Derivation of Equations (10-12)

The left-hand-side of Eq. (8) in the paper can be expressed in a simpler form by first considering the function $\rho(\mathbf{r}_1)$ defined as

$$\rho(\mathbf{r}_1') \equiv \int d\mathbf{1}\ \delta(\mathbf{r}_1 - \mathbf{r}_1') \int d\mathbf{X}\ e^{-\beta[U+u_{\rm c}+u_{\rm o}]}. \tag{1}$$

For a sufficiently large distance, the ligand and the protein are well-separated and the function $\rho(\mathbf{r}_1^*) \equiv \rho(r_1^*, \theta_1^*, \phi_1^*)$ becomes independent of θ_1^* and ϕ_1^*, i.e., $\rho(\mathbf{r}_1^*) = \rho(r_1^*, 0, 0)$. If follows that the fourth term can be written as,

$$\begin{aligned}
\frac{\int_{\rm site} d\mathbf{1} \int d\mathbf{X}\ e^{-\beta[U+u_{\rm c}+u_{\rm o}+u_{\rm a}]}}{\int_{\rm bulk} d\mathbf{1}\ \delta(\mathbf{r}_1 - \mathbf{r}_1^*) \int d\mathbf{X}\ e^{-\beta[U+u_{\rm c}+u_{\rm o}]}} &= \frac{\int_{\rm site} d\mathbf{r}_1\ \rho(\mathbf{r}_1)\ e^{-\beta u_{\rm a}}}{\rho(\mathbf{r}_1^*)} \\
&= \frac{\int_{\rm site} d\mathbf{r}_1\ \rho(\mathbf{r}_1)\ e^{-\beta u_{\rm a}}}{\rho(r_1^*, 0, 0)} \\
&= \frac{\int_{\rm site} d\mathbf{r}_1\ \rho(\mathbf{r}_1)\ e^{-\beta u_{\rm a}}}{\rho(r_1^*, 0, 0) \times \frac{\int d\mathbf{r}_1\ \delta(r_1 - r_1^*)\ e^{-\beta u_{\rm a}}}{\int d\mathbf{r}_1\ \delta(r_1 - r_1^*)\ e^{-\beta u_{\rm a}}}} \\
&= S^* \frac{\int_{\rm site} d\mathbf{r}_1\ \rho(\mathbf{r}_1)\ e^{-\beta u_{\rm a}}}{\rho(r_1^*, 0, 0) \int d\mathbf{r}_1\ \delta(r_1 - r_1^*)\ e^{-\beta u_{\rm a}}} \\
&= S^* \frac{\int_{\rm site} d\mathbf{r}_1\ \rho(\mathbf{r}_1)\ e^{-\beta u_{\rm a}}}{\int d\mathbf{r}_1\ \delta(r_1 - r_1^*)\ \rho(\mathbf{r}_1)\ e^{-\beta u_{\rm a}}} \\
&= S^* \int_{\rm site} dr_1' \left[\frac{\int d\mathbf{r}_1\ \delta(r_1 - r_1')\rho(\mathbf{r}_1)\ e^{-\beta u_{\rm a}}}{\int d\mathbf{r}_1\ \delta(r_1 - r_1^*)\rho(\mathbf{r}_1)\ e^{-\beta u_{\rm a}}} \right] \\
&= S^* \int_{\rm site} dr_1'\ e^{-\beta[\mathcal{W}(r_1') - \mathcal{W}(r_1^*)]},
\end{aligned} \tag{2}$$

where $\mathcal{W}(r_1)$ is a PMF defined as a function of the radial distance r_1 calculated in the presence of the configurational and oriental restraints $u_{\rm c}$ and $u_{\rm o}$,

$$e^{-\beta[\mathcal{W}(r_1') - \mathcal{W}(r_1^*)]} = \frac{\int d\mathbf{1}\ \delta(r_1 - r_1') \int d\mathbf{X}\ e^{-\beta[U+u_{\rm c}+u_{\rm o}+u_{\rm a}]}}{\int d\mathbf{1}\ \delta(r_1 - r_1^*) \int d\mathbf{X}\ e^{-\beta[U+u_{\rm c}+u_{\rm o}+u_{\rm a}]}} \tag{3}$$

and S^* is a surface integral given by

$$\begin{aligned}
S^* &= \int d\mathbf{r}_1\ \delta(r_1 - r_1^*)\ e^{-\beta u_{\rm a}(\theta_1, \phi_1)} \\
&= (r_1^*)^2 \int_0^{\pi} \sin(\theta_1) d\theta_1 \int_0^{2\pi} d\phi_1\ e^{-\beta u_{\rm a}(\theta_1, \phi_1)}.
\end{aligned} \tag{4}$$

Relation to the Standard Double-Decoupling Scheme

The complete calculation of the equilibrium binding constant, as expressed in Eq. (13) in the main text, corresponds to a sequence of well-defined intermediate reversible steps. Alternative computational schemes could readily be derived on the basis of Eq. (4) in the main text by introducing different intermediate configurational integrals Z_i,

$$K_{\mathrm{eq}} = \frac{\int_{\mathrm{site}} d\mathbf{1} \int d\mathbf{X}\ e^{-\beta U}}{Z_n} \times \frac{Z_n}{Z_{n-1}} \times \ldots \frac{Z_3}{Z_2} \times \frac{Z_2}{Z_1} \times \frac{Z_1}{\int_{\mathrm{bulk}} d\mathbf{1}\ \delta(\mathbf{r}_1 - \mathbf{r}_1^*) \int d\mathbf{X}\ e^{-\beta U}}. \tag{5}$$

Here, the denominator and the numerator may be thought of as representing initial and final states of the binding process: the ligand bound to the receptor and the ligand with its center-of-mass at $\mathbf{r}_1^*$ in the bulk away from the receptor while the configurational integrals, Z_i, represent intermediate states that are chosen for their convenience. The design of a practical method consists in wisely choosing those intermediates, such that each separate contributions can be calculated easily. For example, the original double-annihilation method (1) consists in introducing a single configurational integral Z_1 in which the ligand is completely decoupled from its surrounding (protein receptor or bulk solvent). A straightforward application of the double-decoupling scheme is, however, incorrect, as it leads to the problem of the "wandering ligand" (see also the discussion in Ref. 2). Such difficulties are avoided by introducing one additional configurational integral Z_2, corresponding to an intermediate state of a decoupled ligand confined relative to the binding site by an external restraining potential (2–7). Introducing $u_{\mathrm{t}}(r_1, \theta_1, \phi_1)$, a potential restraining the position of the ligand relative to the receptor, and U_0 the potential energy of the molecular system with the interaction of the ligand with its surrounding switched off,

$$\begin{aligned}
\frac{\int_{\mathrm{site}} d\mathbf{1} \int d\mathbf{X}\ e^{-\beta[U+u_{\mathrm{c}}+u_{\mathrm{o}}]}}{\int_{\mathrm{bulk}} d\mathbf{1}\ \delta(\mathbf{r}_1 - \mathbf{r}_1^*) \int d\mathbf{X}\ e^{-\beta[U+u_{\mathrm{c}}+u_{\mathrm{o}}]}} &= \frac{\int_{\mathrm{site}} d\mathbf{1} \int d\mathbf{X}\ e^{-\beta[U+u_{\mathrm{c}}+u_{\mathrm{o}}]}}{\int_{\mathrm{site}} d\mathbf{1} \int d\mathbf{X}\ e^{-\beta[U_0+u_{\mathrm{c}}+u_{\mathrm{o}}+u_{\mathrm{t}}]}} \\
&\times \frac{\int_{\mathrm{site}} d\mathbf{1} \int d\mathbf{X}\ e^{-\beta[U_0+u_{\mathrm{c}}+u_{\mathrm{o}}+u_{\mathrm{t}}]}}{\int_{\mathrm{bulk}} d\mathbf{1}\ \delta(\mathbf{r}_1 - \mathbf{r}_1^*) \int d\mathbf{X}\ e^{-\beta[U_0+u_{\mathrm{c}}+u_{\mathrm{o}}]}} \\
&\times \frac{\int_{\mathrm{bulk}} d\mathbf{1}\ \delta(\mathbf{r}_1 - \mathbf{r}_1^*) \int d\mathbf{X}\ e^{-\beta[U_0+u_{\mathrm{c}}+u_{\mathrm{o}}]}}{\int_{\mathrm{bulk}} d\mathbf{1}\ \delta(\mathbf{r}_1 - \mathbf{r}_1^*) \int d\mathbf{X}\ e^{-\beta[U+u_{\mathrm{c}}+u_{\mathrm{o}}]}} \\
&= \langle e^{-\beta[U-U_0]} \rangle_{(\mathrm{site}, U_0+u_{\mathrm{c}}+u_{\mathrm{o}})} \times \int d\mathbf{r}_1\ e^{-\beta u_{\mathrm{t}}(\mathbf{r}_1)} \\
&\times \langle e^{-\beta[U-U_0]} \rangle^{-1}_{(\mathrm{bulk}, U_0+u_{\mathrm{c}})} \\
&= e^{-\beta G_0^{\mathrm{site}}} \times F_{\mathrm{t}} \times e^{+\beta G_0^{\mathrm{bulk}}}
\end{aligned} \tag{6}$$

where G_0^{site} and G_0^{bulk} are the free energy for inserting the (restrained) ligand into the binding site and the bulk solvent, respectively, and F_{t} is a translational factor with units of volume (note that the influence of the delta function and orientational potential on G_0^{bulk} can be ignored because the bulk is isotropic and uniform). Additional potentials restraining the orientation of the ligand can also be introduced to help the convergence of the individual FEP calculations (2, 6, 7).

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