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A ^{19}F-qNMR-Guided Mathematical Model for G Protein-Coupled Receptor Signaling

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Supplemental Appendix

Equilibrium concentrations of the different receptor species included in Fig. 3 are determined by the following equilibrium constants.

$$K_{AI}=\frac{[A][R_I]}{[AR_I]}\ ,\ \frac{K_{AI}}{\alpha}=\frac{[A][R_{PA}]}{[AR_{PA}]}\ ,\ K_1=\frac{[R_{PA}]}{[R_I]}\ ,\ \alpha K_1=\frac{[AR_{PA}]}{[AR_I]}\ ,\ \frac{K_{AI}}{\beta}=\frac{[A][R_{FA}]}{[AR_{FA}]}\ ,\ K_2=\frac{[R_{FA}]}{[R_I]}\ ,\ \beta K_2=\frac{[AR_{FA}]}{[AR_I]}\ ,$$

$$K_{AP}=\frac{[A][R_{PA}]}{[AR_{PA}]}=\frac{K_{AI}}{\alpha},\ K_3=\frac{[R_{FA}]}{[R_{PA}]}=\frac{K_2}{K_1},\ \gamma=\frac{\beta}{\alpha}$$

The total receptor concentration is defined as

$$[R_T] = [R_I] + [R_{PA}] + [R_{FA}] + [AR_I] + [AR_{PA}] + [AR_{FA}] \qquad (A1)$$

The G protein is implicitly included in the model through the intrinsic efficacies associated to the partially and fully active receptor species. Each receptor species yields a stimulus which is quantified as the product of its concentration times the corresponding intrinsic efficacy. The total stimulus S is the sum of the stimuli of all the active receptor species. Note that the apo receptor is able to generate a stimulus (constitutive receptor activity) and that both the apo and the ligand-bound receptor display distinct states of varying efficacy.

$$S = \varepsilon_{RPA}[R_{PA}] + \varepsilon_{RFA}[R_{FA}] + \varepsilon_{ARPA}[AR_{PA}] + \varepsilon_{ARFA}[AR_{FA}] \qquad (A2)$$

The fractional effect (the ratio between the measured effect E and the maximum possible effect E_m) is obtained from the total stimulus S through a rectangular hyperbola equation that includes the transducer factor K_E, the S value that produces half E_m.

$$\frac{E}{E_m}=\frac{S}{K_E+S}$$

By substituting the stimulus expression by the receptor-dependent expression (eq. 2) and taking into account the total receptor concentration (eq.1), the following expression for the fractional effect E/E_m is obtained

$$\frac{E}{E_m}=\frac{ab}{1+ab}$$

With

$$a=\frac{\chi}{1+K_1+K_2+\frac{[A]}{K_{AI}}(1+\alpha K_1+\beta K_2)}$$

$$b=\varepsilon_{RPA}K_1+\varepsilon_{RFA}K_2+\frac{[A]}{K_{AI}}(\varepsilon_{ARPA}\alpha K_1+\varepsilon_{ARFA}\beta K_2)$$

and $\chi=\frac{[R_T]}{K_E}$ (A3)

When the ligand concentration is expressed in logarithmic form, the E/E_m concentration-effect curve displays two asymptotic values: on the left as log [A] tends to negative infinity ([A] is equal to 0); on the right as log [A] tends to positive infinity ([A] largely increases). The asymptote on the left depicts the basal response of the biological system while the one on the right results from the efficacy of the ligand on the receptor considered.

Asymptotic Values

Basal fractional response: [A] = 0

$$\frac{E}{E_m}=\frac{\chi(\varepsilon_{RPA}K_1+\varepsilon_{RFA}K_2)}{1+K_1+K_2+\chi(\varepsilon_{RPA}K_1+\varepsilon_{RFA}K_2)} \quad (A4)$$

The basal response results from the constitutive receptor activity and this is determined in the present model by the χ parameter (the ratio between total receptor concentration and transduction parameter), K_1 and K_2 constants, regulating the equilibria between inactive and partially active and inactive and fully active states of the apo receptor, respectively, and the intrinsic efficacy of these states. Because the ligand is not present in the system, those parameters related with ligand action are not included.

Maximum fractional response: $\lim_{[A]\to\infty} \frac{E}{E_m}$

$$\lim_{[A]\to\infty} \frac{E}{E_m} = \frac{\chi\left(\varepsilon_{ARPA}\alpha K_1 + \varepsilon_{ARFA}\beta K_2\right)}{1+\alpha K_1+\beta K_2+\chi\left(\varepsilon_{ARPA}\alpha K_1+\varepsilon_{ARFA}\beta K_2\right)} \tag{A5}$$

The maximum fractional response is determined by several factors, including constitutive receptor activity through the χ parameter, and the αK_1 and βK_2 constants, which control the balance between the inactive and partially active, and inactive and fully active states of the receptor when bound to a ligand, respectively. Because the right asymptote comes from ligand-receptor saturating conditions, the intrinsic efficacies of the free receptor species and the equilibrium constant for the binding of the ligand to the receptor do not appear.