Effect of crowding on protein-protein association in diffusion-limited regime

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Abstract.
In the present paper, we establish a theoretical formalism to study the protein-protein association rate in the framework of diffusion-limited theory. To account for the crowding effect due to the presence of polymer, we particularly take into account the deviation of rotational diffusion of proteins from the Stokes-Einstein-Debye relation. Based on fluid mechanics and depletion theory, a new scaling relation for the retardation factor of the rotational diffusion was proposed. Besides, the crowding-induced interaction energy between the proteins has been also properly introduced into the association rate theory. We apply our theory to calculate the association rate constant of proteins β-lactamase and β-lactamase inhibitor protein in poly(ethylene glycol) solutions. Particular attention is paid to the dependence of the association rate constant on the polymer concentration and polymer molecular weight. The deviation from the simple relation based on Stokes-Einstein approximation is well addressed. We find that our theoretical results show good agreements with the experimental data in the whole concentration region, which demonstrates the validity of our theory.

1. Introduction
Protein association is an important center for various cellular processes such as enzyme catalysis, regulation of immune response by cytokines as well as gene regulation, etc [1, 2]. Understanding the physical principles governing association mechanisms and rate constants and furthermore, realistically modeling them, are crucial to study the relationship between the cellular structure and function. To facilitate experimental studies in vitro, synthetic polymers such as polyethylene glycol (PEG), ficoll, dextran, and poly(vinyl alcohol), are commonly used as a means to mimic molecular crowding in the cell [3, 4, 5]. For instance, crowding effects on protein folding, binding, oligomerization, and protein-protein association have been widely investigated by using polymer solutions [6, 7, 5]. In spite of lots of studies so far, a comprehensive understanding of the crowding effect on protein association is still an open and challenging problem.

To better understand the kinetics of association of two proteins (A and B), it is useful to consider the process as a multistep process [8, 9, 10, 11, 12] below (Fig. 1). The initial step is a collision between two proteins in a solution by virtue of translational diffusion. In the next step rotational diffusion will enable the proteins in the collision complex to find the correct orientation to form the transient complex (A ∗ B). At last, the transient complex may evolve to form the final native complex (C) through the transition state, involving desolvation of the interface and exact structural rearrangement. Correspondingly, the overall rate constant of
association is given by

$$k_{\text{on}} = \frac{k_D k_C}{k_{-D} + k_C}, \quad (1)$$

where $k_D$ and $k_{-D}$ represent the rate constants of transient complex formation and dissociation and $k_C$ is the rate constant at which the transient complex evolves to form the final complex. Both the diffusion step and the conformational step can be rate-limiting of protein association. Basically, there is no simple way to determine whether a given protein complex is controlled by diffusion, conformational changes or mixed range. Nevertheless, many experimental [13] observations reveal that in most cases, the conformational rearrangement is fast relative to the dissociation of transient complex (i.e., $k_C \gg k_{-D}$), thus leading to a diffusion controlled regime $k_{\text{on}} \approx k_D$.

So far, great attention has been paid to investigating diffusion-controlled association rate. For simplicity, by neglecting the rotational step, Smoluchowski equation [14] relates $k_D$ and the translational diffusion coefficients according to

$$k_D = 4\pi \bar{r}_h \bar{D}_t, \quad (2)$$

where $\bar{r}_h$ and $\bar{D}_t$ represent the sum of the hydrodynamic radius $r_h$ and translational diffusion coefficient $D_t$ of proteins $A$ and $B$. In simple liquids or dilute agents, $D_t$ can be estimated based on Stokes-Einstein (SE) relation i.e., $D_t = k_B T / 6\pi \eta_{\text{macro}} r_h$ where $\eta_{\text{macro}}$ denotes the macroscopic viscosity of the solution, $k_B$ is the Boltzmann constant and $T$ is the temperature. Further assuming two proteins undergoing association having similar size, it is easy to derive the relative association rate constant obeys the following simple linear relation:

$$\frac{k_{\text{on}}^0}{k_{\text{on}}} = \frac{\eta_{\text{macro}}}{\eta_0}, \quad (3)$$

where "0" denotes the value in pure solvent.

Recently, many experiments [15, 16] have been applied to investigate the protein-protein association in polymer solutions by using stopped-flow fluorescence spectrometer. Interestingly, experimental studies have revealed that the association rate in polymer solutions will seriously deviate from the simple relation based on SE approximation as given by Eq. (3). The deviation is almost negative; i.e., the decrease in association rate with increased viscosity is less than predicted, indicating a faster rate constants in crowding and viscous polymer solutions. Furthermore, the negative deviation increases with increasing polymer concentration and molecular weight. The understanding of such a novel phenomenon can be very important for modeling processes in vivo. To the best of our knowledge, however, such an understanding is still lacking.

Motivated by the above consideration, in the present paper, we will establish a theoretical formalism to analyze the association rate constant for proteins in polymer solutions. The
crowding effect due to the presence of polymer will be properly introduced. Based on the
schematic of the theory of diffusion-limited association (DLA), both translational and rotational
steps are included. The translational diffusion coefficient is assumed to satisfy the SE relation,
while the deviation of rotational diffusion coefficient from the Stokes-Einstein-Debye (SED)
relation will be seriously taken into account. Adopting the fluid mechanics and depletion theory
we proposed a new scaling law for the rotational retardation factor. Furthermore, we introduce
the crowding-induced interaction energy between proteins which can be specified based on the
thermodynamic analysis for the change of free energy for both reactants and transient complex
from dilute to crowding solutions. Finally, we reach the formulation for the association rate
constant, in explicit relationship to the property of polymer solutions and the hydrodynamic
radii of the proteins. We apply our theory to study the association of proteins
(TEM) and β-lactamase inhibitor protein (BLIP) in PEG solutions. The relative association
rate constant in PEG solutions with different PEG molecular weight and varying concentration
will be explicitly evaluated. Through evaluating the dependence of the association rate on the
macroscopic viscosity, we quantitatively analyze the negative deviation from SE estimation. We
compare our theoretical results with the experimental data, and good agreements have been
achieved.

The paper is organized as follows. Firstly, we introduce the DLA theory for evaluating
the protein-protein association rate constant. The crowding effects due to the deviation of rotational
diffusion coefficient from SED relation as well as the crowding-induced interaction energy
between proteins are properly taken into account. Secondly, we apply the theory to
study the association of TEM and BLIP in PEG solutions. The theoretical results will be
compared with the experimental data. At last, we conclude the paper.

2. Model and theory

According to the experimental observations, the protein-protein association rate constant in
most cases occur in the diffusion-controlled regime. Therefore, it is reasonable for us to turn to
DLA theory. The theory starts from the combined rotation-translation diffusion equation for a
pair of particles (spheres) moving in a solution. To account for the nonuniform reactivity of the
proteins and the fact that reaction should occur at the proper mutual orientation, the analytic
expression for the fully DLA reaction rate constant has been well established based on Szabo
approach [17], which yields [18]

$$k_D = 4\pi (D_{t,A} + D_{t,B}) (r_h^A + r_h^B) [F_A \zeta_B \tan(\alpha_B/2) + F_B \zeta_A \tan(\alpha_A/2)],$$

(4)

where $\zeta_i = \sqrt{[1 + D_{r,i}(r_h^A + r_h^B)^2/(D_{t,A} + D_{t,B})]/2}$ where $D_r$ denotes the rotational diffusion
coefficient, and $F_i = \sin^2(\alpha_i/2)$ with $\alpha_i$ being the symmetric reactive region. According to the
above expression, $k_D$ is determined by both translational and rotational diffusion. Note that
recent experimental studies for diffusion of molecular probes in complex polymer solutions, reveal
a very novel decoupling phenomenon between the translational and rotational diffusion [19, 20].
Namely, it was found experimentally that, for diffusion of proteins in polymer solutions, the SE
relation for the translational diffusion remains well satisfied, while rotational diffusion exhibits
large deviations from the traditional SED relation given by $D_r = k_B T/8\pi \eta_{\text{macro}} r_h^3$. Therefore, it
seems reasonable for us to introduce SE relation for $D_t$ and the proper modification for $D_r$ into
Eq. (4). The modification concerning the rotational diffusion coefficient will be provided later.
Furthermore, if we assume $r_h^A \approx r_h^B$ and also $\alpha_A \approx \alpha_B$, we can derive the relative rate constant
(i.e., $k_0^D/k_D$ with $k_0^D$ denotes the value in pure solvent), given by

$$\frac{k_0^D}{k_D} = \frac{\eta_{\text{macro}}}{\eta_0} \left( \frac{5}{2} + \frac{3}{R_{\text{rot}}} \frac{\eta_{\text{macro}}}{\eta_0} \right),$$

(5)
where \( R_{rot} \equiv D_0^0/D_r \) represents the retardation factor of rotational diffusion, i.e., the ratio of rotational diffusion coefficient in pure water to that in polymer solutions. Obviously, the relative rate constant given above is expressed explicitly in terms of both macroscopic theory \( \eta_{macro} \) and the rotational retardation factor \( R_{rot} \). As a limitation case, supposing that SED relation is also valid, such that \( R_{rot} = \frac{\eta_{macro}}{\eta_0} \). Then, we have immediately Eq. (3) which is just the association rate constant under SE approximation.

The macroscopic viscosity for polymer solution is certainly determined by the structure of the polymer solutions, including the polymer size, concentration, and even the statistical length scale, such as mesh length \( \xi \). Here we adopt the promising empirical scaling relation for \( \eta_{macro} \) proposed by Holyst et al. [21],

\[
\frac{\eta_{macro}}{\eta_0} = \exp \left[ \frac{\gamma}{RT} \left( \frac{R_h}{\xi} \right)^\beta \right],
\]

where \( R \) denotes the gas constant, \( \gamma \) is a phenomenological parameter varying from system to system, \( \beta \) is also an empirical parameter, equal to 0.78 for most polymer solutions [22], \( R_h \) is the hydrodynamic radius of the polymer molecule, and \( \xi \) is the correlation length depending on polymer concentration \( \phi \), following

\[
\xi = R_g \left( \frac{\phi}{\phi^*} \right)^{-3/4},
\]

where \( R_g \) is the polymer gyration radius and \( \phi^* \) is the overlap value for crossover from the dilute to semidilute regime, determined by the property of the polymer, according to

\[
\phi^* = \frac{3M_w}{4\pi dN_AR_g^3}
\]

with \( M_w \) being the polymer molecular weight, \( d \) the polymer mass density, and \( N_A \) the Avogadro constant.

The rotational retardation factor \( R_{rot} \) for protein in polymer solutions, which can heavily deviate from SED relation as mentioned above, which therefore will lead to the relative association rate constant as expressed in Eq. (5) rather nontrivial. Based on fluid mechanics [23], \( R_{rot} \) can be evaluated explicitly. When a sphere is rotating in a quiescent fluid, the low Reynolds number flow is solely driven by the shear stress. The fluid velocity field around the particle is assumed to be incompressible and obey the Navier-Stokes equations but now with space-dependent viscosity associated to the depletion effect. Via solving the field equations with proper boundary conditions, we can get the shear stress of the fluid at the probe surface. This allows us to calculate the shear-induced torque \( \Gamma \) exerted on the probe by polymer solutions. Assuming the probe is rotating with angular velocity \( \Omega \), the rotational diffusion coefficient thus follows [24] \( D_r \equiv k_BT\Omega/\Gamma \). As demonstrated in Ref. [25, 26], the rotational diffusion coefficient deviated from the SED relation remarkably. The retardation factor \( R_{rot} \) unexpectedly is not sensitive to the changing of the relative macroscopic viscosity \( \eta_{macro}/\eta_0 \), while is relevant to the thickness of the so-called depletion layer \( \delta \). Moreover, \( R_{rot} \) strikingly exhibits an apparent scaling behavior with respect to the ratio between the probe hydrodynamic radius and the depletion thickness, i.e., \( r_h/\delta \), as shown in Fig. 2. Readers may refer to Ref. [27] for more details. Here, for simplicity, we only quote the final results for the scaling relation of the rotational retardation factor as follows:

\[
R_{rot} = \frac{D_0^0}{D_r} = \exp \left[ a \left( \frac{r_h}{\delta} \right)^b \right],
\]

(9)
Figure 2. Rotational retardation factor $R_{\text{rot}}$ as a function of the length scale $r_h/\delta$, under different macroscopic viscosity $\eta_{\text{macro}}$. The solid line represents the scaling relation as expressed in the figure.

where $a$ and $b$ are fitting parameters given by $a \simeq 0.51$ and $b \simeq 0.56$. We emphasize this scaling law is a general result which is obtained without going into details of any specific information of molecular probes and polymer solutions. The values of the parameters $a$ and $b$ seem robust and universal. Comparing with the retardation factor for translational diffusion, which is determined by the effective hydrodynamics $R_{\text{eff}}$ divided by polymer correlation length $\xi$, i.e., $R_{\text{eff}}/\xi$, $R_{\text{rot}}$ for rotational diffusion, however, is determined by a very different length scale $r_h/\delta$. Note that this scaling can recover the limitations of non-retardation $R_{\text{rot}} \rightarrow 1$ as $\delta/r_h \rightarrow \infty$, and full retardation $R_{\text{rot}} \rightarrow \infty$ as $\delta/r_h \rightarrow 0$. In our opinion, the generality of the scaling relation (9) is basically guaranteed in a certain degree.

The depletion thickness $\delta$ in the formulation (9) is a polymer solution parameter which has a meaning of the depletion thickness near a flat wall in semidilute solutions. As it is well-known, according to many recent experimental and theoretical studies, for colloidal particles suspended in polymer solutions, there appears a depletion layer in the vicinity of the particle surface, introducing a non-homogenous concentration distribution [28, 29]. The depletion thickness characterizes in what extent the non-homogeneity of the polymer concentration sustains until it finally reaches the bulk value. According to the mean-filed approximation [30], $\delta$ can be given by

$$\frac{1}{\delta^2} = \frac{\pi}{4R_g^2} + \frac{1}{\xi_{\text{FH}}^2},$$

where $\xi_{\text{FH}}$ is the Flory-Huggins correlation length. In a good solvent situation, $\xi_{\text{FH}}$ takes the specific form of $\xi_{\text{FH}}^{-2} = -3 \left[ \ln(1 - \phi) \right] \sigma^{-2}$ with $\sigma$ being the statistical segment length of polymer chains (for example, $\sigma \simeq 0.7$ nm for PEG). Clearly, in the dilute limit, $\xi_{\text{FH}} \rightarrow \infty$, such that the depletion thickness $\delta \rightarrow 2R_g/\sqrt{\pi}$. While in the semidilute regime, $\delta$ depends on both polymer gyration radius $R_g$ and polymer concentration $\phi$. Obviously, $\delta$ is a function of both polymer concentration $\phi$ and polymer size. Fig. 3 presents dependence of depletion thickness on $\phi$ and $R_g$. 


Figure 3. The depletion thickness $\delta$ as a function of volume fraction $\phi$ under different polymer gyration radius $R_g$.

Furthermore, taking into account that in the presence of polymer solutions, the association process should be subject to the crowding effect, quantified by crowding-induced interaction energy $\Delta \Delta G^*_c$ between proteins. Thus, the association rate constant in crowding environment will company such an extra energy contribution based on the original diffusion-limited formulation (5). As a result, we can express the relative association rate constant as

$$
\frac{k_{on}^0}{k_{on}} = \frac{k_D^0}{k_D} \exp \left( \frac{\Delta \Delta G^*_c}{RT} \right) = \eta_{macro} \eta_0 \sqrt{\frac{5}{2 + \frac{3}{R_{rot}}} \eta_{macro} \eta_0} \exp \left[ \frac{\Delta \Delta G^*_c}{RT} \right].
$$

(11)

Obviously, the value of crowding-induced interaction energy might depends on the path of the reaction, the properties of the reactants as well as the polymer solutions. Owing to Minton’s work [31], $\Delta \Delta G^*_c$ can be derived based on a reaction cycle as shown in Fig. 4. Briefly, $\Delta \Delta G^*_c$ has the meaning of the difference in transfer free energy between the transient complex and the two separated proteins, from a dilute solution to a crowded solution, i.e.,

$$
\Delta \Delta G^*_c = \Delta G^c - \Delta G^0 = \Delta G^c_{A*B} - \left( \Delta G^dil\rightarrow crd_A + \Delta G^dil\rightarrow crd_B \right),
$$

(12)

where $\Delta G^0$ denotes the free energy for association of A and B in the absence of crowders and $\Delta G^c$ denotes the free energy for that in the presence of crowders. $\Delta G^dil\rightarrow crd_i$ represents the change of free energy for molecular probes from dilute to crowded solutions, which is determined by the relative size of molecular probes and crowders and concentration of crowders. According to the scaled particle theory (SPT) of hard particle fluids proposed by Lebowitz and co-workers [32, 33], we have the following analytical expression for $\Delta G^dil\rightarrow crd_i/RT$:

$$
\frac{\Delta G^dil\rightarrow crd_i}{RT} = - \ln (1 - \phi) + \left( \frac{\phi}{1 - \phi} \right) \left( z_i^3 + 3 z_i^2 + z_i \right) + \left( \frac{\phi}{1 - \phi} \right)^2 \left( 3 z_i^3 + \frac{9 z_i^2}{2 \phi} \right) + \left( \frac{\phi}{1 - \phi} \right)^3 \left( 3 z_i^3 \right),
$$

(13)

where $z_i = r_i^h/R_g$. The expression above provides the explicit relationship between the change of free energy $\Delta G^dil\rightarrow crd_i$ with respect to the polymer concentration, as well as the hydrodynamic radii of both reactant $A$, $B$ and transient complex $A*B$. 


Figure 4. An illustration of the thermodynamic cycle: protein-protein association in dilute and crowded solutions.

Table 1. The basic parameters for PEG solutions.

| PEG  | \( R_g \) (nm) | \( R_h \) (nm) | \( \phi^* \) |
|------|----------------|----------------|-----------|
| 3.35K| 2.44           | 1.49           | 0.081     |
| 6K   | 3.43           | 2.08           | 0.052     |
| 8K   | 4.05           | 2.45           | 0.042     |

Eq. (11) is our main equation. Inserting the macroscopic viscosity \( \eta_{macro} \) Eq. (6), rotational retardation factor \( R^{rot} \) Eq. (9) as well as the crowding-induced interaction energy \( \Delta \Delta G^*_c \) Eq. (12), we can explicitly analyze the protein-protein association rate constant in polymer solutions.

3. Application to PEG systems

We apply the theory to quantitatively evaluate the association rate constants of globular proteins, TEM and BLIP, in PEG solutions. The hydrodynamic radii of TEM and BLIP are roughly equal to be \( r_h \approx 2.2 \) nm. For PEG systems, the gyration and hydrodynamic radii of the coils, i.e., \( R_g \) and \( R_h \) can be estimated according to the molecular weight \( M_w \) via [34, 35] \( R_g = 0.0215M_w^{0.583} \) and \( R_h = 0.0145M_w^{0.571} \). \( \gamma \) is estimated by fitting experimental data, which has been reported to be 4.0 kJ/mol [22]. In addition, the mass density \( \delta = 1126 \) kg/m\(^3\) and the segmental statistical length \( \sigma = 0.7 \) nm [36]. In order to compare our theory with experimental work, we correspondingly consider the association in PEG solutions with several molecular weights \( M_w = 3.35K \) (3.35 kg/mol), 6K (6 kg/mol) and 8K (8 kg/mol) respectively. The polymer concentration varies from 0 to 0.3 or so. For PEG solutions, the basic parameters are listed in Table 1.

In order to specify the theoretical formulation of the association rate constant \( k_{on} \) based
on Eq. (11), we have provided all the necessary parameters except the hydrodynamic radius of the transient complex, i.e., $r_{h}^{AB}$. Note that $r_{h}^{AB}$ is just a model parameter coming from the thermodynamic cycle which is used to determine the change of free energy of the transient complex from dilute to crowded solutions, i.e., $\Delta G_{\text{dil}ightarrow\text{crd}}^{A\ast B}$, according to Eq. (13). Evidently, $r_{h}^{AB}$ stands for an effective hydrodynamic radius, and can be determined only by fitting experiments.

Now we are ready to analyze the association rate constant of TEM and BLIP in PEG solutions. Firstly, in Fig. 5 we plot the relative macroscopic viscosity $\eta_{\text{macro}}/\eta_0$ as a function of polymer concentration $\phi$ according to Eq. (6), for PEG 3.35K, 6K and 8K with $\phi$ ranging from 0 to 0.3. Obviously $\eta_{\text{macro}}$ increases with polymer concentration for solution of PEG with a certain value of $R_g$. Also, it increases with $R_g$ at a fixed value of $\phi$. Then, it is straightforward for us to evaluate the dependence of the association rate constant $k_{\text{on}}$ on the macroscopic viscosity $\eta_{\text{macro}}$ of PEG solutions. By adjusting the single parameter, i.e., the effective hydrodynamic radius of the transient complex $r_{h}^{AB}$, with all other parameters of the system (see Table 1 and the context), we compare our theoretical results with the available experimental data [8]. As a result, we have Fig. 6, where both our theoretical results (solid lines) and the experimental data (symbols) are shown. Herein, the corresponding fitting parameters are estimated as $r_{h}^{AB} \simeq 2.9\text{nm, 2.5\text{nm and 2.2\text{nm}}}$ in PEG 3.35K, 6K, and 8K, respectively, which are in similar size as the proteins TEM and BLIP. Evidently, our theory can agree with the experimental observations very well in the whole concentration region, which demonstrates the validity of our theory in a certain degree.

As shown in Fig. 6, the association rate constant slightly increases with the macroscopic viscosity, and then reaches a plateau at high concentration region. Eq. (3) is also plotted (dashed line). Evidently, the association rate constant deviates from the simple relation based on SE approximation (Eq. (3)), and the deviation is definitely negative indicating the association rate is much faster than the estimation according to SE estimation. Furthermore, the deviation becomes more and more remarkable along with the increase of the polymer concentration (or macroscopic viscosity $\eta_{\text{macro}}$). This phenomena can be well understood by using our model based on Eq. (11), where two important terms, namely rotational retardation factor $R^{\text{rot}}$ and
the crowding-induced interaction energy $\Delta\Delta G_c^*$ between proteins are properly involved. Firstly, as mentioned above, $R_{\text{rot}}$ deviates from estimation of SED relation, leading to a much faster rotational diffusion in PEG solutions due to the depletion effect. Secondly, the presence of $\Delta\Delta G_c^*$ can be another factor which will inevitably enhance the association rate. Therefore, Eq. (11) captures the main factors which characterize the association rate in PEG solutions.

4. Conclusions
In the present work, we proposed a theoretical framework to understand the crowding effect on protein-protein association rate constant $k_{\text{on}}$. Based on the rotation-translation DLA theory Eq. (4), the diffusion-controlled association rate $k_D$ can be determined. We assume the translational diffusion coefficient obeys SE relation, while the rotational diffusion deviates remarkably from SED relation in polymer solutions. We introduce the rotational retardation factor $R_{\text{rot}}$ and then obtain Eq. (5). $R_{\text{rot}}$ follows a new scaling form as demonstrated by Eq. (9) and Fig. 2, which is derived from the fluid mechanics. Furthermore, we consider the modification associated to the crowding-induced interaction energy $\Delta\Delta G_c^*$ between proteins, which can be made specific based on the thermodynamic analysis for the change of free energy $\Delta G_i^{\text{dil-cr}}$ from a dilute solution to a crowded solution for proteins and transient complex, as given by Eq. (13). Finally, we have Eq. (11) which can be used as the basic formulation to evaluate the rate constant $k_{\text{on}}$ for protein-protein association in polymer solutions.

We apply our theory to analyze the TEM-BLIP association in PEG solutions by keeping the hydrodynamic radius $r_{A*B}^h$ of the transient complex as a fitting parameter. The relative
association rate constant under different molecular weights of PEG, and its dependence on polymer concentration or macroscopic viscosity, have been extensively analyzed. As a result, our theoretical results can well reproduce the experimental data in the whole concentration region with very reason fitting parameter $r_{h}^{A+B}$, which demonstrates the validity of our theory. We would like to emphasize our theory might capture the main factors which lead to the large negative deviation from the simple relation of $k_{on}^{0}/k_{on} = \eta_{macro}/\eta_0$. We find that the negative deviation of rotational diffusion coefficient from SED relation due to the depletion effect, as well as the crowding-induced interaction energy, can account for the peculiarly fast protein-protein association rate in the polymer solutions. Since protein association in complex crowding environment is of ubiquitous importance in many cellular processes, we hope our work might be helpful to gain more perspectives in relevant research fields.

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