r_{12}, z)$, instead of $g(r_{12})$, resulting in complicated and poorly defined bound and intermediate states. Secondly, RDF analysis is not applicable for asymmetrical ligands.

#### 6.6.3.1 Binding probability

FIG. 6.10 exemplifies the populations of all four states and the corresponding free energy for a specific parameter setting. The allosteric free energy $c(\beta)$ for a series of $k_{RM}$ values are also plotted. 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 $k_{RR}$ and $k_{RM}$.
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6.6.3.2 Cooperativity $c$ as function of $N_R$ and $k_{RM}$

Defining $\langle c \rangle_\beta$ as

$$\langle c \rangle_\beta = \frac{\int_{\beta_{\text{min}}}^{\beta_{\text{max}}} c \, d\beta}{\int_{\beta_{\text{min}}}^{\beta_{\text{max}}} d\beta} \quad (6.27)$$

allows compressing the simulation results into two-dimensional cooperativity landscapes as a function of the number of TMs $N_R$ and the perpendicular fluctuation harmonic constant $k_{RM}$, and fulfills the two needs mentioned above. Here $\beta = 1/k_B T$ is again the reciprocal temperature. $\beta_{\text{min}}$ and $\beta_{\text{max}}$ are the minimal and maximal $\beta$ values in the corresponding parallel tempering run. In FIG. 6.11 we plot these cooperativity landscapes for both symmetric and asymmetric ligands and three types of lateral fluctuations.

In the case without lateral fluctuation (left column) $\langle c \rangle_\beta$ is positive for $N_R \geq 4$ and $0.4k_B T < k_{RM}\sigma^2 < 40k_B T$. These $k_{RM}$ values correspond on average to moderate perpendicular fluctuations $0.1 \sim 1\sigma$ ($0.25 \sim 2.5\AA$). For higher values of $k_{RM}\sigma^2 \gg 40k_B T$, 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 $p^{bu} = p^{ub} = 0$ and an undefined $c$. Allowing lateral fluctuation (middle column) shifts and increases the maximum of $\langle c \rangle_\beta$ to values of $k_{RM}\sigma^2 > 40k_B T$, but does not alter the position of the maximum in terms of $N_R$. 

Figure 6.11: Cooperativity $\langle c \rangle_\beta$ as function of $N_R$ and $\log_{10}(\beta_0 k_{RM}\sigma^2)$ ($\beta_0$ is a reference temperature to make the log argument dimensionless). Top row: symmetric ligands; Bottom row: asymmetric ligands; Left column: equilibrium ring topology without lateral fluctuation ($k_{RR} = \infty$); Middle column: the effect of deformation from the ring structure together with fluctuations in the neighbor TM distance ($k_{RR}\sigma^2 = 40k_B T$); Right column: the effects of removing the constraint between N- and C-terminal TMs (string topology); Magenta star: parametrization of 240ns MD simulation of bold $\beta 2 AR$ receptor.
6.6 Numerical study of 3D model

Figure 6.12: Cooperativity $\langle c \rangle_\beta$ as function of $N_R$ and $\theta_{cat}$ for ring topology and symmetric ligands. $\beta_0 k_{RR}\sigma^2$ and $\beta_0 k_{RM}\sigma^2$ are 40 and 150 respectively to gain optimal cooperativity shown in FIG. 6.11. Magenta star: parametrization of 240ns MD simulation of bold $\beta 2AR$ receptor

The effect of lateral fluctuations is more dominant than that of normal fluctuations. Moreover, 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 more dominant than the shape deformation of receptor from the equilibrium ring structure. Introducing asymmetry between ligands (lower row) only slightly reduces the altitude of $\langle c \rangle_\beta$ with respect to the symmetric case, but induces a negative $\langle c \rangle_\beta$ for small $k_{RM}$, for all $N_R$.

6.6.3.3 The effect of tilting and rotation on cooperativity

To explore the influence of the fluctuation of TM-tilting and rotation on binding cooperativity, we allow rotation and tilting of TM with respect to the normal vector of the membrane in the simulations. The rotational angle $\varphi$ of TM helices around the normal vector of the membrane is randomly distributed between 0 and $2\pi$. The TM-tilting angle $\theta$ has a uniform distribution between 0 and a cut off angle $\theta_{cut}$.

In FIG. 6.12 a plot the effect of tilting and rotation for a ring receptor with symmetric ligands. We chose $\beta_0 k_{RR}\sigma^2 = 40$ and $\beta_0 k_{RM}\sigma^2 = 150$ as these values correspond to the fluctuations observed in the MD study of a $\beta 2AR$ receptor and to the region with optimal as shown in FIG. 6.11. Notably, increasing the maximally allowed value of the tilt angle $\theta_{cut}$ from $0^\circ$ to $10^\circ$ generally reduces $< c >_\beta$ for all $N_R$ values. For example for $N_R = 6$, $c$ drops from $1.3k_BT$ to $0k_BT$. Thus freedom of rotation decreases the cooperativity of the binding of the two ligands.

6.6.3.4 Mapping the MD simulation on the CG model

We can compare the MD simulation to the CG model results by transforming the scale of fluctuations of the TMs in the MD into CG units which would correspond to $k_{RM}$ and $k_{RR}$.
values (or equivalently the histogram of fluctuations in the unbound state $[R]$), since the MD simulation only represents $[R]$ in our CG model:

$$
\beta_0 k_{RR} \sigma^2 = \frac{\sigma^2}{\langle x^2_{MD} \rangle} \approx 40 \quad (6.28)
$$

$$
\beta_0 k_{RR} \sigma^2 = \frac{\sigma^2}{\langle z^2_{MD} \rangle} \approx 150 \quad (6.29)
$$

These values are plotted as magenta stars in FIG. 6.11 and FIG. 6.12 and fall within the region of optimal positive cooperativity. While the comparison between the MD results and our 3D CG model is indirect, as we cannot measure the cooperativity directly in the MD simulations, these results agree with our hypothesis that naturally occurring receptors have evolved to a region of optimal cooperativity for signal transduction.

Our results are in agreement with Ref. [6] 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 the TM fluctuation (e.g. the normal fluctuation) upon binding.

### 6.7 Conclusions

Our numerical studies of the 2D and 3D model of membrane receptor signaling 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 and rotation) of the TMs 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 bold forms of GPCRs, promising NMR techniques [20], scattering experiments [3] or mutation studies that suppress fluctuations by e.g. introducing chemical bonds.

### 6.8 Appendix - Hill coefficient

The Hill coefficient was first introduced by A. V. Hill in 1910 to describe the binding of oxygen to hemoglobin [11], and subsequently has been widely used in biochemistry, physiology, and pharmacology to analyze binding equilibria in ligand-receptor interactions [28]. Also it is currently commonly used to estimate the number of ligand molecules that are required to bind to a receptor to produce a functional effect, especially for the reactions in which the ligands are identical.

Let $L$ be the identical ligands, and $R$ the receptor. Without knowing the total number of binding sites for $L$ on $R$, we assume the binding reaction happens as follows.

$$
mL + R \rightleftharpoons mL_R \quad (6.30)
$$

where

$$
K = \frac{L_mR}{[L]^m[R]} \quad (6.31)
$$
is the equilibrium constant. \( m \) is an unknown parameter representing the average number of \( L \) bound to \( R \). Considering the total population of \( R \), both with and without \( L \) bound, \( R_{\text{tot}} = [R] + [L_mR] \), we note that the fractional occupancy of \( R \) is

\[
\phi = \frac{[L_mR]}{[L_mR] + [R]} \tag{6.32}
\]

Then we have

\[
\ln \frac{[L_mR]}{[R]} = \ln \frac{\phi}{1 - \phi} = m \ln[L] - \ln K \tag{6.33}
\]

Hill coefficient is defined at the half saturation point (\( \phi=0.5 \)) as

\[
m = \left. \frac{d \ln \frac{\phi}{1 - \phi}}{d \ln[L]} \right|_{\phi=0.5} \tag{6.35}
\]

Note that defining the Hill coefficient at half saturation point is necessary due to the fact that at the mostly "unbound" (\( \phi \approx 0 \)) or "bound" (\( \phi \approx 1 \)) regime, the population of one or more of the extreme states will be so small that large statistical error will be induced to the estimate of cooperativity, or the Hill coefficient, in both simulations and experiments. But at half saturation point, all states have a finite population and no sampling of extremely small population of any state is encountered.

6.8.1 Use & misuse

The Hill coefficient does not apply to cases where ligands are of different species (heterotrophic allostery). Note that the Hill reaction scheme (Eqn. 6.30) is not realistic due to the existence of multiple intermediates in the complex binding reaction. Being only an "effective" number of binding sites, the Hill coefficient can estimate the total number of binding sites on \( R \) only when the population of all intermediates are negligible, i.e. when the binding events are extremely positively cooperative. As pointed out, Hill coefficient provides merely a minimum of the total number of binding sites for \( L \) on \( R \)[28]. Therefore one should not misuse Hill coefficient to predict the number of binding sites on the receptor if the pre-condition of extreme positive cooperativity is not fulfilled.

On the other hand, when the total number of binding sites \( N \) is known, Hill coefficient can be used to analyze binding cooperativity: (I) when \( m = 1 \), there are still only on average one ligand bound to the receptor even though the reaction is in principle a multi-body assembly, cooperativity between the ligands is zero; (II) \( m > 1 \) means positive cooperativity, especially \( m \to N \) indicates extremely cooperative binding among the ligands; (III) \( m < 1 \) indicates negative cooperativity, with \( m \to 0 \) corresponds to infinitive negative cooperativity.

6.8.2 Derivation of the Hill coefficient from the RDF

The Hill coefficient is straightforward to define from the RDF. By identifying the peaks of the RDF, one obtains the level of the cooperative binding, i.e. the probability of the peaks
corresponding to the bound states and the intermediates. Thus integrating the total probability for these peaks defines the fraction of bound states \( \phi = \frac{[L_m R]}{[L_m R] + [R]} \), while integrating the “flat” part \( g(r_{12}) \approx 1 \) yields the fraction of the unbound state \( \frac{[R]}{[L_m R] + [R]} \).

Thus the Hill coefficient \( m = \frac{d \ln \phi}{d \ln [L]} \big|_{\phi=0.5} \) is a function of \([L]\). One can avoid recomputing the bound fraction by realizing that \([L]\) is simply scaling with the box volume and does not alter the integral. As \( r_{12} \to \infty \), \( g(r_{12}) \approx 1 \); \( L_1 \) and \( L_2 \) should tend not to interact with each other, and any further increase of \( r_{12} \) contributes only to the ideal gas component of the total free energy of the system.

Following this idea, we can illustrate the essence of Hill coefficient. The total Helmholtz free energy of the system can be expressed in terms of the ideal gas part and excess part:

\[
A = -\frac{1}{\beta} \ln Z
= A_{id} + A_{ex}
= -\frac{1}{\beta} (\ln(Z_{id}) + \ln(Z_{ex}))
\]  

with

\[
Z_{id} = \int_0^{r_{box}} dr_{12} \zeta(r_{12})
Z_{ex} = \int_0^{r_{box}} dr_{12} \zeta(r_{12}) (g(r_{12} - 1))
\]

Here \( r_{box} \) is the maximal possible distance between \( L_1 \) and \( L_2 \) in the box, \( \zeta(r_{12}) \) is a geometrical correction term defined in SEC. 6.4.2.1. While \( Z_{ex} \) is basically the effective interaction between \( L_1 \) and \( L_2 \), \( Z_{id} \) is effectively the volume \( V \) of the system. In addition, \( Z_{ex} \) is a function of the inverse temperature \( \beta \) and the volume of the system \( Z_{ex}(\beta, V) \). In this context, we use \( Z_{ex} \) to approximate the sum of the final state and the intermediates \([L_m R]\) and \( Z_{id} \) to represent \([R]\). \([L]\) is then equal to \( 1/V \):

\[
m = \frac{d \ln \phi}{d \ln [L]} \big|_{\phi=0.5}
= \frac{d \ln \frac{Z_{ex}(\beta, V)}{Z_{id}}}{d \ln (1/V)} \big|_{Z_{ex}=Z_{id}}
= \frac{d(\ln(V) - \ln(Z_{ex}(\beta, V)))}{d \ln(V)} \big|_{Z_{ex}=V}
= 1 - \frac{d(\ln(Z_{ex}(\beta, V)))}{d \ln(V)} \big|_{\ln(Z_{ex})=\ln(V)}
= 1 + \frac{d(\beta A_{ex}(\beta, V))}{d \ln(V)} \big|_{-\beta A_{ex}=\ln(V)}
\]

Eqn. 6.42 tells that the Hill coefficient is determined by the relation between the ideal gas and excess free energy at the half saturation point: (I) \( m=1 \) if \( A_{ex} \) is independent of \( V \); (II) \( m>1 \) when \( \frac{d(\beta A_{ex})}{d \ln(V)} > 0 \); (III) \( m<1 \) if \( \frac{d(\beta A_{ex})}{d \ln(V)} < 0 \). As \( A_{ex} \) does not depend on the volume in our case, the Hill coefficient is equal to 1.
6.8.3 Derivation of the Hill coefficient from the binding matrix

The Hill coefficient can be derived from our definition of the four states based on binding matrix through considering the influence of the volume change of the system.

With our assumption of the independence of the two volumes separated by the cell membrane, we consider increasing one of the compartments (say the extracellular volume $V_1$) to $V'_1 = V_1 + \Delta V_1$, the partition function $Z$ becomes

$$Z' = \int_{V'_1} dr_1 \int dr_2 \int dr_R e^{-\beta(E_0(r_R) + E_1(r_1, r_R) + E_2(r_2, r_R))}$$  \hspace{1cm} (6.43)

As the first integral in Eqn. 6.43 is over the volume $V'_1$, the integral can be expressed as

$$Z' = Z + \int_{\Delta V_1} dr_1 \int dr_2 \int dr_R e^{-\beta(E_0(r_R) + E_1(r_1, r_R) + E_2(r_2, r_R))}$$  \hspace{1cm} (6.44)

The integral over $\Delta V_1$ is simpler than the full partition function, as the ligand can never bind in that volume, thus $E_1 = 0$.

$$Z' = Z + \frac{\Delta V_1}{V_1^0} \int dr_2 \int dr_R e^{-\beta(E_0(r_R) + E_2(r_2, r_R))}$$  \hspace{1cm} (6.45)

Inserting a $\delta$ function and integrating $r_1$ over the original volume $V_1$ again gives:

$$Z' = Z + \frac{\Delta V_1}{V_1^0} \int dr_2 \int dr_R e^{-\beta(E_0(r_R) + E_2(r_2, r_R))} \delta(E_1)$$  \hspace{1cm} (6.46)

with

$$V_1^0 = \frac{\int r_1 \int dr_2 \int dr_R e^{-\beta(E_0(r_R) + E_2(r_2, r_R))} \delta(E_1)}{\int dr_2 \int dr_R e^{-\beta(E_0(r_R) + E_2(r_2, r_R))}}$$  \hspace{1cm} (6.47)

which is the average volume (in the original system before volume change) in which the energy of the extracellular ligand is zero. The integral in the Eqn. 6.46 is over all states with the outer ligand not bound. Thus, according to Eqn. 6.13 we can replace the integral with the sum over $z_{0j}$

$$Z' = Z + \frac{\Delta V_1}{V_1^0} Z^0$$  \hspace{1cm} (6.48)

with

$$Z^0 = \sum_{j=0}^{N_R} \delta_{j}$$  \hspace{1cm} (6.49)

The new binding probability/populations now follow directly from

$$p'_{uu} = \frac{Z''_{uu} + \Delta V_1 Z'^{0u}}{Z'}$$  \hspace{1cm} (6.50)

$$p'_{ab} = \frac{Z''_{ab} + \Delta V_1 Z'^{0b}}{Z'}$$  \hspace{1cm} (6.51)

$$p'_{bu} = \frac{Z''_{bu}}{Z'}$$  \hspace{1cm} (6.52)

$$p'_{bb} = \frac{Z''_{bb}}{Z'}$$  \hspace{1cm} (6.53)
Now the fraction of occupancy as we define becomes
\[ Z_{0u} = Z \sum_{j=0}^{n-1} z_{0j} \quad (6.54) \]
\[ Z_{0b} = Z \sum_{j=n}^{N_R} z_{0j} \quad (6.55) \]

Thus, the probabilities reduce to
\[ p'_{uu} = \frac{Z_{uu} + \Delta V_i Z_{0u}}{Z + \frac{\Delta V_i}{V_i} (Z_{0u} + Z_{0b})} = \frac{Z_{uu} / Z + \Delta V_i Z_{0u} / Z}{1 + \frac{\Delta V_i}{V_i} (Z_{0u} + Z_{0b}) / Z} = \frac{p_{uu} + \Delta V_i \sum_{j=0}^{n-1} z_{0j}}{1 + \Delta V_i Z^0 / Z} \quad (6.56) \]
\[ p'_{ub} = \frac{Z_{ub} + \Delta V_i Z_{0b}}{Z + \frac{\Delta V_i}{V_i} (Z_{0u} + Z_{0b})} = \frac{Z_{ub} / Z + \Delta V_i Z_{0b} / Z}{1 + \frac{\Delta V_i}{V_i} (Z_{0u} + Z_{0b}) / Z} = \frac{p_{ub} + \Delta V_i \sum_{j=0}^{n} z_{0j}}{1 + \Delta V_i Z^0 / Z} \quad (6.57) \]
\[ p'_{bu} = \frac{Z_{bu} / Z + \frac{\Delta V_i}{V_i} (Z_{0u} + Z_{0b})}{1 + \frac{\Delta V_i}{V_i} (Z_{0u} + Z_{0b}) / Z} = \frac{p_{bu} / Z}{1 + \Delta V_i Z^0 / Z} \quad (6.58) \]
\[ p'_{bb} = \frac{Z_{bb} / Z + \frac{\Delta V_i}{V_i} (Z_{0u} + Z_{0b})}{1 + \frac{\Delta V_i}{V_i} (Z_{0u} + Z_{0b}) / Z} = \frac{p_{bb} / Z}{1 + \Delta V_i Z^0 / Z} \quad (6.59) \]

The one still unknown is \( V_1^0 \), which seems not easy to compute analytically due to the influence from geometry of the particles and the fluctuation in the system. One possible assumption is that \( V_1^0 \approx V_1 \), when the unbound volume is much larger than the volume in which binding is possible to occur. If we use this assumption, the volume dependence follows directly from the binding matrix.

Setting \( V_1' = V_1 + \Delta V_1 \), the above expressions become
\[ p'_{uu}(V_1') = \frac{p_{uu} + \left( \frac{V_1'}{V_1} - 1 \right) \sum_{j=0}^{n-1} z_{0j}}{1 + \left( \frac{V_1'}{V_1} - 1 \right) Z^0 / Z} \quad (6.60) \]
\[ p'_{ub}(V_1') = \frac{p_{ub} + \left( \frac{V_1'}{V_1} - 1 \right) \sum_{j=0}^{N_R} z_{0j}}{1 + \left( \frac{V_1'}{V_1} - 1 \right) Z^0 / Z} \quad (6.61) \]
\[ p'_{bu}(V_1') = \frac{p_{bu}}{1 + \left( \frac{V_1'}{V_1} - 1 \right) Z^0 / Z} \quad (6.62) \]
\[ p'_{bb}(V_1') = \frac{p_{bb}}{1 + \left( \frac{V_1'}{V_1} - 1 \right) Z^0 / Z} \quad (6.63) \]

The above also follows if we replace all binding matrix elements by \( z'_{ij} = z_{ij} + (V_1'/V_1 - 1) \delta_{ij} \). Now the fraction of occupancy as we define becomes
\[ \phi = \frac{Z_{bu} + Z_{bb}}{Z_i(V_1')} = \frac{Z_{bu} + Z_{bb}}{Z_{bu} + Z_{bb} + Z_{ub} + Z_{uu} + (V_1'/V_1 - 1) Z^0} \quad (6.64) \]
\[ = \frac{1}{1 + \frac{Z_{ub} + Z_{uu} + (V_1'/V_1 - 1) Z^0}{Z_{bu} + Z_{bb}}} = \frac{1}{1 + \frac{Z_{ub} + Z_{uu} - Z^0}{Z_{bu} + Z_{bb} + \frac{V_1'}{V_1} Z^0}} \quad (6.65) \]
Using our definition of bound versus unbound states we can get an expression for this derivative that defines the Hill coefficient in Eqn. [6.35]. Setting \( L = 1/V_1 \), with \( V_1 \) the volume of the top compartment, we obtain for the Hill coefficient

\[
m = -V_1 \frac{d(\ln \phi - \ln(1 - \phi))}{d \ln V_1} = -V_1 \left[ \frac{1}{\phi} + \frac{1}{1 - \phi} \right] \frac{d\phi}{dV_1}
\]

(6.66)

The derivative \( d\phi/dV_1 \) can be evaluated directly by

\[
\frac{d\phi}{dV_1} = \lim_{\Delta V_1 \rightarrow \infty} \frac{\phi(V_1 + \Delta V_1) - \phi(V_1)}{\Delta V_1}
\]

(6.67)

Using our definition of bound versus unbound states we obtain for the bound fraction \( \phi(V_1) \)

\[
\phi(V_1) = p_{bu} + p_{bb} = \frac{Z^{bu} + Z^{bb}}{Z}
\]

(6.68)


\[
\frac{d\phi}{dV_1} = \lim_{\Delta V_1 \rightarrow \infty} \frac{1}{\Delta V_1} \left[ \frac{Z^{bu} + Z^{bb} Z^{bu} + Z^{bb}}{Z + \frac{\Delta V_1}{V_1} Z^0} \right] = \lim_{\Delta V_1 \rightarrow \infty} \frac{-(Z^{bu} + Z^{bb}) Z^0}{V_1 Z^2}
\]

where we defined \( Z^0 = Z^{bu} + Z^{bb} \). For the second factor of Eqn. [6.66] we get

\[
\frac{1}{\phi} + \frac{1}{1 - \phi} = \frac{Z}{Z^{bu} + Z^{bb} + Z - (Z^{bu} + Z^{bb})} = \frac{Z^2}{(Z^{bu} + Z^{bb})(Z - (Z^{bu} + Z^{bb}))}
\]

(6.70)

Combining the above equations and realizing that \( Z - (Z^{bu} + Z^{bb}) = Z^{uu} + Z^{ub} \) gives for \( m \),

\[
m = -V_1 \frac{Z^2}{(Z^{bu} + Z^{bb})(Z^{uu} + Z^{ub})} \frac{(Z^{bu} + Z^{bb}) Z^0}{V_1 Z^2} = \frac{Z^0}{Z^{uu} + Z^{ub}}
\]

(6.71)

Now, if \( Z^0 = Z^{uu} + Z^{ub} \), that is, if the unbound states are characterized by the ligand having no interaction with the receptor this simplifies to \( m = 1 \). Hence, the Hill coefficient is always equal to one in our setup, not suitable to indicate the cooperativity between the two binding sites on the two sides of the membrane.
Bibliography


