Allostery in oligomeric receptor models

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We show how equilibrium binding curves of receptor heterodimers and homodimers can be expressed as rational polynomial functions of the equilibrium binding curves of the constituent monomers, without approximation and without assuming independence of receptor monomers. Using a distinguished spanning tree construction for reduced graph powers, the method properly accounts for thermodynamic constraints and allosteric coupling between receptor monomers.

Guanine nucleotide-binding protein (G protein) coupled receptors (GPCRs) are the largest family of signaling proteins in the mammalian genome and targets for therapeutic drugs (1, 2). When GPCRs are activated by extracellular agonists, they interact with heterotrimeric G proteins to regulate downstream second messenger and protein kinase cascades; notably, cyclic-adenosine monophosphate (cAMP), inositol 1,4,5-triphosphate (IP_3), and diacylglycerol (DAG). For example, agonist binding to the β_2-adrenergic receptor (β_2AR) stimulates adenylate cyclase (AC) and generates cAMP. Agonist binding to the β_2AR leads to conformational changes in the 7-transmembrane receptor that destabilize the nucleotide-binding pocket of the G-protein α_s-subunit to release GDP, prior to GTP binding and activation of G proteins. G-protein binding to the 7-transmembrane receptor, in turn, changes the affinity of ligand binding (3).

Equilibrium receptor-occupancy models are used by pharmacologists to quantify changes in ligand affinity and efficacy, and various modes of activation of GPCRs (4–8). Allosteric modulators of GPCRs are of therapeutic interest due to their potential for greater specificity than agonist and antagonist orthosteric ligands (9). Indeed, allosteric modulators hold promise for treating numerous CNS disorders, including schizophrenia (10, 11).

Evidence for clustering of GPCRs has been obtained using various experimental methods, including radioligand binding, coimmunoprecipitation, and fluorescence resonance energy transfer microscopy (FRET) (12–14). Indeed, some GPCRs are obligate dimers (e.g., the $GABA_B$ receptor and taste receptors for sweet and umami responses) (15–17). The physical interactions between monomeric units of GPCR oligomers are likely to be important determinants in the mechanism of receptor activation (18–20). It is widely believed that dimerization and higher-order complexing diversifies GPCR signaling (21–23). Although mathematical analysis has provided specific insights into the complexity of allosteric interactions of receptor oligomers (24, 25), a deeper theoretical understanding of oligomeric signaling could lead to further opportunities for pharmacological intervention.

This paper introduces a novel theoretical framework for understanding allostery and thermodynamic constraints in oligomeric receptor models that are composed of any number of identical monomers. The framework allows equilibrium occupancy measures (i.e., binding curves) of receptor homodimers to be expressed in terms of the properties of constituent monomers, without approximation and without assuming independence of receptor monomers. This is achieved using a distinguished spanning tree construction for reduced graph powers, concepts that are explained in detail below. But first, we briefly review how allosteric parameters arise in receptor models.

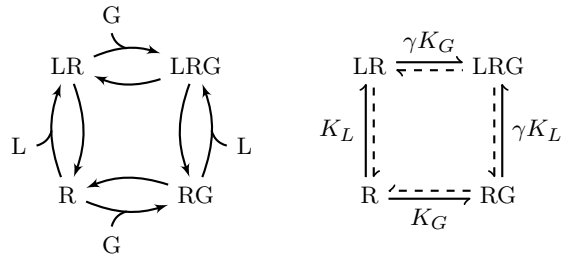


Fig. 1. Ternary complex model of a G-protein coupled receptor (26, 27). For given ligand and G protein concentration ($[\mathrm{L}]$ and $[\mathrm{G}]$), there are three free parameters: κ_ℓ and κ_g (Eq. 1), as well as the allosteric coupling parameter γ.

Thermodynamic constraints in receptor models. There are well-known relationships between cycles in the graph representing receptor model topology, thermodynamic constraints on equilibrium model parameters, and the possibility of allosteric coupling. Consider the aforementioned ternary complex model of ligand (L), 7-transmembrane receptor (R), and G-protein (G) interactions (Fig. 1). The ternary complex model hypothesizes distinct binding sites for ligand (orthosteric) and G-protein (allosteric), 4 receptor conformations (states), and 4 reversible reactions. Microscopic reversibility requires that the product of the transition rates around the four states of the ternary complex model is the same clockwise as counter-clockwise when ligand and G-protein concentrations are independent of receptor state (28). If we consider bimolecular association as the forward reaction, the chemical equilibrium constants are

$$K_L = \frac{[\mathrm{LR}]}{[\mathrm{L}][\mathrm{R}]}\,, \quad K_G = \frac{[\mathrm{RG}]}{[\mathrm{G}][\mathrm{R}]}\,, \tag{1}$$

$\hat{K}_L = [\mathrm{LRG}]/([\mathrm{L}][\mathrm{RG}])$, and $\hat{K}_G = [\mathrm{LRG}]/([\mathrm{G}][\mathrm{LR}])$, where $[\mathrm{L}]$, $[\mathrm{R}]$, etc., represent equilibrium concentrations. The cycle in the ternary complex model leads to the thermodynamic constraint $K_L\hat{K}_G = K_G\hat{K}_L$ and, consequently, the TCM has 3 (not 4) free equilibrium parameters. To emphasize the *cooperativity* of the two binding processes, define an allosteric parameter $\gamma = \hat{K}_G/K_G = \hat{K}_L/K_L$. In that case, the receptor model is specified by two equilibrium association constants (K_L, K_G) and γ, the strength of allosteric cou-

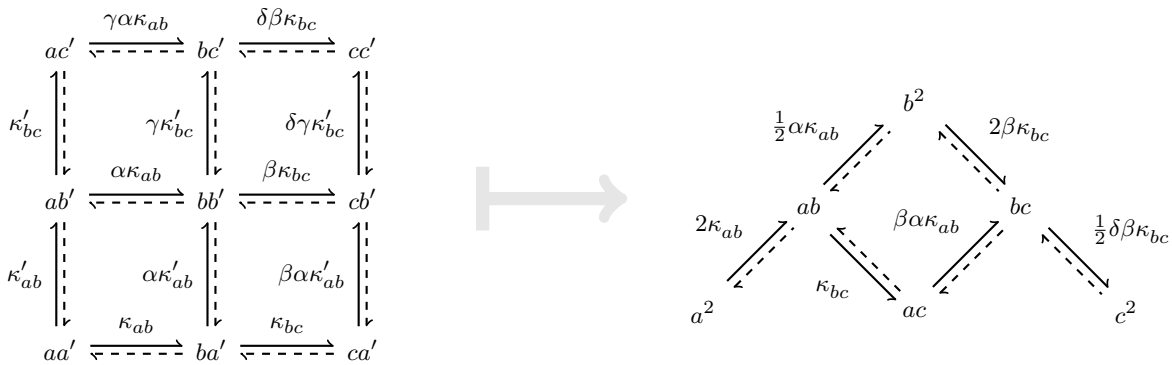


Fig. 2. Left: Equilibrium parameters for a heterodimer composed of two 3-state monomers (same topology, different parameters). There are four Cartesian squares and four allosteric parameters: α, β, γ, and δ. Right: State-transition diagrams for dimer composed of identical and indistinguishable subunits (not necessarily independent).

pling. The ligand affinity is K_L when G protein is unbound, and γK_L when G protein is bound. Thus, $\gamma > 1$ specifies a TCM in which G protein binding increases agonist affinity (as in β_2ARs).

For any [L] and [G], the equilibrium fraction of receptors in each of the four states can be found by expressing each receptor state concentration in terms of [R],

$$[\mathrm{LR}] = K_L[\mathrm{L}][\mathrm{R}] \quad (2)$$
$$[\mathrm{RG}] = K_G[\mathrm{G}][\mathrm{R}] \quad (3)$$
$$[\mathrm{LRG}] = \gamma K_G[\mathrm{G}][\mathrm{LR}] = \gamma K_G[\mathrm{G}]K_L[\mathrm{L}][\mathrm{R}]\,. \quad (4)$$

Solving Eqs. 2–4 simulatneously with the equation for the conserved total receptor concentration,

$$[\mathrm{R}]_T = [\mathrm{R}] + [\mathrm{LR}] + [\mathrm{RG}] + [\mathrm{LRG}]\,,$$

gives the fraction of receptors in each state,

$$[\mathrm{R}]/[\mathrm{R}]_T = 1/z_T \quad (5)$$
$$[\mathrm{LR}]/[\mathrm{R}]_T = K_L[\mathrm{L}]/z_T \quad (6)$$
$$[\mathrm{RG}]/[\mathrm{R}]_T = K_G[\mathrm{G}]/z_T \quad (7)$$
$$[\mathrm{LRG}]/[\mathrm{R}]_T = \gamma K_G[\mathrm{G}]K_L[\mathrm{L}]/z_T \quad (8)$$

where

$$z_T = 1 + K_L[\mathrm{L}] + K_G[\mathrm{G}] + \gamma K_G[\mathrm{G}]K_L[\mathrm{L}]\,. \quad (9)$$

Binding curves for the TCM model are given by $[\mathrm{R}]/[\mathrm{R}]_T$, $[\mathrm{LR}]/[\mathrm{R}]_T$, etc., as a function of [L] and [G] (not shown).

Cooperativity in receptor dimers

Allosteric parameters account for interactions between monomers that compose receptor dimers. To illustrate, consider a monomer with sequential reactions,

$$a \underset{}{\overset{\kappa_{ab}}{\rightleftarrows}} b \overset{\kappa_{bc}}{\rightleftarrows} c\,,$$

where κ_{ab} and κ_{bc} are dimensionless equilibrium constants. For an isolated monomer, the occupancy measures are given by

$$\pi_i = z_i/z_T \quad \text{where} \quad z_T = \textstyle\sum_i z_i\,, \quad (10)$$

$z_a = 1$, $z_b = \kappa_{ab}$, and $z_c = \kappa_{bc}\kappa_{ab}$, that is,

$$\pi_a = 1/(1+\kappa_{ab}+\kappa_{bc}\kappa_{ab}) \quad (11)$$
$$\pi_b = \kappa_{ab}/(1+\kappa_{ab}+\kappa_{bc}\kappa_{ab}) \quad (12)$$
$$\pi_c = \kappa_{bc}\kappa_{ab}/(1+\kappa_{ab}+\kappa_{bc}\kappa_{ab})\,. \quad (13)$$

It is convenient to denote this set of rational functions as

$$[\pi_a : \pi_b : \pi_c] = [1 : \kappa_{ab} : \kappa_{bc}\kappa_{ab}]$$

where it is understood that $[x_1 : x_2 : \cdots : x_n] = [\lambda x_1 : \lambda x_2 : \cdots : \lambda x_n]$ for any $\lambda \neq 0$, and $\lambda = 1/\sum_i x_n$ gives the normalized probability distribution $\pi = (\pi_1, \pi_2, \ldots, \pi_n)$ where $1 = \sum_i \pi_i$.

Heterodimer. A receptor heterodimer model composed of two *distinguishable* monomers with this topology has 9 states, 12 reversible reactions, 4 thermodynamic constraints, and $12 - 4 = 8$ free equilibrium parameters (Fig. 2). Each monomer contributes 2 parameters, for a total of 4 (κ_{ab}, κ_{bc} and κ'_{ab}, κ'_{bc}). The remaining parameters (α, β, γ and δ) encode the strength of 4 2-way allosteric interactions among the monomers (one for each 4-cycle).

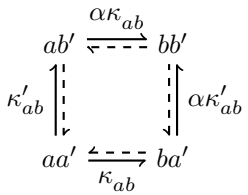
To clarify the meaning of allosteric parameters in Fig. 2, write $\kappa_{ab}[a']$ for the equilibrium constant of the $a \rightleftharpoons b$ reaction of first monomer *occurring in the context of the second monomer being in state* a', and similarly for $\kappa_{ab}[b']$, $\kappa'_{ab}[a]$, $\kappa'_{ab}[b]$.

$$\begin{tikzcd}
ab' \arrow[r, dashed, shift left, "\kappa_{ab}[b']"] & bb' \arrow[l, dashed, shift left] \\
aa' \arrow[u, dashed, shift left, "\kappa'_{ab}[a]"] \arrow[r, dashed, shift left, "\kappa_{ab}[a']"'] & ba' \arrow[u, dashed, shift left, "\kappa'_{ab}[b]"'] \arrow[l, dashed, shift left]
\end{tikzcd}$$

In that case, the allosteric parameter α is, by definition,

$$\alpha := \frac{\kappa_{ab}[b']}{\kappa_{ab}[a']} = \frac{\kappa'_{ab}[b]}{\kappa'_{ab}[a]}\,.$$

Taking states a and a' as reference points, we write $\kappa_{ab} := \kappa_{ab}[a']$, $\kappa'_{ab} := \kappa'_{ab}[a]$. Consequently, $\kappa_{ab}[b'] = \alpha\kappa_{ab}$, $\kappa'_{ab}[b] = \alpha\kappa'_{ab}$, and the equilibrium parameters are



Similar definitions for β, γ and δ lead to state-transition diagram of Fig. 2. The proportion of dimers in each state,

$$\pi = [z_{aa'} : z_{ba'} : z_{ab'} : \cdots : z_{cc'}],$$

can be 'read off' the state-transition diagram, remembering that the equilibrium constants are defined so that $a \to b \to c$ and $a' \to b' \to c'$ are forward reactions (29). Because aa' is not a product of a forward reaction, we assign $z_{aa'} = 1$. The other z_i are given by the product of equilibrium parameters labeling forward reactions on a path from aa' to i. For example, to calculate $z_{cb'}$, we observe the path $aa' \to ab' \to bb' \to cb'$, passes in the forward direction through three reactions with equilibrium constants κ'_{ab} ($aa' \to ab'$), $\alpha\kappa_{ab}$ ($ab' \to bb'$), and $\beta\kappa_{bc}$ ($bb' \to cb'$); the product gives $z_{cb'} = \beta\kappa_{bc} \cdot \alpha\kappa_{ab} \cdot \kappa'_{ab}$. In a similar manner we obtain $z_{ba'} = \kappa_{ab}$, $z_{ab'} = \kappa'_{ab}$,

$$\begin{aligned}
z_{ca'} &= \kappa_{bc} \cdot \kappa_{ab} \\
z_{bb'} &= \alpha\kappa_{ab} \cdot \kappa'_{ab} \\
z_{ac'} &= \kappa'_{bc} \cdot \kappa'_{ab} \\
z_{bc'} &= \gamma\alpha\kappa_{ab} \cdot \kappa'_{bc} \cdot \kappa'_{ab} \\
z_{cc'} &= \delta\beta\kappa_{bc} \cdot \gamma\alpha\kappa_{ab} \cdot \kappa'_{bc} \cdot \kappa'_{ab}.
\end{aligned}$$

Homodimer. A receptor homodimer composed of two *indistinguishable* monomers with the same 3-state topology has 6 states, 6 reversible reactions, 1 thermodynamic constraint, and $6-1=5$ free equilibrium parameters (Fig. 2, right). This time, the monomers, being identical, contribute only 2 parameters (κ_{ab}, κ_{bc}). Three distinct allosteric parameters (α, $\beta = \gamma$ and δ) encode the stength of 2-way interactions.

The receptor homodimer state-transition diagram (Fig. 2, right) is a contraction of the receptor heterodimer (left) obtained by dropping the primes (e.g., $\kappa'_{ab} = \kappa_{ab}$ and $\kappa'_{bc} = \kappa_{bc}$), setting $\beta = \gamma$, and identifying $a^2 \sim aa'$, $ab \sim ba' + ab'$, …, $c^2 \sim cc'$. The fraction of dimers in each state,

$$\pi = [z_{a^2} : z_{ab} : z_{b^2} : z_{ac} : z_{bc} : z_{c^2}], \qquad \textbf{(14)}$$

is given by $z_{a^2} = 1$, $z_{ab} = 2\kappa_{ab}$, $z_{b^2} = \alpha\kappa_{ab}^2$, $z_{ac} = 2\kappa_{ab}\kappa_{bc}$,

$$\begin{aligned}
z_{bc} &= \beta\alpha\kappa_{bc} \cdot \kappa_{bc} \cdot 2\kappa_{ab} = 2\alpha\beta\kappa_{ab}^2\kappa_{bc} \\
z_{c^2} &= \tfrac{1}{2}\delta\beta\kappa_{bc} \cdot \beta\alpha\kappa_{ab} \cdot \kappa_{bc} \cdot 2\kappa_{ab} = \alpha\beta^2\delta\kappa_{ab}^2\kappa_{bc}^2.
\end{aligned}$$

Where the combinatorial coefficient 2 (resp. 1/2) appears as a factor on the transitions out of (resp. into) states a^2, b^2 and c^2. Identifying $\beta = \gamma$ leads to the correspondences $z_{c^2} \sim z_{cc'}$, $z_{bc} \sim z_{cb'} + z_{bc'}$, etc., as expected.

Homodimer allostery. Importantly, the above calculation did *not* assume independent monomers. Rather, the dependence of the monomers in the homodimer has been parameterized by the three allosteric parameters α, β and γ. To see this, transform Eq. 14 to an equivalent expression by dividing each term of Eq. 14 by $(1+\kappa_{ab}+\kappa_{bc}\kappa_{ab})^2$ to obtain

$$\pi = [\pi_a^2 : 2\pi_a\pi_b : \alpha\pi_b^2 : 2\pi_a\pi_c : 2\beta\alpha\pi_b\pi_c : \delta\beta^2\alpha\pi_c^2], \quad \textbf{(15)}$$

where π_a, π_b and π_c are given by Eqs. 11–13.

Eq. 15 is significant. *Without assuming independence*, we have expressed the occupancy measure for a receptor homodimer in terms of

- the occupancy measures of a isolated monomer (π_a, π_b and π_c, determined by κ_{ab}, and κ_{bc}) and
- the allosteric parameters α, β and δ.

In the absence of allosteric interactions, i.e., $\alpha = \beta = \delta = 1$, Eq. 15 simplifies as expected: $\pi_{a^2} = \pi_a^2$, $\pi_{ab} = 2\pi_a\pi_b$, etc.

The remainder of the paper presents a *general theory of allostery in oligomeric receptors composed of any number of identical monomers*. First, we introduce concepts from graph theory that establish the structure of state-transition diagram of receptor homodimers (and oligomers), for any given monomer topology. Next, we characterize cycles and thermodynamic constraints in receptor oligomers in terms of the monomer topology. Third, we show how allosteric interactions in receptor oligomers may be systematically enumerated. Finally, we show how the occupancy measure of a receptor oligomer may always be expressed in terms of the occupancy measures of a isolated monomer and identified allosteric parameters.

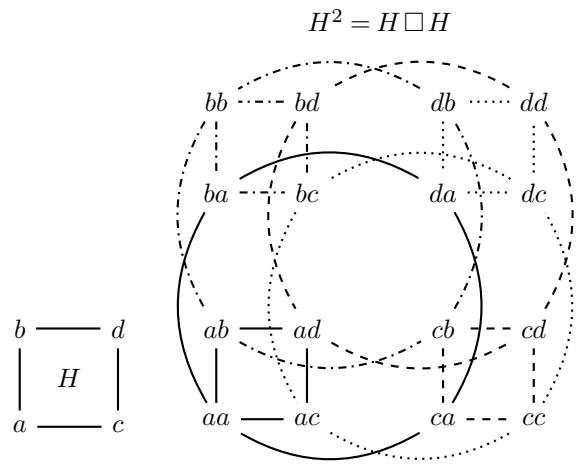


Fig. 3. Topology of a receptor dimer composed of two distinguishable ternary complex monomers (H, see Fig. 1) is given by the Cartesian graph power $H^2 = H \square H$.

Receptor oligomers and reduced graph powers

Let the undirected graph $G = (V(G), E(G))$ represent a receptor subunit model with $v = |V(G)|$ states and $e = |E(G)|$ reversible transitions between these states. We assume G has no loops or multiple edges, and is connected. *What graph corresponds to a receptor homomer composed of k identical subunits with topology given by G?* The answer to this question is the kth *reduced power of G*, denoted by $G^{(k)}$, which is

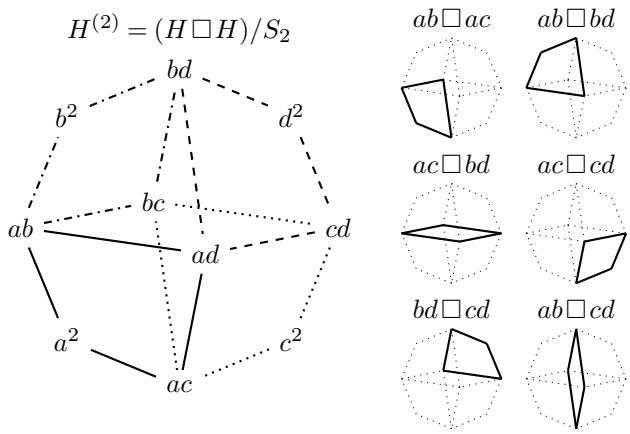


Fig. 4. Left: Topology of a homodimer composed of two *identical and indistinguishable* ternary complex monomers (H, see Fig. 3) is given by the *reduced graph power* $H^{(2)}$ (bottom). For each vertex x of H, the vertices $\{xv \mid v \in V(H)\} \subseteq V(H^{(2)})$ induce a subgraph $Hx \cong H$ of $H^{(2)}$. The subgraph Ha is shown solid in $H^{(2)}$. Right: 6 of 10 4-cycles in $H^{(2)}$ are Cartesian squares.

defined as a contraction of product graphs. We briefly review this construction, following prior work (30).

Recall (31) that the *Cartesian product* of graphs G and H is the graph $G\square H$ whose vertex set is the Cartesian product $V(G)\times V(H)$, and whose edges are

$$\begin{aligned} E(G\square H) \quad = \quad & \{(x,u)(y,v) \mid xy \in E(G) \text{ and } u = v \\ & \textbf{ or } x = y \text{ and } uv \in E(H)\}, \end{aligned}$$

where xy is an abbreviation for a vertex (x,y) of $G\square H$ and, similarly, $uv = (u,v) \in V(G\square H)$. The kth *Cartesian power* of G is the k-fold product $G^k = G\square G\square \cdots \square G$. The *reduced* kth power is the quotient G^k/S_k of G^k by the action of the symmetric group S_k, which acts on G^k by permuting the factors.

In general, say G has vertex set $\{a_1, a_2, \ldots, a_v\}$. Denote by $M_k(G)$ the set of monic monomials of degree k, with indeterminates $V(G)$, and $M_0(G) = \{1\}$. Then $G^{(k)}$ is the graph whose

$$|V(G^{(k)})| = \left(\!\!\binom{v}{k}\!\!\right) = \binom{v+k-1}{k} \tag{16}$$

vertices are the monomials $a_1^{n_1} a_2^{n_2} \cdots a_v^{n_v} \in M_k(G)$. For edges, if $a_i a_j$ is an edge of G, and $f(a_1, a_2, \ldots a_v) \in M_{k-1}(G)$, then $a_i f(a_1, a_2, \ldots, a_v)$ is adjacent to $a_j f(a_1, a_2, \ldots, a_v)$. For each edge $a_i a_j$ of G and each monomial $f \in M_{k-1}(G)$, there is an edge of $G^{(k)}$ from $a_i f$ to $a_j f$; thus, the number of edges of $G^{(k)}$ is

$$|E(G^{(k)})| = e\left(\!\!\binom{v}{k-1}\!\!\right) = e\binom{v+k-2}{k-1}. \tag{17}$$

As an example, consider the graph H shown in Fig. 3, which has the topology of the ternary complex model (Fig. 1). Here $V(H) = \{a,b,c,d\}$ and $E(H) = \{\{a,b\},\{a,c\},\{b,d\},\{c,d\}\}$. The graph power H^2 has $v^2 = 16$ vertices and $2ev = 32$ edges. Fig. 4 shows the *reduced* graph power $H^{(2)}$ that corresponds to the state-transition diagram of a ternary complex receptor dimer composed of identical and indistinguishable monomers. $H^{(2)}$ has

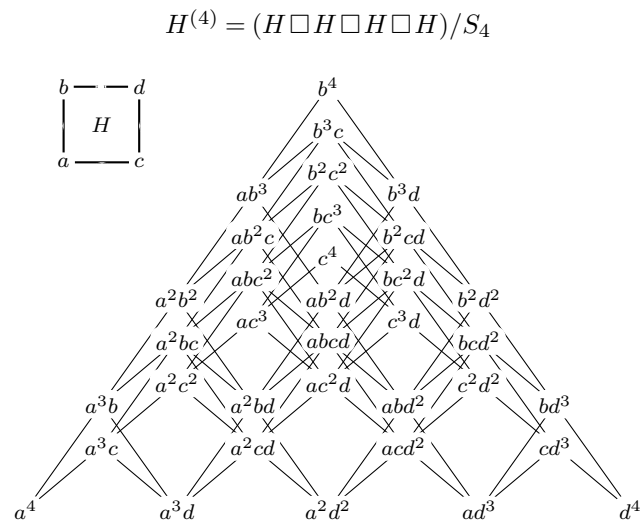


Fig. 5. Topology of a receptor oligomer composed of 4 identical ternary complex monomers (H, see Fig. 1) is given by the reduced power $H^{(4)}$ that contains $\binom{e}{2}\left(\!\!\binom{v}{k-2}\!\!\right) = 60$ Cartesian squares, but many of these are not independent. The cycle space of $H^{(4)}$ has dimension 46.

$\left(\!\!\binom{4}{2}\!\!\right) = 10$ vertices and $4\left(\!\!\binom{4}{1}\!\!\right) = 16$ edges. The edge $(ab, bd) \in E(H^{(2)})$ corresponds to one monomer making an $(a,d) \in E(H)$ transition, i.e, $a \to d$ or $d \to a$, while the other monomer is in state $b \in V(H)$, i.e., an ad transition in the *context* of b. Fig. 5 shows the reduced graph product $H^{(4)}$ that corresponds a receptor oligomer composed of 4 ternary complex monomers. $H^{(4)}$ has $\left(\!\!\binom{4}{4}\!\!\right) = 35$ vertices and $4\left(\!\!\binom{4}{3}\!\!\right) = 80$ edges. The vertices enumerated by expanding the multinomial $(a+b+c+d)^4$ and dropping coefficients, because $V(H^{(4)}) = M_4(H)$. The edge $(b^2cd, bc^2d) = (b,c)\square bcd \in E(H^{(4)})$ corresponds to one monomer of the receptor 4-mer making an $b \rightleftharpoons c$ transition in the context of bcd.

Thermodynamic constraints and cycle space of $G^{(k)}$

The number of thermodynamic constraints in a receptor model is given by *dimension of the cycle space* of the state-transition graph. Following (30), we briefly review this concept.

For a graph G, its *edge space* $\mathcal{E}(G)$ is the power set of $E(G)$ viewed as a vector space over the two-element field $\mathbb{F}_2 = \{0,1\}$, where the zero vector is $0 = \emptyset$ and addition is symmetric difference. Any vector $X \in \mathcal{E}(G)$ is viewed as the subgraph of G induced on X, so $\mathcal{E}(G)$ is the set of all subgraphs of G without isolated vertices. Thus $E(G)$ is a basis for $\mathcal{E}(G)$, and $\dim(\mathcal{E}(G)) = |E(G)|$. For a graph G, its *cycle space* $\mathcal{C}(G)$ is the set of all subgraphs in $\mathcal{E}(G)$ whose vertices all have even degree (that is, the Eulerian subgraphs). Because every such subgraph can be decomposed into edge-disjoint cycles, we see that $\mathcal{C}(G) \subseteq \mathcal{E}(G)$ is spanned by the cycles in G. The dimension of $\mathcal{C}(G)$ is called the *Betti number* of G, and is denoted $\beta(G)$. If G is connected, then

$\beta(G) = |E(G)| - |V(G)| + 1$ (e.g., $\beta(H) = 1$ in Fig. 3). A basis for $\mathcal{C}(G)$ is called a *cycle basis* for G. The *length* of a cycle basis is the sum of the lengths of its cycles. A cycle basis with the least possible length is a *minimum cycle basis* (MCB). See Chapter 29 of (31) for further review.

For a receptor oligomer, the number of thermodynamic constraints is the dimension of the cycle space of the reduced graph product, $\mathcal{C}(G^{(k)})$. Using Eqs. 16 and 17, this is given by the Betti number

$$\beta(G^{(k)}) = e\left(\binom{v}{k-1}\right) - \left(\binom{v}{k}\right) + 1. \qquad \textbf{(18)}$$

The ternary complex 4-mer has $\beta(H^{(4)}) = 80 - 35 + 1 = 46$ thermodynamic constraints (Fig. 5). For receptor homodimers ($k = 2$), Eq. 18 simplifies using $\binom{v+1}{2} = (v+1)v/2$,

$$\beta(G^{(2)}) = ev - \frac{(v+1)v}{2} + 1. \qquad \textbf{(19)}$$

The ternary complex homodimer has $\beta(H^{(2)}) = 16 - 10 + 1 = 7$ thermodynamic constraints (Fig. 4).

Minimal cycle basis of reduced graph powers

Enumerating the $\beta(G^{(k)})$ thermodynamic constraints of a receptor oligomer model is achieved by decomposing cycle space of a reduced power $G^{(k)}$ into the direct sum of two particularly simple subspaces. Following (30), we briefly describe this process.

(1) First, notice that if f is a fixed monomial in $M_{k-1}(G)$, then $x \mapsto xf$ is an embedding $G \to G^{(k)}$. Call the image of this map Gf. Note that Gf is an induced subgraph of $G^{(k)}$ and is isomorphic to G.

(2) Next, we define a special type of cycle in a reduced power. Given distinct edges wx and yz of G and any $f \in M_{k-2}(G)$, there is a square in $G^{(k)}$ with vertices wyf, xyf, xzf, wzf. Let us call such a square a *Cartesian square*, and denote it as $(wx\square yz)f$. Note that a subgraph Gf of $G^{(k)}$ may have squares (4-cycles), but these are not *Cartesian squares*, because they do not have the form specified above. In Fig. 4, $Ha = a^2 + ab + ac + ad \subset H^{(2)}$ is *not* a Cartesian square, whereas the square $ab\square bd = ab + b^2 + bd + ad$ *is* a Cartesian square.

(3) Define the *square space* $\mathcal{S}(G^{(k)})$ to be the subspace of $\mathcal{C}(G^{(k)})$ that is spanned by the Cartesian squares. Any pair of distinct edges wx and yz of G corresponds to a Cartesian square $(wx\square yz)f$, where $f \in M_{k-2}(G)$, so there are $\binom{e}{2}\left(\binom{v}{k-2}\right)$ such squares; however, this set of squares may not be independent. Prior work (30) gives a construction of a *square basis*, i.e., a maximum independent set $\mathcal{B}$ of Cartesian squares in $\mathcal{S}(G^{(k)})$ (see below).

(4) For fixed $f \in M_{k-1}(G)$, the cycle space of reduced kth power of G is the direct sum

$$\mathcal{C}(G^{(k)}) = \mathcal{C}(Gf) \bigoplus \mathcal{S}(G^{(k)}). \qquad \textbf{(20)}$$

Thus, a basis for $\mathcal{C}(G^{(k)})$ may be constructed from independent cycles in $\mathcal{C}(Gf)$ and $\mathcal{S}(G^{(k)})$, as follows. Take a cycle basis $\mathcal{C} = \{C_1, C_2, \ldots, C_{\beta(G)}\}$ for G, and let $\mathcal{B}$ be a square basis for $\mathcal{S}(G^{(k)})$. Fix a monomial $f \in M_{k-1}(G)$ and put $\mathcal{C}f = \{C_1 f, C_2 f, \ldots, C_{\beta(G)} f\}$. Then $\mathcal{C}f \cup \mathcal{B}$ is a cycle basis for $G^{(k)}$. If $\mathcal{C}$ is a minimal cycle basis (MCB) for G, and G has no triangles, then this basis is an MCB for $G^{(k)}$.

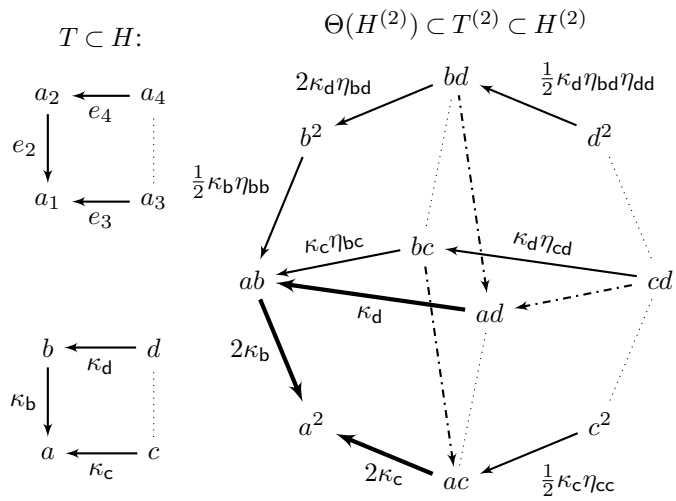


Fig. 6. Rooted spanning tree T of H with indexing respecting a breadth first traversal. The reduced graph product $T^{(2)}$ spans $H^{(2)}$ and $\dim \mathcal{C}(T^{(2)}) = 3$. The subgraph Ta of $T^{(2)}$ is shown thick. Eliminating one edge of each Cartesian square in Υ (dash-dotted lines), the basis of the square space $\mathcal{S}(H^{(2)})$), results in the spanning tree $\Theta(H^{(2)})$ (solid arrows). Specification of three equilibrium association constants and allosteric parameters in the ternary complex homodimer. For expanded notation, make the replacements $\kappa_\mathsf{b} = \kappa_{ab}$, $\kappa_\mathsf{c} = \kappa_{ac}$, $\kappa_\mathsf{d} = \kappa_{bd}$, $\eta_\mathsf{bb} = \eta_{(ab)^2}$, $\eta_\mathsf{bc} = \eta_{ab\square ac}$, $\eta_\mathsf{cc} = \eta_{(ac)^2}$, $\eta_\mathsf{bd} = \eta_{ab\square bd}$, $\eta_\mathsf{cd} = \eta_{ac\square bd}$, and $\eta_\mathsf{dd} = \eta_{(bd)^2}$.

The *square basis* $\mathcal{B}$ may be constructed as follows. Put $V(G) = \{a_1, a_2, \ldots, a_v\}$. Let T be a rooted spanning tree of G with root a_1 with indexing that respects a breadth-first traversal. Any edge of T is thus uniquely determined by its endpoint a_j that is furthest from the root. For each $2 \le i \le v$, let e_j be the edge of T that has endpoints a_i and a_j, with a_j further from the root than a_i. Let $M_{k-2}(a_1, a_2, \ldots a_j)$ denote the monic monomials of degree $k-2$ in indeterminates $a_1, a_2, \ldots, a_j$, with $1 \le j \le v$. Define the following sets of Cartesian squares in $G^{(k)}$:

$$\begin{aligned} \Upsilon &= \{(e_i \square e_j)f \mid \\ &\qquad 2 \le i < j \le v, f \in M_{k-2}(a_1, a_2, \ldots, a_j)\} \qquad \textbf{(21)} \\ \Omega &= \{(a_\ell a_m \square e_j)f \mid a_\ell a_m \in E(G) - E(T), \\ &\qquad 2 \le j \le v, f \in M_{k-2}(a_1, a_2, \ldots, a_j)\}. \qquad \textbf{(22)} \end{aligned}$$

The set $\mathcal{B} = \Upsilon \cup \Omega$ is a basis for the square space $\mathcal{S}(G^{(k)})$.

Identifying thermodynamic constraints

The minimal cycle basis construction for the reduced graph power $G^{(k)}$ suggests a systematic method for enumerating $\beta(G^{(k)})$ edges of a receptor oligomer state-transition graph that correspond to thermodynamic constraints (Eq. 18). The remaining $|V(G^{(k)})|$ edges will be a distinguished spanning tree of $G^{(k)}$, denoted by $\Theta(G^{(k)})$, to which we will assign equilibrium parameters.

(1) Choose a rooted spanning tree of the monomer ($T \subset G$) with indexing that respects a breadth-first traversal (as in

point (3) of the previous section). Because T has no cycles, $\beta(T)=0$ and $\mathcal{C}(T)=\emptyset$. Note that the edges of the spanning tree T are directed reactants $\leftarrow$ products (arrow oriented as the reverse reaction). Let κ_{e_i} denote the equilibrium parameter associated with edge $e_i \in E(T)$.

(2) Construct the reduced graph power $T^{(k)}$, a reduced graph power with the same vertex set as $G^{(k)}$, namely, $M_k(a_1,a_2,\ldots,a_v)$. The edges of $T^{(k)}$ inherit direction from T, because edges of $T^{(k)}$ take the form $e_i f$ where $f \in M_{k-1}$. Note that $T^{(k)}$ spans $G^{(k)}$, $E(T^{(k)}) = E(G^{(k)}) - \Omega$ with Ω as in Eq. 22. Thus, the cycle space of $T^{(k)}$ is its square space, $\mathcal{C}(T^{(k)}) = \mathcal{S}(T^{(k)})$ (Eq. 20), and a basis for $\mathcal{C}(T^{(k)})$ may be constructed from the set Υ (Eq. 21) of independent cycles in $\mathcal{S}(T^{(k)})$.

(3) The distinguished spanning tree $\Theta(G^{(k)})$ we seek has edges

$$E(\Theta(G^{(k)})) = E(T^{(k)}) - \Psi \qquad \textbf{(23)}$$

where Ψ is a set of distinct edges, one from each square in Υ, as follows. Write $e_i = (a_i^-, a_i)$ where a_i^- is the predecessor of a_i in T (and similarly for e_j). Each square $(e_i \square e_j) f \in \Upsilon$ is composed of the four edges (Eq. 21),

$$\begin{aligned}(e_i \square e_j) f \quad &= \quad (a_i^- \square e_j) f + (a_i \square e_j) f \\ &\quad + (e_i \square a_j^-) f + \underbrace{(e_i \square a_j) f}_{\text{exclude}},\end{aligned}$$

where $2 \le i < j \le v$ and $f \in M_{k-2}(a_1,a_2,\ldots,a_j)$. These edges are directed as e_i and e_j,

$$\begin{tikzcd}
a_i^- a_j f \circ \arrow[d, "(a_i^- \square e_j) f"'] & \circ\, a_i a_j f \arrow[l, dashed, "(e_i \square a_j) f"'] \arrow[d, "(a_i \square e_j) f"] \\
a_i^- a_j^- f \circ & \circ\, a_i a_j^- f \arrow[l, "(e_i \square a_j^-) f"]
\end{tikzcd}$$

We include in Φ—and, thus, exclude from $\Theta(G^{(k)})$—the edge $(e_i \square a_j) f$, shown dashdotted above, that is the transition-context pair with context a_j that is last in dictionary order ($a_j^- < a_j$ and $a_j^- < a_i < a_j$). Thus, the set Ψ in Eq. 23 is

$$\begin{aligned}\Psi \quad = \quad & \{(e_i \square a_j) f \mid \\ & 2 \le i < j \le v, f \in M_{k-2}(a_1,a_2,\ldots,a_j)\} \qquad \textbf{(24)}\end{aligned}$$

These edges are distinct, so that $|\Psi| = \beta(T^{(k)})$. In this way, we construct $\Theta(T^{(k)})$, a subgraph of $T^{(k)}$ that is a distinguished spanning tree of $G^{(k)}$. Two examples follow.

Ternary complex dimer. For $H^{(2)}$, shown in Fig. 4, $k=2$ and $M_{k-2}=\{1\}$. Eq. 21 and the rooted spanning tree $T \subset H$ shown in Fig. 6 yields the following basis of the square space $\mathcal{S}(T^{(2)})$,

$$\Upsilon(T^{(2)}) = \{ab \square ac, ab \square bd, ac \square bd\}.$$

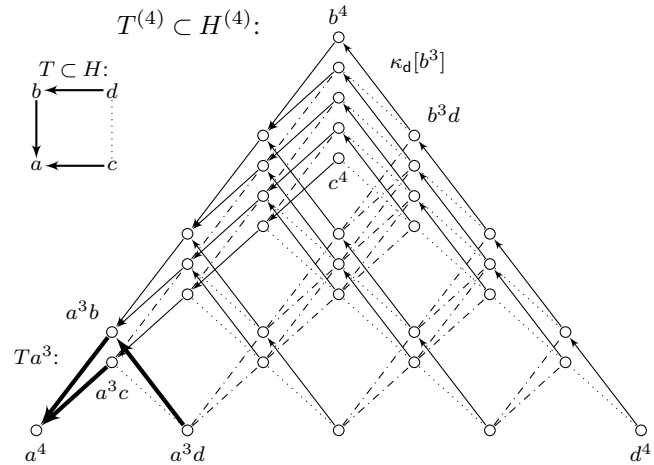


Fig. 7. Topology of a receptor oligomer composed of 4 identical ternary complex monomers (H, see Fig. 1) is given by the reduced power $H^{(4)}$ that contains $\binom{e}{2}\left(\binom{v}{k-2}\right) = 60$ Cartesian squares, but many of these are not independent. The cycle space of $H^{(4)}$ has dimension 46.

The 12 edges of these 3 Cartesian squares are

$$\begin{aligned}
ab \square ac \quad &= \quad a \square ac + b \square ac + ab \square a + ab \square c \\
&= \quad (a^2, ac) + (ab, bc) + (a^2, ab) + \boxed{(ac, bc)} \\
ab \square bd \quad &= \quad a \square bd + b \square bd + ab \square b + ab \square d \\
&= \quad (ab, ad) + (b^2, bd) + (ab, b^2) + \boxed{(ad, bd)} \\
ac \square bd \quad &= \quad a \square bd + c \square bd + ac \square b + ac \square d \\
&= \quad (ab, ad) + (bc, cd) + (ab, bc) + \boxed{(ad, cd)}.
\end{aligned}$$

The edges of $\Phi = \{(ac,bc),(ad,bd),(ad,cd)\}$ are shown dashdotted in Fig. 6.

Ternary complex 4-mer. For $H^{(4)}$, shown in Fig. 5, the Betti number is 46. $T^{(4)} \subset H^{(4)}$ has Betti number $\beta(T^{(4)}) = e\left(\binom{4}{4-1}\right) - \left(\binom{4}{4}\right) + 1 = 3 \cdot 20 - 35 + 1 = 26$ (Fig. 7). The squares that form the basis Υ of the square space (and cycle space) of $T^{(4)}$ are

$$\begin{aligned}\Upsilon \quad = \quad & \{(ab \square ac) g \mid g \in M_2(a,b,c)\} \cup \\ & \{(ab \square bd) f, (ac \square bd) f \mid f \in M_2(a,b,c,d)\}\end{aligned}$$

where

$$\begin{aligned}M_2(a,b,c) \quad &= \quad \{a^2, ab, b^2, ac, bc, c^2\} \\ M_2(a,b,c,d) \quad &= \quad M_2(a,b,c) \cup \{ad, bd, cd, d^2\}.\end{aligned}$$

Using $ab \square c = (ac, bc)$, $ab \square d = (ad, bd)$, and $ac \square d = (ad, cd)$, the $6 + 2 \cdot 10 = 26$ element set Φ, shown dashdotted in Fig. 7, is

$$\begin{aligned}\Phi \quad = \quad & \{(acg, bcg) \mid g \in M_2(a,b,c)\} \cup \\ & \{(adf, bdf), (adf, cdf) \mid f \in M_2(a,b,c,d)\}.\end{aligned}$$

Allosteric parameters in receptor occupancy models

At this point we are prepared to accomplish our primary objective: to assign, in a systematic and general fashion, equilibrium parameters to the edges of the receptor oligomer

state-transition graph $G^{(k)}$. These equilibrium constants are,

$$\kappa_{a_i a_j}[f] \quad \text{where} \quad 1 \le i < j \le v\,,\ f \in M_{k-1}(G)\,,$$

where $a_i a_j$ is the *transition* of a monomer and f is the context (the state of the $k-1$ other monomers). For the receptor homomer $G^{(k)}$, it suffices to assign parameters to the edges of the distinguished spanning tree $\Theta(G^{(k)})$, as constructed in the previous section using the rooted spanning tree $T \subset G$ and the reduced graph power $T^{(k)}$. Because every edge of Θ can be expressed as a transition-context pair with the edge of T (the transition) denoted $e_i = (a_i^-, a_i)$, these equilibrium constants will be denoted as

$$\kappa_{e_i}[f] \quad \text{where} \quad 2 \le i \le v\,,\ f \in M_{k-1}(G)\,.$$

For example, the spanning tree T of the ternary complex monomer has three edges $e_2 = (a_1, a_2)$, $e_3 = (a_1, a_3)$ and $e_4 = (a_2, a_4)$. For readability we write $a = a_1$, $b = a_2$, $c = a_3$, $d = a_4$; thus, $\mathsf{b} = (a,b) = e_2$, $\mathsf{c} = (a,c) = e_3$, and $\mathsf{d} = (b,d) = e_4$. Using this notation, the equilibrium parameters for the spanning tree Θ (Fig. 6) are $\kappa_{\mathsf{b}}[a]$ for the edge (a^2, ab), $\kappa_{\mathsf{b}}[b]$ for (ab, b^2), $\kappa_{\mathsf{d}}[c]$ for (bc, cd), and so on. In $\Theta(H^{(4)})$, the equilibrium constant on the edge $(b^4, b^3 d)$ is denoted $\kappa_{\mathsf{d}}[b^3]$ in Fig. 7.

Enumerating allosteric interactions in receptor homomers

The number of free equilibrium parameters in a receptor oligomer is the number of edges, less the number of thermodynamic constraints, $|E(G^{(k)})| - \beta(G^{(k)}) = |V(G^{(k)})| - 1$, which is the number of edges in the spanning tree $\Theta(G^{(k)})$. For example, the homodimer $H^{(2)}$ is specified by assigning $|E(\Theta(H^{(2)}))| = 9$ parameters (Θ shown solid in Fig. 6). The 4-mer requires $|E(\Theta(H^{(4)}))| = 34$ parameters (Fig. 7, solid arrows).

How should these free parameters for a receptor homomer be specified? Recall that T is a spanning tree of G, Tf for any fixed $f \in M_{k-1}(G)$ is a subgraph of $\Theta(G^{(k)})$ isomorphic to T. Choosing $f = a_1^{k-1}$, we assign the equilibrium parameters associated to the edges of T in the monomer model to the corresponding edges of $Ta_1^{k-1} \subset \Theta(G^{(k)})$.

In the ternary complex homodimer (Fig. 6, thick arrows), these are the directed edges $a^2 \leftarrow ab$, $a^2 \leftarrow ac$, $ab \leftarrow ad$, which are assigned parameters κ_b, κ_c and κ_d, respectively. In the ternary complex 4-mer (Fig. 7), the edges of Ta^3 are $a^4 \leftarrow a^3 b$, $a^4 \leftarrow a^3 c$, $a^3 b \leftarrow a^3 d$.

Next, we assign parameters to remaining edges in a manner consistent with allosteric interactions among monomers. To begin, recall that $v - 1 = |E(T)|$ and define $\left(\!\binom{v-1}{k}\!\right)$ 2-way allosteric parameters

$$\eta_{e_i e_j}[f] := \frac{\kappa_{e_i}[a_j f]}{\kappa_{e_i}[a_j^- f]} = \frac{\kappa_{e_j}[a_i f]}{\kappa_{e_j}[a_i^- f]}\,,$$

where $2 \le i \le j \le v$ and $f \in M_{k-2}(G)$. When $e_i = e_j$, we write

$$\eta_{(e_i)^2}[f] := \frac{\kappa_{e_i}[a_i f]}{\kappa_{e_i}[a_i^- f]}\,.$$

When $(e_i \,\square\, e_j) f$ is a Cartesian square in $G^{(k)}$, we may write $\eta_{(e_i \square e_j) f} := \eta_{e_i e_j}[f]$ to emphasize this fact. For example, the spanning tree $T \subset H$ of the ternary complex monomer has edges, b, c, and d (Fig. 6). The $\left(\!\binom{3}{2}\!\right) = 6$ two-way allosteric parameters for the ternary complex dimer are

$$\eta_{\mathsf{bb}} = \kappa_{\mathsf{b}}[b]/\kappa_{\mathsf{b}}[a] \tag{25}$$
$$\eta_{\mathsf{bc}} = \kappa_{\mathsf{b}}[c]/\kappa_{\mathsf{b}}[a] = \kappa_{\mathsf{c}}[b]/\kappa_{\mathsf{c}}[a] \tag{26}$$
$$\eta_{\mathsf{bd}} = \kappa_{\mathsf{b}}[d]/\kappa_{\mathsf{b}}[b] = \kappa_{\mathsf{d}}[b]/\kappa_{\mathsf{d}}[a] \tag{27}$$
$$\eta_{\mathsf{cc}} = \kappa_{\mathsf{c}}[c]/\kappa_{\mathsf{c}}[a] \tag{28}$$
$$\eta_{\mathsf{cd}} = \kappa_{\mathsf{c}}[d]/\kappa_{\mathsf{c}}[b] = \kappa_{\mathsf{d}}[c]/\kappa_{\mathsf{d}}[a] \tag{29}$$
$$\eta_{\mathsf{dd}} = \kappa_{\mathsf{d}}[d]/\kappa_{\mathsf{d}}[b]\,, \tag{30}$$

where we have used $\mathsf{b} = (a,b)$, $\mathsf{c} = (a,c)$, $\mathsf{d} = (b,d)$ and $f = 1$ ($k = 2$ so $M_{k-2} = \{1\}$). Using the 6 allosteric parameters defined above, and the equilibrium parameters inherited from the monomer model ($\kappa_{\mathsf{b}}[a] = \kappa_{\mathsf{b}}$, $\kappa_{\mathsf{c}}[a] = \kappa_{\mathsf{c}}$, $\kappa_{\mathsf{d}}[a] = \kappa_{\mathsf{d}}$), we will are able to specify the remaining edges of Θ. For example, the parameter on edge (ab, b^2) is formally $\kappa_{\mathsf{b}}[b]$, because this edge is a $\mathsf{b} = (a,b)$ transition in the context of b. Using Eqs. 25–30 we have $\kappa_{\mathsf{b}}[b] = \kappa_{\mathsf{b}}[a]\eta_{\mathsf{bb}} = \kappa_{\mathsf{b}}\eta_{\mathsf{bb}}$. The edge (bd, d^2) is a $\mathsf{d} = (b,d)$ transition in the context of d. The equilibrium constant is $\kappa_{\mathsf{d}}[d] = \kappa_{\mathsf{d}}[b]\eta_{\mathsf{dd}} = \kappa_{\mathsf{d}}\eta_{\mathsf{bd}}\eta_{\mathsf{dd}}$. Repeating this process for all 10 states yields the specification of allosteric parameters in the ternary complex homodimer (Fig. 6). Reading the spanning tree Θ as discussed above, we find,

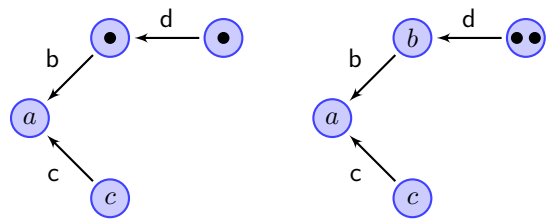
$$\begin{aligned}[\,\pi_{a^2} : \pi_{ab} : \cdots : \pi_{d^2}\,] = [\,&\pi_a^2 : 2\pi_a\pi_b : \pi_b^2\eta_{\mathsf{bb}} \\ &: 2\pi_a\pi_c : 2\pi_b\pi_c\eta_{\mathsf{bc}} : \pi_c^2\eta_{\mathsf{cc}} \\ &: 2\pi_a\pi_d : 2\pi_b\pi_d\eta_{\mathsf{bd}}\eta_{\mathsf{bb}} : 2\pi_c\pi_d\eta_{\mathsf{bc}}\eta_{\mathsf{cd}} : \pi_d^2\eta_{\mathsf{bb}}\eta_{\mathsf{bd}}^2\eta_{\mathsf{dd}}\,]\,.\end{aligned} \tag{31}$$

As promised, without assuming independence of receptor monomers, we have expressed the equilibrium occupancy measure of the ternary complex homodimer in terms of the properties of an isolated monomer (the π_i are functions of κ_{b}, κ_{c} and κ_{d}) and the allosteric parameters (η_{bb}, η_{bc}, etc.). To translating back into our original notation, use $\kappa_{\mathsf{b}} = \kappa_{ab}$, $\kappa_{\mathsf{c}} = \kappa_{ac}$, $\kappa_{\mathsf{d}} = \kappa_{bd}$, $\eta_{\mathsf{bb}} = \eta_{(ab)^2}$, $\eta_{\mathsf{bc}} = \eta_{ab\square ac}$, $\eta_{\mathsf{cc}} = \eta_{(ac)^2}$, $\eta_{\mathsf{bd}} = \eta_{ab\square bd}$, $\eta_{\mathsf{dd}} = \eta_{(bd)^2}$.

Token method for assigning allosteric parameters

The algebraic calculations that assign allosteric parameters to the spanning tree $\Theta(G^{(k)})$ of the receptor oligomer state-transition diagram are systematic. In fact, the allosteric factors may be enumerated using a natural 'token' representation of receptor oligomer states, as follows.

To begin, draw the tree T that spans the state-transition graph G of the monomer. For any given state of the oligomer, put (indistinguishable) tokens in the positions associated with the monomer states. For example, the token graphs associated to states bd and d^2 in the ternary complex dimer are,



To calculate allosteric factor for state bd, we consider the path of each token to the root (the vertex a). These paths yield b for the first token, and $\mathsf{b}+\mathsf{d}$ for the second token. Because the product is $\mathsf{b}(\mathsf{b}+\mathsf{d}) = \mathsf{b}^2+\mathsf{bd}$, the allosteric factor in the term $\pi_b\pi_d$ in Eq. 31 is $\eta_{\mathsf{bb}}\eta_{\mathsf{bd}}$. For state d^2, the path to root for both tokens is $b+d$ and the product is $(\mathsf{b}+\mathsf{d})^2 = \mathsf{b}^2 + 2\mathsf{bd}+\mathsf{d}^2$; thus, the allosteric factor for π_d^2 is $\eta_{\mathsf{bb}}\eta_{\mathsf{bd}}^2\eta_{\mathsf{dd}}$.

Multiway allosteric interactions in receptor oligomers

For receptor oligomers with $k>2$, the situation is complicated by multiway allosteric interactions. Define 3-way allosteric parameters

$$\eta_{e_ie_je_k}[f] := \frac{\eta_{e_ie_j}[a_kf]}{\eta_{e_ie_j}[a_k^-f]} = \frac{\eta_{e_ie_k}[a_jf]}{\eta_{e_ie_k}[a_j^-f]} = \frac{\eta_{e_je_k}[a_if]}{\eta_{e_je_k}[a_i^-f]}$$

where $2\le i\le j\le k\le v$ and $f\in M_{k-3}(G)$. The equalities are shown by expanding the definition,

$$\begin{aligned}\frac{\eta_{e_ie_j}[a_kf]}{\eta_{e_ie_j}[a_k^-f]} &= \frac{\kappa_{e_i}[a_ja_kf]/\kappa_{e_i}[a_j^-a_kf]}{\kappa_{e_i}[a_ja_k^-f]/\kappa_{e_i}[a_j^-a_k^-f]} \\ &= \frac{\kappa_{e_i}[a_ka_jf]/\kappa_{e_i}[a_k^-a_jf]}{\kappa_{e_i}[a_ka_j^-f]/\kappa_{e_i}[a_k^-a_j^-f]} = \frac{\eta_{e_ie_k}[a_jf]}{\eta_{e_ie_k}[a_j^-f]}\,.\end{aligned}$$

In general, n-way allosteric parameters are defined as

$$\eta_{e_{i_1}e_{i_2}\cdots e_{i_n}}[f] := \frac{\eta_{e_{i_1}e_{i_2}\cdots e_{i_{\ell-1}}e_{i_{\ell+1}}\cdots e_{i_n}}[a_{i_\ell}f]}{\eta_{e_{i_1}e_{i_2}\cdots e_{i_{\ell-1}}e_{i_{\ell+1}}\cdots e_{i_n}}[a_{i_\ell}^-f]}\,,\ 1\le\ell\le n\,,$$

where $2\le i_1\le i_2\le\cdots\le i_n\le v$. For a monomer with spanning tree of $v-1$ edges, a receptor composed of k monomers has $\left(\!\binom{v-1}{n}\!\right) = \binom{v+n-2}{n}$ n-way allosteric parameters for $2\le n\le k$. For example, the 4-mer has $\left(\!\binom{3}{2}\!\right) = 6$ 2-way, $\left(\!\binom{3}{3}\!\right) = 10$ 3-way, and $\left(\!\binom{3}{4}\!\right) = 15$ 4-way parameters. Some of these are

$$\begin{aligned}\eta_{\mathsf{bbb}} &= \eta_{\mathsf{bb}}[b]/\eta_{\mathsf{bb}}[a] \\ \eta_{\mathsf{ccd}} &= \eta_{\mathsf{cc}}[d]/\eta_{\mathsf{cc}}[b] = \eta_{\mathsf{cd}}[c]/\eta_{\mathsf{cd}}[a] \\ \eta_{\mathsf{bcd}} &= \eta_{\mathsf{bc}}[d]/\eta_{\mathsf{bc}}[b] = \eta_{\mathsf{bd}}[c]/\eta_{\mathsf{bd}}[a] = \eta_{\mathsf{cd}}[b]/\eta_{\mathsf{cd}}[a] \\ \eta_{\mathsf{bbcd}} &= \eta_{\mathsf{bbc}}[d]/\eta_{\mathsf{bbc}}[b] = \eta_{\mathsf{bbd}}[c]/\eta_{\mathsf{bbd}}[a] = \eta_{\mathsf{bcd}}[b]/\eta_{\mathsf{bcd}}[a] \\ \eta_{\mathsf{ccdd}} &= \eta_{\mathsf{ccd}}[d]/\eta_{\mathsf{ccd}}[b] = \eta_{\mathsf{cdd}}[c]/\eta_{\mathsf{cdd}}[a] \\ \eta_{\mathsf{dddd}} &= \eta_{\mathsf{ddd}}[d]/\eta_{\mathsf{ddd}}[b]\,.\end{aligned}$$

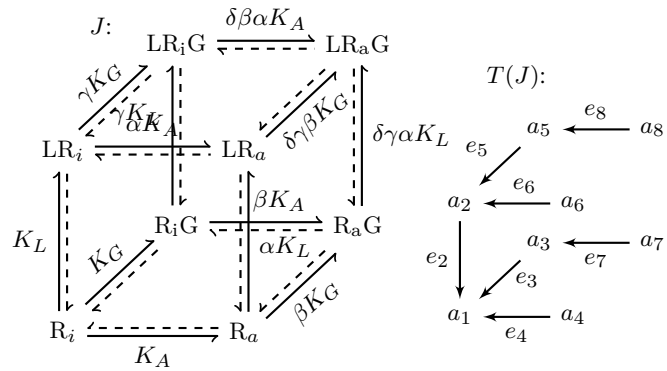


Fig. 8. Cubic ternary complex model of a G-protein coupled receptor (27). There are seven equilibrium parameters: three equilibrium constants (K_L, K_G, K_A) and four allosteric coupling parameter (α, β, γ, δ).

Token method for multiway allosteric parameters

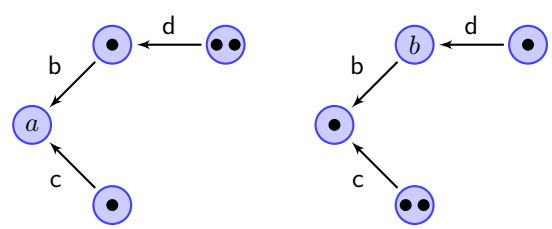
For a receptor k-mer, we may assume $p_1\le p_2\le\cdots\le p_k$ where p_ℓ is the place of the ℓth token. Recall that $e_\ell = (a_\ell^-, a_\ell)$ and define $h(a_\ell)$ recursively,

$$h(a_\ell) = \begin{cases} 0 & \text{for } a_\ell = a_1 \\ e_\ell + h(a_\ell^-) & \text{otherwise.}\end{cases} \tag{32}$$

The n-way interactions are enumerated by the elementary symmetric polynomials in $h_1, h_2, \ldots, h_k$, namely,

$$\varepsilon_n(h_1,h_2,\ldots,h_k) := \sum_{1\le i_1<i_2<\cdots<i_n\le k} h_{i_1}h_{i_2}\cdots h_{i_n}\,. \tag{33}$$

For example, the token graphs associated to states bcd^2 and ac^2d in the ternary complex 4-mer are,



The 2-, 3- and 4-way interactions (Eqs. 32 and 33) are

$$\begin{aligned}\text{2-way} &: h_1h_2+h_1h_3+h_1h_4+h_2h_3+h_2h_4+h_3h_4 \\ \text{3-way} &: h_1h_2h_3+h_1h_2h_4+h_1h_3h_4+h_2h_3h_4 \\ \text{4-way} &: h_1h_2h_3h_4\end{aligned}$$

where

$$\begin{aligned}bcd^2 &: h_1=\mathsf{b} \quad h_2=\mathsf{c} \quad h_3=\mathsf{b}+\mathsf{d} \quad h_4=\mathsf{b}+\mathsf{d} \\ ac^2d &: h_1=0 \quad h_2=\mathsf{c} \quad h_3=\mathsf{c} \quad h_4=\mathsf{b}+\mathsf{d}\,.\end{aligned}$$

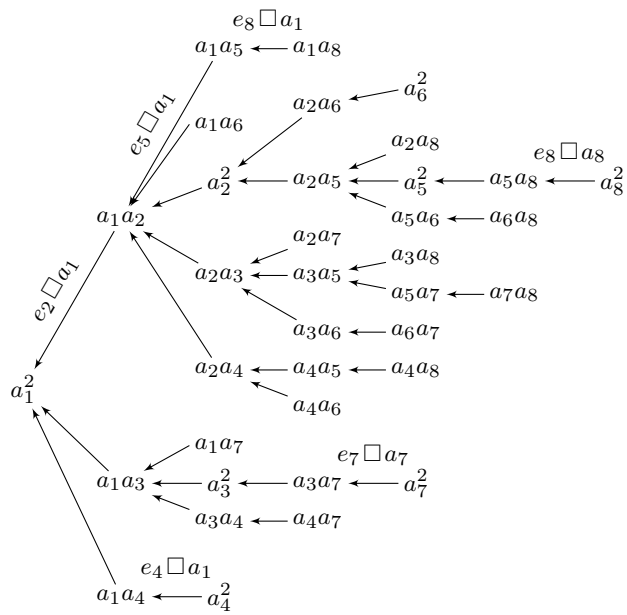


Fig. 9. Spanning tree Θ for the cubic ternary complex homodimer (Fig. 8).

For state bcd^2,

$$\begin{aligned}
\text{1-way} \quad &: \quad 3\mathsf{b}+\mathsf{c}+2\mathsf{d} \\
\text{2-way} \quad &: \quad \mathsf{bc}+2\mathsf{b}(\mathsf{b}+\mathsf{d})+2\mathsf{c}(\mathsf{b}+\mathsf{d})+(\mathsf{b}+\mathsf{d})^2 \\
&\qquad = 3\mathsf{b}^2+4\mathsf{bd}+3\mathsf{bc}+\mathsf{d}^2+2\mathsf{cd} \\
\text{3-way} \quad &: \quad 2\mathsf{bc}(\mathsf{b}+\mathsf{d})+\mathsf{b}(\mathsf{b}+\mathsf{d})^2+\mathsf{c}(\mathsf{b}+\mathsf{d})^2 \\
&\qquad = \mathsf{b}^3+2\mathsf{b}^2\mathsf{d}+3\mathsf{b}^2\mathsf{c}+\mathsf{bd}^2+4\mathsf{bcd}+\mathsf{cd}^2 \\
\text{4-way} \quad &: \quad \mathsf{bc}(\mathsf{b}+\mathsf{d})^2 = \mathsf{b}^3\mathsf{c}+2\mathsf{b}^2\mathsf{cd}+\mathsf{bcd}^2 .
\end{aligned}$$

Thus, the $\pi_b\pi_c\pi_d^2$ term, which has combinatorial coefficient $(1,1,2)! = 12$, has allosteric factors

$$\underbrace{\eta_{\mathsf{bb}}^3\eta_{\mathsf{bd}}^4\eta_{\mathsf{bc}}^3\eta_{\mathsf{dd}}\eta_{\mathsf{cd}}^2}_{\text{2 way}}\ \underbrace{\eta_{\mathsf{bbb}}\eta_{\mathsf{bbd}}^2\eta_{\mathsf{bbc}}^3\eta_{\mathsf{bdd}}\eta_{\mathsf{bcd}}^4\eta_{\mathsf{cdd}}}_{\text{3 way}}\ \underbrace{\eta_{\mathsf{bbbc}}\eta_{\mathsf{bbcd}}^2\eta_{\mathsf{bcdd}}}_{\text{4 way}} .$$

For state ac^2d, a similar calculation gives

$$12\,\pi_a\pi_c^2\pi_d\,\eta_{\mathsf{bc}}^2\eta_{\mathsf{cc}}\eta_{\mathsf{cd}}^2\,\eta_{\mathsf{bcc}}\eta_{\mathsf{ccd}} .$$

Counting allosteric parameters. The method described above for enumerating each allosteric factor in a receptor oligomer model always yields the required $\left(\!\binom{v}{k}\!\right)-1$ parameters. To see this, note that the number of n-way allosteric interactions involving the $v-1=e-\beta(G)$ edges of the spanning tree $T\subset G$ is $\left(\!\binom{v-1}{n}\!\right)$. When $n=1$ this is $\left(\!\binom{v-1}{1}\!\right)=v-1$ (the number of free parameters in a monomer). When these are supplemented with the 2-way through k-way interactions, the correct number of parameters is obtained,

$$\begin{aligned}
v-1+\sum_{n=2}^{k}\left(\!\binom{v-1}{n}\!\right) &= \sum_{n=1}^{k}\left(\!\binom{v-1}{n}\!\right) \\
&= -1+\sum_{n=0}^{k}\left(\!\binom{v-1}{n}\!\right) = -1+\sum_{n=0}^{k}\binom{v+n-2}{n} \\
&= -1+\binom{v+k-1}{k} = -1+\left(\!\binom{v}{k}\!\right) .
\end{aligned}$$

With that said, it is important to note that there is flexibility with regard to the allosteric parameters chosen to express the $\left(\!\binom{v}{k}\!\right)-1$ degrees of freedom. For example, the spanning tree $T\subset H$ chosen to represent the monomer is not unique. There are also easily discoverable identities that relate allosteric parameters, such as

$$\eta_{(ab)^2} = \frac{\eta_{ab\square ac}\eta_{ab\square cd}}{\eta_{ab\square bd}} ,$$

where $\eta_{ab\square cd} := \kappa_{ab}[d]/\kappa_{ab}[c] = \kappa_{cd}[b]/\kappa_{cd}[a]$ (see Eqs. 25–30).

Discussion

The theoretical framework for understanding allostery in receptor oligomers presented here represents an intriguing and novel combination of graph theory and quantitative receptor pharmacology. We began by establishing that the structure of state-transition diagram of a receptor k-mer, for any given monomer topology G, is the reduced graph power $G^{(k)}$. We used a minimal cycle basis construction for reduced graph powers to identify thermodynamic constraints in receptor oligomers *without having to construct* $G^{(k)}$. We showed how allosteric interactions in receptor oligomers may be systematically enumerated. Finally, we show how the occupancy measure of a receptor oligomer may be expressed in terms of the parameters for an isolated monomer and these identified allosteric parameters (Eq. 31).

The concepts and notation introduced here amount to a *general theoretical framework for allostery in oligomeric receptors composed of any number of identical monomers*. For clarity we have used the (perhaps over-simple) ternary complex model dimer and 4-mer as running examples, but the approach is completely general (see Appendix for discussion of the *cubic* ternary complex (27) dimer and 4-mer).

We hope this theoretical framework for receptor homomer allostery will be valuable for subsequent studies of pharmacological alteration of GPCR activity by allosteric modulators, whose action is modeled as a modification of equilibrium constants of one or more receptor monomers. Using a similar approach, an understanding of allosteric interactions in hetero-oligomers is within reach, which could lay a foundation for theoretical analysis of receptor crosstalk, wherein one protomer binds to an agonist, whilst the other unit activates the G protein. Further development of this theory of allosteric modulation in GPCRs may contribute to our understanding of crosstalk between 5-$\mathrm{HT}_{2\mathrm{A}}$ and mGlu_2 heterodimers, and perhaps even the physiology of $\mathrm{G_i}$-$\mathrm{G_q}$ bal-

ance and the mechanism of action of antipsychotic drugs (32, 33).

Appendix: Cubic ternary complex dimer

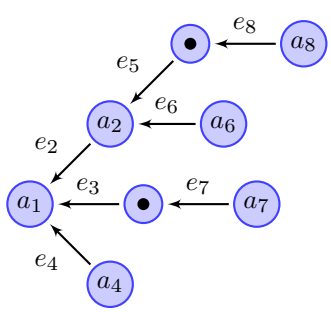
For another example, consider the cubic ternary complex model (denoted J in Fig. 8). There are 12 reversible reactions, but only 7 free equilibrium parameters, because $\beta(G) = e - v + 1 = 12 - 8 + 1 = 5$. In (27), the 5 free parameters are specified as 3 equilibrium constants (K_L, K_G, and $K_A = [\mathrm{R}_a]/[\mathrm{R}_i]$), plus 4 allosteric coupling parameters (α, β, γ, δ). Using a spanning tree with $\mathrm{R}_i \leftrightarrow a_1$ as root (Fig. 8, right), we find

$$\pi = [\, 1 : K_L : K_G : K_A : K_L\gamma K_G \\ : K_L\alpha K_A : K_G\beta K_A : K_L\gamma K_G\delta\beta\alpha K_A \,]$$

where $\pi = [\pi_1 : \pi_2 : \cdots : \pi_8] = [\, [\mathrm{R}_i] : [\mathrm{LR}_i] : \cdots : [\mathrm{LR_aG}] \,]$.

The cubic ternary complex *homodimer* ($J^{(2)}$) has $\binom{9}{2} = 36$ states, $2ev = 192$ edges (it is not a hypercube), and $192 - 36 + 1 = 157$ thermodynamic constraints. There are $192 - 157 = 35$ free equilibrium parameters, and the spanning tree $\Theta(J^{(2)})$ has 35 edges (Fig. 9). The edges removed are $e_i \square a_j = (a_i^- a_j, a_i a_j)$ for $2 \le i < j \le v$. These are $e_2 \square a_3$, …, $e_2 \square a_8$, $e_3 \square a_4$, …, $e_3 \square a_8$, $e_4 \square a_5$, …, $e_4 \square a_8$, $e_5 \square a_6$, …, $e_5 \square a_8$, $e_6 \square a_7$, $e_6 \square a_8$, $e_7 \square a_8$. Fig. 9 shows the edges that remain. Seven parameters are inherited from the monomer model spanning tree that has 7 edges ($e_2, e_3, \ldots, e_8$ in Fig. 8). The remaining parameters are $\left(\!\binom{7}{2}\!\right) = 28$ 2-way allosteric coupling parameters, denoted η_{ij} where $2 \le i \le j \le v$.

The occupation measure for each state can be found using the method described above. For example, the term corresponding to $a_3 a_5$ begins with $2\pi_3\pi_5$. The token diagram is



Here $h_1 = e_3$ and $h_2 = e_2 + e_5$ (the sum of the edge labels from root to token). The 2-way interactions are enumerated by $h_1 h_2 = e_3(e_2 + e_5) = e_2 e_3 + e_3 e_5$, so the allosteric factor is $\eta_{23}\eta_{35}$. Using $h_1 = h_2 = e_4 + e_7$, $h_1 h_2 = e_4^2 + 2e_4 e_7 + e_7^2$, and $\binom{2}{2}$ the term for a_7^2 is found to be $\pi_7^2 \eta_{44}\eta_{47}^2\eta_{77}$. Using a computer algebra system, it is possible automate this procedure to find every term in $\pi = [\, \pi_1^2 : 2\pi_1\pi_2 : \cdots : \pi_7^2\,\eta_{44}\eta_{47}^2\eta_{77} : \cdots : \pi_2\pi_3\,\eta_{23} : \cdots : \pi_8^2\,\eta_{22}\eta_{25}^2\eta_{28}^2\eta_{55}\eta_{58}^2\eta_{88} \,]$ where π_i for the isolated monomer are given above.

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