Self-assembly via anisotropic interactions

Modeling association kinetics of patchy particle systems and self-assembly induced by critical Casimir forces

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Citation for published version (APA):

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The opposing effects of isotropic and anisotropic attraction on dimerisation kinetics

The association and dissociation of particles via specific anisotropic interactions is a fundamental process, not only in biology (proteins), but also in soft matter (colloidal patchy particles). The presence of alternative binding sites can lead to multiple productive states but also to non-productive ‘decoy’ or intermediate states. On top of the anisotropic interactions, particles can experience non-specific isotropic interactions. Here we investigate the effect of one additional non-productive binding site on the association/dissociation kinetics of patchy particles as well as the effect of adding a non-specific isotropic interaction. We find that introducing an additional decoy binding site reduces the association rate constant, independent of the site’s position. In contrast, adding an isotropic interaction increases the association, due to an increased rebinding probability. Introducing non-specific isotropic attraction in a multivalent patchy particle system forming a tetramer yields non-monotonic association kinetics. While this cluster formation might seem almost identical to two-particle binding with a decoy state, the cooperativity of binding multiple particles creates qualitatively different behavior.
6.1 Introduction

Association processes are ubiquitous in biological systems, for instance, proteins binding to DNA, molecular receptor ligands binding to proteins or proteins forming multicomponent complexes \cite{83, 139, 143}. Association and dissociation of proteins is the basic process in many biochemical relevant processes, such as gene regulation, signaling and intercellular communication, where proteins act in concert and not individually \cite{144}. As communication in and between cells is necessarily temporal, knowledge of the association and dissociation rate constants between the relevant proteins is crucial for understanding the balance of the biochemical network and cascade reactions \cite{75}.

Proteins usually bind via specific interaction sites to form a productive target structure. Protein (complexes) can have several similar or identical target sites, leading to multiple productive bound states, such as in multi-site protein modification of kinases \cite{75}. Several types of interactions can be involved in the stabilization of the productive bound target state: hydrophobic interaction, hydrogen bonds, electrostatic interactions (e.g. salt bridges). As these interactions are not uniformly distributed along the surface, but often specifically located close to the target binding sites, they lead to an effective anisotropic interaction with respect to the proteins’ centers of mass. Furthermore, proteins can have anisotropic interactions that do not lead to a productive state, which are sometimes called non-productive interactions. Such non-productive state can be viewed as a decoy state, or as a (metastable) intermediate state toward the target state. While it seems natural to assume that the presence of decoy or intermediate states retards correct association and lower the effective association rate constant, $k_{on}$, the formation of such states might also enhance the possibility of rebinding to the correct productive state. Finally, proteins interact with an overall isotropic potential, for instance, due to van der Waals forces or depletion forces. Because of the anisotropic effective interaction, the possibility of multiple (rebinding) pathways and the presence of isotropic potentials, it is not trivial to predict how the overall association rate constant towards the productive target structure is affected by additional binding possibilities introduced by non-specific sites.

Anisotropic interactions also play a large role in the self-assembly of colloidal patchy particles. Recent experimental breakthroughs allow the synthesis of colloidal patchy particles decorated with anisotropic binding sites \cite{5, 16}. The patchiness and multi-valency of such particles results in different kinetic pathways that systems can take to reach their ground-state and subsequently form higher order phases. \cite{112, 145, 146} Detailed knowledge of association kinetics can help to understand and design complex colloidal self-assembly.

The major question that we address in this chapter is: how does the association kinetics depend on the location, strength, and shape (anisotropic or isotropic) of additional non-productive interactions? To answer this fundamental question we employ a generic patchy-particle model; a simple model that allows us to study several different decoy patch configurations. While patchy particles are commonly used for colloidal particles, several studies have used such simple coarse
grained models also to represent proteins [135]. Indeed, the experimental gas-liquid coexistence curve of lysozyme can be reproduced using a DLVO type potential with screened electrostatic repulsion and attractive ‘patches’ placed on the surface of a sphere [147]. This finding is justified by the generalized law of corresponding states (GLCS), which states that the thermodynamic properties are insensitive to details of the potential and are described by just considering the second virial coefficient and the density [50, 51].

Obtaining accurate (un)binding rate constants is often difficult in simulations due to high free energy barriers that naturally arise in strongly bound particles. Moreover, the entropic barrier for association is large due to the particle alignment required by the anisotropy of the interaction site. Consequently, when brute-force simulations are used, a majority of the simulation time is wasted in the metastable (bound, unbound or intermediate) states, which does neither give information about kinetics nor transition mechanisms. To alleviate the problem of separation of timescales between simulating the microscopic particle dynamics and observing the rare transitions, advanced path sampling techniques have been developed that bias the generation and selection of reactive pathways using unbiased dynamics [103, 148]. Here we use the Single Replica Transition Interface Sampling (SRTIS) method introduced in Ref. [104] and described in section 2.5. Using SRTIS we sample the entire transition network between all important states and extract the full rate matrix, which gives all the necessary information about the kinetics of the system. In addition, mechanistic information is directly available from the path ensemble, see section 2.5 [107, 149]. The combination of the simplified protein model and SRTIS gives us the ability to study many interaction parameters which would otherwise be unavailable. While the resulting rate matrix obtained with SRTIS contains all the dynamical (and thermodynamic) information of a system, it is usually not easy to interpret. Applying concepts from Transition Path Theory (TPT) yields both the overall association kinetics rate constant as well as more insight into which transition mechanisms contributes mostly to the binding.

Here, we first will investigate the effect of one additional non-productive binding site on the association/dissociation rate constant as well as the effect of adding nonspecific isotropic interaction on the association kinetics of two patchy particles. By varying the strength and position of the decoy site, we find that a stronger decoy state always reduces the association rate constant as the pathway between target and decoy state is less probable than unbinding. Surprisingly, the effect of the position of the decoy site on the overall association rate constant is minimal. In contrast, adding a non-specific isotropic interaction in general increases the association rate constant at low decoy site interaction, and the amount of reduction due to the decoy site then does depends strongly on its position. For weak/intermediate decoy strength, there is hardly an effect of the decoy position, whereas for strong decoy strength the association rate constant is suppressed when the decoy position is far from the target site.

Finally, we investigate the dimerization process of more complex shapes. A multivalent patchy particle binding to an existing cluster of three particles to form a tetramer complex could suffer from malformed structures and trapping,
Figure 6.1: Left: cartoon image of the patchy particle model with the distance between decoy and target site, $\psi$, distance between particles $r$, patch vector $p_i$, and corresponding angle $\theta_i$. Middle: states $T$ and $D$ are depicted. Right: potential energy surface as function of the distance between particles, $r$, and the shift in orientation, $\theta$, for $\psi = 120$, $\epsilon_D = 10k_B T$, $\epsilon_T = 15k_B T$, $\epsilon_C = 4k_B T$, $\delta = 20$ showing clearly the two potential minima due to the two patches and additionally the low isotropic attraction.

see chapter $^4$ and $^5$. While one would think that this cluster binding process is almost identical to the two particle binding with a decoy, the effect of cooperativity of binding to multiple particles yields qualitatively different behavior. Adding a non-specific isotropic interaction increases the rate constant of binding and dissociation at first, but when the isotropic interaction rises above $4kT$ the rate constant lowers dramatically. This maximum in association rate constant also changes with concentration, shifting to higher values of nonspecific isotropic interaction.

The remainder of the chapter is organized as follows. In the Methods section we introduce the model and simulation details. We present and discuss the results on the dimer and tetramer systems in the next section, and end with conclusions.

6.2 Methods

Model

For the case of two-particle dimerization, we consider two particles where one particle (1) has only one binding site $b$ whereas the other particle (2) has two binding sites, one target $t$ and one decoy site $d$. We model the interaction between the particles and the patches based on a 24-12 LJ potential. This potential is of shorter range than the standard 12-6 LJ potential. As such the phase behavior exhibits a meta stable liquid vapor coexistence line with respect to the gas solid coexistence $^{137}$, similar to protein solutions.
The total potential is a superposition of a strongly repulsive WCA-like potential\cite{150,151}, an isotropic attractive potential, and the minimal two attractive anisotropic angle dependent potentials:

\[ U_{12}(\mathbf{r}_{12}, \Omega_1, \Omega_2) = U_{\text{rep}}(\mathbf{r}_{12}) + U_{\text{iso}}(\mathbf{r}_{12}) + \min [U_{\text{bt}}(\mathbf{r}_{12}, \Omega_1, \Omega_2), U_{\text{bd}}(\mathbf{r}_{12}, \Omega_1, \Omega_2)] \]

where \( \mathbf{r}_{12} \) is the inter-particle vector and \( \Omega_{1,2} \) the orientations of the particles, stored in quaternion form. The isotropic WCA-like repulsive potential is given by:

\[
U_{\text{rep}}(r_{12}) = \begin{cases} 
4.0 \left[ \left( \frac{\sigma}{r} \right)^{24} - \left( \frac{\sigma}{r} \right)^{12} + \frac{1}{4} \right] & \text{if } r \leq 2^{\frac{1}{12}} \\
0 & \text{if } r > 2^{\frac{1}{12}} 
\end{cases} \tag{6.1}
\]

where \( r = |\mathbf{r}_{12}| \) is the distance between particles, \( \sigma \) determines the size of the particle. The isotropic interaction is given by:

\[
U_{\text{iso}}(r_{12}) = \begin{cases} 
4.0\epsilon_{\text{iso}} \left[ \left( \frac{\sigma}{r} \right)^{24} - \left( \frac{\sigma}{r} \right)^{12} \right] & \text{if } r \leq r_c \\
0 & \text{if } r > r_c 
\end{cases} \tag{6.2}
\]

where \( \epsilon_{\text{iso}} \) is the strength of the isotropic potential and \( r_c \) is the potential cutoff, beyond which the potential vanishes. The anisotropic patchy interaction between \( b \) and \( t \) is given by:

\[
U_{\text{bt}}(\mathbf{r}_{12}, \Omega_1, \Omega_2) = \begin{cases} 
4.0\epsilon_{\text{T}} \left[ \left( \frac{\sigma}{r} \right)^{24} - \left( \frac{\sigma}{r} \right)^{12} \right] S_{\text{bt}}(\mathbf{r}_{12}, \Omega_1, \Omega_2) & \text{if } r \leq r_c \\
0 & \text{if } r > r_c 
\end{cases} \tag{6.3}
\]

where \( \epsilon_{\text{T}} \) is the strength of the patchy interaction between \( b \) and \( t \). The patchy interaction between \( b \) and \( d \) is defined similarly:

\[
U_{\text{bd}}(\mathbf{r}_{12}, \Omega_1, \Omega_2) = \begin{cases} 
4.0\epsilon_{\text{D}} \left[ \left( \frac{\sigma}{r} \right)^{24} - \left( \frac{\sigma}{r} \right)^{12} \right] S_{\text{bd}}(\mathbf{r}_{12}, \Omega_1, \Omega_2) & \text{if } r \leq r_c \\
0 & \text{if } r > r_c 
\end{cases} \tag{6.4}
\]

where \( \epsilon_{\text{D}} \) is the strength of the patchy interaction between \( b \) and \( d \). The continuous patch function \( S_{ij}(\Omega_1, \Omega_2) \) gives a penalty for misalignment:

\[
S_{ij}(\mathbf{r}_{12}, \Omega_1, \Omega_2) = \exp \left( -\frac{\theta_i^2 + \theta_j^2}{2\delta^2} \right) \tag{6.5}
\]

where \( \delta \) defines the patch-width, \( \theta_k = \arccos(\mathbf{p}_k \cdot \hat{r}_{12}) \), with \( \mathbf{p}_k \) the vector defining patch \( p_k \) (with \( k \in \{b,d,t\} \)) on its respective particle (1 or 2), rotated from the particle frame to the system frame along \( \Omega \). The interaction between particles can be easily tuned via the patch-patch interaction strengths \( \epsilon_{\text{T}}, \epsilon_{\text{D}}, \) the isotropic interaction strength \( \epsilon_{\text{iso}} \) and the width \( \delta \). Proteins usually have a specific (narrow) patchy interaction, therefore, the patch-width is chosen to be small, \( \delta = 20 \) degrees. This patch-width was shown to reproduce the gas-liquid curves of protein solutions such as \( \gamma \)-crystallin and lysozyme quite well\cite{138}, albeit with more
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patches. Naturally, an even smaller patch-width is also allowed by the model, however a much smaller patch-width also dramatically restricts the time-step in the dynamics even further. An example of the potential for the dimer is shown in Fig. 6.1.

For the constrained tetramer we employed the same model between each pair of particles of the complex as for the two-particle system. However, there is no additional decoy site defined. Therefore, total energy for the constrained tetramer is given by:

\[
U_{\text{tetr}}^{\text{tot}}(r_{12}, \Omega_1, \Omega_2) = \sum_{i,j} U_{\text{rep}}(r_{12}) + U_{\text{iso}}(r_{12}) + U_{\text{bt}}(r_{12}, \Omega_1, \Omega_2) \tag{6.6}
\]

where the sum is over each particle of the trimer (and corresponding patch) with which the mobile particle interacts with, also see chapter 4 and 5.

We use Dynamic Monte Carlo (DMC) to propagate the system in time, see section 2.3. By using small translation and rotational step sizes, time evolution via MC dynamics solves the Fokker-Planck equations which represents the Brownian movement of proteins in solution [94, 135]. Here we use Single Replica Transition Interface Sampling (SRTIS) to obtain the full (un)binding path ensemble [104, 108], as explained in section 2.5.

Simulation details

**TIS:** For two-particle dimerization, we consider the three possible (meta)stable states: a bound state \( T \), defined when the patchy interaction \( U_{\text{bt}} < 0.9\epsilon_T \), a decoy intermediate state \( D \) defined when the interaction \( U_{\text{bd}} < 0.9\epsilon_D \) and the unbound state when the particles are separated more than \( r_c \). All interfaces around stable states are defined through the energy of the system. For the bound states \( T \) and \( D \) we set interfaces every 1.5\( k_B T \) starting from the boundary of the state until the energy is zero. Interfaces for state \( U \) are set at low values of energy to guide the system towards state \( T \) or \( D \) and to be sure paths with low energy are properly sampled: \([0.0, 10^{-9}, 10^{-3}, 10^{-1}, 0.4, 1.0]\) [135]. An example of a converged simulation (\( \epsilon_D = 8k_B T, \epsilon_{\text{iso}} = 0.0, \psi = 120 \)) showed that \( \log g(\lambda_U) = [0, -0.25, -3.2, -5.5, -6.0, -6.3] \), which validates the use of interfaces with low values as the crossing probability decreases quickly for small values. The interfaces could have been optimized further, however, this would not change the results.

For the constrained tetramer, an additional state \( I \) is defined similarly as in chapter 4. State \( T \) is defined when all three bonds are formed, and \( U_{\text{tetr}}^{\text{tot}} < 2.7\epsilon_T \) and state \( D \) is defined when no bonds are formed and the particle is on the opposite side of the complex, and \( U_{\text{tetr}}^{\text{tot}} < 2.7\epsilon_{\text{iso}} \) and the unbound state when the motile particle is separated more than \( r_c \) to any other particle of the complex. The interfaces of tetramer states are similarly defined as states defined for two particles.

TIS simulations were performed with DMC in a periodic box of 5.7\( \sigma \). A production cycle of \( 5 \times 10^5 \) TIS cycles was performed after the scale factor for the
Wang-Landau biasing was sufficiently low ($< 10^{-5}$), where every cycle consisted of 10 shooting, reversal, replica swap and state swap moves. Averages for the crossing probability and path densities were sampled after each move.

**Potential:** For the two-particle system, the attractive strength and the patch-width of the target site is set to, $\epsilon_T = 15k_B T$ and $\delta_T = 20$ degrees, respectively. For the constrained tetramer complex, the attractive strength and the patch-width of the target sites is set to, $\epsilon_T = 5k_B T$ and $\delta_T = 20$ degrees, respectively. The potential is truncated at $r_c = 2.0\sigma$.

![Figure 6.2: Rate matrix, $K$, for different angles of the decoy patch, $\psi = 60$ (circles), $\psi = 120$ (squares), $\psi = 180$ (triangles). Rate constants $k_{TU}$ and $k_{UT}$ are hardly dependent on $\epsilon_D$ as expected. Moreover, only $k_{TD}$ and $k_{DT}$ are dependent on $\psi$. Elements $k_{DT}$ and $k_{DU}$ show expected Arrhenius behaviour after $\epsilon_D \approx 8k_B T$. However, at low values of $\epsilon_D$, diffusion limits become more dominant.](image-url)
6.3 Results and Discussion

Effect of the decoy binding site on kinetics

Rate matrix and population

We first studied the effect an additional decoy binding site has on the overall binding rate constant. For particle 2, an additional binding site is placed under an angle $\psi$ away from the target site with the same patch-width $\delta_D = 20$ and with attractive strength $\epsilon_D$, see Fig. 6.1. As mentioned above, for this system there are three meta-stable states: a bound state $T$, nonproductive decoy state $D$ and the unbound state $U$. We sample the path ensemble between all states using SRTIS as detailed in the methods section. For each transition we compute the rate constant via Eq. 2.49. It is interesting to note that even though the rate constant is expected to change with different concentration, we can capture a smaller concentration by simply changing the flux out of the unbound state. From

Figure 6.3: Equilibrium population of $T$, $D$ and $U$ as function of decoy interaction strength for different values of $\psi = 60$ (circles), 120 (squares) and 180 (triangles). Clearly, the decoy state only becomes higher populated when $\epsilon_D > \epsilon_T$. Moreover, due to the box size the population of $U$ is always higher. Also note that the populations are not dependent on $\psi$.

Figure 6.4: Overall association (left) and dissociation (right) rate constant calculated via TPT as function of $\epsilon_D$ for different angles of $\psi$. Even though the reactive path density in Fig. 6.5 shows probability from $D$ to $T$, the decoy state has little significant effect on the overall association or dissociation irrespective of the position relative to the target site, due to $k_{DU} > k_{DT}$ for each $\epsilon_D$. At high $\epsilon_D$ the decoy site has a negative effect on the association rate constant which halves at high $\epsilon_D$ almost independent of $\psi$. 

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Eq. 2.41 \( \phi_U = (\langle \tau_0 \rangle + \langle \tau_1 \rangle)^{-1} \) where \( \langle \tau_0 \rangle \) can be calculated from pathways from the minus move and \( \langle \tau_1 \rangle \) from the first interfaces. The flux out of the unbound state can be changed by scaling \( \tau_0 \) with the volume. As \( \tau_0 \) is given by free diffusion when the particles are beyond 2.0\( \sigma \) apart, the dependence of \( \tau_0 \) on volume can be solved analytically and the total flux out of state \( U \) is given by:

\[
\phi_U = \left( \tau_1 + \tau_0 \frac{V}{V^0} \right)^{-1}
\]

where \( V^0 \) is a reference volume with known flux.

We perform the SRTIS simulation for several values of the decoy strength \( \epsilon_D/k_B T = \{2, 4, 6, 8, 10, 12, 14, 16\} \). The resulting rate matrix is plotted in Fig. 6.2. Rate constants \( k_{TU} \) and \( k_{UT} \) are nearly independent of \( \epsilon_D \), as expected as these direct transitions avoid going to \( D \). Only \( k_{TD} \) and \( k_{DT} \) are dependent on \( \psi \), demonstrating that the rebinding probability from state \( D \) to \( T \) is significantly larger for \( \psi = 60 \). Elements \( k_{DT} \) and \( k_{DU} \) show expected Arrhenius behavior for \( \epsilon_D \gtrsim 8k_B T \), whereas at low values of \( \epsilon_D \), the rate constant becomes more diffusion influenced as seen from the nonlinear dependence. From the rate matrix we can obtain the equilibrium population by computing the zeroth eigenvector, or alternatively apply a long time limit of \( p(t) = \exp(Kt) \). These populations are shown in Fig. 6.3. The decoy state only has a larger population than the bound state when \( \epsilon_D > \epsilon_T \). Moreover, due to the size of the box the population of \( U \) is always the highest of the three states. Also note that the populations are independent of \( \psi \). Therefore, the effect of rebinding is only affecting the kinetics of the system, not the thermodynamic equilibrium, as expected. From the rate matrix we can extract the overall association rate constant, \( k_{TPT} \) via Eq. 2.57, shown as a function of \( \epsilon_D \) in Fig. 6.4. Clearly, the overall association rate constant is only slightly retarded due to the presence of the decoy state, as reaching \( D \) will not contribute to association. Additionally, the dissociation rate constant is only minimally decreased at \( \epsilon_D = 16k_B T \) by the presence of the decoy site. One could argue that there is no increase in association rate constant due to the fact that the volume of the box is small. Usual protein concentrations are much lower (e.g. \( \mu\text{molL}^{-1} \)) than what is simulated here (nmmolL\(^{-1}\) when \( \sigma \) is taken as 10nm, a typical protein size). Naively, one would assume that with larger volume the presence of an additional binding site which keeps the particles in close proximity should increase the rate constant relatively to no additional binding site, due to rebinding. However, \( k_{DU} \) is always significantly larger than \( k_{DT} \) (see Fig. 6.2) which shows that when the volume is made bigger, the non-specific site will still not contribute to the association rate constant as the system will more likely go back to the unbound state than progress towards the bound state. It is interesting that the process from \( D \) to \( T \) is apparently more unlikely than \( D \) to \( U \) within these conditions, which is a manifestation of the fact that the requirement of precise alignment to bind due to the patchiness of particles limits the kinetic pathways possible for systems to reach their ground state. Of course, when the decoy binding site moves even closer to the target state, this will change due to lowering of the \( D-T \) barrier.
Free energy and reaction path density

In the first row of Fig. 6.5 we show the free energy landscape for three different values of $\psi$ obtained via Eq. 2.50 with the distance between the centers of the two particles, $R_{12}$, and the angle $\phi = \phi_1 + \phi_2$, where $\phi_i = \arccos(r_{12} \cdot p_i / r)$, as the collective variables that capture the translational and rotational degrees of freedom during the (un)binding process between all three states. The bound state $T$ is clearly visible at $R_{12} = 1.0\sigma$ and $\phi = 0$.

The unbound state is located past the line given by $R_{12} = 2.0\sigma$. The intermediate state $D$ is located at different values of $\phi$ corresponding to $\psi = 60, 120, \text{or } 180$. Mechanistic information can be obtained from the path ensemble by plotting the reactive path density (RPD). In Fig. 6.5 the RPD is plotted for state $D$ defined by Eq. 2.52. The RPD demonstrates that a transition from $T$ to $D$ instead of $U$ is very improbable (has a low density) when $\psi$ is large, as most probably paths end up in $U$ (located at $R_{12} = 2.0\sigma$), which corroborates with the low values of $k_{DT}$. Only for small $\psi$ is there a significant probability to transition from $D$ to $T$.

![Figure 6.5: First row: free energy landscape for distance between the centers, $R_{12}$, and the sum of the angles of patch vectors with the inter-particle vector, $\phi$ for $\epsilon_D = 12k_BT$ and from left to right $\psi = 60, 120$ and 180. All minima due to the stable states are visible where it is clear where the $D$ state is located as $\psi$ is changed. Second row: corresponding reactive path density (RPD) for state $D$. There is only a significant probability from $D$ to $T$ for $\psi = 60$. However, the transition from $D$ to $U$ dominates the reactive transitions out of state $D$. Note that integration of a reactive path distribution does not result in unity.](image-url)
Effect of isotropic non-specific interaction

Rate matrices

Next, we add a non-specific isotropic interaction between the two particles of the dimer, and conduct SRTIS simulations for a range of values $\epsilon_{iso}/k_B T = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, each for different values of $\epsilon_D$.

In Fig. 6.6 the elements of the rate matrices are shown as function of $\epsilon_D$ for $\epsilon_{iso} = 10kT$, while in Figs. 6.7 and 6.8 the rate matrix elements are plotted as function of $\epsilon_{iso}$ for $\epsilon_D = 8k_B T$ and $\epsilon_D = 16k_B T$, respectively.

An isotropic interaction $\epsilon_{iso} = 10k_B T$ increases the binding rate constants $k_{UT}$ and $k_{UD}$ by an order of magnitude relative to the rate constants without the isotropic attraction. Furthermore, there is no difference in the rebinding rate constant $k_{TD}$ for $\psi = 120$ and 180, whereas when $\psi = 60$, $k_{TD}$ increases more sharply.

Naturally, in Fig. 6.7 and 6.8 $k_{TU}$ and $k_{DU}$ show Arrhenius behavior for strong $\epsilon_{iso}$. Interestingly, both $k_{DT}$ and $k_{TD}$ reach a plateau value as function of $\epsilon_{iso}$, showing that after a certain value the process becomes diffusion limited, where the rebinding probability depends on whether $D$ or $T$ is found first. Moreover, for $\psi = 60$ there is hardly any change for $k_{DT}$ and $k_{TD}$ at all with $\epsilon_{iso}$. This indicates that the chance of rebinding is relatively high for small $\psi$, so that the rebinding dominates over the escape at $\epsilon_{iso}/k_B T > 4$. Indeed, the $k_{DT}$ and $k_{TD}$ are relatively high for small $\psi$ compared to large $\psi$. This is caused by an overlap of the patches, lowering the barrier for the $D$ to $T$ transition. For larger $\psi$ angles there is no such effect. Instead, the isotropic interaction now clearly leads to an increase of the chance of rebinding. Note also that Fig. 6.6 shows that changing the decoy strength only changes the exit rate constants from the decoy state N.
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Figure 6.6: Rate matrix, $\mathbf{K}$, for different angles of the decoy patch, $\psi = 60$ (circles), $\psi = 120$ (squares), $\psi = 180$ (triangles) with a non-specific isotropic interaction of $\epsilon_{iso} = 10k_B T$. Rate constants $k_{TU}$ and $k_{UT}$ are not dependent on $\epsilon_D$ and there is no dependency on $\psi$ for $k_{DU}$ as expected. For the rebinding rate constants $k_{DT}$ and $k_{TD}$ there is no difference between $\psi = 120$ or 180, only for $\psi = 60$. 
Figure 6.7: Rate matrix, $K$, for different angles of the decoy patch, $\psi = 60$ (circles), $\psi = 120$ (squares), $\psi = 180$ (triangles) with a decoy interaction of $\epsilon_D = 8k_B T$. Naturally, $k_{TU}$ and $k_{DU}$ show Arrhenius behavior for strong $\epsilon_{iso}$. There is no dependency on $\psi$ for $k_{TU}$, $k_{UT}$ and $k_{DU}$ as expected. Interestingly, for $\psi = 120$ and 180, $k_{DT}$ and $k_{TD}$ level off around $\epsilon_{iso}/k_B T = 4$, whereas for $\psi = 60$ there is no dependency at all on $\epsilon_{iso}$.
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Figure 6.8: Rate matrix, $K$, for different angles of the decoy patch, $\psi = 60$ (circles), $\psi = 120$ (squares), $\psi = 180$ (triangles) with a decoy interaction of $\epsilon_D = 16 k_B T$. Similar trends are visible as in Fig. 6.7 except for $k_{DU}$ and $k_{DT}$ are significantly lower.
In the top row of Fig. 6.9 we show the effect of the non-specific isotropic interaction on the overall association rate constant, $k^{TPT}_{UT}$, computed using Eq. 2.34. Clearly the overall association rate constant increases by more than an order of magnitude with the isotropic interaction for low decoy interaction $\epsilon_D$, and eventually levels off for high attraction, i.e. $\epsilon_{iso} \gtrsim 8$. However, the non-specific isotropic interaction does not change the association rate constant for high non-specific decoy interaction $\epsilon_D$. As the decoy patch becomes more attractive, the...
increase in overall association rate constant gained via the non-specific interaction is lost. It is now just as probable to end up in \( D \) as in \( T \), since both the target and decoy sites become of equal strength, which consequently retards the overall association toward the target state.

The middle row of Fig. 6.9 shows the same rate constant data, as a function of the non-specific decoy interaction \( \epsilon_D \). Here the effect of the decoy strength is clearly to lower the overall association rate constant, for each setting of the \( \epsilon_{iso} \). Note that the retardation effect of the decoy site is stronger when there is non-specific isotropic interaction in contrast to no isotropic interaction all.

In the bottom row of Fig. 6.9, the same data is plotted to show the dependence of the position of the decoy site. The position of the decoy patch only starts to matter for strong decoy interaction in contrast to low decoy interaction. Rebinding from \( D \) to \( T \) increases when the decoy site is close to the target site, but only noticeably at higher \( \epsilon_{iso} \). However, there is almost no difference between \( \psi = 120 \) and \( \psi = 180 \).

Figure 6.10: Flux ratio of \( f_{UT} \) over \( f_{UDT} \) showing the effect of rebinding for different positions of the decoy site for \( \epsilon_D = 14k_BT \) for different values of \( \psi \). Higher rebinding probability for \( \psi = 60 \) (circles) results in lower \( f_{UT}/f_{UDT} \) indicating more reactive pathways from \( U \) to \( T \) via \( D \), in contrast to higher values of \( \psi \).

To clarify this point we computed the net flux using the TPT approach via Eq. 2.56. Fig. 6.10 plot the net flux ratio \( f_{UT}/f_{UDT} \). Clearly, the higher rebinding probability for a low value of \( \psi \) results in more associating pathways via \( D \) than for high values of \( \psi \), i.e. low values of \( f_{UT}/f_{UDT} \). Thus, direct paths are dominant for low \( \epsilon_{iso} \), and high \( \psi \), as rebinding is very rare for these settings. The flux ratio never drops below unity, even when all paths exiting from \( D \) rebind correctly to \( T \), since the chances of going to the \( D \) or \( T \) state from the unbound state \( U \) are about equal.
Reactive path density

The reactive path density for transitions out of $D$ for the system with isotropic interaction is shown in Fig. 6.11. Here one observes an increase in rebinding probability as the isotropic interaction increases, corresponding to the increased flux ratio $f_{UT}/f_{UDT}$ in Fig. 6.10. The rebinding probability changes dramatically between $\epsilon_{iso} = 2$ and $\epsilon_{iso} = 6k_BT$, but saturates for high $\epsilon_{iso} = 12$.

Figure 6.11: Effect of isotropic interaction on reactive path density (RPD) for state $D$ for distance between the centers, $R_{12}$, and the sum of the angles of patch vectors with the inter-particle vector, $\phi$ for f.l.t.r. $\psi = 60, 120$ and $180$. RPD out of state $D$ for $\epsilon_D = 12k_BT$ and $\epsilon_{iso} = 2k_BT, 6k_BT$ and $10k_BT$ showing that the rebinding probability increases with $\epsilon_{iso}$. However, the effect is saturated at high values of $\epsilon_{iso}$.
Constrained tetrahedron formation

Protein complexes require usually more than two proteins. Here, we study the formation kinetics of a model tetramer complex in which each protein has three binding sites \[135\]. (see Fig. 6.12). The rate determining step in the tetramer formation is the addition of single protein to an correctly formed trimer. In chapter 4 and 5 we extensively studied the formation kinetics of this (so-called) constrained tetrahedron. Here, we investigate the effect of adding an isotropic non-specific interaction to each protein. We use the same interaction potential between particles as described in section 6.2, only now the particles have three patches put at the contact points of a perfect tetrahedral arrangement of the particles. For more details see Ref. \[135\] or chapter 4. In this patchy particle system there are four stable states (\(U\), \(T\), \(I\) and \(D\)). Starting in the unbound state \(U\) (consisting of particle far away from the correctly formed trimer) the incoming particle can bind to the fixed trimer correctly by forging all three bonds (the \(T\) state), or could be

Figure 6.12: Cartoon image of states defined for the constrained tetrahedron system with the motile particle in orange depicting the nature of all states defined. Note that the particles in blue are fixed in space and orientation.

Figure 6.13: Left: Overall association rate constant for a constrained tetrahedron for different concentrations. Note that the rate constants are scaled with a factor \(10^x\) to fit in the same Fig., where \(V/V_0 = 1.0\) (black, \(x = 7\)), \(10.0\) (purple, \(x = 8\)), \(10^2\) (green, \(x = 9\)), \(10^3\) (blue, \(x = 10\)), \(10^4\) (orange, \(x = 11\)), \(10^5\) (red, \(x = 11\)). A shift from \(4k_BT\) to \(8k_BT\) is seen for the maximum association rate constant as function of the volume scaling. Right: Same data plotted normalized with the \(\epsilon_{iso} = 0\) rate constant.
trapped in an intermediate state where two frustrated patchy bonds are formed (see Fig. 6.12 for a schematic representation of these states). Note that for this

\[
\begin{align*}
\epsilon_{iso} / k_B T & = 10^{-18} \\
& = 10^{-14} \\
& = 10^{-10} \\
& = 10^{-6} \\
0 & 2 \\
2 & 4 \\
4 & 6 \\
6 & 8 \\
8 & 10
\end{align*}
\]

Figure 6.14: Overall dissociation rate constant for a constrained tetrahedron showing Arrhenius behavior as function of $\epsilon_{iso}$.

system the patch interaction set to $\epsilon_T = 5k_B T$ is already yielding the same total energy for state $T$ as for the dimer (see Section 6.3). The additional non-specific isotropic potential results in a fourth (meta)stable state, $D$, where the incoming particle is trapped on the opposite side of the trimer, forming no specific patchy bonds, but only interacting with the isotropic potential. In this state the attaching protein forms three non-specific bonds with the fixed trimer simultaneously. Therefore rearrangement into the productive state only occurs by breaking at least one non-specific bond. As the trimer can be seen as a rigid body with a (3-valent)

\[
\begin{align*}
\epsilon_{ISO} & = 10 \\
\epsilon_D & = 8 \\
\epsilon_D & = 4 \\
\epsilon_D & = 0 \\
0 & 2 \\
2 & 4 \\
4 & 6 \\
6 & 8 \\
8 & 10 \\
10 & 12 \\
12 & 14 \\
14 & 16 \\
16 & 0
\end{align*}
\]

Figure 6.15: The overall association rate constant from Fig. 6.9 presented again, but now in a 2D heat map for $\psi = 60$ (left), 120 (middle) and 180 (right) to show the connection to the constrained tetramer. The diagonal line drawn through each heat map gives an indication how cooperativity affects the overall assembly similarly how it occurs for the formation of the constrained tetrahedron. The diagonal cut shows that for decoy states close to the target state, a higher maximum overall association rate constant is reached.
Two opposing effects of isotropic and anisotropic attraction

Figure 6.16: Overall association rate constant similarly as in Fig. 6.9, however now the concentration is decreased a hundredfold according to Eq. 6.7. The retardation effect of the decoy state is relatively decreased. Moreover, the higher rebinding probability for small $\psi$ is also visible.

binding site, and the $D$ state as a decoy state, at first sight this situation seems very similar or (almost) identical to the binding of 2 particles with a decoy state, as discussed in section 6.3. To investigate the similarity/difference between these cases, we compute the $4 \times 4$ association rate matrix $K$ via SRTIS, for several values of the attractive isotropic interaction, which are shown in Fig. 6.18. In Fig. 6.13 we plot the overall TPT association rate constant as a function of $\epsilon_{iso}$. Strikingly, the rate constant increases first, then decreases with non-specific interaction. We also report the curves for different box volume where the rate constants are scaled according to Eq. 6.7. Interestingly, for all cases the rate constant increases first, then decreases with non-specific interaction. Interestingly, for all cases the rate constant increases first, then decreases with non-specific interaction. A shift from $4k_BT$ to $8k_BT$ is seen for the maximum in association rate constant for decreasing concentration. Note that the behavior of the dissociation rate constant is as expected (see Fig. 6.14), i.e. it follows roughly an Arrhenius law. One would think that the case of the constrained tetrahedron would be almost identical to the non-specific decoy case with only a slightly different geometry. However, the behavior is rather different due to the cooperativity of the non-specific interaction in the trimer. To investigate whether this behavior is robust with respect to protein concentration (or system volume), we compare in Fig. 6.16 the volume dependency of the protein dimer formation with the constrained tetrahedron, in where the concentration is decreased hundredfold using the data in Fig. 6.9. Note that these curves again do not show a maximum, although as the concentration is decreased all curves tend to overlap, indicating that the retardation effect due to the decoy state is decreased.

This unexpected difference between the tetramer and the dimer systems can be reconciled by realizing that for the tetramer the decoy (malformed) state potential is not fixed, but changes with $\epsilon_{iso}$, ignoring the configurations in which the attaching protein is bound to two particles as $U_N = 3\epsilon_{iso}$. This corresponds for the dimer to $U_D = \epsilon_{iso} + \epsilon_D$ so that the two systems behave similarly for approximately $\epsilon_D = 2\epsilon_{iso}$. This relation specifies a diagonal cut through the parameter space of Fig. 6.9. We plotted this diagonal in the 2D heat map representation of Fig. 6.9 in Fig. 6.15. Indeed, there is a maximum as function of $\epsilon_{iso}$, thus explaining the behavior of the tetramer formation.
6.4 Conclusion

We studied the effect of an additional binding site and non-specific interaction on the kinetics of simple patchy particle dimer formation and constrained tetrahedron formation. Using SRTIS to obtain the complete rate matrix, we showed that the presence a decoy state retards the association, and the overall assembly changes with position of the decoy patch. By adding a nonspecific interaction to the particles the overall association rate constant can increase by an order of magnitude due to rebinding from the decoy state, while the dissociation rate constant is showing Arrhenius behavior. If the decoy patch has a strong interaction, the overall association rate constant does not increase despite a strong nonspecific isotropic interaction. Moreover, the rebinding probability also changes clearly with decoy site position.

Furthermore, we fine-grained the model, i.e. studied a constrained tetrahedron, which, at first sight, does not differ significantly from the simple patchy-particle model. The small differences in this model do not change the thermodynamics. However, they qualitatively alter the kinetics of association.

These findings indicate that the rebinding effect due to the isotropic potential enhances association for dimer systems, but can suppress it for larger complexes. More generally, we conclude that additional anisotropic potentials suppress overall association kinetics, while isotropic potential enhances it. This can be understood in energy landscape terms. A smooth energy landscape as represented by an isotropic potential will be easy to navigate for the dimer. A rougher energy landscape, such as induced by (deep) potential minima causes kinetic trapping, hampering the search for global minima. Surprisingly, the enhancement that is gained by the isotropic potential is completely vanished for kinetic traps of more than $8 \ k_B T$. Our prediction is thus, that natural protein association can accommodate binding traps up to that strength, but not much higher.

One caveat here is that the threshold of $8 \ k_B T$ does depend on the potential shape. Here, we used a narrow binding site angle of the 20 degrees. The value of the threshold will probably depend on the potential shape of the potential, as the total binding strength is determined by the depth of the potential as well as its binding volume. A more narrow anisotropic potential will move the threshold up, a broader one will move it down.

Note that non-specific isotropic interactions of more than a few $k_B T$ will lead to non-specific aggregation of large amounts of particles. The short ranged nature of the 24-12 LJ potential leads to a metastable vapor-liquid coexistence line with respect to the fluid-solid line [137]. In fact, at the used concentrations a system with a strong isotropic 24-12 LJ potential, the system will likely crystallize. For high $\epsilon_{\text{iso}}$ crystallization can only be avoided for very low concentration. Our results are robust against lowering the concentration, as is shown in Fig. 6.16. Furthermore, by using a potential with an even shorter range, e.g. a 100-50 LJ potential, the fluid-solid line will decrease significantly, whereas the effect of the non-specific isotropic interaction on the association kinetics will not change qualitatively.

These results are important to understand protein association, and soft matter
Two opposing effects of isotropic and anisotropic attraction

Self assembly. For instance, our result suggest that if optimal associating kinetics is important, e.g. for signaling or cellular response, evolution should tend to smooth the energy landscape for binding. If such optimal binding kinetics is selected for, one would even expect for a smooth energy landscape with a gradient toward the binding site. These insights can also be used as a design principle for enhancing soft matter self assembly by dressing patchy particles with a smooth non-specific isotropic attraction, and ensuring that nonproductive patchy interactions are not too strongly binding.

Finally we would like to note that our methodology allows to evaluate rate matrices up to moderate complexity (up to tens of stable states) for arbitrary potentials for proteins dimers. This is important for the multi-scale modeling of biochemical networks. Indeed our results directly give the intrinsic rate constants for association and dissociation that are required for a MD-GFRD multi-scale simulation [152], which is able to evolve the long time dynamics of complex biochemical networks.

Appendix

Normalized association rate constant: decoy and isotropic attraction

Figure 6.17: Normalized overall association rate constant, \( k_{\text{UT norm}} = \frac{k_{\text{TPT UT}}(\epsilon_D, \epsilon_{\text{iso}})}{k_{\text{TPT UT}}(\epsilon_D = 0, \epsilon_{\text{iso}})} \) for the dimer system as function of \( \epsilon_D \) for different isotropic interactions with \( \epsilon_{\text{iso}}/k_B T = 0 \) (purple), 2 (green), 4 (light blue), 6 (orange) and 8 (yellow), 10 (blue).

In Fig. 6.17 we plot the same data as in Fig. 6.9 but now the overall association rate constant is normalized according to: \( k_{\text{UT norm}} = \frac{k_{\text{TPT UT}}(\epsilon_D, \epsilon_{\text{iso}})}{k_{\text{TPT UT}}(\epsilon_D = 0, \epsilon_{\text{iso}})} \). It demonstrates that at a higher isotropic attraction, the decoy interaction interestingly has relatively a stronger retarding influence on the association rate constant. This could serve as an explanation when upon naively increasing the isotropic interaction (e.g. increasing the concentration of polymers for stronger depletion forces) does not result in a (significantly) higher association rate constant.
Rate matrices constrained tetramer

A graphical representation of the rate matrices used in the analysis of the con-
strained tetramer formation.

Figure 6.18: Rate matrix, $K$ for constrained tetrahedron formation as function of
$\epsilon_{iso}$. Note that for rate constants from and to $D$ are only defined for $\epsilon_{iso} > 0$. 