Effect of rotation on the diffusion-controlled rate of ligand–protein association

(Eyring rate theory/diffusion frequency factor/surface-hindered rotation)

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Contributed by Terrell L. Hill, October 10, 1975

ABSTRACT The rate of binding of a fairly large ligand molecule to a protein is reduced below the usual diffusion-controlled rate by the requirement of a certain rotational orientation. A simple, approximate treatment of this effect is given for special cases of spherical and ellipsoidal ligands. As the center of an ellipsoidal ligand approaches a protein surface, there is an effective repulsive potential between ligand and surface owing to restricted rotation of the ligand. The frequency factor $kT/h$ of the Eyring rate theory is replaced in these reactions involving diffusion in solution by $D/RA$, where $D =$ diffusion coefficient of ligand, $A =$ thermal deBroglie wavelength of ligand, and $R =$ “capture” distance around the binding site on the protein.

This paper presents an elementary, approximate, transition-state theory approach to ligand–protein interactions for cases in which the ligand is a large enough molecule to involve significant rotational effects. These effects tend to reduce the diffusion-controlled rate of ligand–protein association.

As examples, in the myosin–actin–ATP kinetic system, association interactions of this type enter at two different levels: ATP or ADP (ligands) + S1 moiety of myosin; and S1 (ligand) + F-actin. This paper is the second in a series of three (1) concerned primarily with the myosin system.

The first two sections, on monatomic molecules (or ions) as ligands, present necessary background material. The last two sections are then concerned with several examples of the binding of fairly large ligand molecules treated as rotating rigid bodies.

A point of perhaps special interest is that for diffusion-controlled association and dissociation reactions (in solution), the frequency factor $kT/h$ of the Eyring rate theory (for gases) is replaced by a new factor $D/RA$ (see below). Other examples in which the same type of frequency factor occurs will be discussed in a separate paper.

Adsorption-desorption of monatomic gas

In this section we show (see also ref. 1, p. 200) that the Eyring theory gives correct results for this reference system. The model we choose is the simplest possible. A dilute gas mixture contains three components: monatomic ligand molecules each with partition function (p.f.) $q_l$; relatively large adsorbent molecules, each with one empty site for binding one ligand molecule, with p.f. $q_0$; and adsorbent molecules with the binding site occupied by a ligand, with p.f. $q_1$. The potential energy of interaction $u$ (the “potential surface”) between one ligand and one “empty” adsorbent molecule is zero for large separations but drops suddenly to a minimum value $u_0$ ($u_0 < 0$) as the ligand enters the binding site. The transition state in the binding process is a cross-sectional area (or “window”) $S$, around the site, on which $u = 0$: a molecule passing through this window is trapped by the potential well, and thus bound. A ligand molecule approaching the adsorbent surface outside of the site region $S$ is reflected by the surface ($u \to \infty$) and not bound.

The surface is taken as the $xy$-plane ($x = y = 0$ at the site center), with $z$ increasing outward from the site. The p.f.s can be written (1)

$$q_1 = \Lambda^2 V, \quad q_0 = qV, \quad q_1 = q_{0}, q_{1} e^{-kT}$

where $\Lambda$ is the so-called thermal deBroglie wavelength $h/(2\pi m k T)^{1/2}$ (ref. 1, p. 76), $V =$ volume, $m =$ mass of ligand, $q =$ p.f. of empty adsorbent molecule (except for the factor $V$), and $q_0, q_1$ are the three one-dimensional vibrational p.f.s of a ligand bound in the site. Any perturbation of the adsorbent by the bound ligand can be included formally in $e^{-kT}$.

An activated complex (1) has two-dimensional translational motion of the ligand on the area $S$ (this is in lieu of the more common vibrational motion, at the saddle point, normal to the reaction coordinate), with p.f.

$$q^* = q\Lambda^2 SV.$$

The rate at which ligand molecules are bound is $\alpha'c_L c_0$ while the rate at which molecules escape is $\beta c_1$, where $c =$ concentration ($N/V$). Using Eyring’s theory (ref. 1, p. 197), we find then

$$\alpha = (kT/h)(q^2/V)(q_j/V)(q_j/V) = (kT/(2\pi m)^{1/2}S$$

or, for the corresponding first-order rate constant,

$$\alpha = \alpha'c_L = \alpha'p_0/kT = p_0S/(2\pi m k T)^{1/2}$$

where $p_0 =$ partial pressure. This agrees with a well-known result in the kinetic theory of gases (collision rate on a surface). Since simple rate theory thus gives $\alpha'$ correctly, it necessarily provides the correct $\beta$ as well, because equilibrium statistical mechanics tells us that (1)

$$K = \alpha' / \beta = c_1/c_0 = (q_1/V)/(q_j/V)/(q_j/V)$$

$$= q_{0, q_{1}, e^{-u_0 kT}} = \Lambda^2.$$

Hence

$$\beta = (kT/h)(q^2/V)/(q_j/V)$$

$$= (kT/h)\Lambda^2 Se^{-u_0 kT}/q_{0, q_{1}}.$$

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The potential barrier in this process of ligand escape is $-u_0$. In ligand binding, the only "barrier" is one of restricted geometry ($S$).

Adsorption-desorption of monatomic ligand from solution

This example provides further necessary background. The model is essentially the same as above except that the three components are now dilute solutes in a solvent. To be explicit, we assume first that the window of area $S$ around the binding site on the $xy$-plane is a hemisphere of radius $R$, so that $S = 2\pi R^2$. The ligand and adsorbent molecules interact with the solvent, of course. But Eqs. 1 and 2 are still appropriate if the zero of ligand free energy is suitably chosen to leave $q_0$ as it stands and if $u(x,y,z)$ is replaced by the potential of mean force $w(x,y,z)$ between ligand and an empty adsorbtion molecule. This potential is found by holding a ligand and an adsorbent molecule fixed, with relative coordinates $x,y,z$ as before, and averaging over all possible configurations of all solvent molecules. Formally (1, 2),

$$e^{-w(x,y,z)/kT} = \int e^{-U(x,y,z)/kT} \, dx$$

where $U = \text{total potential energy of solvent + one ligand molecule + one adsorbent molecule and } r = \text{all coordinates of all solvent molecules}$. The zero of $U$ is chosen to give $w = 0$ for large separations $x,y,z$ (1, 2).

Although Eqs. 1 and 2 (with $w_0$ in place of $u_0$) still have essentially the same significance, Eqs. 3 and 4 for $\alpha'$ and $\beta$ cannot be used because the frequency factor $kT/h$ in Eyring's theory is not applicable when solvent is present. Our procedure will therefore be to obtain $\alpha'$ independently from diffusion theory and from this result deduce a new frequency factor. This frequency factor will then be carried over to the more complicated cases of the next two sections.

The diffusion problem here is well-known (3). We use spherical coordinates. At steady state, $\nabla^2 \psi = 0$ and the ligand flux $J = -D_0 \nabla \psi$, where $D = \text{ligand diffusion coefficient}$. The boundary conditions are $c = 0$ ("absorption") on the surface $S (r = R)$, $c = c_j$ at $r = \infty$, and $J = 0$ ("reflection") on the $xy$-plane outside of $S$. Thus $c$ is a function of $r$ only. One finds easily

$$c = c_j(1 - \frac{R_i}{R}), \quad \alpha = (2\pi R)(D_0 c_j/R)$$

and hence $\alpha' = 2\pi DR$, where $\alpha$ is the total rate of ligand capture by $S$ (compare Eq. 4). In $\alpha$ and $\alpha'$, $D$ is to be replaced by (4) $D + D_ad$, if the adsorbent diffusion coefficient is not negligible.

If we return now to Eq. 3, use the same expressions for $q^4$, $q_0$, and $q_0$, and introduce $\alpha' = 2\pi DR$, we find $D/RA$ for the new frequency factor. That is,

$$\alpha' = (D/RA)(q^4/V)/(q_1/V)(q_0/V).$$

The rate constant for escape from the binding site is then (compare Eq. 6)

$$\beta = (D/RA)(q^4/V)/(q_0/V) = 2\pi DRe^{-u_0/kT}/q_0^2 q_j = \alpha'/K.$$ [10]

The frequency factor $D/RA$ is not "universal", as $kT/h$ is, for it depends on the geometry (see below) and on the nature of ligand, solvent, and site. To examine orders of magnitude, let us anticipate application to a larger ligand with, say, molecular weight 200, $D = 5 \times 10^{-6}$ cm$^2$ sec$^{-1}$, $R = 4 \text{Å}$, and $T = 293^\circ\text{K}$. Then, for this example, $\lambda = 0.072 \text{Å}$, $D/RA = 1.73 \times 10^{11}$ sec$^{-1}$, $kT/h = 6.10 \times 10^{12}$ sec$^{-1}$, and $\alpha' = 2\pi DR = 7.6 \times 10^{10}$ sec$^{-1}$. In the dilute gas case (i.e., using $kT/h$ in place of $D/RA$) $\alpha' = 4.2 \times 10^{10}$ sec$^{-1}$.

Diffusion in External Field

In the above model we have taken $w = 0$ except for the potential well of depth $-w_0$ in the region $r < R$ and the reflection ($w \to \infty$) on the $xy$-plane outside of $S$. To generalize, suppose we introduce $w(r)$ in place of $w = 0$, where $w(r)$ increases monotonically from $w(\infty) = 0$ to $w(R) \equiv \infty > 0$ at $r = R$. Thus, there is a potential barrier in binding ($w^\dagger$) as well as in escape ($w^\iota - w_j < 0$). For example, there might be electrostatic repulsion between ligand and site. This is again a well-known diffusion problem. The boundary conditions are unchanged but now

$$J = -D(\nabla c + c\nabla(w/kT)), \quad 0 = \nabla \cdot J$$ [11]

where again $c$ is a function of $r$ only. One finds

$$\alpha' = 2\pi DR{e^{-u_0/kT}}$$ [12]

and

$$e^{-u/kT} = R \int_R^\infty e^{-r/kT} r^{-2} \, dr.$$ [13]

This quantity is an average of $e^{u/kT}$ over $r$ with weighting $r^{-2} dr$ so the region near $r = R$ (where $w \approx w^\dagger$) is emphasized. But necessarily $e^{-u/kT} \leq e^{w/kT}$.

If we apply transition-state theory (Eq. 9) to this problem, as an approximation, $q^4$ (Eq. 2) contains a new factor $e^{-u/kT}$, so we find $\alpha' = 2\pi DR{e^{-u/kT}}$. This is a smaller value than given (correctly) by Eq. 12. Similarly, in Eq. 10 for $\beta$, $w_0$ is replaced by $w_0 - w^\dagger$ in the correct expression but by $w_0 - w^\iota$ in the transition-state approximation.

Planar Capture Window

If the binding region (site) on the adsorbent is in the shape of a cavity, a planar capture window (transition state) may be more realistic than a protruding hemisphere. Suppose the planar window is bounded by a circle of radius $R$ in the $xy$-plane with center at $x = y = 0$. Boundary conditions are: $c = 0$ on the window; $c = c_j$ far from the window; and $J = 0$ on the $xy$-plane outside of the circle. Oblate spheroidal coordinates (ref. 4, p. 168) are natural here. The equation of the spheroids (on which $c = \text{constant}$) is

$$\frac{z^2}{R_0^2} + \frac{x^2 + y^2}{R^2(\xi^2 + 1)} = 1$$ [14]

where $0 \leq \xi \leq \infty$. As $\xi \to \infty$, $R \xi \to r$. One finds, after a short calculation,

$$c = (2c_j/\pi) \tan^{-1} \xi, \quad \alpha' = 4\pi R.$$ [15]
Incidentally, in an external field \( w(\alpha) \), \( \alpha^\prime = 4DRe^{-w^*/kT} \) where
\[
e^{-w^*/kT} = \frac{2}{\pi} \int_0^\infty e^{-x^2/kT} dx. \quad [16]
\]

**Free Energy Changes.** If we write \( \mu_0^a + kT \ln \epsilon_0 \) for the chemical potential \( \mu_0 \) of each solute species, then (ref. 1, p. 178) \( \mu_0^a = -kT \ln (\epsilon_0/V) \). Hence the standard free energy change for the binding process \( L + 0 \rightarrow 1 \) is, from Eq. 1,
\[
\mu^a = (\mu_0^a + \mu_0^b) = w_0 - kT \ln q_a q_b - kT \ln \Lambda^a. \quad [17]
\]
The "basic free energy change" (7) in adsorbent molecules for the same process is
\[
\mu^a = [\mu_0^a + \mu_0(b)] = w_0 - kT \ln q_a q_b - kT \ln \Lambda^c. \quad [18]
\]
The latter equation illustrates the fact that, except for qualitative purposes, the potential surface change (here \( w_0 \rightarrow 0 \)) cannot be used by itself for the basic free energy change.

**Binding of a spherical ligand on a protein**

Consider a ligand in solution that can be approximated as a rigid sphere of radius \( a \) and principal moments of inertia \( A=B=C \). We suppose that a site on the surface of this sphere may "attach" to a complementary binding site on the surface of a macromolecule, e.g., a protein. The latter surface is approximated by a plane. The center of the sphere has coordinates \( x, y, z \).

Both translational and rotational diffusion are involved in the binding process. As in the preceding section, insofar as translational motion is concerned, the ligand is captured by the protein binding site when the ligand center is within a small hemisphere \( r = R \) about the origin.

The potential of mean force \( w \) is now a function of \( x, y, z \) and also of the Eulerian rotational angles \( \theta, \phi, \psi \). That is, in Eq. 7, six relative coordinates are held fixed in the integration. When the ligand center is outside of \( r = R \), we take \( w = 0 \). The depth of the binding potential well is again \( -w_0 \), and there is reflection \( (w \rightarrow \infty) \) of the ligand center from the \( xy \)-plane \( (z = 0) \) outside of the hemisphere. (The actual protein surface is the plane \( z = -a \).

In order for capture (binding) to occur, not only must \( x, y, z \) be within the hemisphere \( r = R \) but a similar condition must be met simultaneously by the Eulerian angles. That is, we assume that the ligand sphere must be within specified deviations from the optimal rotational orientation for attachment. Let \( XYZ' \) be fixed axes in the ligand sphere, with origin at the center. Let \( X'Y'Z' \) be spatial axes, parallel to \( XYZ \) already used above, but with origin also at the center of the sphere. \( (XYZ) \) are fixed relative to the binding site on the protein. When the sphere rotates without translation, \( X'Y'Z' \) remain fixed but \( XYZ' \) rotate with the sphere. The axes \( XYZ' \) and \( X'Y'Z' \) coincide in the optimal rotational orientation, where we take \( \theta = 0, \phi = 0 \). In an arbitrary orientation, \( \theta \) is the angle between the \( Z' \) and \( Z \) axes; \( \phi \) is the azimuthal angle locating the \( Z' \) axis as it rotates around the fixed \( Z \) axis with \( \theta = \) constant, and \( \psi \) is the angle of rotation of the sphere around the \( Z' \) axis itself.

The condition on \( \theta \) and \( \phi \) for capture to occur can be specified by requiring the \( Z' \) axis to be within a limited solid angle \( 4\pi f_\theta^+ \) (where \( 0 < f_\theta^+ < 1 \)) about the optimal orientation. The most obvious choice is \( 0 \leq \phi \leq 2\pi \) (i.e., no restriction) and \( 0 \leq \theta \leq \theta^\dagger \), where \( \theta^\dagger \) is the limiting value of \( \theta \). Hence
\[
f_\theta^* = \frac{1}{4\pi} \int_0^{\theta^\dagger} \sin \theta d\theta = \frac{1}{2} (1 - \cos \theta^\dagger). \quad [19]
\]

Similarly, the condition on \( \psi \) is that it lie within a limited range \( 2\pi f_\psi^\dagger \) (where \( 0 < f_\psi^\dagger < 1 \)) about \( \psi = 0 \), say \( -\pi f_\psi^\dagger < \psi < \pi f_\psi^\dagger \). Likely orders of magnitude for \( f_\theta^\dagger \) and \( f_\psi^\dagger \) will be discussed below.

To be more explicit now about \( w \); \( w = 0 \) or \( w \rightarrow \infty \) (see above) except within the six-dimensional capture region \( 0 < r < R, 0 \leq \theta \leq \theta^\dagger, -\pi f_\psi^\dagger < \psi < \pi f_\psi^\dagger \) where there is a potential well of depth \( -w_0 \). The proper but difficult way to handle this problem is to consider the simultaneous translational and rotational diffusion of a sphere (7-9) in six dimensions with absorption on a portion of the five-dimensional surface of a hyperellipsoid and reflection from \( z = 0 \) otherwise. Instead, for present order-of-magnitude purposes, we use the transition-state approximation. In this approximation, translation and rotation are not treated equivalently as they should be. We start with the translational Eqs. 9 and 10 and take rotation into account by introducing rotational p.f.s. into \( q_a, q_b \), and \( q_1 \). The rate theory approximation concerns \( q_1 \), not \( q_a \) or \( q_b \). The approximation amounts to assuming an equilibrium (rather than steady-state) distribution over rotational angles at and near the transition state.

In place of Eqs. 1 and 2, we now have
\[
q_L = \Lambda^{-3} q_a q_b, \quad q_0 = qV; \quad q_1 = q_a q_b q_0 e^{-w_0/kT} \quad [20]
\]
\[
q^* = q_a q_b q_0^* \Lambda^{-2} SV \quad [21]
\]

In the potential surface \( w \), at the transition state (where \( w_0 = 0 \)), there are now five dimensions (the "window") normal to the reaction coordinate. The corresponding ligand p.f. (part of \( q^* \)) is \( q_1 \Lambda^{-2} \) where \( q_1 \) is a rotational p.f. over the limited angular ranges mentioned above. The integration (ref. 6, p. 194) over \( \sin \theta d\theta d\\phi d\\psi \) that contributes a factor \( 2\pi f_\theta^+ \) to \( q_1 \) contributes here \( 2\pi f_\theta^+ f_\psi^\dagger \). Therefore \( q_1^* = f_\theta^+ f_\psi^\dagger q_1 \). Final-

**FIG. 1. Schematic Ellipsoidal ligand bound to protein.** (a) Prolate ellipsoid with \( c > b = a \). (b) Oblate ellipsoid with \( a = b > c \).
ly, the p.f. $q_a$ in $q_1$ is the three-dimensional ”rocking” (vibrational) p.f. into which $q_a$ degenerates when the ligand is bound to the protein site. Just as we are using, in effect, a “square-well” approximation to the potential (rather than, say, a five-dimensional parabolic well) in writing $q_1 \sim \Lambda^{-2\delta}$, we can also employ $q_{\alpha} \sim f_{\alpha} f_{\phi}$, where $f_{\alpha} f_{\phi}$ has the same significance for a bound ligand that $f_{\alpha} f_{\phi}$ has for an activated complex. We would of course expect $f_{\alpha} < f_{\alpha}^1$ and $f_{\phi} < f_{\phi}^1$.

On using the above relations in Eqs. 5, 9, and 10, we obtain the modifications

$$K = \alpha'/\beta = q_{\alpha} q_{\phi} e^{-\Delta \mu / K T} \Lambda f_{\alpha} f_{\phi} \quad [22]$$

$$\alpha' = 2 \pi D R f_{\alpha} f_{\phi}^* \quad [23]$$

$$\beta = 2 \pi D R e^{\Delta \mu / K T} (f_{\alpha}^* f_{\phi}^*/f_{\alpha} f_{\phi}) q_{\alpha} q_{\phi} \Lambda^3. \quad [24]$$

Thus $K$ and $\alpha'$ may be reduced considerably by the new rotational factors while $\beta$ would be increased. The main qualitative kinetic effect is clearly due to the more stringent geometrical requirements (at the transition state)—rotational as well as translational—that must be satisfied in order for binding to occur.

In Eqs. 17 and 18 for the free energy changes, $\Lambda^3$ should be replaced by $\Lambda f_{\alpha} f_{\phi}$ since $\Delta \mu = -K T K N$ (compare Eqs. 5 and 23).

Order of Magnitude Estimates. Choice of a particular model would allow us to pursue Eqs. 22 to 24 further. Instead of this, we give an argument here that should suffice to estimate orders of magnitude of $f_{\alpha}$ and $f_{\phi}$ in a number of cases. For this purpose we assume that the translational capture distance $R$ is also operable in determining angular allowances for capture. Thus, starting with the optimal rotational orientation $\theta = 0, \psi = 0$, we first rotate the axis $Z'$ through an angle $\chi = \theta$ chosen so that the site on the ligand surface moves a straight-line distance $R$ from its original position. Next, we rotate about $Z'$ an angle $\chi f_{\phi}$ such that the original position of the site moves a straight-line distance $R$ (the site itself remains fixed on the $Z'$ axis). Therefore $\sin(\chi / 2) = R / 2a$ and $f_{\phi} f_{\phi} = 2 \pi / \phi$. From Eq. 19, then,

$$f_{\alpha} f_{\phi} = R^2 / 4a^2 < 1, \quad f_{\alpha} f_{\phi}^* = 1 / 3, \quad f_{\alpha} f_{\phi} f_{\phi}^* = R^2 / 12a^2. \quad [25]$$

(Alternative but similar arguments lead, instead, to $f_{\phi}^* = 1 / \pi$.) As might be expected, the larger the ligand sphere, the larger the rotational effect on the rate constant $\alpha$ (Eq. 23): $f_{\phi} f_{\phi}^* f_{\phi}^* \sim 1 / a^2$ with $R \cong$ constant.

As a numerical example, if we take $R / a = 1 / \pi$ and $s_{\phi} f_{\phi}/f_{\phi} = 50$, the new factor in K (Eq. 22) is $4.2 \times 10^{-4}$, in $\alpha'$ (Eq. 23) it is $2.1 \times 10^{-3}$, and in $\beta$ (Eq. 24) it is $50$. In the numerical example following Eq. 10, $\alpha'$ is reduced to $1.6 \times 10^{-7}$ sec$^{-1}$ M$^{-1}$, a common order of magnitude (10).

Binding of an ellipsoidal ligand on a protein

We consider two special cases only: (a) the ligand is a prolate ellipsoid of revolution with its site at one end (Fig. 1a); and (b) the ligand is an oblate ellipsoid of revolution with its site centrally located (Fig. 1b). That is, in both cases (using the same labeling of axes as above), the site on the ligand surface and also on the $Z'$ axis, which is the symmetry axis. The center of the ellipsoid is at $x, y, z$ relative to the origin shown in Fig. 1. The surface of the protein is the $z = -c$ plane. In the prolate case, the semi-axes of the ellipsoid are $c > b = a$. In the oblate case, $a = b > c$.

(a) Prolate ellipsoid. We assume, as in the case of spheres, that translational capture occurs on the surface $r = R$. But here the center of the ellipsoid may be in the negative region $a - c < z < 0$. We take this into account, approximately, by using for the capture area that part of the surface $r = R$ in $a - c < z < 0$. That is, $S = 2 \pi R(R + c - a)$. If $c - a \geq R, S = 4 \pi R^2$. This is an approximation because, even without absorption on $R = R$, the concentration of ligand centers in $a - c < z < 0$ is less than $cL$ owing to restricted rotation [see subsection (b), below].

Other necessary changes in the treatment of spherical ligands above, are: the translational diffusion coefficient $D$ is now an average (7-9); in Eq. 21, $A^{3/2}$ becomes $A^{1/2}$; in Eqs. 23 and 24, $R$ is replaced by $R + c - a$ (or by $2R$ if $c - a > R$); and in Eq. 25, $a$ is replaced by $c$. Eq. 22 for $K$ is unchanged, as is the frequency factor $D/R A$ in Eqs. 9 and 10 ($\alpha', \beta$, and $q^2$ are all proportional to the modified $S$).

In summary: as in the spherical case, the primary rotational obstacle to binding here is proper orientation of the long axis $Z'$ (i.e., $f_{\phi}^*$); $f_{\alpha}^*$ is less important.

(b) Oblate ellipsoid. In this case, $Z'$ is the short axis (Fig. 1b). In Eq. 21, $A^{3/2}$ again becomes $A^{1/2}$. The nearest center of the ligand can be to the protein surface ($z = -c$) is the plane $z = 0$. When $0 < z \leq a - c$, there is restricted rotation owing to collision of the ligand with the protein surface. This is encountered before binding can occur if $R > a - c$. The translational capture area is again, as in the spherical case, the hemisphere $S = 2 \pi R^2$.

Eqs. 22 to 24 apply without formal change. As for estimates of $f_{\alpha}$ and $f_{\phi}$, we take, as above, $f_{\phi} = 1 / \pi$ and also $f_{\phi} = R^2 / 4c^2 < 1$ provided that $R \geq a - c$ (rotation when $z = R$ is in this case unrestricted by the protein surface); however, if $R < a - c$, the situation as regards $f_{\phi}$ is complicated somewhat, as follows.

With the ligand center at $x = 0, y = 0, z = R$, we let $\theta$ increase from $\theta = 0$ towards the allowed angle for capture $2 \sin^{-1}(R / 2c)$ (see the text above Eq. 25). But this is an upper limit for $\theta$ because the ligand may hit the protein surface $z = -c$ first. Let $\alpha$ be the value of $\theta$ at which an ellipsoid (Fig. 1b) with center at $z$ just touches the surface $z = -c$. Then it is easy to show that

$$\cos \alpha = [(a^2 - (z + c)^2) / (a^2 - c^2)]^{1/2}. \quad [26]$$

Here we put $z = R$. Thus, when $R < a - c$, $f_{\phi}^*$ is estimated as the smaller of $f_{\phi} = R^2 / 4c^2$ < 1 and (see Eq. 19)

$$f_{\phi}^* = \frac{1}{2} \left[1 - \left[\frac{a^2 - (R + c)^2}{a^2 - c^2}\right]^{1/2}\right]. \quad [27]$$

It follows that Eq. 27 is the applicable expression if

$$(a/c)^2 > (4 + 2s - s^2)/s(2 - s), \quad [28]$$

where $s = R/c < 2$. For example, if $R = c$, this condition is $a/c > 5/\sqrt{2}$. Incidentally, whenever Eq. 28 is satisfied, $R < a - c$ (i.e., $a/c > s + 1$) is also.

As an appendix to this subsection, we comment further on the restricted rotation encountered by an oblate ellipsoid (ligand) when it is near the protein surface (at equilibrium; without binding), i.e., in $0 \leq z \leq a - c$. The potential of mean force $w(z, x, y, z, \theta, \phi, \psi)$ is zero except when the ellipsoid touches the surface, in which case $w = \infty$. In $0 \leq z \leq a - c$, the rotational p.f. $q_r(z)$ (see $q_r$ in Eq. 20) is reduced below
the value \( q_r = q_r(\infty) \) because \( \theta \) has a limited range: \( 0 \leq \theta \leq \alpha \) and \( \pi - \alpha \leq \theta \leq \pi \), where \( \cos \alpha \) is given by Eq. 26. Thus

\[
q_r(z) = \left[ 1 - \cos \alpha(z) \right] q_r, \quad (0 \leq z \leq a - c). \tag{29}
\]

There will be a proportional reduction in ligand concentration near the surface since, at equilibrium, \( c_l(z) \sim q_r(z) \sim q_r(z) \).

Instead of using \( q_r(z) \) in \( q_r \), an alternative but equivalent procedure is to introduce a new potential of mean force, as follows. If \( e^{-w(z)/kT} \) (with \( w = 0 \) or \( \infty \)) is integrated over \( \sin \theta d\theta d\phi \) (see Eq. 7), a spatial (3) \( e^{-w(z,y,z)/kT} \) is obtained (actually, a function of \( z \) only). We can then write (Eq. 20)

\[
q_r(z) = \Lambda^{-1} q_r e^{-w(z)/kT v}
\]

where

\[
u(z)/kT = -\ln \left[ q_r(z)/q_r \right] = -\ln \left[ 1 - \cos \alpha(z) \right] \geq 0.
\]

That is, the surface presents an effective external (repulsive) field \( w(z) \) to ligand molecules because of enforced hindered rotation. Unless \( R \geq a - c \), this barrier must be surmounted before binding can occur (as in Fig. 1b). In addition, a factor of 1/2 must be included in \( q_r \) (as in Eq. 27) because the correct side of the ligand must be facing the protein surface.

Just as Eq. 12 is a refinement on transition-state theory, the external field in Eq. 31 could be made (together with Eq. 11) the basis of a refinement in Eqs. 23, 27, etc. But this would not be simple. Note also that, even in this "refinement," rotation would still be treated as at equilibrium.

Although a barrier to binding is not involved (see Fig. 1a), we remark that in the prolate case, in the region \( a - c \leq z \leq 0 \), the effective potential is found to be

\[
u(z)/kT = -\ln \left[ \frac{(z + c)^2 - a^2}{c^2 - a^2} \right] \geq 0. \tag{32}
\]