

# From Induced Fit to Conformational Selection: A Continuum of Binding Mechanism Controlled by the Timescale of Conformational Transitions

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**ABSTRACT** In receptor-ligand binding, a question that generated considerable interest is whether the mechanism is induced fit or conformational selection. This question is addressed here by a solvable model, in which a receptor undergoes transitions between active and inactive forms. The inactive form is favored while unbound but the active form is favored while a ligand is loosely bound. As the active-inactive transition rates increase, the binding mechanism gradually shifts from conformational selection to induced fit. The timescale of conformational transitions thus plays a crucial role in controlling binding mechanisms.

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Conformational transitions usually accompany receptor-ligand binding. In the simplest case, one can assume that the receptor switches from one conformation in the unbound state to another in the bound state (while the ligand remains rigid). The two conformations are referred to here as the inactive form and active form, respectively. From a mechanistic point of view, it is of interest to ask when the conformational transition occurs during the binding process.

Two extreme scenarios can be envisioned (Fig. 1 *a*). In the first, known as conformational selection, the unbound receptor makes rare excursions to the active form, to which the ligand then binds, resulting in the formation of the final receptor-ligand complex. In the second scenario, known as induced fit, the ligand first binds loosely to the receptor while it is still in the inactive form; the loosely bound ligand then induces the change to the active form, leading to the formation of the final complex.

Whether binding can be characterized as conformational selection or induced fit has been the focus of many recent studies (1–9). This letter presents an analytically solvable model to analyze the interplay between conformational transition and ligand binding. The main finding is that, for any given energy landscapes of the receptor in the unbound and loosely bound states, there exists a continuum of binding mechanism, which is tunable by the timescale of the conformational transitions relative to the timescale of receptor-ligand relative diffusion. Conformational selection is manifest when the conformational transitions occur slowly, whereas induced fit appears under fast conformational transitions.

The receptor and ligand are modeled here as spherical particles (Fig. 1 *b*). The interparticle distance is denoted as  $r$ , which takes value  $R$  at contact. The two particles undergo diffusion, with relative diffusion constant  $D$  (in the Supporting Material, I further consider the case where the diffusion constant differs when the receptor is in the inactive or active form). Formation of the final complex can occur only when the particles come into contact while the receptor is in the active form. When

the interparticle distance is between  $R$  and  $R + \Delta \equiv R_1$ , the receptor-ligand pair forms a loose complex. The inactive-active transition rates differ when the ligand is unbound ( $r > R_1$ ) and when the ligand is loosely bound ( $R < r < R_1$ ):

$$r > R_1 : \quad \text{inactive} \xrightleftharpoons[\omega_-]{\omega_+} \text{active}, \quad (1)$$

$$R < r < R_1 : \quad \text{inactive} \xrightleftharpoons[\omega_-]{\omega_+} \text{active}. \quad (2)$$

When loosely bound, the ligand experiences an interaction potential  $U_g$ ,  $g = a$  or  $i$ , with the active or inactive receptor; while unbound the interaction potentials are zero for both forms of the receptor. Detailed balance dictates that

$$\omega_+/\omega_- = (\omega_{0+}/\omega_{0-})\exp[-(U_a - U_i)/k_B T],$$

where  $k_B$  is Boltzmann's constant and  $T$  is the absolute temperature. Of particular interest are cases with transition rates such that the equilibrium probability of the active form in the unbound state,

$$p_{0a} = \omega_{0+}/(\omega_{0+} + \omega_{0-}),$$

is close to 0 whereas that in the loosely bound state,

$$p_a = \omega_+ / (\omega_+ + \omega_-),$$

is close to 1.

The above dual-transition rates model is based on one introduced by Szabo et al. (10) but differs by the presence of the loosely bound state, which allows one to account for the change in the energy landscape of the receptor by interactions with the ligand. Another related model is one for the binding of a ligand to a buried site in a receptor, which is

accessible only through a lid that undergoes open-closed transitions (11,12). These transitions, like in the model of Szabo et al., were assumed to be unaffected by ligand binding. Agmon (13) has modeled the conformational transition of an enzyme as diffusion along a continuous coordinate, but the energy landscape is unaffected by substrate binding.

The study's main interest is the receptor-ligand binding rate constant,  $k_{\text{on}}$ , which is determined by the steady-state distribution functions,  $\rho_a(r)$  and  $\rho_i(r)$ , of the receptor-ligand pair. They satisfy the reaction-diffusion equations

$$D\nabla^2\rho_i(r) - \omega_+\rho_i(r) + \omega_-\rho_a(r) = 0, \quad (3)$$

$$D\nabla^2\rho_a(r) + \omega_+\rho_i(r) - \omega_-\rho_a(r) = 0, \quad (4)$$

in the region  $R < r < R_1$  and similar equations in  $r > R_1$ , with the transition rates there given by  $\omega_{0\pm}$ . Collision of an inactive receptor with the ligand does not form the final complex; correspondingly, there is a reflecting boundary condition at contact

$$d\rho_i(r)/dr = 0 \text{ at } r = R. \quad (5)$$

Collision of an active receptor with the ligand can lead to the final complex; that process is modeled with a partially reflecting boundary condition at contact:

$$4\pi D_a R^2 d\rho_a(r)/dr = \kappa\rho_a(r) \equiv k_{\text{on}} \text{ at } r = R. \quad (6)$$

The solution for  $k_{\text{on}}$  is outlined in the [Supporting Material](#). The final result is given by

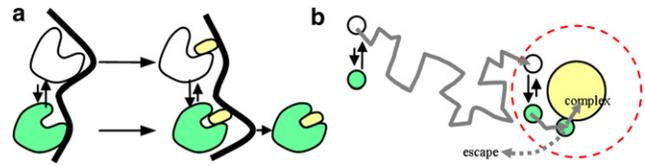
$$\begin{aligned} & 4\pi DR_1 p_{0a} e^{-U_a/k_B T} (B_+ C_- + B_- C_+) / k_{\text{on}} \\ &= p_a (A_+ C_- + A_- C_+) + p_a E (B_+ C_- + B_- C_+) \\ & - (p_a p_{0i} - p_i p_{0a}) \times [4 + (p_a p_{0i} / p_i - p_{0a}) (B_+ e^{\lambda\Delta} + B_- e^{-\lambda\Delta})] \end{aligned} \quad (7)$$

where

$$\begin{aligned} \lambda &= [(\omega_+ + \omega_-)/D]^{1/2} \\ \lambda_0 &= [(\omega_{0+} + \omega_{0-})/D]^{1/2} \\ A_{\pm} &= 1 \pm (1 + \gamma \pm \lambda R) / p_a \gamma \lambda R, \\ B_{\pm} &= \pm (1 \pm \lambda R) / \lambda R_1, \\ C_{\pm} &= [1 \pm \lambda R_1 - (1 + \lambda_0 R_1) p_{0i} / p_i \exp(-U_a/k_B T)] \exp(\mp \lambda \Delta), \\ \lambda &= \kappa / 4\pi DR, \\ E &= p_{0a} \exp(-U_a/k_B T) + p_{0i} \exp(-U_i/k_B T) - 1, \\ p_i &= 1 - p_a, \text{ and} \\ p_{0i} &= 1 - p_{0a}. \end{aligned}$$

The binding rate constant in the limits of slow and fast conformational transitions is of particular importance. Note that the transition rates appear in three dimensionless parameters:  $\lambda\Delta$ ,  $\lambda R$ , and  $\lambda_0 R_1$ . These parameters measure the timescale of the conformational transitions relative to the timescale of receptor-ligand relative diffusion. In the limit of slow conformational transitions, i.e., when  $\lambda\Delta$ ,  $\lambda R$ , and  $\lambda_0 R_1 \ll 1$ , one finds that

$$k_{\text{on}} = p_{0a} k_{\text{on}0}, \quad (8)$$



**FIGURE 1** Model of receptor-ligand binding. The inactive and active receptors are shown in white and green, respectively; the ligand is in yellow. In the loosely bound state, an active receptor can form the final complex (or escape to the unbound state). (a) Conformational selection versus induced fit. The energy landscapes (*black curves*) of the receptor differ in the unbound and loosely bound states. Conformational selection follows the down and then right path, whereas induced fit follows the right and then down path. (b) The dual-transition-rates model. The ligand is immobilized at the origin; the loosely bound state is formed when the receptor is inside the dashed circle.

where  $k_{\text{on}0}$  is the rate constant if the receptor stays in the active form

$$k_{\text{on}0} = \frac{4\pi DR \times \kappa e^{-U_a/k_B T}}{4\pi DR + \kappa (R e^{-U_a/k_B T} + \Delta) R_1}. \quad (9)$$

In Eq. 8, the binding rate constant is scaled down from  $k_{\text{on}0}$  by the equilibrium probability,  $p_{0a}$ , of the active form in the unbound state. This result can be understood as follows. Consider a receptor that initially is far away from the ligand. In the limit of slow conformational transitions, the receptor does not have time to undergo active-inactive transitions before it comes into contact with the ligand. If the receptor started out in the active form, the binding rate constant would be  $k_{\text{on}0}$ . If the receptor started out in the inactive form, it would not be able to bind with the ligand at all and the binding rate constant would be 0. Now the probability for the receptor to be initially in the active form is  $p_{0a}$ . Therefore, the binding rate constant is  $p_{0a} k_{\text{on}0}$ . The above scenario corresponds to conformational selection; correspondingly, the result given by Eq. 8 will be denoted as  $k_{\text{CS}}$ .

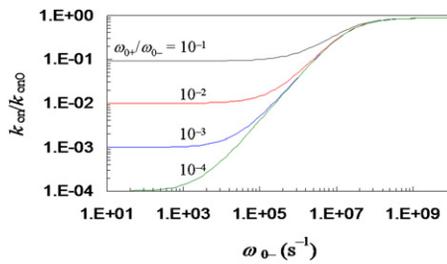
In the fast conformational-transition limit, specifically, when  $\lambda\Delta$  and  $\lambda R \gg 1$ , one finds that

$$k_{\text{on}} = \frac{4\pi DR \times p_a \kappa e^{-U_{\text{eff}}/k_B T}}{4\pi DR + p_a \kappa (R e^{-U_{\text{eff}}/k_B T} + \Delta) / R_1}. \quad (10)$$

This result is the same as  $k_{\text{on}0}$  but with  $\kappa$  replaced by  $p_a \kappa$  and the interaction potential  $U_a$  replaced by an effective potential defined by

$$\exp(-U_{\text{eff}}/k_B T) = p_{0a} \exp(-U_a/k_B T) + p_{0i} \exp(-U_i/k_B T).$$

When the conformational transitions are fast, the receptor-ligand pair enters the loosely bound state while the receptor is predominantly in the inactive form. The receptor then quickly switches to the active form, allowing the formation of the final complex. This scenario corresponds to induced fit; correspondingly, the result given by Eq. 10 will be denoted as  $k_{\text{IF}}$ .

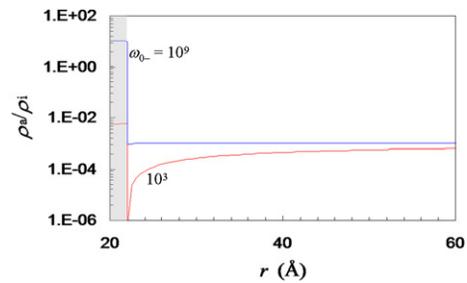


**FIGURE 2** Results for  $k_{\text{on}}$  over a range of  $\omega_{0+}/\omega_{0-}$ , when  $\omega_{+}/\omega_{-}$  is fixed at 10. Other parameters are  $\gamma = 1$ ,  $\Delta/R = 0.1$ ,  $\omega_{-} = \omega_{0-}$ ,  $D = 10 \text{ \AA}^2/\text{ns}$ ,  $R = 20 \text{ \AA}$ , and  $U_l = 0$ . The lower and upper limits of  $k_{\text{on}}$  give  $k_{\text{CS}}$  and  $k_{\text{IF}}$ , respectively.

Fig. 2 displays sample results for the binding rate constant. These results show that, as the transition rates increase,  $k_{\text{on}}$  gradually shifts from the slow limit  $k_{\text{CS}}$  to the fast limit  $k_{\text{IF}}$ . At a fixed equilibrium probability of the active form in the loosely bound state, the operating range of the conformational-selection scenario expands as the active form becomes more favored in the unbound state. In contrast, Fig. S1 in the Supporting Material shows that, at a fixed equilibrium probability of the active form in the unbound state, the operating range of the induced-fit scenario expands as the active form becomes more favored in the loosely bound state. The Supporting Material also presents results when the diffusion constants differ between the active and inactive forms.

Further insight into the conformational-selection and induced-fit scenarios is provided by the pair distribution functions. Fig. 3 displays the population ratio,  $\rho_a(r)/\rho_i(r)$ , as a function of the interparticle distance  $r$ . In the conformational-selection scenario, as represented by the curve with  $\omega_{0-} = 10^3 \text{ s}^{-1}$ , the population ratio stays close to the equilibrium value  $\omega_{0+}/\omega_{0-}$  of the unbound state even after the ligand is loosely bound. In contrast, in the induced-fit scenario, corresponding to the curve with  $\omega_{0-} = 10^9 \text{ s}^{-1}$ , the population ratio abruptly changes from the equilibrium value  $\omega_{0+}/\omega_{0-}$  of the unbound state to the equilibrium value  $\omega_{+}/\omega_{-}$  of the loosely bound state upon loose binding of the ligand.

In conclusion, the dual-transition-rates model highlights the role that the timescale of conformation transitions plays in controlling binding mechanisms. Excursions to the active form in the unbound state do not necessarily mean the conformational-selection scenario. If the inactive-to-active transition rate is sufficiently high in the loosely bound state, then the receptor that eventually forms the final complex may still come into the loosely bound state predominantly in the inactive form. The resulting mechanism of binding would be induced fit. Conversely, loose binding of the inactive form does not necessarily mean induced fit. If the inactive-to-active transition is slow, the loosely bound receptor will diffuse into the unbound state instead of converting into the final complex. The complex will then have to be formed from the receptor that enters the loosely bound state in the active form, i.e., via conformational selection.



**FIGURE 3** The pair distribution functions. The values of  $\omega_{0-}$  in  $\text{s}^{-1}$  are displayed. Other parameters are  $\gamma = 1$ ,  $\Delta/R = 0.1$ ,  $\omega_{0+}/\omega_{0-} = 10^{-3}$ ,  $\omega_{-} = \omega_{0-}$ ,  $\omega_{+}/\omega_{-} = 10$ ,  $D = 10 \text{ \AA}^2/\text{ns}$ ,  $R = 20 \text{ \AA}$ , and  $U_l = 0$ . The loosely bound state is represented by the shaded region.

## SUPPORTING MATERIAL

The derivation of Eq. 7 and two additional figures are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495\(09\)01758-5](http://www.biophysj.org/biophysj/supplemental/S0006-3495(09)01758-5).

## ACKNOWLEDGMENTS

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## REFERENCES and FOOTNOTES

- Eldridge, A. M., and D. S. Wuttke. 2008. Probing the mechanism of recognition of ssDNA by the Cdc13-DBD. *Nucleic Acids Res.* 36:1624–1633.
- Eletsky, A., A. Kienhöfer, ..., K. Pervushin. 2005. Investigation of ligand binding and protein dynamics in *Bacillus subtilis* chorismate mutase by transverse relaxation optimized spectroscopy-nuclear magnetic resonance. *Biochemistry.* 44:6788–6799.
- Espinoza-Fonseca, L. M. 2009. Reconciling binding mechanisms of intrinsically disordered proteins. *Biochem. Biophys. Res. Commun.* 382:479–482.
- Hammes, G. G., Y. C. Chang, and T. G. Oas. 2009. Conformational selection or induced fit: a flux description of reaction mechanism. *Proc. Natl. Acad. Sci. USA.* 106:13737–13741.
- Latham, M. P., G. R. Zimmermann, and A. Pardi. 2009. NMR chemical exchange as a probe for ligand-binding kinetics in the theophylline-binding RNA aptamer. *J. Am. Chem. Soc.* 131:5052–5053.
- Okazaki, K., and S. Takada. 2008. Dynamic energy landscape view of coupled binding and protein conformational change: induced-fit versus population-shift mechanisms. *Proc. Natl. Acad. Sci. USA.* 105:11182–11187.
- Rea, A. M., V. Thurston, and M. S. Searle. 2009. Mechanism of ligand-induced folding of a natively unfolded helixless variant of rabbit I-BABP. *Biochemistry.* 48:7556–7564.
- Sullivan, S. M., and T. Holyoak. 2008. Enzymes with lid-gated active sites must operate by an induced fit mechanism instead of conformational selection. *Proc. Natl. Acad. Sci. USA.* 105:13829–13834.
- Swift, R. V., and J. A. McCammon. 2009. Substrate induced population shifts and stochastic gating in the PBCV-1 mRNA capping enzyme. *J. Am. Chem. Soc.* 131:5126–5133.
- Szabo, A., D. Shoup, ..., J. A. McCammon. 1982. Stochastically gated diffusion-influenced reactions. *J. Chem. Phys.* 77:4484–4493.
- Zhou, H.-X. 1998. Theory of the diffusion-influenced substrate binding rate to a buried and gated active site. *J. Chem. Phys.* 108:8146–8154.
- Zhou, H. X., S. T. Wlodek, and J. A. McCammon. 1998. Conformational gating as a mechanism for enzyme specificity. *Proc. Natl. Acad. Sci. USA.* 95:9280–9283.
- Agmon, N. 1985. A diffusion Michaelis-Menten mechanism: continuous conformational change in enzymatic kinetics. *J. Theor. Biol.* 113:711–717.