Biophysical Journal, Volume *111*

Supplemental Information

Biased Allostery

Stuart J. Edelstein and Jean-Pierre Changeux

SUPPORTING MATERIAL

Biased Allostery

Stuart J. Edelstein and Jean-Pierre Changeux

As originally developed the allosteric model described the properties of feedback-inhibited enzymes (1) based on two conformational states, T and R. For receptors the states were renamed B and R, respectively (2)—see below. The original model distinguished between homotropic and heterotropic interactions. For homotropic interactions, cooperative binding of the substrate or a substrate analog was characterized in terms of the fractional occupancy, $\overline{Y}$:

$$\overline{Y} = \frac{\alpha(1+\alpha)^{N-1}+Lc\alpha(1+c\alpha)^{N-1}}{(1+\alpha)^{N}+L(1+c\alpha)^{N}} \quad (1)$$

along with the state function to give the fraction of molecules in the R state, $\overline{R}$:

$$\overline{R} = \frac{(1+\alpha)^{N}}{(1+\alpha)^{N}+L(1+c\alpha)^{N}} \quad (2)$$

These equations are expressed in terms of the affinity of the substrate or other ligand, X, normalized to the R state, α = [X]/K_R. The allosteric constant, L, specifies the relative stability of the T and R states in the absence of ligand, L = [T]/[R]. The ratio of the affinities of the two states for the ligand is given by c, where $c = K_R/K_T$. For heterotropic interactions the modulation of L by binding of positive or negative allosteric modulators is defined by L' (3):

$$L' = L\left[\frac{(1+d\beta)(1+e\gamma)}{(1+\beta)(1+\gamma)}\right]^{N} \quad (3)$$

with heterotropic modulators classified as either inhibitors ($d = K_R/K_T > 1$) or activators ($e = K_R/K_T < 1$) present at normalized concentrations of β or γ, respectively, relative to the corresponding value of K_R.

An important assumption for the above analysis is distinct allosteric sites for the positive and negative modulators. For example, the above equations were applied to the enzyme aspartate transcarbamylase to describe the regulatory effects in the presence of both the allosteric inhibitor CTP and the allosteric activator ATP (4). However, subsequent crystallographic studies revealed that the two families of compounds bind to the same regulator site on the enzyme (5). When two different effector ligands bind competitively, the equations must be reformulated to take into

account competitive binding between different effector ligands to the same site. The competition of two ligands for the same site modifies the basic allosteric equations. For two ligands X and Z that bind to separate sites on an allosteric protein with relative affinities β and γ, all possible species, S_i for binding to a particular conformational state are given by the expansion of the equation:

$$\sum S_i = (1 + \beta)^N (1 + \gamma)^N \quad (4)$$

whereas for competition of the two ligands for the same sites, the various species are given by:

$$\sum S_i = (1 + \beta + \gamma)^N \quad (5)$$

As a result, competitive allostery changes the equations $\overline{Y}$ and $\overline{R}$. For example, in the case of two competitive ligands X and Z the competitive forms of equations 1-3 are modified to give:

$$\overline{Y} = \frac{(\beta+\gamma)(1+\beta+\delta)^{N-1} + L(d\beta+e\gamma)(1+d\beta+e\gamma)^{N-1}}{(1+\beta+\gamma)^N + L(1+d\beta+e\gamma)^N} \quad (6)$$

$$\overline{R} = \frac{(1+\beta+\delta)^N}{(1+\beta+\gamma)^N + L(1+d\beta+e\gamma)^N} \quad (7)$$

$$L' = \left[\frac{(1+d\beta+e\gamma)}{(1+\beta+\gamma)}\right]^N \quad (8)$$

Following the principles described above for competitive allostery, the mathematical representation of biased allostery for GPCRs can be presented in terms of a global partition function for receptors in basal state B, active states A_{blue} and A_{red}, that interact with transfer molecules G-protein and β-arresting, and potentially biased agonists. In this case the sum of all states, $\sum S_i$, is given by the equation below with fixed equilibrium constants defined in Table 1 and variable concentrations of agonist, [Ag]; G-protein [Gp]; and β-arrestin [Ar]. Where $\sum S_i = \sum S_A + \sum S_B$, the two latter terms calculated separately for the A and B states are:

$$\sum S_A = (1+\frac{[Ag]}{A_{K_{Ag\_red}}})\left\{(1 + \frac{[Gp]}{A_{K_{Gp\_red}}} + \frac{[Ar]}{A_{K_{Ar\_red}}})\right\} + M_A(1 + \frac{[Ag]}{A_{K_{Ag\_blue}}})\left\{(1 + \frac{[Gp]}{A_{K_{Gp\_blue}}} + \frac{[Ar]}{A_{K_{Ar\_blue}}})\right\} \quad (9)$$

$$\sum S_B = L_{red}(1+\frac{[Ag]}{B_{K_{Ag\_red}}})\left\{(1 + \frac{[Gp]}{B_{K_{Gp\_red}}} + \frac{[Ar]}{B_{K_{Ar\_red}}})\right\} + M_B(1 + \frac{[Ag]}{B_{K_{Ag\_blue}}})\left\{(1 + \frac{[Gp]}{B_{K_{Gp\_blue}}} + \frac{[Ar]}{A_{K_{Ar\_blue}}})\right\} \quad (10)$$

For the simulations in the present study a single B state was considered, but in principle discrete B_{blue} and B_{red} could be present in parallel with the A_{blue} and A_{red} states, as would be encountered for biased inverse agonism. In order to accommodate this hypothetical situation, equation (10) for $\sum S_B$ allows for B_{blue} and B_{red}, (where $M_B = [B_{blue}]/[B_{red}]$). For the simulations presented here the two potential B states were combined by using identical values for the relevant parameters and only the combined constants are presented in Table 1.

For agonist binding to the full system, the equation for the agonist binding function $\overline{Y}$ is obtain by calculating the appropriate numerator (Y_N) and dividing by $\sum S_i$ to yield $\overline{Y} = Y_N/\sum S_i$, where $Y_N = Y_{N\_A} + Y_{N\_B}$.

$$Y_{N\_A} = \left(\frac{[Ag]}{{}^{A}K_{Ag\_red}}\right)\left\{\left(1 + \frac{[Gp]}{{}^{A}K_{Gp\_red}} + \frac{[Ar]}{{}^{A}K_{Ar\_red}}\right)\right\} + M_A\left(\frac{[Ag]}{{}^{A}K_{Ag\_blue}}\right)\left\{\left(1 + \frac{[Gp]}{{}^{A}K_{Gp\_blue}} + \frac{[Ar]}{{}^{A}K_{Ar\_blue}}\right)\right\} \quad (11)$$

$$Y_{N\_B} = \left(\frac{[Ag]}{{}^{A}BK_{Ag\_red}}\right)\left\{\left(1 + \frac{[Gp]}{{}^{B}K_{Gp\_red}} + \frac{[Ar]}{{}^{B}K_{Ar\_red}}\right)\right\} + M_B\left(\frac{[Ag]}{{}^{B}K_{Ag\_blue}}\right)\left\{\left(1 + \frac{[Gp]}{{}^{B}K_{Gp\_blue}} + \frac{[Ar]}{{}^{B}K_{Ar\_blue}}\right)\right\} \quad (12)$$

For the A state, the $\overline{A}$ functions are defined separately with respect to A state complexes with either G-proteins or β-arrestins:

$$\overline{A}_{Gp} = \left[\left(1 + \frac{[Ag]}{{}^{A}K_{Ag\_red}}\right)\left\{\left(\frac{[Gp]}{{}^{A}K_{Gp\_red}}\right)\right\} + M_A\left(1 + \frac{[Ag]}{{}^{A}K_{Ag\_blue}}\right)\left\{\left(\frac{[Gp]}{{}^{A}K_{Gp\_blue}}\right)\right\}\right] / \sum S_i \quad (13)$$

$$\overline{A}_{Ar} = \left[\left(1 + \frac{[Ag]}{{}^{A}K_{Ag\_red}}\right)\left\{\left(\frac{[Ar]}{{}^{A}K_{Ar\_red}}\right)\right\} + M_A\left(1 + \frac{[Ag]}{{}^{A}K_{Ag\_blue}}\right)\left\{\left(\frac{[Ar]}{{}^{A}K_{GAr\_blue}}\right)\right\}\right] / \sum S_i \quad (14)$$

An additional contribution to the total $\overline{A}$ is made by A-state molecules with agonist bound, but neither G-protein nor β-arrestin bound.

$$\overline{A}_{Ag\_red} = \left[\left(1 + \frac{[Ag]}{{}^{A}K_{Ag\_red}}\right) + M_A\left(1 + \frac{[Ag]}{{}^{A}K_{Ag\_blue}}\right)\right] / \sum S_i \quad (15)$$

$$\overline{A}_{Ag\_blue} = \left[\left(1 + \frac{[Ag]}{{}^{A}K_{Ag\_red}}\right) + M_A\left(1 + \frac{[Ag]}{{}^{A}K_{Ag\_blue}}\right)\right] / \sum S_i \quad (16)$$

Fractions of the molecular population in red and blue states, f_A_{red} and f_A_{blue}, respectively, calculated from the $\sum S_i = \sum S_A + \sum S_B$ in equations (11) and (12) by separating terms to give $\sum S_{Red}$ and $\sum S_{Blue}$ to yield $f\_A_{red} = \sum S_{Red}/\sum S_i$ and $f\_A_{blue} = \sum S_{Blue}/\sum S_i$. Equations (9) – (16) apply to momomeric receptors, but appropriate versions bases on equations (1) – (8) may be readily derived for oligomeric receptions with integer values of N > 1.