Self-assembly via anisotropic interactions

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

Newton, A.C.

Publication date
2017

Document Version
Final published version

License
Other

Citation for published version (APA):
Self-assembly via anisotropic interactions

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

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus prof. dr. ir. K.I.J. Maex
ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
op donderdag 26 januari 2017 te 14:00 uur

door

Arthur Cecil Newton

geboren te Bonaire, Nederlandse Antillen
The research described in this thesis was carried out at the Van ’t Hoff Institute for Molecular Sciences, University of Amsterdam, The Netherlands.

ISBN: 978-94-028-0485-0

The work described in this thesis was financially supported by the Foundation for Fundamental Research on Matter (FOM), which is part of The Netherlands Organisation for Scientific Research (NWO).
1 Introduction

1.1 Self-assembly

Self-assembly is a key concept in soft condensed matter science. It is the non-dissipative spontaneous formation of structural order due to reversible interactions between individual components [1, 2]. A multitude of examples can be found in nature and include micelle or vesicle formation, folding of proteins, signaling networks via protein-protein binding, virus assembly, fibrilization of amyloidogenic peptides and compaction of DNA into chromosomes, see Fig. 1.1 for cartoon impressions of some of these processes. These examples show that the size and nature of the components and the interactions are diverse. Amphiphilic molecules assemble into micelles and vesicles, whereas entire proteins called capsomeris form the virus capsid protecting the genetic material, and the list could go further with length-scales reaching for the stars.

Figure 1.1: Examples of self-assembly in cartoon form where particles interact via the attractive purple patches. Left is an example of particle polymerization which could be used to depict fibrilization of amyloidogenic peptides [3]. Middle is part of a Kagome lattice built out of tri-block patchy particles of which the successful self-assembly has been reported [4]. Right is an image of particles clustering in micellar or tubular structures which Kraft et al. and Granick have shown to be realizable with patchy colloids [4, 5].

Components typically do not change character before and after integration into the self-assembled structure. However, this is not always true as building blocks can also have changing internal structure. For example, amyloidogenic proteins
folded into their native 3D structures do not form fibrils. It is the (partially) unfolded state of these proteins with the hydrophobic patches exposed that fibrilize into long chains. However, what is distinctive of self-assembly is that the individual components are not irreversible glued to each other such as a carbon atom in graphite. A bond is considered reversible if the energy of the bond is on the order of the thermal energy, $k_B T$, where $k_B$ is the Boltzmann constant and $T$ the temperature, as a single bond then still has considerable probability to break simply by thermal agitation, \textit{i.e.} the ceaseless random motion of molecules that is associated with heat. In the self-assembly examples mentioned above weak reversible interactions are crucial for shape and function. Proteins are allowed to diffuse through the lipids of cell membranes. Wrongly folded proteins or mistakes made in virus assembly can be fixed. Compacted DNA can be unwound when its necessary to read the genetic code.

We can find many more examples in a wide variety of scientific fields where systems 'spontaneously' organize into ordered patterns: in Belousov-Zhabotinsky reactions oscillating patterns emerge, in Rayleigh-Bénard convection regular structures of convections cells develop, in bacterial colonies cells grow in fractal shapes and in the desert sand dunes form undulations. However, in these examples continuous input of energy or reactants is necessary and these systems when forming these structures and patterns are therefore never in equilibrium. If the energy input to these systems is cut off, the formed structure would decay over time. We reserve the term self-organization to these type of non-equilibrium processes, and self-assembly specifically when no energy is dissipated and the system remains essentially in equilibrium. Of course, self-assembling systems can still be out of equilibrium. For example, it is the relaxation from a dilute solution stable at a high temperature towards the self-assembled ground-state structure when the temperature is lowered which is the process of self-assembly.

Although historically the term self-assembly was used in the study of virus capsids, when it was recognized that viruses self-assemble into mono-disperse finite-sized structures out of only a small set of subunits \cite{7–9}, today the principles of self-assembly hold great potential to be used for making building blocks for functional materials in nanotechnology, and thus the term has permeated into not only biology, physics and chemistry, but also materials science.

\section*{1.2 Colloidal patchy particles}

This thesis discusses the self-assembly of colloidal particles. The term colloidal spans the group of particles with dimensions between 1 nm and 1 \(\mu\)m. Therefore, we can in principle view globular proteins and micrometer cells also as colloidal particles, or shorter, as colloids. Colloids have various applications and can be found in everyday materials such as in milk or mayonnaise as microscopic fat particles or in paint as tiny bits of pigment.

Another way to specify a particle as colloidal, is when in solution it is randomly buffeted around and its trajectory can be appropriately described by Brownian
motion, the erratic motion of particles due to constant collisions with solvent molecules. Due to Brownian motion, colloids are in principle able to explore configurations of the system autonomously. This is in stark contrast, of course, with larger particles such as sand grains which sediment and do not show Brownian motion. For these particles to form structural order input of energy is required. Additionally, colloids are large enough to be observed under a microscope. As such, well-defined mono-disperse colloidal systems have played a big role in the advancement of physics of soft condensed matter and nowadays serve as model systems to study phase transitions, e.g. nucleation, freezing, etc.

Colloids can also play the role of "atoms" and "molecules" in hierarchical structures for tomorrow's materials. Much is known about how atoms form molecules, namely through retrosynthetic analysis where almost every large complex molecule can be constructed out of smaller cheaper molecules [10, 11]. Additionally even to some extent how molecules assemble into supramolecular structures, such as crown ethers, cryptands, catenanes and rotaxanes [12]. However, design principles for the assembly of objects on the (sub)micrometer scale with targeted functional properties remain largely unclear, due to the hierarchical assembly of a large number of constituents it requires.

However, even though the blueprint of self-assembly for microscopic particles is not known, buildings blocks are numerous. Aside to naturally occurring colloids, recent advances in colloidal synthesis make it possible to create almost mono-disperse (sub)micrometer particles in various shapes and sizes: spheres, cubes, rods, plates, ellipsoids, tetrahedron, stars or snowman [13, 14]. Moreover, it has also become possible to selectively change part of the surface of colloids, creating anisotropy not through changing the shape, but by changing the physiochemical properties along the surface of particles [4, 15, 16]. Over the years, 'patchy particles' have become the umbrella term for these type of particles. Returning to the natural colloids, such as proteins, virus capsids or even cells, we see that they are also not isotropic. Capsomeres are globular proteins with attractive hydrophobic patches exposed on the surface. Amphiphilic molecules are anisotropic in shape due to a hydrophilic head and a hydrophobic tail. As such, anisotropic interactions is what drives self-assembly into finite size or open structures. The reason for this is twofold.

Firstly, adding anisotropy to microscopic particles helps in the design of building blocks for complex structures. Endowing particles anisotropy can result in discrete directed bonds, i.e. valency, between particles where the connection to molecular bond formation through electronic orbitals is quickly established. As seen in Fig. 1.1 colloids with two opposing binding sites or valences should form one-dimensional colloidal polymers, three valencies in one plane and each pair separated by 120 degrees will form a two-dimensional beehive and a tetrahedral valency quickly reminds us of carbon and in principle can assemble in the three dimensional colloidal diamond structure which is a holy grail for photonic crystals with a band-gap in the visible region. However, it turns out that the virtuality of the drawing board is far removed from the reality of the lab. Although certain successes have been reported [4, 5, 16–19], it remains problematic to actually de-
sign and observe targeted supracolloidal structures and discoveries are still mostly fortuitous. One such example is the Kagome crystal whose experimental successful formation was reported in 2011 [4] and a theoretical explanation of the stability of the crystal was given in 2013 [20]. Predictions from computer simulations and theory are, therefore, beneficial in the design of supracolloidal structures.

Secondly, anisotropic interactions are imperative for particles to cluster into finite size structures pre-empting bulk phase separation. Although maybe trite, it is important to realize that isotropic particles can not form finite size clusters before a liquid or solid phase kicks in. This stems from the fact that for finite size clusters to be stable, a strong interaction is necessary between colloids. For example, to induce dimer formation for colloids that interact with a range on the order of a tenth of the diameter, the bond strength should be on the order of ten times the thermal energy. However, at these bond energies, particles that solely bind via isotropic interactions have already long before condensed into a bulk phase. Therefore, it is critical for self-assembly that bulk phase separation is suppressed by lowering the temperature of condensation by means of lowering the bonding volume possible for interaction, i.e. creating valencies. Of course, when particles interact both anisotropically and isotropically, it is possible that finite sized structures form out of a condensed phase [3, 21].

Successful self-assembly of microscopic particles often relies on simple interactions such as hard sphere interactions, Van der Waals forces, depletion interactions, electrostatic, magnetic, sticky DNA, hydrophobic forces or critical Casimir forces [4, 5, 16, 18, 19, 22–26]. Kraft et al. have shown that by creating snowman shaped particles with one smooth and one rough lobe with differing depletion force strengths causes finite size micelle formation [5]. Moreover, by adding a second rough lob tubular structures are formed [6]. Janus particles, where half of the surface is repulsive or inert and the other half is attractive due to hydrophobic forces, dispersed in water arrange into micellar and tubular structures [17]. By adding another valency, a tri-block particle has shown to arrange into the Kagome lattice [4]. Additionally, Evers et al. have used the combination of anisotropically shaped micrometer particles, polymer repulsion and hydrophobic attractive forces to make virus like shells [19]. Finally, developments in partial surface functionalization with DNA, have opened up routes for very precise control due to the precise specificity of DNA base-pairs [16, 25].

1.3 Critical Casimir forces and patchy particles

Recently it has been shown that particles suspended in a critical binary liquid also experience a force [27–31]. This effective force was first recognized by Fisher and de Gennes in 1978 and is nowadays coined the critical Casimir force, reminiscent of the quantum Casimir force which occurs between parallel conducting plates in vacuum which confine zero-point fluctuations of the electromagnetic field when they are brought close to each other [32]. According to Fisher and de Gennes a thermodynamic analogue exists where an effective force between two surfaces
arises due to the confinement of solvent fluctuations (in density or in composition) between the two boundaries. When the correlation length (the effective size of the fluctuations) becomes of the order of the separation between these boundaries, the critical Casimir force becomes significant. The critical Casimir force lends its name due to the fact that this will be the case near the critical point of a binary liquid, at which the correlation length of the fluctuations diverges. Only recently was this force experimentally realized with colloids due to the advent of single particle manipulations with optical tweezers and advanced microscopy tools. Direct measurement using a single colloidal sphere and a planar surface immersed in a binary liquid mixture of water and 2,6-lutidine, observed that while approaching the critical temperature, the interaction increased both in range and in depth [28]. Additionally, Hertlein et al. showed that if the two opposing surfaces, from the sphere and the wall, preferentially adsorb different components of the solvent, a repulsive effective force arises. Interestingly, it has been demonstrated that the critical Casimir forces can also induce bulk phase transitions of colloids, even when the solvent mixture is prepared off critical [33–37]. By using refractive and density matched particles, 3D reconstructed confocal microscopy images show that isotropic sub-micrometer particles condense reversibly from a gas state first into a liquid state and subsequently into a FCC crystal upon approaching the critical point. Combined with a computational study it is demonstrated that this force can be modeled with a combination of an electrostatic repulsion and an attraction which is temperature dependent due to the fact that the range and the strength are dependent on the correlation length [34].

As mentioned above, if both surfaces preferentially adsorb the same component of the binary liquid, an attraction arises. In contrast, the surfaces repel when they have opposing chemical preferences. Therefore, critical Casimir forces highly depend on the boundary conditions imposed by the surface of the submerged particles. It opens up the possibility to use Casimir forces combined with patchy particles where the physicochemical properties, and thus the boundary conditions, can change along the surface due to the patches of the particle. The critical Casimir force is universal and does not require further functionalization of the surface similar to sticky DNA colloids, but is dependent on the nature of the surface of the particles. Moreover, Casimir forces take maximum advantage of the solvent fluctuations, and as such are temperature dependent and reversible. Thus critical Casimir forces can also be employed to induce self-assembly of patchy particles by tuning the temperature, mimicking molecular bonding at the colloidal scale, see Fig. 1.2.

It is interesting to note that there is considerable theoretical debate on the origin of the critical Casimir forces and the applicability of effective potentials to describe such interactions [38–41]. On the one hand there is the original work of Fisher and de Gennes which is analogous to the quantum version of the Casimir force, where the fluctuations in composition cause the interactions between surfaces. Several theoretical studies have shown how the universal scaling function behaves under different conditions, and these have been measured experimentally [42–47]. On the other hand, an attraction also arises by preferential adsorption
and the free energy gain in mixing adsorption profiles of two surfaces, which are necessarily also dependent on the correlation length. Especially when the mixture is prepared off-critical, the main driving force of the Casimir interaction is elusive and very much open to debate. The similarity between these two explanations, both depending on the solvent correlation length, makes it very difficult to study experimentally which explanation is closer to the truth. In this thesis we adopt a heuristic approach, which entails that we are interested in the effective result of the Casimir force and the applicability to self-assembly of anisotropic colloids. As such we do not give an answer to this open question. However, this discussion on the origin of the critical Casimir force being explained by either wetting effects or confined critical fluctuations does not detract from the fact that solvent mediated forces are highly promising for assembly of patchy particles.
1.4 Molecular simulation and simple models

Although the position of colloids can be observed over time under a microscope, the link between structure formation and the interactions between particles is not trivially understood. Molecular simulation often gives additional, explanatory or predicting information to experimental studies as it has access to detail still inaccessible to modern experimental techniques [48].

In molecular simulation we need to input all the interactions between all particles and integrate the equations of motion or use Monte Carlo to sample configuration space. At the most fundamental level, all matter and interactions should be described using quantum mechanics. However, for systems with particles large in size and number it becomes close to impossible to solve the multi-body time-dependent Schrödinger equation. Therefore, we make use of effective potentials, obtained through coarse graining, where most of the degrees of freedom of the system are effectively integrated out. Effective potentials keep the most essential ingredients of the interactions underlying the phenomena under study. Although it seems a limitation of computer simulations, distilling out the essential physics from a problem and incorporating it into a model that explains the trends quantitatively (or even qualitatively), leads to more understanding than simply using all positions and momenta of each atom as input into a black box and solving the Schrödinger equation. In this respect, globular proteins and synthetic colloids are closely related. By coarse graining proteins by leaving out most of the atomic details, they start to resemble colloidal particles synthesized in the lab. Of course, viewing proteins as rigid colloidal frameworks only works well for globular proteins whose internal structure does not change by for example unfolding of the native protein structure. When considering virus assembly, capsomer proteins that make up the virus typically remain globular. In chapter 4 and 5 we use this property of capsomers and model the proteins as inert spheres with attractive patches on the surface.

![Figure 1.3: Picture of models used for simulation of colloidal suspensions. On the left isotropic particles interact through a square well potential where they repel when the hard cores (blue and orange) overlap and only attract if the purple regions overlap. In the case of the Kern-Frenkel potential for patchy particles in the middle, the bonding volume is reduced by an angular modulation which decreases the purple regions. A more realistic model can also be used where the patches continuously fade as the particles misalign gradually. A different way of incorporating directionality is by depositing one or multiple smaller particles on the bigger centre sphere. All constituents then interact through isotropic potentials.](image-url)
For colloidal systems, the solvent is typically left out of the calculation as the effective interaction between colloids is usually not dependent on the state of the solvent. Note however, that this assumption is still under debate for the critical Casimir force. A famous example where colloidal particles have successfully been modeled is the DLVO theory that describes the isotropic effective interaction in colloidal suspensions as a combination of an isotropic electrostatic repulsion and an isotropic van der Waals attraction [49]. Moreover, the depletion potential and the critical Casimir force has also been similarly modeled with a simple pair-potential [34, 35]. In theory and computer simulation, the simple square well potential is also extensively used, relying on the fact that thermodynamic properties of the system are usually not dependent on the microscopic details of the potential as dictated by the generalized law of correspondent states (GLCS) [50, 51]. Since these models are fundamentally isotropic in nature and the 'usual' potentials act on the centre of mass of the two particles, a modification is necessary to describe the interaction between anisotropic particles.

There are (at least) two different ways of making effective interactions directional. Firstly, an isotropic effective potential, $U_{iso}(r)$, such as the square well or Lennard-Jones potential, can be multiplied with a factor, $f(r, \Omega)$, which is dependent on the relative orientation of particles.

$$U(r, \Omega) = U_{iso}(r)f(r, \Omega) \quad (1.1)$$

Typically, $f(r, \Omega)$ is a switching function which depends on the surface overlap of two opposing patches. Systematic studies have been performed with the Korn-Frenkel model, which extends the square well potential to patchy particles by excluding discrete parts of the bonding volume where the degree of exclusion is given by a patch width [52–60], see Fig. 1.3. When patchy interactions depend continuously on the surface overlap of patchy particles, continuous switching functions are employed (e.g. Gaussian) [61–64]. The advantage of these type of potentials is that the range and patch width can be varied independently. A disadvantage is that a particle pair can only form one bond assuming that patches are not allowed to overlap. A second way of adding directionality is by using a more molecular viewpoint where a particle is built out of two or more constituents where the constituents can in principle vary in size. All constituents interact through an isotropic potential which can depend on what type of pair of constituents, $\alpha\beta$, is considered:

$$U(r, \Omega) = \sum_{\alpha,\beta} U_{\alpha\beta}(r, \Omega) \quad (1.2)$$

This is especially useful when the particle is anisotropic in shape [5, 6, 65]. However, spherical particles with patches on the surface can also be represented by one or more small particles deposited on the surface of a larger center particle [66, 67]. A disadvantage of this type of model is that the effective patch width is coupled to the range with which the small particle interacts as shown in Fig. 1.3 however no angles need to be calculated which simplifies the calculation significantly especially when using Brownian dynamics. Deciding which type of model gives the most natural potential highly depends on the patchy particle of interest.
1.5 Kinetics of self-assembly

Numerous theoretical and numerical work have studied the thermodynamic phase behavior of patchy particles, predicting not only interesting building blocks for novel functional materials, but also demonstrating new physical properties \[55, 66, 68, 69\]. These studies assume a more thermodynamic viewpoint of self-assembly, in which the system will always reach the global minimum of the free energy on going from a collection of disordered particles to the final structure. All potential energy interactions between particles, \(U\), determine the weight of configurations whereas the number of configurations at this energy determines the entropy, \(S\). The sum is the free energy, \(F = U - TS\). Despite the fact that the free energy is indispensible information to understand the principles of self-assembly, in experiments of synthetic colloids the global free energy minimum, \(i.e.\) the thermodynamic stable state, is not always found. Due to the fact that prior to complete assembly a system can reach meta-stable states from which escape can be rare, it becomes very difficult to reach the target state \[70, 74\]. From this we can appreciate the fact that

\[
\begin{align*}
  k_{GA} &< k_{GC} \\
  k_{AC} &< k_{CG} \\
  k_{AG} &< k_{GA} \\
  k_{GC} &< k_{GC}
\end{align*}
\]

Figure 1.4: A cartoon image depicting how particles can assemble properly into the ordered structure, \(C\), or into the random aggregate, \(A\), out of the disordered state, \(G\) with the rate constants, \(k_{\alpha \beta}\), that gives the timescale of each process. In order for \(C\) to assembly in an appropriate length of time, \(k_{GC}\) needs to be bigger than \(k_{GA}\) as \(k_{AC}\) and \(k_{AG}\) is usually very low for kinetically trapped states. Naturally, more intermediate states are possible in between each transition. In this thesis we study simple association and dissociation processes that should give insight into the complete assembly problem.

biological systems have found a way to circumvent this problem. When considering for example virus assembly, it is remarkable that a collection of protein subunits
Introduction

self assemble into impressive mono-disperse superstructures as seen in Hepatitis, Ebola or the Tobacco virus. To understand such assembly we should ask what are the barriers and rate constants between stable states? Which transient intermediates are important and lead to proper structure formation and which intermediates are dead ends? How do additional isotropic or anisotropic interactions between particles change the assembly pathway and the rate of formation? What effect do rotational dynamics have in exploring configuration space?

Clearly, knowing the kinetics of the system is just as important as it explains on which time-scale a system relaxes to the ground-state and determines how long we have to wait in the lab for thermodynamic equilibrium to set in. Moreover, the prediction of time-scales of associating proteins via specific binding sites is also important in understanding the temporal response of bio-chemical networks [75].

Fundamental to growth and nucleation of supracolloidal structures are association and dissociation processes and the rate constants between possible bound states, see Fig. 1.4.

Modeling association and dissociation of particles has a long tradition in physics and chemistry [67, 76–86]. We can start by considering a simple assembly process, the binding of one particle to a binding site. The binding of a diffusive particle $A$ to another particle $R$ in a volume $V$ is denoted by the reaction $A + R \rightleftharpoons AR$. The reaction rate equations are:

$$\frac{d[AR]}{dt} = +k_+[A][R] - k_- [AR], \quad \frac{d[A]}{dt} = -k_+[A][R] + k_- [AR]$$

(1.3)

where $[X]$ denotes the concentration of component $X$ and $k_+$ and $k_-$ are the association and dissociation rate constants, respectively. Alternatively we can describe this as a two state problem, denoting the bound state $AR$ as $B$ and the unbound state $A + R$ by $U$. The rate equation then becomes:

$$\frac{dp_B}{dt} = -k_{BU}p_B + k_{UB}p_U, \quad \frac{dp_U}{dt} = +k_{BU}p_B - k_{UB}p_U$$

(1.4)

where $p_B$ and $p_U$ denote the population (or probability) of finding the system in the bound state $B$ or unbound state $U$, respectively. The two equations are related by noting that $p_B/p_U = [AR]/[A]$, $[A] = [R]$ and $k_+[R] = k_{UB}$ and $k_- = k_{BU}$. It is now easy to see, that we can generalize Eq. 1.4 to any number of states and write down a master equation which is a generic gain-loss equation for the probabilities for each stable state $I$, $p_I$, where the rate constant between state $I$ and $J$ is given by $k_{IJ}$:

$$\frac{p_I(t)}{dt} = \sum J k_{JI}p_J(t) - k_{IJ}p_I(t)$$

(1.5)

by choosing an appropriate rate constant matrix, $K$, we can rewrite Eq. 1.5 as:

$$\dot{p}(t) = Kp(t)$$

(1.6)
where \( \mathbf{p}(t) \) is the vector of components \( p_I(t) \) and \( \dot{p}(t) \) is its time derivative. The formal solution of Eq. 1.6 with given initial \( \mathbf{p}(0) \) is as follows:

\[
\mathbf{p}(t) = e^{Kt} \mathbf{p}(0)
\]  

(1.7)

Although this expression is convenient, without knowledge of \( K \), it does not help to find \( \mathbf{p}(t) \) explicitly [87]. Obtaining all the rate matrix elements is thus the crux of the problem. Note that if \( K \) is known, also the stationary solution, \( \mathbf{p}^{eq}(t) \), in the long-time limit, \( t \to \infty \), can be found which leads to the detailed balance equation:

\[
k_{IJ} p^{eq}_I = k_{JI} p^{eq}_J
\]  

(1.8)

Although analytical techniques exist to calculate the (dis)association rate constant [78, 81], this is usually only possible for isotropic particles. When intermediates formed during the assembly process are non-spherical, analytical solutions become difficult to find. Instead of trying to solve the problem analytically, Brownian dynamics simulations have therefore been extensively used to study association kinetics of anisotropic particles, typically in the context of proteins or other biomolecules. Northrup et al. in Ref. [83] developed a method to calculate the association rate constant between two proteins based on Brownian dynamics in conjunction with simple models, where trajectories are initiated from random configurations where particles are separated by a distance \( \sigma \) and a trajectory is terminated when the particles collide in the correct orientation or are separated enough that recollision is negligible. By monitoring many of these trajectories, the probability that particles starting at a distance \( \sigma \) associate rather than diffuse away and escape into the bulk can be calculated which is related to the effective association rate constant.

Although anisotropy can be taken into account in these techniques [67], when multiple pathways via strongly bound intermediates become important, these methods based on the free diffusive behavior of particles become highly inefficient, especially when dissociation and rebinding are important processes to consider. For this purpose, recently developed path sampling techniques are used which are able to alleviate problems of long lived stable intermediates [88, 89].

### 1.6 Outline of the thesis

In this thesis different questions in self-assembly are studied considering not necessarily the thermodynamic behavior of patchy particles, but how by changing the underlying potential, multivalency or dynamics, structure formation is influenced. The first part of the thesis (chapter 3) deals with extraction of a pair-potential from experimental microscopy data on colloidal systems consisting of anisotropic particles. And in the second part (chapter 4, 5, 6) we study the association and dissociation of patchy particles with one or multiple binding sites and discuss how it affects the overall (un)binding rate constants and assembly mechanism. Moreover, we also study how changing the dynamics, to be specific the rotational
motion of particles, influences the self-assembly pathway of three-patch particles forming a tetrahedron.

Chapter 2 briefly reviews computational techniques, such as Monte Carlo and Brownian Dynamics, used to sample the configurational space of colloidal particles. Moreover, the calculation of radial distribution functions, virial coefficients, translational and rotational diffusion constants will be discussed. Furthermore, this thesis extensively makes use of path sampling methods developed to simulate so-called rare events necessary to study the kinetics of self-assembly. Several technical details on path sampling methods are explained. Chapter 3 reports on modeling of experiments on colloidal self-assembly induced via the critical Casimir force with a simple temperature dependent pair-interaction between particles. We present a framework for optimizing a potential based on a simple model via experimental microscopy images. The optimized potential from experimental data predicts correctly the onset of aggregation for these particles and gives a possible explanation for the collapse of network structures formed via a non-equilibrium experimental protocol very near the critical point by extrapolating the model to temperatures very close to the critical point. The remainder of the thesis is concerned with the dynamics and kinetics of (dis)association of patchy particles. In chapter 4 we investigate how changing the dynamics of particles, in contrast to their interaction can also influence the kinetics of self-assembly of multivalent patchy particles. We show that when rotational diffusion is lowered, experimentally possible by changing the environment of particles via molecular crowders or external fields, the tendency to form trapped structures is decreased as rotational motion induces the exploration of intermediate frustrated structures. We present a simple master equation model to further exemplify this observation. In chapter 5 we show that multivalency of patchy particles does not changes the thermodynamics of polyhedron structure formation, but affects the kinetics. Multivalency affects both the frequency of visiting trapped states and the residence time within frustrated states during association. Chapter 6 presents a study of the association kinetics of dimer formation of patchy particles where one particle is decorated with additional binding sites other than the target binding site. We discuss how these decoy binding sites affect the kinetics by rebinding and how adding an isotropic interaction further affects the rebinding probability. Moreover, we show how the isotropic interaction between particles forming a multicomponent structure can also lead to cooperativity in binding, qualitatively changing the association kinetics.

It is evident from the underlying motivations of these chapters that when discussing the (dis)association or self-assembly of anisotropic particles a multitude of diverse questions arises, from how to extract effective anisotropic potentials from experimental data to how rotational dynamics and multivalency influences kinetics. Since, as soon as the interactions between particles become anisotropic, the number of degrees of freedom to study increases, such as the number of patches per particle, the arrangement of these patches, the width or size of the patches, surface mobility of the patches, the nature of interaction per patch and possibly even the shape of the patches. Together with the possibility of changing the shape of the particle itself, the possibilities are close to infinite, all of which nowadays is
not just a theoretical abstraction anymore but in principle can be achieved experimentally. Of course, in this thesis only a small subset of questions are selected and even the system sizes are limited to a minimum in most chapters, however the topics discussed can transcend these limitations and the findings apply more generally. The method of extracting potentials described in chapter 3 also extends to other patchy particles that can be modeled by a rigid framework similar to molecules and does not necessarily depend on the use of critical Casimir forces. Chapter 4 demonstrates how not only the interaction between particles matter for successful assembly, but also which pathway over the free energy landscape is taken and how this can be influenced by rotational diffusion. Furthermore, the prediction of time-scales for particles with additional binding sites in chapter 6 can be used to study bio-chemical networks via multi-scale simulation techniques. Finally, the use of path sampling techniques and technical details discussed in chapters 4 to 6 are applicable to other patchy particle systems, also much larger in size. To summarize, this thesis reflects the different faces of self-assembly of patchy particles and provides generic insight in the relevant association processes, which can be used in future research.
2 Computational methods

2.1 Ensemble averaging

In statistical thermodynamics a system in the canonical ensemble consists of \( N \) particles in a volume \( V \) at a temperature \( T \), where a configuration of all particles is denoted as \( \mathbf{x} = \{ \mathbf{r}_1, \mathbf{r}_2, \ldots, \mathbf{r}_N \} \) with \( \mathbf{r}_i \in \mathbb{R}^3 \) the coordinates of the \( i \)th particle. If the particles interact with a pair potential, \( U(\mathbf{x}) \), the equilibrium probability of a configuration is given by the Boltzmann weight:

\[
P(\mathbf{x}) = \frac{\exp(-\beta U(\mathbf{x}))}{Z}
\]

where \( \beta = 1/k_B T \) and the partition function \( Z = \int d\mathbf{x} \exp(-\beta U(\mathbf{x})) \) normalizes the relative probability, where \( \int d\mathbf{x} \) is a proper integral over every configuration \( \mathbf{x} \). In computer simulation averages can be calculated based on the coordinates of particles. The ensemble average of an observable, \( \mathcal{O}(\mathbf{x}) \), is given by:

\[
\langle \mathcal{O} \rangle = \frac{\int d\mathbf{x} \mathcal{O}(\mathbf{x}) \exp(-\beta U(\mathbf{x}))}{\int d\mathbf{x} \exp(-\beta U(\mathbf{x}))}
\]

The explicit evaluation of the integral is typically not feasible for systems with a large number of particle due to the huge number of configurations possible. The same problem underlies the calculation of the free energy, from which all thermodynamic properties can be determined:

\[
F = -k_B T \log Z
\]

A simple Monte Carlo integration which calculates Eq. 2.2 by generating random configurations of particles, calculating the corresponding Boltzmann weight and adding to the running average is extremely inefficient in getting correct results, as it is extraordinarily more likely to generate configurations with a very low weight than not.

Instead of generating configurations with uniform probability, configurations could be generated proportional to their Boltzmann weight. As such, configurations with a high Boltzmann weight would simply occur more frequently during
Computational methods

sampling than configurations with a low weight. In doing so, the statistical weight
of each configuration is then taken into account in the generation probability and
therefore ensemble averages can be calculated as unweighted averages:

$$\langle O \rangle \approx \frac{1}{m} \sum_{k=1}^{m} O(x_k)$$

(2.4)

where on the right hand side the estimate of the integral as we can compute it in
computer simulations is shown when generating $m$ configurations and computing
the value of $O$ for configuration $x_k$, which is now generated with a probability
proportional to its Boltzmann weight.

Unfortunately, we can not use the same method to calculate $F$ as it explicitly
depends on the phase space volume. More advanced techniques need to be
employed, such as thermodynamic integration, umbrella sampling, etc. [48].

Below we discuss two techniques which ensure the generation of new configu-
rations with the proper relative Boltzmann weight. One is doing a Monte Carlo
simulation according to the Metropolis algorithm and the other is using Molecular
dynamics.

2.2 Monte Carlo

In the Metropolis Monte Carlo (MC) technique new configurations are not gen-
erated randomly, but are generated according to the relative Boltzmann weight
between old and new configurations. In practice this means that starting from a
given configuration of particles, $x_o$, a trial move is performed which changes the
system to a new configuration, $x_n$. This trial move is rejected or accepted on
the basis that it should preserve the equilibrium distribution once it is reached.
Usually, an even stronger condition, detailed balance, is imposed:

$$p(x_o)T(x_o \rightarrow x_n) = p(x_n)T(x_n \rightarrow x_o)$$

(2.5)

This process is iterated $m$ times, where after $m$ iterations a sufficient number of
configurations is collected. In doing so, we ensure that the most relevant configu-
rations are sampled and from this ensemble of configurations the correct equilibrium
properties are calculated according to Eq. 2.4.

Monte Carlo trial moves

Several Monte Carlo moves are used in the following chapters to sample config-
uration space [48]. As the particles are anisotropic in shape and/or potential,
translational and rotational moves are used. In a translation move we translate
a randomly chosen particle, $i$, by a random shift in the range of $[-\delta r_{max}; \delta r_{max}]$.
The probability of accepting the move is:

$$P_{acc}(x_o \rightarrow x_n) = \min [1, \exp (-\beta \Delta E)]$$

(2.6)
where $\Delta E = E(n) - E(o)$ is the energy difference between the new and old configuration.

The rotation move is a bit more involved as we need a way to change the orientation of particles randomly. Rotations of objects could be done via the three Euler angles. However, when converting the Euler angles to rotation matrices, they would also require the use of trigonometric functions which are expensive and not numerically stable. Therefore, we make use of quaternions to represent the orientation. Quaternions are an extension to complex numbers first described by William Rowan Hamilton in 1843 and take the form:

$$q = q_0 + q_1 i + q_2 j + q_3 k$$ (2.7)

where the complex numbers, $i$, $j$ and $k$, follow the relation:

$$i^2 = j^2 = k^2 = ijk = -1$$ (2.8)

Importantly, if we only use unit length quaternions we can represent quaternions as rotations. In three dimensions, any rotation of an object can be seen as a single rotation around an unit axis $\hat{u}$ over an angle $\theta$. Quaternions can encode this exact rotation as follows:

$$q = \begin{pmatrix} q_0 \\ q_1 \\ q_2 \\ q_3 \end{pmatrix} = \begin{pmatrix} \cos\left(\frac{\theta}{2}\right) \\ \hat{u}_x \sin\left(\frac{\theta}{2}\right) \\ \hat{u}_y \sin\left(\frac{\theta}{2}\right) \\ \hat{u}_z \sin\left(\frac{\theta}{2}\right) \end{pmatrix}$$ (2.9)

In this case, we also make use of trigonometric functions. It is, however, less than using Euler angles. To actually rotate the rigid object we could convert the quaternion into a rotation matrix without the use of trigonometric equations as follows:

$$A = \begin{pmatrix} q_0^2 + q_1^2 - q_2^2 - q_3^2 & 2q_1q_2 - 2q_0q_3 & 2q_1q_3 + 2q_0q_2 \\ 2q_1q_2 + 2q_0q_3 & q_0^2 - q_1^2 + q_2^2 - q_3^2 & 2q_2q_3 - 2q_0q_1 \\ 2q_1q_3 - 2q_0q_2 & 2q_2q_3 + 2q_0q_1 & q_0^2 - q_1^2 - q_2^2 + q_3^2 \end{pmatrix}$$ (2.10)

and rotate a vector $\mathbf{p}$ to $\mathbf{p}'$ using $\mathbf{p}' = A\mathbf{p}$. Alternatively, we can directly use the quaternion and rotate the vector $\mathbf{p}$ by $\mathbf{p}' = \mathbf{q}\mathbf{p}\mathbf{q}^{-1}$, where $\mathbf{q}^{-1}$ is the complex conjugate of $\mathbf{q}$. Note that the vector $\mathbf{p}$ first needs to be converted to a quaternion: $\mathbf{p} = (0, \hat{p}_x, \hat{p}_y, \hat{p}_z)$. A random unit quaternion which uniformly samples a sphere can be generated by randomly choosing an angle $\theta$ in range of $[0; \delta \theta_{max}]$ and a random unit vector $\hat{u}$. A rotational move is then performed by again choosing a random particle, choosing a random orientation and accepting the move according to Eq. [2.6]

To ensure that configurations decorrelate and the samples become independent, $\delta r$ and $\delta \theta$ should be chosen carefully. However, as systems studied here have a low density, two versions of the rotational and translational moves are used, one with no limit to $\delta r$ or $\delta \theta$ and one where the acceptance ratio is somewhere between 0.3 and 0.7.
These two moves only change the old configuration to the new one, by changing only a single particle. If strongly binding particles are considered clusters of different sizes are formed quickly. However, the subsequent relaxation of these clusters is limited by only using single particle moves. In order to get further relaxation, we also employ moves where not only entire clusters but also parts of clusters are translated and rotated. In this thesis the cluster move was used as explained in Ref. [90].

2.3 Molecular dynamics for colloidal particles

Molecular Dynamics is a simulation technique where uncle Isaac Newton’s equations of motion are solved:

$$\vec{F} = m\ddot{x} \quad (2.11)$$

where $\vec{F} = -\partial U/\partial r$. Typically, this would entail the calculation of forces between each and every particle in the system. However, we make use of effective potentials where we have integrated out the degrees of freedom of the solvent particles. To describe Brownian motion which colloidal particles exhibit due to the now ‘disappeared’ solvent molecules, the Langevin equation can be used:

$$\vec{F} - \gamma \dot{x} + \sqrt{2\gamma k_B T} \zeta = m\ddot{x} \quad (2.12)$$

where the friction term, $\gamma$, is related to the diffusion constant via the Einstein relation, $D = \gamma k_B T$. The random collisions with the solvent are captured by the random force, $\zeta$, which satisfies the following relations:

$$\langle \zeta(t) \rangle = 0$$
$$\langle \zeta(t)\zeta(t') \rangle = \delta(t - t') \quad (2.13)$$

which is referred to as white noise. Here we further assume that the inertia of particles is negligible relative to the friction and random forces, i.e. the overdamped limit, $m\ddot{x} \approx 0$. This leads to the following equation for over-damped Langevin dynamics or Brownian Dynamics (BD):

$$\beta D\vec{F} + \sqrt{2D} \zeta = \dot{x} \quad (2.14)$$

Eq. 2.14 can easily be integrated for isotropic particles, however, for patchy particles both the position and orientation need to be evolved over time. An elementary algorithm developed for rigid patchy particles that updates the orientation of particles by the use of quaternions is described in Ref. [91]. If BD is used, some potentials, especially when anisotropic are not possible or difficult to convert to forces. A list of expressions for the calculation of forces and torques is given in Ref. [92]. However, in this thesis Dynamic Monte Carlo is used to evolve particles in time.
Dynamic Monte Carlo

Dynamic Monte Carlo (DMC) simulations can be used to study dynamics of simple systems, because in the limit of very small displacements and when no unphysical moves are used, it is equivalent to Brownian dynamics (BD) [74, 93, 94]. DMC is, however, easier to implement in the case of patchy particles, especially in the case of step potentials, lattice models, ‘reactive’ patches or other exotic potentials from which the derivative of the energy is hard to calculate. A disadvantage of DMC is that collective motion is suppressed if anything but infinitesimal steps are being used. However, if collective motion is not important then this is no problem. Additionally, recent DMC methods also incorporate cluster moves in a dynamically correct way, which makes hierarchical assembly dynamics also possible, in exchange for a more complex algorithm [74, 93].

In molecular simulation we make use of reduced units, also for time. In BD a time-step is related to physical time via the diffusion constant at infinite dilution, $D^0_T$:

$$\Delta t = \frac{\Delta t_{BD} \sigma^2}{D^0_T}$$

where $\sigma$ is the diameter of the particle. Therefore, to convert time-steps from a Brownian Dynamics simulation to physical time, the diffusion constant of the particles need to be known. In DMC there is no integration cycle with a well defined time-step, but MC cycles with a given set of displacements. In what follows, a MC cycle will consist of on average $N$ translations and $N$ rotations, $N$ being the number of particles. We need to connect the displacements in a MC cycle to translational and rotational diffusion constants, and thus Brownian time. To achieve this, we should obtain the ‘translational time’, $\Delta t_{trans}$, and ‘rotational time’, $\Delta t_{rot}$, per MC cycle.

A translation move is performed by randomly choosing a shift in position in a range of $[-\delta r, \delta r]$ for each Cartesian axis. We start the derivation by using the relation between mean square displacement and the unit of time at infinite dilution, $D^0_T$:

$$\langle \Delta r^2 \rangle = 6D^0_T$$

where $a_{trans}(\delta r)$ is the acceptance ratio for translation. Combining equation 2.16 and 2.17 we get the translational time corresponding to a MC cycle:

$$\Delta t_{trans} \approx \frac{\delta r^2 a_{trans}}{6D^0_T}$$

A rotational move is performed by choosing a random unit vector and a random
angle between \([0, \delta \theta]\). Just like in Eq. \ref{eq:2.16}, the requirement is:

\[
\frac{\langle \Delta \theta^2 \rangle}{\Delta t} = 6 D_R^0
\]  

(2.19)

where \(\theta\) is measured in radians. The mean squared rotational displacement (mrd) per cycle:

\[
\frac{\langle \Delta \theta^2 \rangle}{\text{cycle}} = \frac{1}{\delta \theta} \int_0^{\delta \theta} a_{rot}(\theta) \theta^2 d\theta \approx \frac{\bar{a}_{rot} \delta \theta^2}{3}
\]  

(2.20)

The rotational time per cycle is then given by:

\[
\Delta t_{rot}^{\text{cycle}} = \frac{\delta \theta^2 \bar{a}_{rot}}{18 D_R^0}
\]  

(2.21)

The argument of Romano \cite{Romano1994} to bootstrap the algorithm is by saying that in order for both ‘translational and rotational time’ to flow evenly, \(\Delta t_{trans}\) and \(\Delta t_{rot}\) per MC cycle have to be equal on average (plus having a high acceptance probability):

\[
\frac{\Delta t_{trans}^{\text{cycle}}}{\Delta t_{trans}^{\text{cycle}}} = \frac{\Delta t_{rot}^{\text{cycle}}}{\Delta t_{rot}^{\text{cycle}}}
\]  

(2.22)

Using Eq. \ref{eq:2.21} and \ref{eq:2.18} and rearranging, we find that the ratio between \(\delta r\) and \(\delta \theta\) should be:

\[
\frac{\delta r}{\delta \theta} = \sigma \sqrt{\frac{a_{rot}}{a_{trans}} \frac{f_{SE}^2 D_T}{3 D_R}}
\]  

(2.23)

In Ref. \cite{Romano1994}, it is shown that for DMC to correspond to BD, it is necessary that \(a_{rot} > 0.7\) and \(a_{trans} > 0.7\). For spherically shaped particles, the Stokes-Einstein relation between translational and rotational diffusion constants can be used, \(D_R^0 = 3 D_T^0 / \sigma^2\). However, a constant, \(f_{SE}\), is added to the ratio that can take non-Stokes-Einstein conditions into account.

During the simulation several diagnostics should be checked. For instance the ratio \(\langle \Delta \theta^2 \rangle / \langle \Delta r^2 \rangle\) should be equal to the state dependent ratio \(D_r / D_t\) in the diffusive limit. In Ref. \cite{Romano1994} it is shown that this ratio is the same for MD and DMC for several packing fractions.

If one wants to compare simulations to experiments, \(D_T^0\) and \(D_R^0\) have to be known a priori from experiments. In this way the reduced unit of time in simulations can be converted or the experimental values can be converted to Brownian units.

### 2.4 Structural and dynamical properties

Both MC and MD (DMC) can be used to obtain thermodynamic properties of the system by collecting a statistically relevant number of configurations for systems of anisotropic particles. Here, we discuss radial distribution functions which need special attention when considering anisotropic particles, and how to calculate the second virial coefficient for anisotropic particles which gives information about when particles tend to aggregate into clusters.
Radial distribution functions

The radial distribution function or pair correlation function, \( g(r) \), gives the probability of observing a particle at a distance \( r \) from a reference particle. Therefore, it characterizes the local structure around a particle. The \( g(r) \) can be measured experimentally via either neutron and X-ray scattering for molecular fluids or light-scattering for colloidal suspensions. Furthermore, \( g(r) \) is important for many theories of simple liquids. For isotropic particles and in the low density limit, \( g(r) \) is related to the pair potential \( U(r) \) via a simple Boltzmann inversion: 
\[
\beta U(r) = -\log(g(r)).
\]
Therefore, measuring \( g(r) \) in experiment can give direct information on the microscopic interactions between particles.

However, for anisotropic particles this relation naturally does not hold. For this purpose, we adopt the approach explained in Eq. 1.2. Here a particle is considered to be a rigid framework made up of different spheres, comparable to how atoms form a molecule. A radial distribution is then defined where the trivial neighbor contributions, the contributions from the same ‘molecule’, are ignored. This is called the site-site radial distribution and will be further explained in chapter 3. The site-site radial distribution is typically used for molecular liquids, however, in chapter 3 we use this correlation function to characterize the structure of anisotropically shaped colloids.

Second virial coefficient

Particles interacting with a relatively short ranged isotropic interaction obey a generalised law of correspondence states (GLCS) which states that thermodynamic properties of these type of systems are insensitive to details of the potential, but are only dependent on the density and the second virial coefficient \( B_2 \). A condition for this phenomenon to arise is that each bond contributes independently and equally to the partition function which is usually true for a short ranged interaction, \[51\]. Conveniently, it is shown that GLCS is also true for particles with anisotropic interactions \[51\]. In general, when \( B_2 < -1.5 \) the system will aggregate \[95\]. Sometimes \( B_2 \) can be calculated analytically such as in Ref. \[52\]. However, it can also be calculated numerically. The general definition for the virial coefficient in three dimensions without an assumption about the potential is:

\[
B_2 = \frac{1}{2} \int_0^{2\pi} \int_0^\pi \int_0^{r_c} d\phi d\theta dRR^2 \sin(\theta) \left[ 1 - e^{\beta U_{eff}(R)} \right]
\]

(2.24)

However, for anisotropic particles we also need to integrate over every possible orientation:

\[
B_2 = \frac{1}{2} \int_0^{2\pi} \int_0^\pi \int_0^{r_c} d\phi d\theta dRR^2 \sin(\theta) \left[ 1 - \int_0^{2\pi} \int_0^\pi \int_0^{2\pi} d\alpha_1 d\alpha_2 d\beta_1 d\beta_2 \sin(\alpha_1) \sin(\alpha_2) e^{\beta U(\Omega_1, \Omega_2, R)} (4\pi)^{-2} \right]
\]

(2.25)
where the Euler angles $\alpha_i$ and $\beta_i$ properly define every possible orientation of both particles. Note that symmetry arguments can reduce the number of integrations necessary, e.g. in the case of anisotropic but linear particles.

**Diffusion constants of patchy particles**

From MC we can only obtain static properties such as the quantities described above. However, from MD or DMC we can also calculate dynamic transport properties related to the diffusion of particles. When considering anisotropic particles, both translational and rotational diffusion constants are important quantities. Note that $D_T$ and $D_R$ are input parameters in Brownian dynamics, but we need to check whether the implemented dynamics is consistent.

**Translational diffusion constant**

To calculate the translational diffusion constant, the mean square displacement (msd) can be measured. The msd grows in time without bound as follows:

$$\langle \Delta r^2(\Delta t) \rangle = \left\langle [r(t + \Delta t) - r(t)]^2 \right\rangle$$  \hspace{1cm} (2.26)

where $r(t)$ is the position of the particle at time $t$. If we take the limit:

$$\lim_{\Delta t \to \infty} \langle \Delta r^2(\Delta t) \rangle = 2dD_T\Delta t$$  \hspace{1cm} (2.27)

where $d$ is the number of dimensions. It follows that the translational self-diffusion constant $D_T$ can be easily calculated by taking the slope of the msd in the long time limit. Note that for particles with anisotropic shape, each cartesian component in the reference frame can show different diffusion constants which can be taken into account by using a diffusion tensor with differing diagonal components.

$D_T$ can in principle also be calculated from the velocity autocorrelation function (vacf) using linear response theory via the Green-Kubo relation:

$$D_T = \int_0^\infty d\tau \left\langle v_x(\tau)v_x(0) \right\rangle$$  \hspace{1cm} (2.28)

However, as particles considered in this thesis have no inertia and thus no velocity because they are considered to be completely over-damped (Brownian), the vacf can not be calculated.

**Rotational diffusion constant**

The rotational diffusion constant, $D_R$, can be calculated by measuring the mean square angular displacement, msad, which quantifies the rotational motion of the unit vector $\hat{u}$ that defines the orientation of the particle. Rotation of a particle in a time difference $\Delta t$ can be envisioned as the rotation of $\hat{u}$ by an angle $\theta_{rot} = \arccos (\hat{u}(t) \cdot \hat{u}(t + \Delta t))$ around a rotation vector defined as the cross product $u_{rot} = \hat{u}(t) \times \hat{u}(t + \Delta t)$.  

26
If simply the angle is used to construct the msad, the msad will be bound as particles will eventually have rotated full circle towards the initial orientation \( \hat{u}(0) \), which would seem as it has returned to the initial orientation. This would limit the time over which one can measure the msad. To measure a msad which is unbound just like the msd described above, a more involved calculation is necessary. A rotational displacement is defined, \( \delta \phi(t) \), whose magnitude is given by \( \theta_{rot} \) and direction is given by \( u_{rot} \). The total angular displacement is given by:

\[
\phi(t) = \int_0^t \delta \phi(t') dt'
\]  

The unbound msad is then given by:

\[
\langle \Delta \phi^2(\Delta t) \rangle = \langle [\phi(t + \Delta t) - \phi(t)]^2 \rangle
\]

The long time limit is as follows:

\[
\lim_{\Delta t \to \infty} \langle \Delta \phi^2(\Delta t) \rangle = 4D_R \Delta t
\]

from which it follows similarly to \( D_T \), that \( D_R \) can be calculated by taking the slope of the msad even when measured over long times.

**Digression:**
Note that in Eq. 2.19 \( d = 3 \), whereas in Eq. 2.31 \( d = 2 \). It depends on how rotation is actually measured. Rotation can be measured by the diffusion of a rotating unit vector \( \hat{u} \) which is described by a two-dimensional diffusion on a spherical plane and it leads to a pre-factor of 4 as in Eq. 2.31. However, if rotation is measured by the angular displacement we need three dimensions as we have three independent Euler angles to consider, which leads to a pre-factor of 6 as in Eq. 2.19.

### 2.5 Simulating self-assembly rare events

Rare events are transitions between stable regions of phase space that are extremely infrequent due to the presence of large free energy barriers. Typical examples in chemical physics are the folding of proteins, homogeneous nucleation or chemical reactions. In self-assembly of patchy particles, transitions between stable states where particles are clustered can also be viewed as rare events. Due to the reduction of binding volume for patchy particles relative to isotropic particles, high binding energies are necessary for stable clusters, which causes unbinding events to be rare. Moreover, due to the small binding volume, the binding transition of patchy particles also encounter significant entropic barriers.

Although the ground-state is known to be an ordered structure, a system can still become kinetically trapped in states where particles are clustered into disordered aggregates. Therefore, knowing how the dynamics and the interactions of particles affect the association or dissociation rate constants and transition between intermediate states becomes important, besides when the thermodynamic phase behavior is known.
However, obtaining accurate (un)binding rate constants is often difficult in simulations due to the same high free energy barriers that naturally arise in strongly bound particles. When brute-force simulations are used, a majority of the simulation time is wasted inside the stable state which does not give information about the transitions. To alleviate this problem of separation of timescales between simulating the particle dynamics and the macroscopic binding rate constants, advanced path sampling techniques have been developed that bias the sampling of reactive pathways.

In this section we will discuss what path sampling method is used in this thesis and technical details concerning the calculation of rate constants and path densities.

From TPS to SRTIS

A method developed to generate reactive pathways without biasing the dynamics such as umbrella sampling or metadynamics, is Transition Path Sampling (TPS) \[88, 97–99\]. In TPS reactive pathways are generated through a Monte Carlo scheme which samples the reactive path ensemble between two or multiple states. Similarly to conventional Monte Carlo, where configurations are visited in proportion to the Boltzmann distribution, TPS samples reactive pathways according to their proper weight. The advantage of TPS and related methods is that the entire unbiased reactive path ensemble can be sampled, from which the mechanism of otherwise elusive reactive pathways can be analyzed in a statistically meaningful way. Moreover, a reaction coordinate is not needed, only a definition of states, i.e. the basins of attraction of a reactant and a product. In principle, also the reaction rate constant can be calculated via TPS.

After the development of TPS, many advances has been made in the field of path sampling methods. Transition Interface Sampling (TIS) was introduced as a more efficient approach to calculate the rate constant between states. It does so by defining interfaces around states through an order parameter that gives an indication on the progress of the reaction \[100\]. The rate constant calculation is based on the effective positive flux through these dividing surfaces. In TIS, path ensembles are simulated for each interface separately. These paths no longer only need to be reactive, but can also consist of \(A \rightarrow A\) paths. Moreover, in the RETIS approach the concept of replica exchange was introduced, by allowing neighbouring interfaces to swap pathways if both pathways obey the conditions imposed by the interfaces \[101, 103\]. This greatly increased the convergence of the path simulations as pathways can decorrelate quicker. A disadvantage of RETIS however, is that the number of interfaces can easily increase to an enormous amount when for instance multiple states need to be defined, limiting the practical implementation as all interfaces need to be sampled simultaneously. Recently, the Single Replica Transition Interface Sampling (SRTIS) method was developed by Du and Bolhuis \[104\] where, similar to simulated tempering, only a single replica walks through all the interfaces set by the multiple state TIS network, in contrast to RETIS where each interface is sampled individually and simultaneously. As such, the
large increase in the number of interfaces is not as much of a problem, as there is only one single replica in memory. In the following chapters, the SRTIS method is used to sample path space for different patchy particle systems.

**Paths, interfaces and indicator functions**

Before continuing on to explain what path moves are actually used and how the rate constant is calculated, it is convenient to introduce how a path is conceptualised in path sampling.

A path is thought of as a discretized sequence of configurations, \( x^L = [x_0, x_1, \ldots, x_L] \), where \( x_i \) are phase space points usually defined by coordinates \( r \) and momenta \( p \) of the \( N \)-particle system. However, in following chapters the momenta are left out due to the use of DMC. Each configuration \( x_k \) is separated by a time \( \Delta t \), such that the total time duration of the path \( T = L \Delta t \).

An unbiased trajectory \( x^L \) has probability:

\[
P[x^L] = \rho(x_0) \prod_{i=0}^{L-1} p(x_i \to x_{i+1})
\]  

where \( \rho(x_0) \) is the steady state distribution for the first configuration of the path, \( p(x_i \to x_{i+1}) \) is the Markov probability to move from \( x_i \) to \( x_{i+1} \). Akin to the Boltzmann probability for configurations, also the path probability is normalized by a partition function:

\[
Z = \int \mathcal{D}x^L P[x^L]
\]  

where \( \mathcal{D}x^L \) defines a suitable integral over all paths.

In the multiple state TIS framework we define a set \( S \) of \( M \) states. Each state \( I \) has its own interface set \( \Lambda_I \) of \( m + 1 \) interfaces, \( \Lambda_i^I \), defined through an order parameter \( \lambda_I(x) \), where the boundary of interface \( i \) of state \( I \) is set by \( \lambda_i^I \). The boundary of a state is denoted as \( \lambda^0_I \). No interfaces of the same set are allowed to intersect. However, interfaces that belong to different states of course are allowed to overlap.

In order to define the path probability for TIS path ensembles it is convenient to introduce indicator functions which define when a path belongs to the ensemble of \( \Lambda_i^I \):

\[
h_i^I[x^L] = \begin{cases} 1 & \text{if } x_0 \in I \land x_L \in S \land \forall\{j|0 < j < L\} : x_j \notin S \land \exists\{j|0 < j < L\} : \lambda(x_j) > \lambda_i^I \\ 0 & \text{otherwise} \end{cases}
\]  

where the third line indicates that there has to exists a slice \( x_j \) which crosses interface \( \Lambda_i^I \). We then define the path probability to observe a path \( x^L \) in replica \( i \) as:

\[
P_{\Lambda_i^I} = h_i^I[x^L] P[x^L]/Z
\]
In this thesis states are defined through the topology of the structure formed, number of bonds and energy of the system. Interfaces are defined based on the energy of the system which ensures that paths are biased both radially and orientationally away from stable states, which avoids hysteresis.

Path MC moves

The main path sampling move is the shooting move, where usually from a (randomly) chosen time slice of the current path, a new path is generated. Due to the stochastic nature of the dynamics the newly generated path will sample a different part of path space. Here a time slice is not chosen randomly, but we use constrained one-way shooting from the current interface, $\lambda^I_i$ where the new path is always accepted as long as it ends up in a stable state \[103]. The acceptance probability of the shooting move is:

$$P_{acc} [x^I_L(0) \to x^I_L(n)] = h^I_i[x^I_L]$$ (2.36)

Note that the usual TIS length criterion $\min \left[ 1, \frac{L_0}{L_n} \right]$ does not appear in Eq. 2.36. This term should be used when the generation probability of a shooting point is uniform along the path, because then the generation probability is dependent on the length of the current and new path.

In order to walk through replicas, a replica swap is used, where an attempt is made to change the sampling from the current interface to a neighbouring interface which is only possible when the path crosses both interfaces. Naturally, as stable states are strong attractors, this would lead to oversampling near the state and undersampling of any pathways that lead far away from state $I$. Therefore, we employ a Wang Landau bias on the replica swapping probability to enforce uniform sampling between replicas \[104, 105]:

$$P_{acc}(x^I_L; \lambda^I_i \to \lambda^I_j) = h^I_j[x^I_L] \min \left[ 1, \frac{g(\lambda^I_j)}{g(\lambda^I_i)} \right]$$ (2.37)

where $g(\lambda^I_i)$ is the density of paths, which is updated upon visiting $\Lambda^I_i$ via a scale-factor $f_{WL}$ which is set to an arbitrary value at the beginning of the simulation. When during the simulation all replicas have been sampled uniformly within a certain threshold, $f_{WL}$ is halved until it has converged to a sufficiently low number \[104, 105]. For a converged TIS simulation, $g(\lambda^I_i)$ should become proportional to the crossing probability, because to obtain uniform sampling over each interface, the acceptance in Eq. 2.37 should be biased with the ratio of the naturally occurring probability for pathways, which is the ratio of crossing probabilities between the interfaces to be swapped (see also section 2.5).

To allow exchange between paths starting from different states, a state-swap move is employed. The current initial state is changed to a different state and the direction of the path is reversed which is only possible when the path connects
Figure 2.1: A schematic demonstrating the SRTIS path moves. From top left to top center: a shooting move is attempted which shoots the current path from the current interface $\Lambda^1_I$ and eventually also crosses interface $\Lambda^2_I$. From top center to top right: as the current path now also crosses $\Lambda^2_I$, we can swap the current replica $\Lambda^1_I$ with $\Lambda^2_I$ and start collecting pathways for this path ensemble. From top right to bottom right: after a shooting attempt and a replica swap, a reactive path is generated which connects states $I$ and $J$. From bottom right to bottom center: a state swap is performed which reverses the direction of the path. Also the current interface set is changed from $\Lambda_I$ to $\Lambda_J$. From here a replica swap can be performed according to the Wang-Landau bias, $\frac{g(\lambda^i_J)}{g(\lambda^i_I)}$. From bottom center to bottom left: again a shooting move is performed, and subsequently a time-reversal which changes the direction of the path.

state $I \to J$ with $J \neq I$. The acceptance probability for the state swap is:

$$P_{\text{acc}}(x^L; \lambda^i_I \to \lambda^j_J) = h^j_J[\hat{\mathbf{x}}^L] \min \left[ 1, \mathcal{N} \frac{g(\lambda^i_I)}{g(\lambda^j_J)} \right]$$  \hspace{1cm} (2.38)

where $\hat{\mathbf{x}}^L$ is denoted to indicate $\mathbf{x}^L$ in reverse order, and $\mathcal{N}$ is unity if a state swap is only performed between the same two interfaces $\lambda^i_I$ and $\lambda^j_J$, and it is the fraction of the number of replicas of states, $\frac{m_I}{m_J}$ if all interfaces between states are allowed to swap. Although not commonly used as in most studies all states have the same number of interfaces, especially when states are nested within interfaces, it can be advantageous to allow all interfaces to state swap. Note that $g(\lambda^j_J)$ is also used for the state swap. This ensures that each replica, across all states is sampled uniformly.

To randomize within the state, we also employ the so-called minus move \[102\]. In the minus move, a path is generated that begins at the interface boundary of $\Lambda^1_I$ and is subsequently evolved within the stable state $I$ instead of away from it. First of all, this makes sure different exits out of state $I$ are sampled. Second, these paths can be used to calculate the flux out of state $I$ as shown in Eq. \[2.41\].

Moreover, in order to achieve further decorrelation between pathways, we also use the time-reversal move where the order of the path is reversed. Acceptance
probability for this move is simple due to the fact that microscopic reversibility ensures that both directions of paths are equally probable:

$$P_{acc}(\lambda^i_I; \mathbf{x}^L \rightarrow \mathbf{x}^L]) = h^i_I[\mathbf{x}^L]$$ (2.39)

which indicates that only $I \rightarrow I$ paths are accepted. In Fig. 2.1 a graphical summary is presented of all the moves. Note that other path moves are also possible, but these moves are able to efficiently sample the entire path ensemble for self-assembling systems.

**Digression:**

Although TIS as described above and used in the following chapters is a very efficient method to obtain the entire path ensemble between all states, it might not be the best method in general for studying self-assembly. The main reason is that self-assembly intrinsically consist of two different types of processes. One is association where the energy decreases due to bond formation and the other is dissociation where energy increases due to bond breaking. In the TIS setup described here, we only use one set of interfaces for each state defined by the energy of the system that can only bias the generation of pathways in one direction of the reaction coordinate, up or down in energy. Therefore, it is not possible to effectively bias the generation of pathways corresponding to both types of processes. An obvious solution to this setback, is that each state gets two or more sets of interfaces, each corresponding to a distinct process. Multiple Interface Set TIS (MISTIS) is designed to handle different sets of interfaces per state [106]. Specifically for self-assembly transitions, two different interfaces can be defined. One for association, $\lambda_A^{ass} = \min[r_{cut} - d_{ij}]$ where $d_{ij}$ are all distances between possible binding sites and $r_{cut}$ is the distance where two binding sites are considered to be completely unbound, and where the min function returns the minimum of all pairs. Another interface set would be for dissociation, $\lambda_A^{diss} = \min[d_{ij}]$. Now, in contrast to the TIS setup described above, when the system resides in an intermediate state, both
associating and dissociating pathways can be generated, see Fig. 2.2. Note that the rate constant calculation is a bit more involved than TIS with one interface set [106].

## Rate equation

One of the main reasons to perform a TIS simulation, is that one is interested in calculating rate constants. From a TIS calculation, the rate constant can be obtained via [100]:

$$k_{IJ} = \phi_I P(\lambda^0_J | \lambda^1_I)$$ (2.40)

where the flux, $\phi_I$, is defined as the number of positive crossings per unit of time out of the stable state $I$ through the first interface $\lambda^1_I$ and $P(\lambda^0_J | \lambda^1_I)$ is the crossing probability from the first interface of state $I$ to the first interface of state $J$, which can be factorized as $P(\lambda^0_J | \lambda^1_I) = P(\lambda^m_J | \lambda^1_I)P(\lambda^0_I | \lambda^m_I)$. The flux is calculated on the fly as follows:

$$\phi_I = \left( \langle \tau^0 \rangle + \langle \tau^1 \rangle \right)^{-1}$$ (2.41)

where $\langle \tau^0 \rangle$ is the average path-length in the minus interface and $\langle \tau^1 \rangle$ is the average path-length from the first interface. Typically, $P(\lambda^0_J | \lambda^m_I)$ can also be calculated on the fly from the outermost interface:

$$P(\lambda^0_J | \lambda^m_I) = \frac{n_{IJ}(\lambda^m_I)}{\sum_J n_{IJ}(\lambda^m_I)}$$ (2.42)

where $n_{IJ}(\lambda^m_I)$ is the number of pathways from $I$ to $J$ for the outermost interface $\lambda^m_I$ and the sum is over all states. Note that both $\phi_I$ and $P(\lambda^0_J | \lambda^m_I)$ could also efficiently be calculated from a brute force MD run. The crossing probabilities, $P(\lambda^0_J | \lambda^1_I)$, are usually very small in the case of rare events and therefore, difficult to obtain via brute-force MD. However, because in TIS crossing histograms are accumulated for each interface, we can obtain good statistics on the crossing probability by performing histogram reweighting (WHAM) on the individual crossing probabilities for every interface which is described next.

## Crossing probability and WHAM

Crossing histograms can be constructed by monitoring the maximum order parameter value a path has reached away from state $A$ as follows:

$$P_A(\lambda | \lambda^i) = \int D\mathbf{x}^L \mathcal{P}_{\Lambda^i_A}[\mathbf{x}^L] \mathcal{H}(\lambda_{max}[\mathbf{x}^L] - \lambda)$$ (2.43)

where $\mathcal{P}_{\Lambda^i_A}[\mathbf{x}^L]$ is the probability of a path, $\mathbf{x}^L$, when sampling pathways in replica $i$, and $\mathcal{H}(x)$ is the Heaviside step function.

In order to obtain the full unbiased crossing histogram, each individual crossing histogram obtained by sampling pathways for each replica $\Lambda^i_A$ has to be combined
and given its proper weight. This is done via WHAM and the combined crossing probability is defined as follows:

$$P_A(\lambda|\lambda^i_A) = \sum_{i=1}^{n=1} \bar{w}_A^i \mathcal{H}(\lambda^i_A - \lambda) \mathcal{H}(\lambda - \lambda^i_A) \sum_{j=1}^{i} P_A(\lambda|\lambda^j_A)$$  \hspace{1cm} (2.44)$$

where $\bar{w}_A^i$ are given by:

$$\bar{w}_A^i = \frac{1}{\sum_{j=1}^{i}(w^j_A)^{-1}}$$  \hspace{1cm} (2.45)$$

where $w^j_A$ are the optimized WHAM weights for each interface crossing histogram. 

**Digression:**

Now, Eq. 2.45 looks at first sight a bit magical. Why wouldn’t each interface be given the weight $w^i_A$ which comes directly from WHAM? It is basically constructed such, that it avoids over counting of paths.

However, the following simple example might give some more comfort. Imagine a state $A$ with three interfaces. Imagine the WHAM weights for each interface are $w^1_A = 1.0$, $w^2_A = 0.5$ and $w^3_A = 0.1$, which according to Eq. 2.45 would lead to $\bar{w}_A^1 = 1.0$, $\bar{w}_A^2 = \frac{1}{3}$ and $\bar{w}_A^3 = \frac{1}{13}$. If we perform a TIS simulation for this system and generate a thousand pathways for each replica, we could make the following histogram which gives the total number of pathways starting from replica $\Lambda^i$ (row) and has crossed replica $\Lambda^j$ (column) with $j \geq i$, $n_{IJ}(\lambda)$, which would resemble the continuous crossing histogram as in Eq. 2.43:

<table>
<thead>
<tr>
<th>$n_{IJ}(\lambda)$</th>
<th>$\Lambda^1$</th>
<th>$\Lambda^2$</th>
<th>$\Lambda^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Lambda^1$</td>
<td>1000</td>
<td>500</td>
<td>100</td>
</tr>
<tr>
<td>$\Lambda^2$</td>
<td>1000</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>$\Lambda^3$</td>
<td></td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>1500</td>
<td>1300</td>
</tr>
</tbody>
</table>

As the distribution of pathways from $\Lambda^1$ represents the distribution of the natural ensemble, we want the total number of pathways given in the last row after reweighting to match the distribution of $\Lambda^1$. If we multiply the weights $\bar{w}_A^i$ with $n_{IJ}(\lambda)$ we obtain precisely that!

However, in the above histogram we did not give a path a particular weight as a path can belong to all three replicas. It seems more intuitive and clean to assign a path one certain weight, i.e. it belongs to only one replica. To assign pathways only one certain weight we histogram the pathways differently (which will also be used in the Reweighted Path Ensemble below). We will create a histogram where a path is only assigned to a replica which it has maximally crossed, $n_{IJ}(\lambda^{max})$:

<table>
<thead>
<tr>
<th>$n_{IJ}(\lambda^{max})$</th>
<th>$\Lambda^1$</th>
<th>$\Lambda^2$</th>
<th>$\Lambda^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Lambda^1$</td>
<td>500</td>
<td>400</td>
<td>100</td>
</tr>
<tr>
<td>$\Lambda^2$</td>
<td>800</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>$\Lambda^3$</td>
<td></td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>1200</td>
<td>1300</td>
</tr>
</tbody>
</table>
Mindbogglingly, the same weights $\bar{w}_i A$ multiplied with $\sum_{I \in S} n_{I,J}(\lambda^{max})$ also returns the distribution of $\Lambda_i$! Note that this reweighting does not depend on the fact that we have uniformly sampled across each replica, as $w_i A$ would scale accordingly, which becomes important for SRTIS. A more formal proof is given in Ref. [107].

Reweighted path ensemble

In SRTIS we obtain the Wang-Landau biased path ensemble. To reweight the path ensemble to match it to the natural path ensemble, i.e. give each sampled pathway its proper weight, we can use the crossing probabilities obtained from WHAM [107]. The reweighted path ensemble (RPE) can be calculated as follows:

$$P[x^L] = \sum_{I \in S} c_I \left[ w_i A P_{\Lambda_i}^- [x^L] + \sum_{j=1}^{n-1} P_{\Lambda_j} [x^L] W_j [x^L] \right],$$

where $P_{\Lambda_i}^- [x^L]$ is the probability to sample path $x^L$ in the minus interface, $P_{\Lambda_j} [x^L]$ is the probability to obtain path $x^L$ while sampling $\Lambda_j$, $W_j [x^L] = \sum_{i=1}^{n-1} \bar{w}_i \mathcal{H}_i [x^L]$ where $\mathcal{H}_i [x^L]$ is used to select the correct weight $\bar{w}_i$ for a path that has its maximum $\lambda$ between interface $j$ and $j + 1$: $\mathcal{H}_i [x^L] = \mathcal{H}(\lambda_{max}[x^L] - \lambda_i)\mathcal{H}(\lambda_{i+1} - \lambda_{max}[x^L])$.

In SRTIS we strive to sample all interfaces across all states uniformly, which is also done via the WL bias, $g(\lambda_i)$ in the state swap move. To get the proper weight between states, we use the constants $c_I$. If we would not use any bias for the state swap, we only allow swaps between replicas $\Lambda_i^m$ and $\Lambda_j^m$, we would obtain the natural ensemble between these two interfaces, meaning these two interfaces should have the same weight. We can achieve this by scaling each weight $w_i^m,nat = w_i^m / w_i^m$, which sets the weight of the outermost interface of each state, $w_i^m$, to the same value. If no bias is used in state swap, $c_I$ would not be necessary. However, we do. For this reason, we should after scaling all weights with $w_i^m$, also scale with $g(\lambda_i^m) / g(\lambda_i^m)$ where $R$ is a reference state. Therefore, $c_I = g(\lambda_i^m) / g(\lambda_i^m)$. Note that $c_I$ can also be obtained by matching rate constants method described in Ref. [103]. Via the RPE we can calculate the free energy landscape, path densities and reactive currents.

Rate calculation nested states

The rate constant calculation for the systems with only two states is given by Eq. 2.40. In a multiple state system, where states can be nested in between interfaces of other states, Eq. 2.40 is not valid anymore as it assumes that all transitions from $I$ cross the outermost interface $\lambda_i^m$, which is not necessarily the case for systems which are nested in between interfaces. If Eq. 2.40 is used for states nested within interfaces, many transitions could be missed in the rate constant calculation, see Fig. 2.3 for example. One can circumvent this problem by calculating the rate
constant via the path-type numbers introduced in ref. [108]. A path-type number is defined as \( n_{IJ}^i(\lambda^k_I) \), which is the number of paths in replica \( i \) joining states \( I \) and \( J \) that have crossed at maximum interface \( \lambda^k_I \). The superscript indicates that the paths should obey the condition of replica \( i \) in the ensemble. Because we have set the maximum interface, we can reweight these numbers with the WHAM weights obtained with the reweighting of the crossing probability as follows:

\[
\tilde{n}_{IJ}(\lambda^k_I) = \bar{w}_k^I \sum_{i=1}^{m} n_{IJ}^i(\lambda^k_I), \tag{2.47}
\]

where \( \bar{w}_k^I = \left( \sum_{i}^{k} \frac{1}{w_i^I} \right)^{-1} \) is the WHAM weight for paths that have crossed interface \( \lambda^k_I \) at maximum (note that also the path-type numbers themselves can be reweighted, however, this is more difficult and should be the same as the weights obtained via the crossing probability anyway). Now we have the reweighted number of paths that join state \( I \) with state \( J \) that have crossed interface \( \lambda^k_I \). Subsequently summing over all \( k \) replicas gives the reweighted number of paths coming from state \( I \) and ending in state \( J \):

\[
\tilde{n}_{I,J} = \sum_{k=1}^{m} \tilde{n}_{I,J}(\lambda^k_I), \tag{2.48}
\]

Because the Wang-Landau scheme biases the simulation to sample all states equally via the state-swap bias, the path-numbers for each state need to be corrected for this bias. In an unbiased ensemble there are as many reactive \( IJ \) path as \( JI \) paths. Therefore, we split the obtained path-type matrix, \( \tilde{n}_{IJ} \), into \( M \) matrices and symmetrize the \( Ith \) matrix: \( \tilde{n}_{J,I} = \tilde{n}_{I,J} \) and setting all other entries of the \( Ith \) matrix to zero, resulting in \( M \) different matrices with only a nonzero \( Ith \) row and
a nonzero $I$th column. Subsequently, all $M$ matrices are joined via WHAM giving the individual weights for each state (these weights can also be used to calculate the coefficients, $c_I$, in RPE). This leads to a $M \times M$ transition matrix, $\tilde{n}_{IJ}$. Normalizing the matrix with the total numbers of paths going out of a state $\sum_J \tilde{n}_{IJ}$ and multiplying with the flux gives the generalized rate matrix for multiple state systems:

$$k_{IJ} = \phi_I \frac{\tilde{n}_{IJ}}{\sum_J \tilde{n}_{IJ}}. \quad (2.49)$$

### Free energy landscape

From the RPE we can calculate the free energy landscape for a chosen set of collective variables simply by projection:

$$F(q) = -k_B T \log p(q) + C$$

$$p(q) = C \int D x^L \, p[x^L] \sum_{k=0}^L \delta[q(x_k) - q], \quad (2.50)$$

where $C$ is an arbitrary constant, $q(x_k)$ are the collective variables at time step $x_k$ and $P[x^L]$ is the reweighted path probability as given in Eq. 2.46.

One could histogram all the free energies after the simulation using saved pathways after one has obtained the proper weights. However, as every path that has reached a certain maximum interface based on $\lambda_{max}$ is reweighted with the same weight, it is convenient to histogram on the fly for each interface separately, and subsequently reweight and sum all the histograms. As such, paths do not necessarily need to be stored for subsequent analysis.

### Reactive path density

In Eq. 2.50, $p(q)$ gives the probability of each configuration, $q(x_k)$. However, the mechanism of a process can sometimes be obscured by the dominance of certain metastable configurations. To get more insight into the mechanism, the reactive path density is useful. We can define two different path densities, per transition or per state.

The reactive path density for each transition separately is defined as:

$$n_{AB}^r(q) = \int D x^L \, P[x^L] h_A(x_0) h_B(x_L) h_q(x^L). \quad (2.51)$$

where $h_A(x_0) h_B(x_L)$ picks out all the reactive trajectories between the appropriate states $A$ and $B$ and $h_q(x^L)$ is unity if the path visits $q$.

The reactive path density out of state $A$ is defined as:

$$n_A^r(q) = \int D x^L \, P[x^L] h_A(x_0) h_q(x^L). \quad (2.52)$$
where now \( h_A(x_0) \) picks out all reactive trajectories out of state \( A \). The additional information that this type of path density offers, is that also the relative probability of reactive pathways out of state \( A \) can be seen.

Note that a path density does not add up to unity as the density is not normalized by the number of configurations used to construct the final histogram, but normalized by the number of paths.

**Reactive path current**

In self-assembly certain configurations are dead ends, which are still projected in \( n_r(q) \). We can also average out these dead ends by calculating the reactive path current. The reactive path current is defined as follows [109]:

\[
J_{BU}(q) = C \int D x^L \mathcal{P}[x^L] h_A(x_0) h_B(x_L) \sum_{k=0}^{L} \delta[q(x_k)] - q_k \dot{q}(x_k) \tag{2.53}
\]

where \( \dot{q}(x_k) \) is the estimated velocity of \( q \): \( \dot{q}(x_k) \approx \frac{q(x_{k+1}) - q(x_{k-1})}{2\Delta t} \). This current has the same properties as the reactive path density, except for the fact that dead ends are averaged out, which results in the mean direction a path will follow.

**Transition Path Theory**

From SRTIS we can obtain the full rate matrix, \( K \), between states. However, essential questions important to understanding self-assembly are not answered directly by looking at \( K \) [110]. How can patchy particles with different initial configurations find the correct ground state? Through which sequence of intermediate states do the building blocks pass through towards the final structure before possible unbinding? Are there multiple routes possible? What is the overall rate constant of the process if all possible routes are considered? Transition Path Theory (TPT) is a convenient framework designed to help answer these questions [109, 110]. Similarly to TIS, TPT requires state space to be separated in states which specify the (self-assembly) process. Therefore, it seems very suited to use TPT from TIS results. In what follows we describe a transition from \( A \) to \( B \) with possible intermediate states \( I \) along the way.

**Committer**

The starting point in TPT analyses in this thesis usually start from the committor, \( q_i^+ \), defined as the probability that the system, currently in \( i \), will reach \( B \) before it reaches \( A \). The commitment probabilities are computed from the transition matrix, \( T \), where \( T_{ij} \) gives the probability to reach \( j \) from \( i \) within a certain lag time, \( \tau \) by solving the following linear set of equations:

\[
q_i^+ = \sum_{k \in B} T_{ik} + \sum_{k \in I} T_{ik} q_k^+ \tag{2.54}
\]
with the committor \( q_A^+ = 0 \) and \( q_B^+ = 1 \) as boundary conditions. We get the transition matrix from the rate matrix obtained via TIS by \( T = \exp(K\tau) \).

We can also define the reverse committor probability \( q_i^- = 1 - q_i^+ \), which is the probability to reach \( A \) before \( B \) when currently in \( i \).

### Flux through states

As discussed above the question through which sequence of intermediate states do the building blocks pass through towards the final structure can also be deduced from TPT. For this purpose we can calculate the fluxes, \( f_{ij} \), from which the dominant sequence of states during self-assembly can be deduced. The effective flux \( f_{ij} \) is given by:

\[
 f_{ij} = \pi_i q_i^- T_{ij} q_j^+ 
\]

where \( \pi_i \) is the equilibrium population of state \( i \). Note that this definition of \( f_{ij} \) still contains all recrossings between intermediate states. To get rid of these recrossings, we can consider the net flux which will give a more clear picture of the sequence of states taken in the overall process:

\[
 f_{ij}^+ = \max[0, f_{ij} - f_{ji}] 
\]

Note that detailed balance dictates that the net flux for the reverse process is given by the transpose: \( f_{ji}^- = (f_{ij}^+)^T \).

### Overall rate constant

TPT not only gives information about the mechanism of the process, one can also calculate the overall rate constant between states considering all possible routes between \( A \) and \( B \). The overall rate constant is given by:

\[
 k_{AB} = \frac{\sum_{J \neq A} \pi_A T_{AJ} q_J^+}{\tau \sum_{J=0}^{M} \pi_J q_J} 
\]

where the denominator is necessary because the numerator does not take into account the probability of being in a forward \( A \to B \) transition. Therefore, the overall rate constant is divided by the probability that the system while being in any state \( I \) was last in \( A \) and not in \( B \).
3 Modeling critical Casimir force induced self-assembly experiments on colloidal dumbbells

Colloidal particles suspended in a binary liquid mixture can interact via solvent mediated interactions, known as critical Casimir forces. For anisotropic colloids this interaction becomes directional, which leads to rich phase behavior. While experimental imaging and particle tracking techniques allow determination of isotropic interaction effective potentials via Boltzmann inversion, the modeling of effective interaction in anisotropic systems is non-trivial precisely because of this directionality. In this work we provide a framework for extracting effective interaction potentials for non-spherical dumbbell particles from observed radial and angular distributions, by employing Reference Interaction Site Model (RISM) theory and direct Monte Carlo simulations. For colloidal dumbbell particles dispersed in a binary liquid mixture and interacting via induced critical Casimir forces, we determine the effective site-site potentials for a range of experimental temperatures. Using these potentials to simulate the system for strong Casimir forces, we reproduce the experimentally observed collapse, and provide a qualitative explanation for this behavior.
3.1 Introduction

Colloidal particles suspended in a binary liquid mixture of water and picoline (3-methylpyridine) experience an effective attraction when the temperature is raised from below towards the coexistence line of the binary mixture. As this universal attraction between colloids occurs close to the critical point of the binary liquid, it is commonly referred to as the critical Casimir force \[28, 32\]. The solvent mediated interaction is highly dependent on temperature, with the range of the attraction increasing as the temperature approaches the critical point. In contrast to more common colloidal interactions such as electrostatic or depletion interactions, this temperature dependence gives precise control over the interactions between colloids. Previous work employing critical Casimir forces showed that spherical colloids can spontaneously phase separate into different colloidal phases over a small range of temperature \[34–36\].

Recent breakthroughs have made it possible to synthesize anisotropic colloids \[4, 5, 16, 111\] and study their behavior in binary liquids. These particles have an anisotropic shape, and/or interact via directional potentials. Particles of the latter category, known as ”patchy particles”, are able to form colloidal molecules \[16\], and open crystal structures \[4\]. Suspensions of anisotropic colloidal particles have, therefore, great potential for the assembly of novel nano and micron-scale structures. In a recent paper we showed that the critical Casimir force can induce directional interactions between anisotropic dumbbell colloids \[112\]. Controlling the temperature leads to different structural morphologies, including small clusters, strings, and aggregates. At temperatures very close to the binary liquid coexistence line, a sudden collapse transition was observed \[112\]. The aim of this work is to provide an effective pair potential that is able to describe the behavior of the dumbbell system.

Modeling colloidal systems via effective pair potentials has a long history. A famous example is the DLVO theory that describes the effective interaction in colloidal suspensions as a combination of electrostatic repulsion and van der Waals attraction \[113, 114\]. More recent examples include the depletion potential, induced by non-adsorbing polymers, and the critical Casimir force itself \[35, 115\]. These potentials are fundamentally isotropic in nature, allowing a description of the interaction as a potential acting on the centers of mass of the two particles. This symmetry allows extracting the effective pair potential by Boltzmann inversion of the radial distribution function obtained from experiments via advanced optical imaging and particle tracking techniques. The situation is more complex for anisotropically interacting particles where such symmetry does not exist. One choice is to make the potential angular dependent, which is for example done in models such as the Kern-Frenkel model \[116\]. Another option, which we follow here, is to view the anisotropic particle as a rigid construction consisting of several particles that each interact via an isotropic potential. This is essentially a molecular viewpoint, where the constituents of the molecule are isotropic spheres, glued together to form a rigid anisotropic colloidal building block. This viewpoint is suitable for the dumbbell system that we consider here. We develop a simple
model for the dumbbell interactions based on an isotropic form of the effective critical Casimir potential, which we then optimize, employing both Reference Interaction Site Model (RISM) theory \[117-119\] and direct Monte Carlo simulations, in order to reproduce variants of experimental radial and angular distribution functions. The obtained potential reproduces the different morphologies observed far from the critical point remarkably well. In addition, the optimized potential gives an explanation for the structural collapse observed in the experiments when the temperature approaches the coexistence line of the binary fluid.

The remainder of the chapter is as follows. In section 3.2 we explain the experimental setup, introduce the model and theoretical and simulation methods. In Section 3.3 we present and discuss the results. We end with concluding remarks.

3.2 Methods

Experiments

The colloidal patchy dumbbell particles, composed of sterically stabilized PMMA spheres with a core fluorescently labelled with NBD-MAEM and a non-fluorescent shell, were prepared following the procedure laid out in Ref. [112]. The particles were suspended in a binary liquid mixture of heavy water and picoline (3-methylpyridine, 3MP), with a weight fraction $c_{3MP} = 0.25$, below the critical composition, $c_c = 0.28$ [120]. The hydrophobic spherical ends prefer 3MP, while the hydrophilic shells prefer the water component. Glass capillaries were filled with suspensions at colloid volume fraction of 0.2\%, and flame sealed to prevent any composition change due to evaporation. Critical Casimir interactions were induced by heating the suspensions to temperatures $\Delta T = T_{CX} - T$ below the coexistence temperature $T_{CX} = 38.55^\circ C$ where the two solvent components 3MP and water still form a homogeneous mixture. To minimize equilibration times, we first kept the suspension at $\Delta T = 5^\circ C$, where critical Casimir interactions are negligible and the particles do not aggregate. As the suspension is not density matched, particles sediment to the bottom of the capillary resulting in a quasi-2D system.

We follow particle aggregation directly in real space by imaging individual particles in an area of $104 \mu m \times 104 \mu m$ using confocal microscopy. For each measurement, a series of at least 3000 images was recorded for sufficient statistics. To ensure the same initial conditions (no clusters formed inside the capillary) for all measurements, we always equilibrated the system at $\Delta T = 5^\circ C$ for at least 15 minutes before raising the temperature to the final desired value close to $T_c$. The images were analyzed using the particle tracking software IDL [121] and the positions of the centers of the fluorescently labelled spherical ends are determined with an accuracy of 0.03\,$\mu m$ in the horizontal, and 0.05\,$\mu m$ in the vertical direction. These data sets underwent an identical analysis as the data obtained from simulations, for consistency. The colloidal surface charge and Debye length for the prepared suspension were determined using electrophoresis and conductivity
experiments. For more details on the experiments we refer to Ref. [112].

**Dumbbell model and interaction potentials**

Previous work has demonstrated that the phase behavior of spherical colloids in near critical binary liquids can be modeled via a superposition of the Casimir attraction and an electrostatic repulsion [34, 35]. We extend this model towards dumbbells by modeling the particles as two touching fused hard spheres with radius $R = 1.15\mu m$. The distance between the centers of mass of the spheres is thus equal to $2R$. Every pair of spheres not on the same dumbbell interacts via a simple potential:

$$u(r) = u_{rep}(r) + u_{att}(r),$$

(3.1)

where $r = r_{i\alpha} - r_{j\gamma}$ is the center-center distance between two spheres not belonging to the same dumbbell: sphere $\alpha = 1, 2$ of dumbbell $i$ and sphere $\gamma = 1, 2$ of dumbbell $j$. We assume that the repulsion between particles is dominated by electrostatic repulsion and a hard-sphere mechanical repulsion, so that $u_{rep}(r) = u_{HS}(r) + u_{el}(r)$, with the hard sphere $u_{HS}(r)$ potential defined as

$$u_{HS} = \begin{cases} 0 & \text{if } r > 2R \\ \infty & \text{if } r \leq 2R, \end{cases}$$

(3.2)

and the electrostatic repulsion as [122]:

$$u_{el}(r) = \frac{2\pi R\sigma^2l_D^2}{k_BT\epsilon\epsilon_0}e^{-(r-2R)/l_D},$$

(3.3)

where $\sigma$ is the surface charge density, $l_D$ is the Debye length, $\epsilon$ is the dielectric constant of the solvent ($\epsilon = 69$), $\epsilon_0$ is the electric permittivity and the unit of energy is in $k_BT$. The surface charge, $\sigma$, and Debye length, $l_{Deb}$, have been measured via electrophoretic experiments which yielded $\sigma = -0.188\mu C/cm^2$, and $l_D = 24.3nm$. As the diameter of the particle is roughly $2.3\mu m$, these numbers correspond to a high repulsion ($\epsilon_{Deb} = \frac{2\pi R\sigma^2l_D^2}{k_BT\epsilon\epsilon_0} = 6.9 \times 10^3k_BT$) that decays relatively quickly ($l_D = 0.0211R$).

The attraction between two spherical particles is given by the temperature dependent Casimir interaction which at short distances is argued by Fisher and de Gennes to be of the form (using the Derjaguin approximation) [32, 35]:

$$u_{att}(r) = u_{Cas}(r) = -\frac{2\pi R}{\xi}e^{-(r-2R)/\xi},$$

(3.4)

where $\xi$ is the correlation length of the fluctuations in composition of the near critical binary liquid. Note that the pre-factor is of the form $\xi^{-1}$. A consequence of this is that the pre-factor diverges for small correlation lengths, which is slightly counter-intuitive as the general observation is that particles become more strongly aggregated close to the critical point (large $\xi$) suggesting a stronger attraction between particles. However, due to the strong electrostatic repulsion described
above only the long range effects of the Casimir attraction are important, which cancels the diverging part of the Casimir potential.

The correlation length close to the critical point of the binary liquid, \([\rho_C, T_C]\) scales with the temperature as follows [35]:

\[
\xi(\Delta T) = \xi_0 \left( \frac{\Delta T}{T_C} \right)^{-\nu},
\] (3.5)

where \(\xi_0\) is the correlation length at \(T = 0\)K, \(T_C\) is the critical temperature, \(\Delta T = T_C - T\) is the distance between the experimental temperature and the critical temperature, and \(\nu = 0.63\) is the relevant critical exponent. Clearly, the correlation length diverges as the critical point is approached. Even when the binary liquid is prepared slightly off-critical, this scaling of the correlation length usually does approximate the behavior well [123]. For our system the coexistence temperature \(T_{CX} = 38.55^\circ\)C. Therefore, our simple model potential is completely defined by the parameters \(R, \sigma, l_D, \Delta T\) and \(\xi_0\). The value of \(\xi_0\) is not known from experiments, and we therefore fit this value in order to describe how the potential develops when approaching the coexistence temperature.

Note that we do not claim that this potential form is the best way to describe the critical Casimir forces. Especially when the binary liquid is prepared off-critical, the scaling of the correlation length is also dependent on the distance to the critical composition [40, 46, 47]. Here, we do not take this into account as we do not aim to have a complete descriptions of the Casimir forces, but a sufficient one to describe the overall aggregation behavior.

Due to the size of the particles and because the system is not density matched, the particles easily sediment to the bottom of the sample. To mimic this situation we added a gravitational potential in the simulations

\[
u_{grav}(r) = r_z/l_{grav},
\] (3.6)

with \(r_z\) the vertical height above the bottom of the box and \(l_{grav} = k_B T/\Delta \rho V_{db} g\) with \(\Delta \rho = 0.19\)gcm\(^{-3}\) for PMMA in water/3MP mixture, \(V_{db} = \frac{8}{3} \pi R^3\) and \(g = 9.81\)ms\(^{-2}\). Based on the buoyancy of the particles, we estimated the gravitational length \(l_{grav} \approx 0.15R\). Periodic boundaries were only applied in the \(x\) and \(y\) directions, so that particles cannot sediment below \(z = 0\). In addition, in the experiments the glass surface can also induce critical Casimir forces and can restrain particles to the surface [28]. In this way, the system becomes effectively quasi two dimensional, as is observed in the experiments. To model this effect we added a square well potential

\[
u_{surf}(r) = \begin{cases} 0 & \text{if } r_z > \Delta \\ -\epsilon_{surf} & \text{if } r_z \leq \Delta, \end{cases}
\] (3.7)

with the range \(\Delta = 1.7R\) and depth \(\epsilon_{surf} = 10k_B T\). This choice of parameters roughly reproduced the observed average number of dumbbells sticking out of the plane. Moreover, if a weaker wall interaction is used spurious peaks arise in
Modeling critical Casimir forces

the $\theta$-$\phi$-distributions explained in Eq. 3.12 below, which are not present in the experimental distributions.

For the Monte Carlo simulations the total potential energy of the dumbbell system is thus given by

$$U_{\text{tot}} = \sum_{j=1}^{N} \sum_{i<j}^{N} \sum_{\alpha,\gamma=1}^{2} u_{\text{rep}}(r_{i\alpha,j\gamma}) + u_{\text{att}}(r_{i\alpha,j\gamma})$$

$$+ \sum_{i=1}^{N} \sum_{\alpha=1}^{2} u_{\text{grav}}(r_{i\alpha}) + u_{\text{surf}}(r_{i\alpha}),$$

(3.8)

where $r_{i\alpha,j\gamma} = r_{i\alpha} - r_{j\gamma}$ is the center-center distance between two spheres not belonging to the same dumbbell.

Potential optimization

Although the parameters of the potential are connected to physical properties of the system as indicated in Eqs. 3.3 and 3.4, the system is not solely determined by these properties. Additional effects such as van der Waals forces and polymer repulsion could also play a role in the interaction of colloids, especially since these dumbbell particles are not synthesized to minimize these effects. Previously in Refs. [34] and [36] the potential for spherical particles was obtained via fitting the potential of mean force obtained via inversion of the radial distribution function $g(r)$. At low density this inversion is simply $u(r) = -k_B T \ln g(r)$. However, several problems arise for anisotropic particles. While the one-to-one mapping between radial distribution function and the effective pair potential still holds [119, 124], the simple inversion of the radial distribution function is not valid anymore. Additional complications arise due to the fact that not all particles are accounted for, because the visualization via the microscope is limited to a plane. Moreover, the intrinsic error of the tracking of the particles causes an error in the particle positions. Finally, the polydispersity of the spheres and the variation in the distance between spheres of the dumbbell, play a role. Clearly, we can not use a direct fitting procedure to obtain the anisotropic potential. However, we can try to predict the radial distribution function for anisotropic particles by simulation or theory, and compare these predicted distributions to the experimental one, and determine which potential parameters give the most optimal match.

For this purpose we propose several distribution functions to compare between simulations and experiment. From the experimental imaging techniques we determine the 2D site-site radial distribution $g_{\alpha\gamma}(r)$ between sphere $\alpha = 1,2$ on dumbbell $i$ and sphere $\gamma = 1,2$ on a different dumbbell $j$, defined as

$$g_{\alpha\gamma}(r) = \rho^{-2} \langle N(N-1)\delta(r_{i\alpha})\delta(r_{j\gamma} - r) \rangle,$$

(3.9)

where $\delta(r)$ denotes the Dirac delta function, $\rho$ is the 2D particle density (the number of particles per area), and the angular brackets denote an ensemble average over all configurations. The probability to find a site $\gamma$ a distance $r$ away from site
\(\alpha\) (with \(\alpha\) and \(\gamma\) on different molecules) is thus \(2\pi \rho g_{\alpha\gamma}(r)\). This distribution can be connected with the well-known reference interaction site model (RISM) integral equation theory for molecular fluids \[119, 125, 126\]. This distribution is estimated from the experimental data by evaluating

\[
g_{\alpha\gamma}(r) \approx \frac{1}{n_f} \sum_k \frac{1}{N_k(N_k-1)} \sum_{i<j} \frac{2}{N_k} \frac{\delta(r_{i\alpha,j\gamma} - r)}{f(r_{i\alpha}, r_{j\gamma})},
\]

(3.10)

with \(n_f\) the number of frames, \(N_k\) the number of dumbbells in frame \(k\), \(f(r_{i\alpha}, r_{j\gamma})\) denotes the fraction of the circle centered at \((r_{i\alpha}\) and goes through \(r_{j\gamma}\)\) that falls inside the microscope image. This fraction corrects for the fact that particles outside the image do not contribute.

In a similar way we also measure the minimum distance radial distribution, \(g_{md}(r)\), where only the minimum distance between all dumbbell pairs is counted. This distribution is estimated from the experimental data by evaluating

\[
g_{md}(r) \approx \frac{1}{n_f} \sum_k \sum_{i<j} \frac{\delta(\text{min}(r_{i1,j1}, r_{i1,j2}, r_{i2,j1}, r_{i2,j2}) - r)}{N_k(N_k-1)f(r_{i\alpha}, r_{j\gamma})},
\]

(3.11)

where the \(\text{min}()\) function returns the lower of its arguments. These versions of the radial distribution minimize the effect of the trivial neighbour.

We can also use a slightly different approach and focus solely on the orientations the particles have with respect to each other when they bind, by measuring the bond-angle distributions, which should be less dependent on the standard error of determining distances given by the system. For neighboring dumbbells we define two different bond angles, \(\phi_{ij} = \arccos(p_i \cdot v_{ij})\) and \(\theta_{ij} = \arccos(p_i \cdot p_j)\) where \(v_{ij}\) is the vector between the center of mass of the spheres (on different dumbbells) closest to each other, so that \(|v_{ij}| = \min(r_{i1,j1}, r_{i1,j2}, r_{i2,j1}, r_{i2,j2})\), and \(p_i\) is the dumbbell vector of which the direction is given by the sphere that defines \(v_{ij}\) (see Fig. 3.1). Through these angles different particle orientations can be discerned and analyzed. The angular distribution distribution is given by

\[
p(\theta, \phi) = \frac{1}{n_f} \sum_k \sum_{i<j\in nn} \delta(\theta_{ij} - \theta)\delta(\phi_{ij} - \phi),
\]

(3.12)
where the second sum is over the bonded neighbors only.

In contrast to the radial distribution function of spherical particles in a dilute suspension, none of the three distributions Eqs. [3.10][3.12] are directly related to the effective pair potential. Therefore, we perform many Monte Carlo simulations with a predefined set of potential parameters, \( \{\xi_0\} \), and subsequently find the distribution which fits best with the appropriate experimental distribution. To obtain a degree of similarity between two distributions we use the Jensen-Shannon divergence:

\[
d_{JS} = \sum_i P(i) \log \frac{P(i)}{M(i)} + \sum_i Q(i) \log \frac{Q(i)}{M(i)},
\]

(3.13)

where \( \sum_i \) is the sum over every bin \( i \), \( P(i) \) and \( Q(i) \) are the distributions from simulations and experiments respectively, and \( M(i) = \frac{1}{2}(P(i) + Q(i)) \). The potential parameters that give the smallest \( D_{JS} = \sum \Delta T d_{JS}(\Delta T) \) (where the sum goes over all temperatures measured experimentally), determine the optimized pair-potential in Eq. 3.1, where the sum goes over the set of temperatures used in experiment. Note that \( g_{md}(r) \) and \( g_s(r) \) are not normalized, so that the value for \( d_{JS} \) is not bound by \( \ln 2 \).

RISM theory

We employed the RISM theory as a first approximation to match the observed site-site radial distribution functions to an underlying effective potential between the dumbbell particles. In this approach we followed Talbot and Tildesley, who applied the RISM theory to 2D dumbbell systems [126]. Since the experimental system is only quasi 2D we do expect some differences.

The 2D site-site correlation function is given by

\[
g_{\alpha\gamma}(r) = \rho^{-2} \langle N(N-1)\delta(r_{1\alpha})\delta(r_{2\gamma} - r) \rangle,
\]

(3.14)

where \( \delta(r) \) denotes the Dirac delta function, \( \rho \) is the 2D particle density (the number of particles per area), and the angular brackets denote an ensemble average over all configurations. The probability to find a site \( \gamma \) a distance \( r \) away from site \( \alpha \) (with \( \alpha \) and \( \gamma \) on different molecules) is thus \( 2\pi r \rho g_{\alpha\gamma}(r) \).

Setting the total correlation function \( h_{\alpha\gamma}(r) = g_{\alpha\gamma}(r) - 1 \), the RISM theory defines the direct correlation function \( c_{\alpha\gamma}(r) \) by a (2x2) Ornstein-Zernike matrix equation

\[
\hat{h}(k) = \hat{\omega}(k)\hat{c}(k)\hat{\omega}(k) + \rho\hat{\omega}(k)\hat{c}(k)\hat{h}(k),
\]

(3.15)

where the hats denote 2D Fourier transforms, also known as Hankel transforms,

\[
\hat{f}(k) = 2\pi \int_0^\infty f(r)J_0(kr)rdr \tag{3.16}
\]

\[
f(r) = \frac{1}{2\pi} \int_0^\infty \hat{f}(r)J_0(kr)dk, \tag{3.17}
\]

48
with \( J_0(x) \) denoting the zeroth order Bessel function. The elements of the \( \omega \) matrix are

\[
\omega_{\alpha\gamma}(k) = \delta_{\alpha\gamma} + (1 - \delta_{\alpha\gamma})J_0(kl),
\]

with \( l \) the intramolecular distance between the centers of mass of particles in one dumbbell. Because dumbbells are homo dimers all site-site correlations are identical and the RISM equation reduces to a single scalar equation:

\[
\hat{h}(k) = \hat{\omega}(k)\hat{c}(k)\hat{\omega}(k) + 2\rho\hat{\omega}(k)\hat{c}(k)\hat{h}(k),
\]

where \( \hat{\omega}(k) = 1 + J_0(kl) \) and the density is now the sphere density rather than the dumbbell density. Rearrangement leads to an expression for the direct correlation function

\[
\hat{c}(k) = \frac{\hat{h}(k)}{\hat{\omega}(k)^2 + 2\rho\hat{\omega}(k)\hat{h}(k)}.
\]

Like in any integral equation approach this equation needs a closure relation. Since we consider particles with long range interactions (relative to the hard sphere interaction), a reasonably good closure is the hypernetted-chain (HNC) approximation

\[
g(r) = h(r) - 1 = e^{-\beta u(r) + h(r) - c(r)}.
\]

Eq. 3.20 and 3.21 form a closed set, and allow the computation of the \( g(r) \) from a given \( u(r) \). The solution is obtained numerically, by iteration, mixing in the previous solutions of \( h(r) \) at each iteration step. The \( u(r) \) is optimized using the Jensen-Shannon divergence of the computed final \( g(r) \) and the experimental \( g(r) \). For comparison of the computed and experimental distributions, the computed \( g(r) \) is convoluted with a Gaussian function of width \( w \), to mimic the experimental error. During the optimization \( \xi_0 \) as well as the width \( w \) were varied.

### Simulation details

**Optimization potential via MC**

For the optimization via Monte Carlo simulations, we placed \( N = 100 \) dumbbell particles in a cubic box and applied periodic boundaries in the \( x-y \) direction. The number of particles was chosen to correspond roughly to the experimentally observed number of dumbbells. The gravitational and square well potential acted in the \( z \)-direction, as describe above. The size of the box was set to \( L = 64R \), chosen such that the number density of the cubic box is \( \rho = 0.0004R^{-3} \), and the 2D number density \( \rho = 0.025R^{-2} = 0.02\mu m^{-2} \). In order to minimize hysteresis during aggregation we employed an annealing procedure. Starting far from \( T_C \), at \( \Delta T = 1.6K \) where the attraction is very shallow, we performed \( 10^6 \) equilibration and \( 10^6 \) production cycles, where a cycle consists of moving each particle once on average, before increasing the temperature with 0.05K. This procedure was repeated until \( \Delta T = 0.85K \), corresponding to a strong Casimir attraction. The Monte Carlo moves were comprised of single particle translation and/or rotation.
moves, augmented with cluster moves [128]. A MC cycle consists of $N$ particle rotations or translations, and 1 cluster move. Cluster moves were performed using the algorithm in Ref. [90]. The maximum translation and rotation were optimized to achieve an acceptance ratio between 20% and 70%. We performed this scheme for values of the zero temperature correlation length $\xi_0 = 9.2 \times 10^{-4}\mu m$ to $\xi_0 = 20.7 \times 10^{-4}\mu m$ with increments $\Delta \xi_0 = 1.15 \times 10^{-5}\mu m$.

**Modeling the network collapse**

For the diffusion limited aggregation (DLA) simulations to study the collapse transition from a network structure, we first start with cluster moves only, so that clusters do not relax internally. A particle is considered part of a cluster if it is within $0.23\mu m$ within any of the particles of a cluster. We then relaxed the obtained DLA network structure using single particle moves and rotations with very small maximal translation and rotation displacements, until the local energy minimum was reached.

### 3.3 Results and Discussion

#### Potential optimization

**RISM Approach**

As an initial step we used the RISM approach to optimize the potential, using the Jensen-Shannon divergence between the experimental and predicted $g_{a\gamma}(r)$. Note that in order to properly compare experimental distribution to either theoretical predictions or simulations, the experimental error needs to be taken into account. This experimental error arises from uncertainty in measuring the positions of the center of the spheres, polydispersity in particle size and variation in the intramolecular distance between the dumbbell spheres. As the size of the particles is around $2.3\mu m$, the expected range of the attraction far from the coexistence temperature ($\Delta T > 1K$, is small relative to the size of the particle. In contrast, the broad first neighbor shell peak in the experimental $g_{md}(r)$ and $g_{a\gamma}(r)$ would suggest a larger attractive range. However, the observed fluctuations in bond distance of two bound dumbbells in the experimental image sequence are commensurate with the expected width of the potential, and not with the broad first peak in the radial distribution. This suggests that the broadness of the peak is introduced by the polydispersity in particle size and shape, and the measurement error. To mimic this error, the computed $g_{md}(r)$ and $g_{a\gamma}(r)$ is convoluted with a Gaussian function of width $w$. The number density in the plane is set to $\rho = 0.025\mu m^{-2}$.

The results of the RISM optimization are shown in Fig. 3.2. Fig. 3.2c shows the Jensen-Shannon divergence for the different $\xi_0$ and $w$ values. The global minimum is obtained around $\xi_0 = 16.3 \times 10^{-4}\mu m$ and $w = 0.16R$. The potentials corresponding to the minimum in $D_{JS}$ are shown in Fig. 3.2a. Note that the potentials
are relatively short ranged compared to the particle size. Far from the coexistence temperature $\Delta T = 1.55K$ the attraction has a minimum of about $0.05k_B T$. As the temperature increases, the potential gradually deepens and widens. The predicted and broadened $g_{\alpha\gamma}(r)$s are shown in Fig. 3.2b together with the experimental measurements. There are some differences with the experimental rdfs, e.g. in the height of the first peak, and some of the more detailed features. However, the location of the peaks and the qualitative behavior is in agreement. Note that the fit is global, and optimizes all potentials simultaneously.

Figure 3.2: (a) optimized potentials obtained from the measured $g_{\alpha\gamma}(r)$ using RISM. (b) comparison of predicted with experimental $g_{\alpha\gamma}(r)$. (c) Jensen-Shannon divergence for different $\xi_0$ and $w$ values. Note that the $w$ values are in units of diameter of the particle.

**Simulation approach**

Next, we used direct Monte Carlo simulations to globally fit the potentials to the entire set of measured distributions for the minimum distance radial distribution function $g_m d(r)$ as well as the site-site radial distribution function $g_{\alpha\gamma}(r)$. These simulations are expected to be more accurate compared to the RISM results, as they are performed in 3D, and mimic the experimental situations to a much better degree. Again the predicted radial distribution functions were convoluted with a
Figure 3.3: (a) Optimized potential for 5 values of $\Delta T$ for $\xi_0 = 16.5 \times 10^{-4} \mu m$. As the critical point is approached, a minimum is developed. Note that the short ranged nature of the potential due to the relative size of the correlation length and the size of the particles. Inset shows the divergence of the correlation length close to $T_C$. (b) Sum over temperatures, $D_{JS}$, as a function of $\xi_0$ when fitting $g_{md}(r)$ demonstrating that the minimum is indeed at $\xi_0 = 16.5 \times 10^{-4} \mu m$ ($D_{JS}$ is not bounded by the usual value of $\ln 2$ due to the fact that the radial distributions are not normalized). The sharp increase at higher $\xi_0$ is due to the fact that complete aggregation occurs for these values. (c) Fitted $g_{md}(r)$ and (d) fitted $g_s(r)$ for $\xi_0 = 16.5 \times 10^{-4} \mu m$ demonstrating that for this value of $\xi_0$ simulations (lines) reproduce the main features and trend of the experimental distributions (points) very well. Note that the $w$ values are in units of diameter of the particle.

Gaussian of width $w$ to take into account the effect of the experimental measurement errors.

In Fig. 3.3b the $D_{JS}$ values found for various $\xi_0$ and $w$ are plotted when using $g_{md}(r)$ to optimize which demonstrates that $\xi_0 = 16.5 \times 10^{-4} \mu m = 1.65 nm$ and $w = 0.18R$ yields the best fit. This value defines the effective pair sphere-sphere potential given by Eq. 3.1 over the entire temperature range. Fitting to $g_{\alpha \gamma}(r)$ also yields a fitted value of $\xi_0 = 16.5 \times 10^{-4} \mu m$. The value of $\xi_0$ is on the same order as has been found in previous work [31]. The site-site effective pair potentials $u_{ij}(r)$ are plotted in Fig. 3.3a clearly showing the weak interaction at low temperature $\Delta T = 1.55K$ which deepens and develops a longer range when the temperature is
Figure 3.4: (a) Experimental and (b) simulation $\theta$-$\phi$ angle probability distributions for from left to right $\Delta T = 1.55K$, 1.15K, 0.95K (for experiment and simulation) and 0.05K (for experiments) and 0.85K (or simulations). Note that the optimized potential at $\Delta T = 0.05K$ also shows the same distribution. On the x-axis is $\theta$ angle and on the y-axis is $\phi$ angle. While there are differences in the angles sampled for experiments and simulation, the main features of the experimental distributions are clearly reproduced in simulations. Far from the critical point linear configurations dominate, indicated by the population $\theta = 180^\circ$ and $\phi = 180^\circ$. As $T_C$ is approached, the population shifts to $\theta = 0^\circ$ and $\phi = 70^\circ$ or $\phi = 110^\circ$. At high temperature experiment and simulation do show exactly the same features, demonstrating that for fully aggregated particles no distinction can be made between temperatures based on the $\theta$-$\phi$ distribution.

The corresponding $g_{md}(r)$ and $g_{\alpha\gamma}(r)$ are presented in Fig. 3.3c and 3.3d. As in the case for the RISM optimization, a reasonable match is found for most temperatures. However, there are also differences with the RISM results, due to the approximations made in the theory. For instance, it seems that the optimized potential is too weak for $\Delta T = 1.55K$ and $\Delta T = 1.35K$. While a very good match is found for higher temperatures for both $g_{md}(r)$ and $g_{\alpha\gamma}(r)$, while in the RISM optimization it is the other way around. Also the second peak in $g_{md}(r)$ is well reproduced at high temperatures. Several features in $g_{\alpha\gamma}(r)$, e.g. near $r = 3.2\mu m$ and $r = 3.9\mu m$, are not well reproduced, possibly because the model potential, although capable of describing the main features of the dumbbell particles, cannot account for every signature in the radial distributions as the particle shape can deviate from the simple modeled one. Moreover, the experimental data set itself is of limited size.
Validation of the optimized potential

To validate the optimized potential, we compare experimental and simulation snapshots as well as the simulation predictions of the angular distributions with experimental ones, and compute the location of the condensation transition with the second virial coefficient approach.

In Fig. 3.4 the angular distributions are shown. The features of the distributions significantly change with temperature. At low temperature, $T = 37.00\,^\circ C$, where the attraction is minimal, the majority of the population is in the top right corner at high $\theta$ and high $\phi$, which corresponds to bonds being formed in a linear orientation. As temperature increases, the degree of aggregation also increases and the population shifts from high $\theta$ and high $\phi$, to two peaks at $\theta = 0^\circ$ and $\phi = 70^\circ$ or $\phi = 110^\circ$, which corresponds to a more closed packed bond formation. The corresponding optimized angular distributions obtained via simulation are also presented. The simulated distributions show the same trend as the experiments, i.e. the population shifts from the linear orientation towards more close packed configurations. The two most right pictures differ in temperature for experiment ($\Delta T = 0.05K$) and simulation ($\Delta T = 0.85K$), but do show exactly the same features. This demonstrates that when the particles are fully aggregated, no distinction can be made between temperatures based on the $\theta$-$\phi$ distribution.

Images from experiments in Fig. 3.5, showing the aggregation of particles at several temperatures, are qualitatively well reproduced by simulation. While no aggregation is observed for temperatures far from $T_C$, particles start to aggregate into clusters as the temperature is increased toward the binary liquid coexistence line. The experimental aggregation temperature is found to be around $\Delta T = 0.95K$, something that is reproduced in the simulations.

Since the optimized potential is relatively short ranged, it is not so easy to predict this onset of aggregation. A simple but effective predictive measure for aggregation is the second virial coefficient, $B_2$ [50, 129]:

$$B_2 = \frac{1}{2} \int dr \int d\Omega (1 - e^{-\beta u_{eff}(r, \Omega)}),$$  \hspace{1cm} (3.22)

where $r$ is now the inter particle vector between centers of mass of two dumbbells, $d\Omega$ denotes the relative orientation of the dumbbells in 3D, and the inter-molecular potential is $u_{db}(r, \Omega) = \sum_{\alpha,\gamma=1}^2 u(r_{\alpha\gamma}(r, \Omega))$, with $u(r_{\alpha\gamma})$ the effective site-site pair potential, and $r_{\alpha\gamma}$ the distance between the centers of mass of the dumbbell spheres $\alpha$ and $\gamma$ each on different dumbbells. Positioning one of the particles in the origin with its intramolecular vector along the $z$-axis, the integration is thus over all positions of orientations of the second dumbbell. Of special importance is the reduced second virial coefficient, $B^*_2 = B_2/B^H_{2DB}$ where $B^H_{2DB}$ is the hard dumbbell fluid which is known exactly [126]. This reduced second virial coefficient provides a rough indication of the aggregation behavior. When $B^*_2$ becomes negative, particles begin to attract each other. When $B^*_2 < -1.5$ it indicates a transition to a condensed phase [50, 129]. In Fig. 3.6 the numerically integrated $B_2^*$ for the optimized potential is shown. $B_2^*$ becomes negative around $\Delta T = 1.0K$.
and smaller than $-1.5$ below $\Delta T = 0.95\text{K}$ which is indeed the aggregation temperature in experiments. Further decrease of $\Delta T$ causes a fast drop of the $B_2^*$ indicating that the system is undergoing a condensation/aggregation transition in this temperature region.

Figure 3.5: Snapshots of (a) the experiment and (b) simulation for from left to right $\Delta T = 1.55$, 1.15, 0.95 and 0.55K, demonstrating that the correct morphology for every temperature is obtained.

Figure 3.6: Numerical calculation of $B_2^* = B_2/B_2^{HDB}$ for the optimized potential. The $B_2^*$ becomes negative around $\Delta T = 0.95\text{K}$ which is also the aggregation temperature in experiments. Further decrease of $\Delta T$ causes a fast drop of the $B_2^*$ indicating that the experiments are very close to the fluid transition in this region.

**Collapse from network to compact state**

Critical Casimir forces can clearly induce aggregation between anisotropically shaped colloids. Although this aggregation is in principle reversible with temper-
Figure 3.7: (a) Optimized potential for $\Delta T$ close to $T_C$, $\Delta T = 0.2K$ (blue), 0.05K (yellow), 0.03K (red), 0.01K (purple), showing that due to the pre-factor in Eq. 3.4 the potential becomes very long ranged and flatter for small $\Delta T$, leading to the particles becoming more motile. Inset shows how the potential minimum increases close to $T_C$. (b) Relaxation of the total energy divided by minimum of attractive well over time for $\Delta T = 0.01K$ (red), 0.02K (orange), 0.04K (cyan) and 0.3K (blue) starting from five different trapped network structures. (c) Sudden increase close to $T_C$ in average $E_{\text{tot}}(U_{\text{min}}N)^{-1}$ at the final time-step for each $\Delta T$ which is a measure for the number of interactions per dumbbell. (d) Slope of $E_{\text{tot}}$ divided by $U_{\text{min}}$ over time demonstrating that relaxation of energy is significantly higher close to the critical point.

ature, the morphology of the final structure still highly depends on the protocol of the experiment. When a temperature ramp is used that increases the temperature from $\Delta T = 5.0K$ in a short time-frame to $\Delta T = 0.5K$, thus inducing a strong attraction, the resulting structure can become (partially) kinetically trapped in a metastable state from which it is usually very difficult to escape. In general one can anneal such a trapped state by weakening the attraction again (assuming the attraction is reversible in practice) so that the particles unbind again and the system can escape from the metastable trapped state.

While the range of the Casimir attraction monotonically increases upon approaching the coexistence temperature, due to the functional form of Eq. 3.4 the minimum of the potential does not behave monotonically with temperature, and in fact reaches a maximum depth before becoming more shallow again (see the inset of Fig. 3.7a ). We argue that this particular feature of the potential leads to
Figure 3.8: (a) Series of snapshots for a network-like structure close to the critical point. From left to right, we can see the structure relaxing towards a more compact structure facilitated by the long range nature of the Casimir force. (b) Series of snapshots for a network like structure from simulations at $\Delta T = 0.01K$ modelling the collapse seen in experiments. Note that in experiments the collapse is observed at 0.05K. However, the temperature sensitivity is also on this order. Clearly, the structure fully collapses when the correlation length diverges at $\Delta T = 0.01K$. At this point the range of the Casimir attraction is long enough such that the particles globally find their minimum. If the correlation length remains small, the relaxation is not observed on this time-scale.

The observed experimental behavior of a structural collapse for temperatures close to the coexistence line. In Fig. 3.8 a sequence of snapshots is shown of such a collapse transition, from an extended network to a more compact structure. The system relaxes over several minutes, where the initial configuration on the left is stuck in a metastable state with more linear chain-like structures. For temperatures very close to $T_c$, ($\Delta T \approx 0.05K$), the structure collapses towards a more compact structure as shown on the right in Fig. 3.8.

We can model this collapse transition by preparing a non-equilibrium network like structure using a diffusion limited aggregation scheme (see Methods), in which we only perform MC cluster moves which are always accepted provided there is no hard core overlap. A cluster is defined as a set of contiguous particles, where we consider two dumbbells to be contiguous when any two spheres of the dumbbell are closer than $0.2R$. Starting from a random gas like configuration, this procedure quickly results in a network structure in which the particles have not been allowed to equilibrate due to the fact that only cluster moves are used. Subsequently, this network structure is quenched into a local energy minimum dictated by the
optimized pair potential at $\Delta T = 0.5K$, employing only local single particle MC moves with small translation and rotation step sizes, such that the system becomes truly trapped in this network structure (see Fig. 3.8). Repeating this procedure five times generates five independent initial structures which are then relaxed via single particle translation and rotation MC moves with small step sizes ($\delta_t = 0.02R$, $\delta_r = 0.03$ rad) for $10^7$ MC cycles, where every MC cycle has $N_{\text{part}}$ translation and rotation moves. This roughly corresponds to an experimental relaxation of several minutes [94]. Snapshots of the resulting collapse structures are shown in Fig. 3.8.

The analysis of these simulations is summarized in Fig. 3.7. Fig. 3.7b. shows the relaxation of the total energy versus simulation time for several values of $\Delta T$. It is clear that when the attraction is still short ranged, the potential does not allow equilibration of this structure to a more compact configuration, because the dumbbells only interact with their local neighborhood. However, as the correlation length quickly increases at very small $\Delta T$, the pair interaction also becomes longer ranged. Therefore, the particles have more ‘wiggle’ room within the potential well, allowing the Casimir force to induce a full collapse of the structure, thereby lowering the energy of the system significantly. The energy per particle normalized to the minimum ($E_{\text{tot}}/(U_{\text{min}}N)^{-1}$), is plotted in Fig. 3.7c and clearly shows that the structure collapses only for low $\Delta T$, and thus for longer ranged attractions at small $\Delta T$, a particle thus effectively interacts with more neighbors. The relaxation of the potential energy over time is shown in Fig. 3.7d, also demonstrating that indeed the energy relaxes faster for small $\Delta T$.

### 3.4 Conclusions

We have demonstrated that the experimentally observed Casimir induced self-assembly of colloidal dumbbells [112] can be modeled with a relatively simple potential form. We optimized this potential to reproduce both the minimum distance and site-site radial distribution function. The good agreement between simulation and experimentally observed radial and angular distributions and structures suggests that the effective pair potential sufficiently accurately describes the interaction between colloidal dumbbells, at least for short-ranged potentials. For temperatures very close to the coexistence line, where the correlation length increases drastically, probably an effective pair potential is insufficient to describe the interaction as many body effects become important. Additionally, we have shown that the divergence of the correlation length, together with the weakening of the potential depth is able to explain the experimentally observed collapse of locally stuck network structures into more compact configurations. These results are quite robust as in fact it only requires that the attractive force becomes shallower when the range of interaction increases, which makes the finding more general. The universal nature of the Casimir force offers opportunities for other anisotropic colloidal systems. Future prospects entail the further modification of the particle shape and physio-chemical surface properties to induce more complex structure formation.
Rotational diffusion affects the self-assembly pathways of patchy particles

Predicting the kinetics of the self-assembly of particles with anisotropic interactions, for instance, colloidal patchy particles or proteins with multiple binding sites, is important for the design of novel high-tech materials as well as for understanding biological systems such as viruses or regulatory networks. Often stochastic in nature, such self-assembly processes are fundamentally governed by rotational and translational diffusion. Whereas the rotational diffusion constant of particles is usually considered to be coupled to the translational diffusion via the Stokes-Einstein relation, in the past decade it has become clear that they can be independently altered by e.g. molecular crowding agents or via external fields. Because virus capsids naturally assemble in crowded environments such as the cell cytoplasm but also in aqueous solution in vitro, it is important to investigate how varying the rotational diffusion with respect to translational diffusion alters the kinetic pathways of self-assembly. Using recently developed path-sampling techniques, we can sample and analyze the entire self-assembly kinetic network of simple patchy particle systems. For assembly of a designed cluster of patchy particles we find that changing the rotational diffusion does not change the equilibrium constants, but significantly affects the dynamical pathways, and enhanced (suppresses) the overall relaxation process and the yield of the target structure, by avoiding (encountering) frustrated states. Besides insight, this finding provides a design principle for improved control of nanoparticle self-assembly.
4.1 Introduction

In nature, self-assembled complex structures and networks often provide function. Prime examples are virus capsides, where capsomer proteins with specific interaction sites self-assemble into various structures, such as icosahedrons and decahedrons. Protein complexes can also spontaneously form in the living cell, e.g. in signal transduction networks. Self-assembly of small designed building blocks also can provide novel (bio)materials with desired properties. Such building blocks can consist of proteins, synthetic polypeptides, but also of colloidal particles. Particularly, the advent of novel synthesis routes for colloidal particles with a valence, so called “patchy particles” opened up avenues for designing colloidal superstructures. Numerous experimental, theoretical and numerical studies have enabled understanding the phase behaviour of these particles, predicting not only interesting building blocks for new functional materials, but also demonstrating new physics [4, 13, 55, 68].

Design principles for colloidal superstructures can predict which structure is the most thermodynamically favorable state [59]. However, the fact that kinetics often trumps thermodynamics can hamper such design of colloidal superstructures. Strong directional binding and slow dissociation can kinetically trap patchy particle systems in a malformed state, rendering it unable to reach the designed equilibrium (ground) state [5]. Controlling the self-assembly of colloidal particles thus requires understanding how the system evolves towards equilibrium, which is dictated by the kinetic network between all the states the system can occur in. Several studies have demonstrated that the assembly towards the final ground state is affected by changing the interaction between patchy particles [70, 130], which affects both the thermodynamics and kinetics of the system. In contrast, here we study how the pathways change upon changing the dynamics only. Colloidal dynamics is often stochastic, and well described by over-damped Langevin (Brownian) dynamics. The rotational and translational diffusion constant of anisotropic particles is under standard conditions coupled via the Stokes-Einstein relation. However, in environments with high molecular crowding or in external fields the Stokes-Einstein relation is not necessarily valid anymore [131-133]. Depending on the molecular crowder, the ratio between the rotational and translation diffusion constant can go up or down. In this work we investigate how varying this ratio influences the equilibrium kinetic network for small self-assembled clusters of colloidal patchy particles. Such particles provide a simple model for self-assembly protein complexes such as in viruses or signal-transduction networks. Understanding and prediction of the colloidal self-assembly mechanisms requires the rate constants and pathways for all possible dissociation and association events in the kinetic network. However, on the time-scale of the dynamics of the microscopic particles, binding and certainly dissociation processes are usually rare events due to high free energy barriers caused by strong directional binding. As straightforward dynamical simulation is extremely inefficient, we employed the Single Replica Transition Interface Sampling (SRTIS) algorithm to collect all possible (un)binding trajectory ensembles relevant to the patchy colloid assembly [104]. Surprisingly, even for
the dimerization of a 1-patch particle we already find an effect of the rotation on the formation dynamics. Next, we investigate a dimerization of 2-patch particles, which exhibits an intermediate state and multiple pathways of formation. However, the effect of the rotation becomes truly important if metastable intermediates are possible, such as in tetrahedron formation of a 3-patch particle. Here we find that varying rotational diffusion favors one pathway over the other, without changing the equilibrium constants. Finally, we investigate the entire nine-state kinetic network of a tetrahedron cluster, and show that a change in the rotational diffusion shifts the preferred self-assembly pathways significantly. While for low rotational diffusion the overall rate constant of tetrahedron formation decreased, frustrated states are avoided, leading to significantly less kinetic trapping. Controlling the kinetic network would offer an extra control in the design of new self-assembled functional materials. Including the interplay between rotational and translational diffusion in the self-assembly design of new supra-colloidal structures could open up new opportunities for controlling the bottom-up synthesis of functional materials. Moreover, this work help to understand how rotational diffusion influences self-assembly processes in naturally occurring crowded environments such as the biological cell. As it has been established that the crowded environment in a cytoplasm decreases the ratio of rotational diffusion over the translation diffusion [133], our simulations provide an additional explanation why protein complex assembly does not suffer more from kinetic trapping as one would naively expect.

4.2 Methods and Simulation details

Model Patchy Particles

We model the particles as patchy hard spheres with diameter $\sigma$. For a center-to-center distance, $\sigma < R_{ij} < 2\sigma$, the patch potential is:

$$u_{ij}(R_{ij}, \Omega) = -\epsilon \sum_{p_{ij}} e^{-kr_{ij}} e^{-(\theta_i^2+\theta_j^2)/w^2}$$  (4.1)

where $\Omega$ denotes the orientations of the particles, $\sum_{p_{ij}}$ is the sum over all patch pairs, $k$ controls the range of the interaction, $r_{ij}$ is the distance between patch centres, $\theta_\alpha$ is the angle between patch vector of particle $\alpha$ and the vector connecting the centre of $\alpha$ and the centre of the patch on the other particle and $w$ is a parameter that controls the patch width (see Fig.4.2). Note that the arccosine is needed for the angle calculation. As the patch widths in this work are all small, we used an approximation for the arccosine in order to speed up the simulation significantly. The model in this chapter is different than in chapter 5 and 6. This is to accommodate the need for multiple bonds between particle pairs in section 4.4. In our simulations we used Dynamic Monte Carlo (DMC) to evolve the system in time via translational and rotational MC moves, as described in chapter 2.
Simulation and SRTIS settings

One-patch dimer. Two hard spheres of radius $\sigma$ are each decorated with one patch of range $k^{-1} = 4.0\sigma$ and width $w = 0.7$. Note that for this value of $w$, the potential depth $\epsilon = 20k_B T$ has to be sufficiently high for a stable dimer. The dimer is put in a periodic cubic box of size $5\sigma$, which sufficiently stabilizes the unbound state. Only two different states are defined, the bound state ($B$), when the energy of the system, $E_{\text{sys}} = \sum_{i<j} u_{ij}$, is lower than $-12k_B T$, and the unbound state ($U$), when the particles are separated more than $2\sigma$, which is also where we truncate the potential to zero. For the bound state we choose the first interface at the stable state definition and the rest of the interfaces are separated by $3k_B T$ until maximum energy is reached. For the unbound state it is necessary to define many interfaces close to the zero point energy: $\{0, 0, 10^{-14}, 10^{-12}, 10^{-10}, 10^{-8}, 10^{-6}, 10^{-4}, 10^{-2}\}$, to bias the system towards $B$ also when the particles are not properly aligned.

Two-patch dimer. The potential parameters are identical to one patch case, except that the patch width is decreased ($w = 0.1$) to ensure the two patches do not overlap. The state and interface definition are identical to the one-path system, except more interfaces surround the bound state, since the bound state is lower in energy. Tetramer. Model parameters for this system are $\epsilon = 12k_B T$, $w = 0.1$ and $k = 4.0\sigma^{-1}$. To decrease the diffusive pathway for the four particles, we altered the box size for this system to $4.0\sigma$. The bound state is defined as $E_{\text{sys}} < -20k_B T$, with three correct bonds formed, and the intermediate state as $E_{\text{sys}} < -10k_B T$, with two bonds formed in a frustrated state. Again, we use the system energy as the order parameter for the interfaces. The interfaces for each state are separated by $3k_B T$ starting from their minimum energy. The same interfaces and state definition for the unbound state as before are used. Each state state is recognized according to their appropriate topology defined by their bonds.

4.3 Dimer of one patch particles

Two hard spheres of radius $\sigma$ with a single, relatively narrow, attractive patch, analogous to a binding site on a globular protein, can form a dimer. Although, due to the simplicity of the system, no energetically frustrated trap can exist, it is insightful to understand whether and how rotational diffusion can change the dynamical pathway taken during dissociation or association. We sample the path ensemble for this two-state binding process with SRTIS. The interfaces around stable states are defined by the energy of the system. We construct the free energy landscape, as well as the (un)binding rate constants, see Eqs. 2.50 and 2.40. Fig. 4.2 shows the free energy as a function of the distance between the particles, $R_{12}$, and the sum of the angles $\phi = \phi_1 + \phi_2$, with $\phi_{1,2}$ the angle between the patch vectors and the inter particle vector $\vec{R}_{12}$. The bound state is the free energy minimum at $\phi = 0$ and $R_{12} = \sigma$. At larger distances, the orientational part of the free energy becomes symmetric with respect to $\phi$, indicating particles can freely rotate in the unbound state where the interaction vanishes. Between these minima
Figure 4.1: Cartoon of the patchy dimer with parameters used in the potential, and order parameters used for the free energy and reactive path density plots.

| \( f_{SE} = 0.1 \) | \( \langle \phi_{0I} \rangle \) | \( P(\lambda_m|\lambda_{1I}) \) | \( P(\lambda_{0I}|\lambda_m) \) | \( k_{IJ} \) |
|---|---|---|---|---|
| B | 3.58_1 \times 10^{-3} | 4.03 \times 10^{-5} | 0.223_0 | 3.31 \times 10^{-8} |
| U | 1.29_1 \times 10^{-3} | 3.58 \times 10^{-2} | 0.0059_1 | 3.01 \times 10^{-7} |

| \( f_{SE} = 1.0 \) | \( \langle \phi_{0I} \rangle \) | \( P(\lambda_m|\lambda_{1I}) \) | \( P(\lambda_{0I}|\lambda_m) \) | \( k_{IJ} \) |
|---|---|---|---|---|
| B | 5.22_4 \times 10^{-3} | 8.862 \times 10^{-5} | 0.216_1 | 1.001 \times 10^{-7} |
| U | 1.312_9 \times 10^{-3} | 4.516 \times 10^{-2} | 0.015_3 | 9.41 \times 10^{-7} |

| \( f_{SE} = 10.0 \) | \( \langle \phi_{0I} \rangle \) | \( P(\lambda_m|\lambda_{1I}) \) | \( P(\lambda_{0I}|\lambda_m) \) | \( k_{IJ} \) |
|---|---|---|---|---|
| B | 1.669_1 \times 10^{-2} | 1.508 \times 10^{-4} | 0.043_3 | 5.067 \times 10^{-7} |
| U | 1.47_2 \times 10^{-3} | 5.100 \times 10^{-2} | 0.039_3 | 5.059 \times 10^{-6} |

Table 4.1: Flux, crossing probabilities and rate constants TIS one patch dimer assembly in MC cycle units

an (entropic) binding free energy barrier is visible. Naturally, the free energy landscape does not change with the rotational diffusion as it solely depends on the interactions between the particles. In contrast, varying the rotation diffusion ratio \( f_{SE} = D_0^s \sigma^2/3D_t^0 \) significantly alters the dynamical pathways between B to U. Fig. 4.2 visualizes the projection of these dynamical paths, i.e. the reactive path density \( n_r(q) \), see Eq. 2.51 in the \( R_{12}, \phi \) plane. The path density broadens for higher rotational diffusion, and significantly changes shape, as indicated by the red curve of maximum path density connecting U to B. The reactive path density also yields the orientational escape distribution as a function of \( \phi \) at the boundary of the unbound state (see Fig. 4.2). This distribution indicates the probability of orientation at dissociation, and is skewed to lower values of \( \phi \) for low rotational diffusion, while becoming symmetric for high rotational diffusion. The enhancement at \( \phi = \pi \) is due to the projection. The change in reactive pathways is also visible in the reactive current (see Fig. 4.2) which gives the average velocity
Rotational diffusion affects self-assembly

Figure 4.2: The free energy (top left), reactive path densities (top right), escape distribution (bottom left) and reactive currents (bottom right) for the one patch particle system for different values of $f_{SE}$ as a function of the distance between particles, $R_{12}$, and the angle $\phi = \phi_1 + \phi_2$, with $\phi_{1,2}$ the angles between the patch vectors and the inter-particle vector. The binding pathway clearly changes with rotational diffusion constant, from more reactive pathways via translation for slow rotational diffusion toward reactive pathways more via rotation for fast rotational diffusion, without changing the free-energy landscape.

...at which the particles move along the reactive pathways, and hence does not contain off-pathway excursions. Both the reactive path density and the reactive current reveal that for low $f_{SE}$, i.e. slow rotational diffusion, the particles follow a straight pathway along the radial coordinate. For higher $f_{SE}$, and faster rotational diffusion, they follow a more curved association route from the unbound state into the attractive well. Note that due to microscopic reversibility, dissociation pathways are the reverse of the association trajectories. The difference in behavior can be understood by realizing that particles need to be aligned for binding. In the case of slow rotational diffusion, particles that are not aligned when close together will diffuse away transitionally before binding can occur.

The rate constants are given by $k_{IJ} = \phi_I P(\lambda_{0,I} | \lambda_{1,I}) = \phi_I P(\lambda_{m,I} | \lambda_{1,I}) P(\lambda_{0,I} | \lambda_{m,I})$, where $\phi_I$ is the flux out of state $I$, and $P(\lambda_{m,I} | \lambda_{1,I})$ and $P(\lambda_{0,I} | \lambda_{m,I})$ are crossing probabilities defined in section 2.5. The measured fluxes out of the stable states, the crossing probabilities and the rate constants are given in table 4.1. Increasing rotational diffusion by two orders of magnitude, enhances the absolute rate constants by a factor ten. Nevertheless, the ratio between the rate constants does not change, as this is determined by thermodynamics. Note that while the bond energy is $20k_B T$, the ratio $k_{BU}/k_{UB} \approx 0.1$ indicating that $U$ is still relatively...
stable with respect to $B$. Of course, this is also dependent on the width of the attractive patch and the simulation volume (concentration of the colloids).

### 4.4 Dimer of two patch particles

We subsequently performed SRTIS of dimer formation for particles with two patches, where association occurs via a multiple step mechanism. Figure 4.3 shows the free energy and reactive path density as a function of the bond distances, $r_{1,2}$ (the bonds are identified in the bound state). This projection yields a more detailed assembly picture. Again, the free energy is independent of $f_{SE}$ and shows a minimum at $r_1 = r_2 = 0$. Here the two particles do not concertedly assemble into the dimer state, but first form one bond, followed by a rotation around this bond into the dimer state. The two peaks near the dimer state around $r_{12} = 0.25$ in the reactive path density indicate both dimer permutations have been sampled. Note
that the low path density at the origin is caused by the truncation of the paths as they reach the bound state. Increasing the rotational diffusion slight changes the reactive path density toward a broader single-bond peak, as the requirement for alignment is relaxed, and hence 'concertedness' decreases. To summarize, for fast rotational diffusion misalignment will still lead to binding, whereas less so for slow rotational diffusion.

4.5 **Tetrahedron assembly**

To understand the effect of rotational diffusion on a more complex kinetic network, we study the formation of a tetrahedron consisting of particles with three patches. The patch vectors are set at an angle of $60^\circ$ w.r.t each other, so that the patches form an equilateral triangle on the surface of the particle. The ground state of this system is a tetrahedron. As a first step, we study the final transition in the self-assembling process, namely the addition of the fourth particle to a preformed trimer cluster, while keeping the trimer constrained.

Besides the bound state $B$ and unbound state $U$, there is now also an intermediate state $I$ where the fourth particle 'wrongly' binds with two bonds to the trimer. To escape from this frustrated intermediate state, a bond has to be broken before the complete tetrahedron can form. We study how the kinetic network between these three states changes when the rotational diffusion is changed. The rate constant calculation is more intricate than the simple dimer system, see Eq. 

![Figure 4.4: Population ratio for the intermediate state over the unbound state, as the system relaxes starting from a fully populated bound state, for different rotational diffusion constants. It is clear that for fast rotational diffusion, the intermediate state is populated relatively more than for slow rotational diffusion. For all rotational diffusion constants the system equilibrates towards the same thermodynamic state.](image)
The inset in Figure 4.4 shows the kinetic network. We per-

Figure 4.5: Top: Net flux graph for the tetrahedron assembly. The arrow size indicates the relative magnitude of the flux. For faster rotational diffusion, the system avoids the trimer state \( Tr \) more than for slow diffusion, and follows a more frustrated pathway via states \( T_f1, T_f2 \) and \( T_f3 \), an extension of the principle shown for the constrained tetrahedron system. Middle: cartoon images of all states defined except for the unbound state. Bottom left: Population ratio for the trimer state, \( Tr \), over the frustrated tetramer state, \( T_f2 \), as the system relaxes starting from a fully unbound state for different rotational diffusion constants, demonstrating how the system equilibrates during assembly. For fast rotational diffusion the trimer state is more populated during the equilibration. For all rotational diffusion constants the system equilibrates towards the same thermodynamic state. Bottom right: Committors to \( T_d \), \( q_i^+ \), for all intermediate states as a function of the rotational diffusion factor, \( f_{SE} \). The committor for the frustrated tetramers decreases with decreasing \( f_{SE} \). In contrast, the committor of \( Tr \) increases.
Rotational diffusion affects self-assembly

formed SRTIS and computed the rate matrix $K$ for different values of $f_{SE}$, and computed the time evolution of the population by $p(t) = p(0)e^{-Kt}$. To show how $f_{SE}$ influences the assembly pathway we consider the ratio $P_I/P_U$ of the intermediate state population over the unbound state. Figure 4.4 shows this ratio as a function of time, for a system initially in the bound state. While all curves decay eventually to the same equilibrium population, at short times the population ratio $P_I/P_U$ scales roughly linearly with the rotational diffusion ratio $f_{SE}$. Rotational diffusion thus significantly influences the pathways for self-assembly, or in this case disassembly.

Next, we examine the self-assembly of four free particles into a tetrahedron. We define a set of 9 states: {Unbound state ($U$), one dimer ($D_1$), two dimers ($D_2$), trimer ($Tr$), frustrated trimer ($Tr_f$), frustrated tetramer states ($T_{f1}$, $T_{f2}$, $T_{f3}$) and the fully assembled tetrahedron state ($T_d$)} (see Figure 4.5 for graphical representations). Again we do not define states that can transit to one of the nine defined states by barrier-less rotation around bonds. Performing a SRTIS simulation results in a complete rate matrix $K$. Applying transition path theory to the computed rate matrices results in flux matrices, as well as committors for each state for the transition from $U$ to $T_d$, see Eq. 2.55 \[110\]. Fig. 4.5 shows a graphical summary of this information for different values of $f_{SE}$. While the net flux generally decreases with decreasing rotational diffusion, the thickness of arrows correspond to the normalized net flux, in order to emphasize the differences in self-assembly pathways taken. For slow rotational diffusion $f_{SE} = 0.1$, the $U - D - Tr - T_d$ pathway carries most of the flux. For higher $f_{SE}$, the $Tr$ state is avoided, as was the case in the constrained tetrahedron system. Therefore, at high rotational diffusion, the transition between $U$ and $T_d$ occurs preferably via frustrated states, $T_{f1}$, $T_{f2}$ and $T_{f3}$, which are then more accessible. Transitions along $Tr_f$ are favourable, even for low $f_{SE}$, probably due to combinatorial entropy since there are more ways to (dis)assemble into $Tr_f$ than into $Tr$. Another way to demonstrate the influence of rotational diffusion on the assembly is to show the population dynamics of the trimer state, $Tr$, over the frustrated tetramer state, $T_{f2}$ (see Fig. 4.5). The population of the trapped intermediate state $T_{f2}$ is initially significantly less populated for slow than fast rotational diffusion, indicating that indeed for assembly with fast rotational diffusion particles aggregate more easily towards frustrated states. Indeed, this also implies that for larger systems beyond the tetramer the frustrated states will lead to an even stronger trapping effect. We show that the correctly formed tetrahedron pathway dominates for slow rotational diffusion by analyzing the rate matrices using such strong traps.

In Fig. 4.5 the committor $q^+_i$, defined as the probability that from state $i$ state $T_d$ will be reached before reaching $U$, is shown as function of $f_{SE}$. The committor for the frustrated tetramers decreases with decreasing $f_{SE}$. In contrast, the probability for $Tr$ increases, indicating again that for slow rotational diffusion a less frustrated pathway is followed.
Population dynamics of a trapped system

In principle, the gain in relative flux towards correctly formed structures should result in a better yield of the designed ground state, and in less kinetic trapping. While in our examples the intermediate states are not truly kinetic traps, we envision that for more complex target structures, which compete against completely disordered aggregates, the price paid for slowing down the dynamics is outweighed by taking the correct self-assembly route and could lead to higher relative yields. We tried to simulate this situation with our measured rate matrices to clarify this line of reasoning. To model kinetic trapping we have changed the rate matrix such that every tetramer state is a sink, where no population can escape from. This simulates the situation in which reaching a malformed tetramer state will catastrophically end up in a disordered aggregate, or adversely that a properly formed tetrahedron will not disassemble again. Additionally, we set that the only correct binding pathway is via $Tr$, so that there is only one good assembly pathway. We can clearly see in Fig. 4.6 that indeed the relative population of the correctly formed tetrahedron versus all other tetramer states increases with decreasing rotational diffusion. Note that now the populations will not end up at the same value, because population gets lost in the trapped/frustrated Tetramer states.

Figure 4.6: An effort to replicate kinetic trapping due to frustrated states by performing population dynamics with the computed rate matrices, however, with all tetramer states as sinks. Here we show the relative population of the fully formed tetrahedron versus the sum of populations of the frustrated states $T_f$. The yield of $T_d$ increases for slower rotational diffusion.
4.6 Analysis of simple master equation model

To illustrate how our main finding extends to large systems we have analyzed a simple abstract model using a master equation population analysis. This abstract model consists of a number of states, shown in Fig. 4.7 in which the first digit in the index of the states denotes the stability index, e.g. in terms of bonds between particles, while the second digit denotes the frustration index, e.g. the number misaligned bonds. We assume that the initial state 11 can jump to state 21, which can then become frustrated, or misaligned, by jumping to state 22. The rate constants $k^+_{tt}$ and $k^-_{tt}$ denote, respectively, the associating and dissociation rate constants, for making such particle bonds. The $k^+_{tr}$ and $k^-_{tr}$ denote the rotational move that causes misalignment of bonds. We set $k^-_{tr} = 0.1k^+_{tr}$, to mimic that the number of possible frustrated states is increasing with the number of misaligned bonds. We choose $k^-_{tr} = 0.01k^+_{tr}$, such that the assembly into the ground-state 51 is favorable with respect to the trapped state. Figure 4.7 shows a maximum of five bonded particles, but of course this model can handle any number of bonds in the system, by simply extending this figure. We solve the corresponding master equation of this model for two settings: $k^+_{tr} = k^-_{tr} = 10^{-5}$ corresponding of fast rotation dynamics, and $k^+_{tr} = 10^{-6}$ and $k^-_{tr} = 10^{-5}$, corresponding to slow rotation dynamics. We monitor the ratio of population of the final state with respect to all other states $p_{final}/(1 - p_{final})$. This ratio is shown in Fig. 4.8 for the slow and fast diffusion case for a model with 15 bonded states, i.e.. 15 particles

![Diagram of abstract simple model](image_url)
in the assembly. Clearly, the slow diffusion case rises much more quickly than the fast. This is because the direct route, without frustration is preferred to the trapped state, since the moves into the frustrated states are less likely. We can also look at the relative increase of the slow rotational diffusion ratio versus the fast rotational diffusion ratio. This is shown in the right panel of the Fig. 4.8. The yield of the final state for slow \( k_r^+ \) is more than a 100 times larger than for the fast rotational diffusion. This ratio only becomes larger for assembly of a larger number of particles. The increased yield eventually decreases again, because the system with fast rotational diffusion will eventually relax to the equilibrium state, where the yields should be equal. This simple analysis shows that our finding is generic and can be extended to larger systems.

4.7 Conclusion

Our path-sampling simulations demonstrate the proof-of-concept that altering the rotational diffusion can affect the kinetic network and hence the mechanism of self-assembly for simple patchy particle systems. For the dimer system of one-patch and two-patch particles the mechanism of reactive pathways changes significantly with rotational diffusion, from a translational reactive pathway for slow rotational diffusion, to a rotational reactive pathway for fast rotational diffusion. The pathways do not need to follow the free energy landscape, and avoid the saddle point when the dynamics are changed. In the assembly of a constrained tetrahedron of four particles decorated with three patches, faster rotational diffusion increases the likelihood of a route via the intermediate state. This effect is even stronger for the full tetrahedron assembly, where the frustrated states and kinetic traps are avoided when rotational diffusion is suppressed. We argue that these results hold beyond the tetramer, and can be seen as a generic effect. We support this argument by analyzing the population dynamics of a simple Markov model that
mimics a much larger assembly process. This analysis clearly shows that slower rotational diffusion results in less frustration and in orders of magnitude higher yields. This generalization makes our finding into a genuine novel principle for controlling and understanding complex self-assembly kinetics.

Appendix

Crossing probability one patch system

In Fig. 4.9 we show the crossing probability as a function of the order parameter used in the TIS simulations, the energy of the system, for both the bound and unbound state for the one patch system described in the main text. Clearly, the crossing probability of $B$ and $U$ for low rotational diffusion is always lower than for fast rotational diffusion. The points are the Wang-Landau density of paths, $\log g(\lambda_I)$, which have become equal to the crossing probability. There is a steep drop at low energies for the crossing probability of $U$ after which $P(\lambda)$ only slowly decays with decreasing energy, which makes it necessary to set interfaces at low values of the energy.

![Crossing probability One Patch dimer format](image)

Figure 4.9: Left: the logarithm of the crossing probability as a function of the energy of the system for both $B$ (solid line) and $U$ (dashed line) for the one patch system described in the main text for $f_{SE} = 0.1$ (red), 1.0 (green) and 10 (blue) after a converged TIS simulation. Right: the same crossing probability for $U$ now versus $\log \lambda = \log(-E)$. The points are the Wang-Landau density of paths, $\log g(\lambda_I)$, which are equal to the crossing probability.

Typical diffusive paths for the one-patch dimer

In Fig. 4.10 we depict typical diffusive paths from the path ensemble on top of their respective reactive path density for $f_{SE} = 0.1, 1.0$ and 10. Note that for low rotational diffusion the path is mostly sampling horizontal (translational axis), while for the high rotational diffusion the paths are exploring much more in a vertically (rotation axis), as expected. For these high values of $f_{SE}$ many rotations of the particle pair can be made in one path before binding.
Figure 4.10: The reactive path densities for the one patch particle system for \( f_{SE} = 0.1, 1.0 \) and 10 (left to right) as a function of the distance between the particles, \( R_{12} \), and the vector inner product between the patch vectors of both particles, \( \phi \). A typical diffusive path is shown on top of the density plots.

**Overall rate constants for the full tetrahedron assembly**

The overall forward and backward rate constants for the transition between the bound tetrahedron \( T_d \) and the fully unbound state \( U \) as computed from TPT according to Eq. 2.57 are shown in Fig. 4.11. Note that both forward and backward rate constants scale roughly linearly with \( f_{SE} \), such that the ratio of the two stays constant.

Figure 4.11: The overall rate constants from the fully assembled tetrahedron to the unbound state (top) and from the unbound state to the fully assembled tetrahedron (bottom) as a function of rotational diffusion factor \( f_{SE} \).

**Scaling with the unbound state dwell time**

Decreasing the rotational diffusion via \( f_{SE} \) does increase the timescale of all relaxation processes (see Fig. 4.12). Also the unbound state dwell time increases. We can therefore investigate whether the overall assembly relaxation times trivially depends on this unbound state dwell time. In Fig. 4.12 we show the population of the tetrahedron state with the time axis rescaled by the time spent in the unbound state.
state. When all relaxation processes in the assembly scale in the same way, all curves should collapse on top of each other. This is clearly not the case. In fact,

![Figure 4.12](image)

Figure 4.12: Left: population of the fully formed tetrahedron with respect to the equilibrium population. Right: Relative population of the fully formed tetrahedron when the time-step is rescaled with the time spent in the unbound state.

the ordering of the curves remains the same. We can understand this by realizing that the timescale in the unbound state is dominated by the translational diffusion, and less so by rotation. Changing the rotational diffusion thus has some effect on the unbound timescale, but not dramatically so.

### Time step set by rotational diffusion constant

When the rotational diffusion is lowered by decreasing $f_{SE}$, the overall dynamics naturally also slows down (see Fig. 4.12). However, as we could also have changed the translational diffusion instead of the rotational diffusion, we can take the same data and scale the rate constants such that the time-step is now set by the rotational diffusion constant. As Fig. 4.13 clearly shows, the tetrahedron population grows fastest when the translational is faster than the rotation.

![Figure 4.13](image)

Figure 4.13: Population of the fully formed tetrahedron when the time-step is set by the rotational diffusion constant. The tetrahedron population grows fastest when the translation is faster than the rotation.
5 The role of multivalency in the self-assembly kinetics of patchy particle complexes

Association and dissociation of particles are elementary steps of many natural and technological relevant processes. For many of such processes the presence of multiple binding sites is essential. For instance, protein complexes and regular structures such as virus shells are formed from elementary building blocks with multiple binding sites. Here we address a fundamental question concerning the role of multivalency of binding sites in the association kinetics of such complexes. Using single replica transition interface sampling simulations, we investigate the influence of the multivalency on the binding kinetics and the association mechanism of patchy particles that form polyhedral clusters. When the individual bond strength is fixed, the kinetics naturally is very dependent on the multivalency, with dissociation rate constants exponentially decreasing with the number of bonds. In contrast, we find that when the total bond energy per particle is kept constant association and dissociation rate constants turn out rather independent of multivalency, although of course very dependent on the total energy. The association and dissociation mechanism, however, depends on the presence and nature of the intermediate states. For instance, pathways that visit intermediate states are less prevalent for particles with five binding sites compared to the case of particles with only three bonds. The presence of intermediate states can lead to kinetic trapping, and malformed aggregates. We discuss implications for natural forming complexes such as virus shells and for the design of artificial colloidal patchy particles.
5.1 Introduction

Particles with multiple binding sites are ubiquitous in natural and synthetic systems. Proteins naturally form complexes by binding via multiple sites, virus shells are assembled from capsid proteins with multiple interactions, and nanostructures can be built from multivalent patchy particles. Indeed, breakthroughs in colloid synthesis enable colloids to bind anisotropically via multiple binding sites or patches [4, 5, 16, 17]. Such multivalent patchy colloids can form not only crystal phases, but also lead to chains, lamellar structures, colloidal micellar structures and even colloidal hollow shells that are reminiscent of viral shells.

When complex structures are self-assembling from elementary building blocks, their ground state structure should be both thermodynamically stable and at the same time kinetically accessible [61, 63, 70, 134]. When many building blocks are involved the self assembly usually occurs via multiple intermediate states before reaching the final ground state. Such intermediate structures can be short-lived, but also very long-lived. Long lived intermediates occur typically when non-native bonds are formed, yielding a frustrated intermediate, that can only reach the ground state by breaking those frustrated bonds again. Clearly, such frustrated states can restrict the formation of the ground-state cluster within a viable time-frame, also because such malformed bonds can lead to larger kinetically trapped random aggregates. It is therefore, in fact, rather surprising that in nature certain self-assembly processes are so successful, e.g. virus assembly. Indeed, previous studies have shown that the conditions for successful self assembly of multivalent particles rely on a fine tuning of interaction parameters such as the bonding strength or bonding volume [5, 63, 70]. When the interaction strength is too strong there is no binding at all, when too large, the system becomes frustrated. When the bonding volume (patch width) is too narrow, there is no binding, when too wide, the system forms random aggregates. Moreover, there is an important role for non-specific binding interactions, which allows the particles to bind weakly first, before finding the final correct target configuration [21]. These findings are universal self-assembly principles that can lead to rational design rules for artificial molecular or colloidal superstructures.

The question we would like to address here is what role multivalency plays in association and dissociation processes, and what influence it has on the kinetics and mechanism of these processes. Multivalent particles can form multiple bonds with other particles or previously formed complexes, yielding a correctly formed structure, or possibly a malformed intermediate state. How does the formation mechanics and kinetics of correctly formed structures depend on the number of bonds the particles can form? To address this question we study a simple model system of spherical particles with multiple binding sites or patches. In chapter 4 we studied the formation of a tetramer from its four constituent particles with three binding sites [135]. In this chapter we systematically extend this study to particles with four and five binding sites. Such particles, can form symmetrical octahedrons and icosahedrons. Since the number of intermediates rises combinatorially with the number of constituent building blocks we will investigate as a first step the
final assembly process, the association/dissociation of the last particle, which is a uniquely defined step in the assembly as it leads to and from the fully formed symmetric polyhedral cluster. As such, all other particles already in the cluster are constrained. We study three different types of clusters, a tetrahedron, an octahedron and an icosahedron where each particle forms three, four or five bonds, respectively (see Fig. 5.1 for a schematic representation of the clusters). For each of these clusters three conformations are defined: a fully bound (B), an unbound state (U) and a partially bound cluster (I) where certain bonds result in a rotationally frustrated state.

While previous work focused mostly on the thermodynamic stability as well as the kinetic accessibility [21, 61, 70, 136], here we are more concerned with the actual kinetics, the rate constants of assembly and disassembly, and the reactive pathways of association and disassociation. Due to the high binding energies necessary to obtain stable clustered structures from patchy particles, obtaining reactive pathways in a statistically meaningful way by brute force molecular dynamics is extremely inefficient. Recently, path sampling techniques have been developed to solve this problem by biasing the generation of reactive pathways without altering the underlying dynamics. We apply the Single Replica Transition Interface Sampling (SRTIS) framework to study rare association/dissociation events involved in the final steps toward the fully formed polyhedron [104, 108].

By analyzing the rate matrix obtained from SRTIS all kinetic information on the overall association dissociation process can be retrieved. A convenient framework is Transition Path Theory, which gives insight in relevant quantities such as commitment probabilities and the net flux through intermediate states [110]. The multivalency has a trivial way of influencing the thermodynamics of binding and the associated kinetics: for a fixed patch binding interaction the binding equilibrium constant increases exponentially with the number of bonds. When the bond strength is fixed, the kinetics also is very dependent of the multivalency, with dissociation rate constants exponentially decreases with the number of bonds. This makes it hard to study the influence of just the number of binding sites. Therefore, we compare not the individual patch strength, but the total binding strength per particle upon complete binding. Remarkably, when the total bond energy is kept constant, the association and dissociation rate constants are rather independent of multivalency. Of course, the kinetics is very dependent on the total binding energy of all particles. The association and dissociation mechanism however depend on the presence and nature of the intermediate states. Pathways that visit the intermediate states are less prevalent for higher five-fold multivalent particles, compared to particles with only three bonds. Such intermediate states can lead to kinetic trapping, and malformed aggregates. We discuss implications for natural forming complexes such as virus shells.

We show that the intermediates have an effect on the overall association or dissociation process and as such the mechanism. A difference is found between the frequency of visiting intermediate states given by TPT, and the actual residence time in the intermediate state given by the time-evolution of the populations.

The remainder of the chapter is as follows. In section 5.2 we explain the
Role of multivalency in self-assembly kinetics

Figure 5.1: Cartoon image of the three polyhedrons considered in this work where the orange particle is the motile particle and the patches are depicted in purple. From top to bottom: tetrahedron, octahedron, icosahedron. From left to right are the three different states: the ground state $B$, a realization of a frustrated intermediate state $I$ and the unbound state $U$.

patchy particle model, briefly explain Dynamic Monte Carlo, and end with a small summary of TPT and steady state analysis. In section 5.3 we present the main results of the paper. We end with concluding remarks.

5.2 Methods and Simulation details

Patchy particle model

Similarly to the study of Wilber et.al. [61], we use a simple patchy particle model. The potential between particles is defined as:

$$u(r_{ij}, \Omega_i, \Omega_j) = u_{rep}(r_{ij}) + u_p(r_{ij}, \Omega_i, \Omega_j)$$

where $r_{ij}$ is the inter-particle vector and $\Omega_{i,j}$ the orientations of the particles, stored in quaternion form. The isotropic WCA-like repulsive potential is given by:

$$u_{rep}(r_{ij}) = \begin{cases} 4.0\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^6 + \frac{1}{4}\right] & \text{if } r \leq 2\frac{1}{\sqrt{2}}\sigma \\ 0 & \text{if } r > 2\frac{1}{\sqrt{2}}\sigma \end{cases}$$

(5.1)
where \( r = |\mathbf{r}_{ij}| \) is the distance between particles, \( \sigma \) determines the size of the particle. The anisotropic patchy interaction is given by:

\[
 u_p(\mathbf{r}_{ij}, \Omega_i, \Omega_j) = \begin{cases} 
 4.0 \epsilon \left[ \left( \frac{\sigma}{r} \right)^{24} - \left( \frac{\sigma}{r} \right)^{12} \right] S(\mathbf{r}_{ij}, \Omega_i, \Omega_j) & \text{if } r \leq r_c \\
 0 & \text{if } r > r_c 
\end{cases}
\] (5.2)

where \( \epsilon \) defines the strength of the interaction. 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 continuous patch function \( S(\Omega_i, \Omega_j) \) gives a penalty for misalignment:

\[
 S(\mathbf{r}_{ij}, \Omega_i, \Omega_j) = \exp \left( -\frac{\theta_i^2 + \theta_j^2}{2\delta^2} \right) 
\] (5.3)

where \( \delta \) defines the patch-width and \( \theta_\alpha \) is the angle between patch vectors \( \alpha \) and \( \mathbf{r}_{ij}/r \) where \( \alpha \) is the patch vector which minimizes the angle. Therefore, only the patches on each particle that are closest to \( \mathbf{r}_{ij} \) interact. We consider particles with narrow patches (\( \delta = 10 \) degrees) such that every bond is well defined and there are no multiple overlaps between patches. A small patch-width as used here was shown to reproduce the gas-liquid curves of protein solutions such as \( \gamma \)-crystallin and lysozyme quite well [138], albeit with more patches. Note that this patchy model is somewhat simpler than in chapter 4 as now we no longer require multiple bonds per particle pair.

**Dynamics**

Overdamped Langevin (Brownian) dynamics are typically used to propagate protein or colloidal systems in time [83, 130]. However, due to the constraint on the immobile particles, the force calculation is rather difficult. Therefore, we use Dynamic Monte Carlo because of the ease of implementation without losing the necessary dynamical information, see section 2.3 [94]. In each DMC cycle a translation and rotation move is tried. A translation move consists of translating a particle by a randomly chosen shift between \([-\delta r, \delta r]\) for each Cartesian axis. A rotation move is done by choosing a random unit vector and rotating the orientation over a randomly chosen angle between \([0, \delta \theta]\). The main disadvantage of DMC is the fact that collective motion of clusters is suppressed [74, 93]. However, collective motion is not important for this system as we only consider one particle to move freely. The rotational and translational step sizes are fixed following the Stokes-Einstein relation, \( \delta r = 0.01 \sigma \) and \( \delta \theta = 0.03 \text{rad} \), see chapter 2 for more information on DMC.
Single replica Transition Interface Sampling

Single replica transition interface sampling (SRTIS) is used to sample path space and thus obtain the rate matrix \[104, 108\]. Three states are defined: the fully bound state (B), the unbound state (U) and an intermediate state (I) where two bonds are formed, and the system is frustrated such that the remaining unbound patches are unable to form bonds via barrier-less rotation. When the mobile particle is at least \[r_U\] away from the centre of the polyhedron, the system is in the unbound state, where \[r_U\] is chosen such that when in \(U\), the mobile particle is at least \(2.0\sigma\) away from any particle.

The other two states are defined by the correct topology and when the energy of the system is lower than \(-0.9n_b\epsilon\) where \(n_b\) is the number of bonds that defines the state. The interfaces around each state are defined by the energy of the system minus the ground state energy. For B and I the interfaces are equally spaced from the state boundary by \(1.5k_BT\) until the maximum possible energy is reached. The interfaces for the unbound state should be defined carefully. If one defines the interfaces based on the same energy spacing as for the bound states, particles could become stuck in a region where there is little binding energy and the system is subsequently not pushed towards the bound states as the transition is mostly entropic in nature. Therefore, we choose to define many interfaces close to the unbound state: \([10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 0.4, 1.0]\). This choice of interfaces will guide towards the bound states.

TPT analysis

Although the full rate matrix \(K\) gives all kinetic information, the overall rate constant from \(B\) to \(U\) including the direct and indirect pathway via \(I\) can be calculated via TPT [110]. As given by Eq. 2.54, the commitment probability is the start of a TPT analysis. In this study there is only one intermediate state and therefore, the commitment probability for state \(I\) is simply given by:

\[ q_I^+ = \frac{T_{IU}}{T_{IU} + T_{IB}} \]  \hspace{1cm} (5.4)

where \(T_{IJ}\) is the transition probability to go from \(I\) to \(J\) in a certain lag time \(\tau\). From the committor we can calculate two interesting properties. Firstly, the overall rate constant for the dissociation process can be calculated explicitly:

\[ k_{TPT}^{BU} = \frac{p_BT_{BU} + p_BT_{BI}q_I^+}{\tau(p_B + p_Iq_I^-)} \]  \hspace{1cm} (5.5)

where \(p_I\) is the equilibrium probability of state \(I\), and \(q_I^-\) is the backward-committor, \(1 - q_I^+\).

Secondly, the effective flux \(f_{IJ}\) gives frequency of a reactive pathway directly from \(B\) to \(U\) or indirectly \(B\) to \(U\) via an intermediate state \(I\) which can be generally calculated via Eq. 2.55. To calculate the net flux without any recrossings one computes: \(f_{IJ}^+ = \max[0, f_{IJ} - f_{JI}]\). For these simplified systems \(f_{UB} = p_UT_{UB}\) and \(f_{UIB} = p_U T_{UI} q_I^-\), where \(q_I^- = 1 - q_I^+ = \frac{T_{IB}}{T_{IB} + T_{IU}}\).
Steady state approximation

We can also approximate the overall rate constant analytically by performing an analysis of the master equation: \( \frac{dp_I}{dt} = -\sum_J p_I k_{IJ} + \sum_J p_J k_{JI} \) and assume a steady state approximation \( \frac{dp_I}{dt} = 0 \). This leads to an overall dissociation rate constant:

\[
k_{ss}^{BU} = k_{BU} + \frac{k_{BI} k_{IU}}{k_{IB} + k_{IU}}
\]

which is very similar to Eq. 5.5 if we use \( k_{IJ} = T_{IJ}/\tau \), except for the term \( p_I q_I \) in the denominator of Eq. 5.5 which drops out due to the assumption of a steady state where it is considered that \( p_I \ll p_B \).

Simulation details

TIS simulations were performed with DMC in a periodic box of size 2\( r_U \). 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.

5.3 Results and discussion

We consider particles with narrow patches (\( \delta = 10 \) degrees) such that every bond is well defined and there are no multiple overlaps between patches. A small patch-width as used here was shown to reproduce the gas-liquid curves of protein solutions such as \( \gamma \)-crystallin and lysozyme quite well [138], albeit with more patches. From SRTIS we obtain the full rate matrix, \( K \), for each polyhedron which we show in Fig. 5.2 as a function of the patch-patch attraction, \( \epsilon \). Rate constants, \( k_{BU} \) and \( k_{IU} \) show clear Arrhenius behavior, i.e. \( k_{IJ} \propto \exp(-\beta \Delta G^\dagger_{IJ}) \), especially at high \( \epsilon \), where \( \Delta G^\dagger_{IJ} \) is the free energy barrier between \( I \) and \( J \). For low \( \epsilon \) rate matrix elements \( k_{BI} \) and \( k_{IB} \) flatten off slightly, because these processes become more diffusion limited at very low \( \epsilon \). Naturally, the rate constant out of the bound state to the unbound state is lowest for the icosahedron as it has the highest number of bonds (Note that we compare here the situation for constant fixed patch interaction \( \epsilon \)). For each polyhedron, the intermediate state has two bonds, and therefore each rate constant out of state \( I \) is very similar. Rate constants from \( k_{UB} \) and \( k_{UI} \) do not show Arrhenius behavior at all. Clearly, the rate limiting step in these types of transitions is the alignment of patches which is mostly a diffusive process.

Although the individual rate constants describe in principle the full association process, they are not intuitive. An interesting quantity for self-assembling systems is the overall rate constant which in part is responsible for the overall polyhedron yield. In Fig. 5.3 we show the overall association and dissociation rate constants as a function of total energy \( E_{tot} = -n_b \epsilon \) to compare for equi-energetic clusters where we see that the overall rate constants are very much the same and there is
Role of multivalency in self-assembly kinetics

Figure 5.2: Rate matrix, $K$, for tetrahedron (light blue), octahedron (green) and icosahedron (purple) plotted as function of $\epsilon$; $k_{BU}$ (top left), $k_{UB}$ (top right), $k_{BI}$ (center left), $k_{IB}$ (center right), $k_{IU}$ (bottom left), $k_{UI}$ (bottom right).
only a small difference between different polyhedra. The association rate constant of the tetrahedron differs from that of the octahedron and icosahedron, although this could be due to different volumes available to the unbound state and geometry of the system.

Clearly, the dissociation rate constant depends mostly on the total energy and is less dependent on the number of bonds or the intermediate states. This can be understood if we assume that two pathways contribute to the dissociation: direct dissociation from $B$ to $U$ and indirect dissociation via the intermediate state. As derived via steady state approximation in Eq. 5.6, the direct dissociation rate constant should be proportional to $k_+^{dir} \propto \exp(-n_b \epsilon)$ where $n_b$ is the number of bonds of the system. The indirect dissociation could be approximated by the steady state solution: $k_+^{ind} = k_{IU}k_{BI}/(k_{IB} + k_{IU})$. Based on simple fits to the Arrhenius relation from Fig. 5.2, the following relations should approximately hold: $k_{IU} \propto \exp(-2\epsilon)$, $k_{BI} \propto \exp(-(n_b-1)\epsilon)$, $k_{IB} \propto \exp(-\epsilon)$. The steady state solution becomes:

$$k_+^{ind} = \frac{\exp(-(n_b+1)\epsilon)}{\exp(-\epsilon) + \exp(-2\epsilon)}$$

if we further approximate that $\exp(-\epsilon) \gg \exp(-2\epsilon)$ which is valid for high $\epsilon$, the dissociation rate constant for every polyhedron becomes $k_+ = \exp(-n_b \epsilon)$. Therefore, as shown by Fig. 5.3, the total dissociation rate constant, $k_{BU}^{TPT}$, is mostly dependent on the total energy given by $E_{tot} = -n_b \epsilon$. Vibrational and rotational entropy naturally also plays a role, however, it seems less significant especially at high patch attraction. Although it seems fairly trivial, this does mean that apparently the number of bonds a particle is bound to does not make a difference if we compare the same total energy. Note that the result would be the same if state $I$ has a different number of bonds.

Figure 5.3: Effective association (left) and dissociation (right) rate constants calculated via TPT for the tetrahedron (blue triangles), octahedron (green squares) and icosahedron (purple circles). The effective association rate constants do not vary dramatically with the total energy. Differences can be attributed to the volume available to the unbound state, and the actual geometry of the cluster. Effective dissociation rate constants do depend on the total energy following roughly the Arrhenius law (except at low binding energy), and do not differ much between polyhedra for the same total energy.
Although the rate constants are not different, the mechanism of assembly or disassembly can be different, which in turn impacts the overall assembly. If a system resides longer in the intermediate state, frustrated dangling bonds can lead to kinetically trapped states, when other particles from the bulk attach to a growing structure. Moreover, if during assembly intermediate states are frequently visited, the probability of trapping naturally also increases. More information about the mechanism can be distilled from the TPT analysis. For comparison we plot the ratio $f_{UIB}/f_{UB}$ in Fig. 5.4. This ratio demonstrates whether the direct or the in-
via equal patch strength, but equal total energy. In Fig. 5.6 the relaxation of the
population ratio $P_I/P_B$ is shown for $E_{tot} = -40k_B T$ which demonstrates that the
tetrahedron state clearly has relatively the highest population in the intermediate
state when the systems are compared with equal total energy. Note that for all
three systems the population of the bound state is almost unity and that the full
relaxation is not shown. The ordering is different than for the $f_{UIB}/f_{UB}$ shown in Fig. 5.4
demonstrating that there is a difference between the equilibrium net flux and the
out-of-equilibrium relaxation pathways. Moreover there is a clear
difference between the frequency of visiting intermediate states, as predicted by
TPT analysis, and the dwell time in the intermediate state, which is important
for the population dynamics.

From the SRTIS path ensemble we can also extract the mechanism of self-
assembly and dissociation by projecting reactive pathways on collective variables.
Here we focus on the dissociation pathways from the bound state $B$. The reactive
path density is defined as in Eqn. 2.52

$$n^r_B(q) = \int Dx^L P^r_B[x^L] h_q(x^L).$$

(5.8)

where $Dx^L$ is a proper integral over all pathways, $P^r_B[x^L]$ is the probability of a
reactive pathway out of state $B$ and $q(x_k)$ is the collective variable of choice. In
Fig. 5.7 we show the reactive path density for dissociation from state $B$ mapped
onto the distance of the motile particle to the polyhedral cluster, $R$ and total
Role of multivalency in self-assembly kinetics

Figure 5.6: Ratio of the intermediate population over the bound state relaxing from the unbound state for $E_{\text{tot}} = -40k_B T$ demonstrating that the tetrahedron stays the longest time in the intermediate state, due to the fact that it is harder to break a bond in the tetramer than in the octahedron or icosahedron.

energy, $E_{\text{tot}}$. Clearly, in case of the tetrahedron reactive pathways from $B$ are dominated by the $B \rightarrow I$ transition. This is a reflection of Fig. 5.4 that shows that the intermediate frustrated state is frequented often during the (dis)assembly of a tetrahedron complex. In contrast, the intermediate state is mostly avoided for the octahedron and icosahedron, where clearly the highest probability follows the $B \rightarrow U$ transition. Note also that the path probability from $B \rightarrow I$ is indeed slightly higher for the icosahedron than for the octahedron as indicated by Fig. 5.4.

Conclusions

We studied the effect of multivalency on the kinetics and mechanism of self-assembly of patchy particles forming polyhedral clusters. By using SRTIS we were able to obtain the complete rate matrix and thus the full kinetic picture of these simple patchy particle systems. No difference in kinetics is found when the total energy of the ground-state is fixed which corroborates with a simple steady state analysis. In contrast, the mechanism of self-assembly does depend on the properties of the intermediate state. All studied multivalent particles system can associate into a metastable intermediate state, in which two bonds are formed, and the others are not. From this intermediate state the system can relax into the correctly formed fully bound state. Pathways that visit the intermediate states are less prevalent for five-fold multivalent particles compared to particles with only three bonds. Instead the association pathways are more likely to navigate directly from unbound to bound without visiting the intermediate states. Long sojourns in the intermediate state can lead to kinetic trapping, and malformed aggregates.
Figure 5.7: Reactive path density obtained from the reweighted path ensemble, representing the dissociation mechanism starting from correctly bound state \( B \). The path density is mapped onto the distance, \( R \), between the motile particle and the polyhedral cluster and the binding total energy, \( E_{\text{tot}} \). For the tetrahedron (left), the most dominant escape from the bound state, located at \( E_{\text{tot}} = -40k_BT \) ends up via diffusion in the one bond state located for the tetrahedron at \( E_{\text{tot}} = -13.3k_BT \), in state \( I \) located at \( E_{\text{tot}} = -26k_BT \). Clearly, direct dissociation to the unbound state is not very probable. In contrast, escape from \( B \) for the octahedron (middle) and icosahedron (right) clearly does end up in \( U \) and not only in the frustrated state \( I \). Moreover, the icosahedron shows to have more reactive path probability to the \( I \) state than the octahedron which is also reflected in the flux ratio \( f_{UIB}/f_{UB} \) in Fig. 5.4. Note that due to microscopic reversibility this analysis also holds for assembly, rather than disassembly.

It is therefore kinetically favorable for self-assembling systems to have a ground state where particles form many bonds which stabilize the ground state instead of only a few which would result in strongly frustrated intermediate states.

Furthermore, we found a difference between the equilibrium net-flux and the out-of-equilibrium relaxation pathways. In the net flux analysis, long sojourns in intermediate states are not taken into account, simply only the number of times a certain state is visited along (dis)assembly. The number of times a state is visited of course does not necessarily depend on the stability of this state, but on the kinetic accessibility of the state which depends on the number of microstates a macrostate entails. The icosahedron has more microstates in \( I \) (50) than the octahedron (16). This difference is also reflected in the net flux ratio.

Although the studied systems are too simple to represent real proteins or even colloidal particles, some general observations about self-assembly of complexes can be made. The generic conclusion that particles with many weak bonds in the ground state are able to self-assemble in a more defect-free way than particles with a few strong bonds, might be interesting for the experimental design of colloid particles to form desired structures. While our evidence is restricted to the symmetry cases that we used here (tetrahedron, octahedron, icosahedron) we speculate that
other symmetries also show similar features. Our general conclusion implies that natural occurring systems that spontaneously form complex structures with high fidelity from multivalent particles such as virus shells, would show a tendency to have more rather less binding sites.

Naturally, it would be even more informative to study the full unrestricted dynamics of particles for these different type of clusters, i.e. to investigate the assembly pathways starting from 12 unbound particles in case of the icosahedron. However, with increasing motility also comes an exponentially increasing number of intermediate states which might become intractable. An adaptive scheme can be considered to automatically find only the important intermediate states such as adaptive TIS or a Markov State Model [71, 104].
6 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 nonspecific 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 \[83, 139, 143\]. 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 \[144\]. 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 \[75\].

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 \[75\]. 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 \[5, 16\]. 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. \[112, 145, 146\] 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 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,
Two opposing effects of isotropic and anisotropic attraction

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, an isotropic attractive potential, and the minimal two attractive anisotropic angle dependent potentials:

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

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:

\[ U_{rep}(\mathbf{r}_{12}) = \begin{cases} 4.0 \left( \left( \frac{\sigma}{r} \right)^{24} - \left( \frac{\sigma}{r} \right)^{12} + \frac{1}{4} \right) & \text{if } r \leq 2^{1/6} \\ 0 & \text{if } r > 2^{1/6} \end{cases} \] (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:

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

where \( \epsilon_{iso} \) 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:

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

where \( \epsilon_T \) is the strength of the patchy interaction between \( b \) and \( t \). The patchy interaction between \( b \) and \( d \) is defined similarly:

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

where \( \epsilon_D \) 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:

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

where \( \delta \) defines the patch-width, \( \theta_k = \arccos(\mathbf{p}_k \cdot \hat{\mathbf{r}}_{12}) \), 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 \( \epsilon_T, \epsilon_D \), the isotropic interaction strength \( \epsilon_{iso} \) 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 [138], albeit with more
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:

\[ U_{\text{tot}}^{\text{tettr}}(\mathbf{r}_{12}, \Omega_1, \Omega_2) = \sum_{i,j} U_{\text{rep}}(\mathbf{r}_{12}) + U_{\text{iso}}(\mathbf{r}_{12}) + U_{\text{bt}}(\mathbf{r}_{12}, \Omega_1, \Omega_2) \]  

(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 \( 1.5k_B T \) 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 \( U_{\text{tot}}^{\text{tettr}} < 2.7\epsilon_T \) and state \( D \) is defined when no bonds are formed and the particle is on the opposite side of the complex, and \( U_{\text{tot}}^{\text{tettr}} < 2.7\epsilon_{\text{iso}} \) 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 $r_c = 2.0 \sigma$.

![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.](#)

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.
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.

![Figure 6.3](image)

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](image)

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 $\psi$. 
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:

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

(6.7)

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 (\( \text{mmolL}^{-1} \) 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 $\phi_i = \arccos(\mathbf{r}_{12} \cdot \mathbf{p}_i/r)$, 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 $\psi = 60, 120$, 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)
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 $\epsilon_{iso}/k_BT = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, 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 $\epsilon_D = 8k_BT$ and $\epsilon_D = 16k_BT$, respectively.

An isotropic interaction $\epsilon_{iso} = 10k_BT$ 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 $\epsilon_{iso}/k_BT > 4$. 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.
Two opposing effects of isotropic and anisotropic attraction

Figure 6.6: Rate matrix, $K$, 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 $\epsilon_D = 8k_BT$. 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 $\epsilon_{iso}/k_BT = 4$, whereas for $\psi = 60$ there is no dependency at all on $\epsilon_{iso}$.
Two opposing effects of isotropic and anisotropic attraction

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 $\epsilon_D = 16k_B T$. Similar trends are visible as in Fig. 6.7 except for $k_{DU}$ and $k_{DT}$ are significantly lower.
Figure 6.9: Different cuts through the parameter space for the overall association rate constant $k_{UT}^{TPT}(\psi, \epsilon_{iso}, \epsilon_D)$. Top Row: Overall association rate constant for f.l.t.r. $\psi = 60, 120, 180$ for different decoy strengths, $\epsilon_D/k_BT = 2$ (purple), 4 (green), 6 (light blue), 8 (orange), 10 (yellow), 12 (dark blue), 14 (red) and 16 (black), as function of $\epsilon_{iso}$. The association rate constant clearly increases with non specific isotropic interaction, however, at high $\epsilon_D$ values the rate constant decreases. Middle row: Overall association rate constant for f.l.t.r. $\psi = 60, 120, 180$ for different isotropic strengths, $\epsilon_{iso}/k_BT = 0$ (purple), 2 (green), 4 (light blue), 6 (orange) and 8 (yellow), 10 (blue) as function of $\epsilon_D$. The association rate constant clearly increases with non specific isotropic interaction, however, at high $\epsilon_D$ values the rate constant decreases. Bottom row: Overall association rate constant for f.l.t.r. $\epsilon_D/k_BT = 2$, 8 and 14 for different positions of the decoy binding site $\psi = 60$ (circles), 120 (squares) and 180 (triangles) seen as function of $\epsilon_{iso}$. Clearly, the position of the decoy patch start to matter for strong decoy interaction in contrast to low interaction. Rebinding from $D$ to $T$ increases when the decoy site is close to the target site, however, there is no difference between $\psi = 120$ and 180. Note that the scale of the y-axis differs.

In the top row of Fig. 6.9, we show the effect of the non-specific isotropic interaction on the overall association rate constant, $k_{UT}^{TPT}$, computed using Eq. 2.54. 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 $\psi$.](image)

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 $\epsilon_{iso}$.
Two opposing effects of isotropic and anisotropic attraction

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 for-

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.

mation 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 6.13: 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

\[ k_{TU} \]

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)

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

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 $4k_B T$ to $8k_B T$ 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, $k_{UT}^{norm} = k_{UT}^{TPT}(\epsilon_D, \epsilon_{iso})/k_{UT}^{TPT}(\epsilon_D = 0, \epsilon_{iso})$ for the dimer system as function of $\epsilon_D$ for different isotropic interactions with $\epsilon_{iso}/k_B T = 0$ (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: $k_{UT}^{norm} = k_{UT}^{TPT}(\epsilon_D, \epsilon_{iso})/k_{UT}^{TPT}(\epsilon_D = 0, \epsilon_{iso})$. 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 constrained tetramer formation.

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

111
References


114


[51] G. Foffi and F. Sciortino, “On the possibility of extending the noro-frenkel
generalized law of correspondent states to nonisotropic patchy interactions,”


[53] F. Romano, E. Sanz, and F. Sciortino, “Phase diagram of a tetrahedral

[54] F. Romano and F. Sciortino, “Colloidal self-assembly: patchy from the bot-

[55] F. Smallenburg and F. Sciortino, “Liquids more stable than crystals in par-

[56] F. Romano and F. Sciortino, “Patterning symmetry in the rational design


[58] T. Vissers, Z. Preisler, F. Smallenburg, M. Dijkstra, and F. Sciortino, “Pre-

[59] Z. Preisler, T. Vissers, G. Munaò, F. Smallenburg, and F. Sciortino, “Equi-
librium phases of one-patch colloids with short-range attractions,” *Soft Mat-


[61] A. W. Wilber, J. P. Doye, A. A. Louis, E. G. Noya, M. A. Miller, and
P. Wong, “Reversible self-assembly of patchy particles into monodisperse

H. C. Kok, and R. Lyus, “Controlling crystallization and its absence: pro-


List of publications

Publications related to this thesis:

- **Chapter 3**
  Newton, A. C. ², ⁴, ⁵; Nguyen, A. ³; Veen, S. J. ³; Kraft, D. J. ³; Schall, P. ¹, ⁵; Bolhuis, P. G. ¹, ⁴, ⁵, ⁶; *in review*, Modeling critical Casimir force induced self-assembly experiments on patchy colloidal dumbbells

- **Chapter 4**
  Newton, A. C. ², ⁴, ⁵; Groenewold, J. ¹; Kegel, W. K. ¹, ⁵; Bolhuis, P. G. ¹, ⁴, ⁵, ⁶; *Proceedings of the National Academy of Sciences*, ¹¹², 15308, 2016, Rotational diffusion affects the dynamical self-assembly pathways of patchy particles

- **Chapter 5**
  Newton, A. C. ², ⁴, ⁵; Groenewold, J. ¹; Kegel, W. K. ¹; Bolhuis, P. G. ¹, ⁴, ⁵, ⁶; *in preparation*, The role of multivalency in the association kinetics of patchy particle complexes

- **Chapter 6**
  Newton, A. C. ¹, ², ⁴, ⁵; Kools, R. ²; Swenson, D. W. H. ¹, ⁵; Bolhuis, P. G. ¹, ⁵, ⁶; *in preparation*, The opposing effects of isotropic and anisotropic attraction on dimerization kinetics

¹ Design research
² Performed research (simulations)
³ Performed research (experiments)
⁴ Analysis data
⁵ Preparation manuscript
⁶ Project supervision
Summary

Self-assembly, the non-dissipative spontaneous formation of structural order spans many length scales, from amphiphilic molecules forming micelles to stars forming galaxies. This thesis mainly deals with systems on the colloidal length scale where the size of a particle is between a nanometer and a micrometer. As such, this thesis focuses on the self-assembly of colloidal particles made in the laboratory forming supracolloidal structures in a capillary and making the link to proteins forming complexes or virus shells. Whereas retrosynthetic analysis gives a handle on how atoms form molecules and subsequently how molecules form even bigger molecules, similar design principles are lacking for assembling micrometer particles. Last decade has witnessed great advances in the synthesis of micrometer particle building blocks. It is currently possible to make colloids anisotropic in shape, or anisotropic in surface properties, so-called patchy particles. Patchy particles show great promise in the design of new building blocks, possibly applicable in novel functional materials. Moreover, patchy particles have also shown to be good models for globular proteins. This thesis discusses mainly two topics using advanced computer simulation techniques. The first part of this thesis deals with the extraction of an effective potential for anisotropic colloidal dumbbell particles interacting through the critical Casimir force. The second part deals with how the kinetics and mechanism of formation of simple colloidal or protein structures are influenced by changing the interaction between or the dynamics of patchy particles.

In chapter 3 a modeling study is presented to understand experiments performed on colloidal dumbbells submersed in a binary liquid which self-assemble via solvent mediated interactions when the critical point is approached, the so-called critical Casimir forces. We define a new correlation function that characterizes the local structure of anisotropic colloids. Instead of the site-site radial distribution function, important in the RISM theory for molecular liquids which we also apply to colloidal dumbbells, we define a minimum distance radial distribution function which excludes contributions of trivial neighbors that obscure the signatures of the interaction. A framework is presented where via matching experimentally obtained correlation functions to correlations functions obtained through Monte Carlo simulation using a simple model for colloidal dumbbells interacting through critical Casimir forces, an effective potential is extracted. We find that the ex-
tracted potential, even though it is based on a very simple model, can reproduce the correct morphologies as seen in microscopy images, can predict the onset of aggregation and at the same time give an explanation for the sudden collapse of a network structure made of colloidal dumbbells very near the critical point.

The second part of this thesis studies the colloidal self-assembly mechanisms and rate of (dis)assembly and thus requires the rates and pathways for all possible dissociation and association events in the kinetic network. However, on the time-scale of the dynamics of the microscopic particles, binding and certainly dissociation processes are usually rare events due to high free energy barriers caused by strong directional binding. As straightforward dynamical simulation is extremely inefficient, in chapters 4 through 6 we employed the Single Replica Transition Interface Sampling (SRTIS) algorithm to collect all possible (un)binding trajectory ensembles relevant to the patchy colloid assembly.

In chapter 4 we investigated how varying the ratio between rotational and translational diffusion influences the equilibrium kinetic network for small self-assembled clusters of colloidal patchy particles. Already for the dimerization of a 1-patch particle or a 2-patch particle an effect of the rotation is found on the formation dynamics. The (dis)association mechanism moves from a pathway along more translational degrees of freedom, to a more unconcerted pathway along rotational degrees of freedom upon increasing the rotational diffusion from ten times slower to ten times faster normal Stokes-Einstein conditions. We have also studied a tetrahedron system of a 3-patch particle where metastable states are possible. For the unconstrained tetrahedron, the entire nine-state kinetic network was sampled, and we demonstrated that a change in the rotational diffusion shifts the preferred self-assembly pathways significantly. While for low rotational diffusion the overall rate of tetrahedron formation decreased, frustrated states are avoided, leading to significantly less kinetic trapping. Including the interplay between rotational and translational diffusion in the self-assembly design of new supra-colloidal structures could open up new opportunities for controlling the bottom-up synthesis of functional materials. Moreover, this work helps to understand how rotational diffusion influences self-assembly processes in naturally occurring crowded environments such as the biological cell.

In chapter 5 the effect of multivalency is studied in the self-assembly of patchy particles. We found that multivalency has a trivial way of influencing the thermodynamics of binding and the associated kinetics, namely that for a fixed patch binding interaction the binding constant increases exponentially with the binding strength. When the bond strength is fixed, the kinetics also is very dependent of the multivalency, with dissociation rates exponentially lower with the number of bonds. Alternatively, when the total bond energy is kept constant, association and dissociation is rather independent of multivalency, although of course very dependent on the total energy. The association and dissociation mechanism however depend on the presence and nature of the intermediate states. For higher five-fold multivalency, the intermediate states are relatively avoided with respect to particles with only three bonds. Such intermediate state can lead to kinetic trapping, and malformed aggregates. We showed that the intermediates have an effect on
the overall association or dissociation process and as such the mechanism.

In chapter 6 we investigated the effect of one additional non-productive binding site on the association/dissociation rate as well as the effect of adding nonspecific isotropic interaction. The effect of the depth as well as the position of the decoy site on the binding kinetics is studied, and it is found that stronger decoy state always reduces the association as the pathway between target and decoy state is less probable than unbinding. In contrast, adding a non-specific isotropic interaction in general increases the association rate at low decoy site interaction, and the amount of reduction then does depends strongly on the position and strength of the decoy site. For weak/intermediate decoy strength, there is hardly an effect of the position, whereas for strong decoy position away from the target site, the association is suppressed. We also studied the effect of non-specific decoy binding on particles forming complexes. Adding a non-specific isotropic interaction to a multivalent particle forming a tetramer complex with an already formed trimer of particles, increases the rate of binding and dissociation at first, but when the isotropic interaction rises above 4 kT the rate lowers dramatically. This maximum in association rate also changes with concentration, shifting to higher values of nonspecific isotropic interaction. While one would think that this cluster binding is almost identical to the two particle binding with decoy, the cooperativity of binding multiple particles creates qualitative different behavior.
Samenvatting

Zelforganisatie, de spontane formatie van structurele orde, zie je terug op verschillende ordes van grootte, van amfifiele moleculen die micellen vormen tot sterren die zich in stelsels organiseren. Een van de eerste publicaties die de term zelforganisatie gebruikt dateert uit 1962 in het artikel "Physical Principles in the Construction of Regular Viruses" van Donald Caspar en Aaron Klug. Een openstaande vraag toentertijd ging over de precieze structuur en aard van viruses. Hoe wordt een virus opgebouwd? Waaruit bestaat de schil van een virus? Het was zeer verassend dat de complexe structuren die de schillen van viruses aan kunnen nemen maar bestonden uit enkele eiwitten. Dit heeft natuurlijk praktisch nut (voor het virus). De informatie over de schil van het virus is immers ook vastgelegd in het relatief korte DNA (of RNA) van de virus. Het is gunstig als zo min mogelijk informatie nodig is om dit te coderen. Alleen staat dan de vraag open hoe uit slechts enkele bouwstenen zulke complexe structuren gevormd kunnen worden. Caspar en Klug introduceerden in het artikel de term zelforganisatie. De eiwitten vormen door middel van spontaan gevormde bindingen de schil van het virus. In plaats van een productielijn waar elk component zorgvuldig geplaatst moet worden door een extern mechanisme, is het assemblage proces voor deze moleculaire structuren als het ware in de componenten zelf geprint. De componenten kunnen daardoor spontaan een complexe hiërarchische structuur vormen. Alsof alle losse stalen componenten van de Eiffeltoren in de lucht gegoooid kunnen worden, en elk component van de Eiffeltoren weet welke positie in de uiteindelijke structuur het zal innemen. Met dit concept in gedachte, wordt het duidelijk dat veel structuren in de natuur tot op zekere hoogte spontaan vormen (micellen, sterrenstelsels, mensen).


In tegenstelling tot moleculen, waarvan door retrosynthetische analyse vaak bepaald kan worden hoe ze opgebouwd kunnen worden, zijn gelijksoortige ontwerpprincipes voor de assemblage van structuren op de micrometerschaal gebrekkig. Er
is aan microscopische bouwstenen daarentegen geen gebrek. In de laatste decennia zijn grote sprongen gemaakt in de synthese van colloïdale deeltjes. Allerlei vormen deeltjes zijn mogelijk: bollen, staafjes, kubussen, stervormige, etc. Ook is het mogelijk om alleen specifiek gedeelte van het oppervlak van deeltjes gedeeltelijk te veranderen. Dit soort deeltjes worden patchy particles genoemd. Deze patches kunnen er voor zorgen dat twee deeltjes zich alleen via deze patches kunnen binden. De analogie met moleculen wordt snel gemaakt, en daardoor lijken patchy particles goeie kandidaten om mogelijk nieuw soort materialen te maken. Ook worden patchy particles gezien als goeie modelsystemen voor eiwitten die vaak hydrofobe oppervlaktes hebben die zorgen voor precieze binding.

Aangezien de mogelijkheden eindeloos lijken, wordt in deze thesis computer simulaties gebruikt om tot beter inzicht te komen hoe we patchy particles kunnen gebruiken voor zelforganiserende systemen. Hierin bestuderen we twee hoofdonderwerpen. Het eerste gedeelte gaat over hoe we de drijvende kracht achter de assemblage van anisotrope deeltjes kunnen modelleren aan de hand van experimenteel verkregen data. Hierbij gebruiken we de kritische Casimir kracht om de deeltjes te laten aggregeren. Het tweede gedeelte bestudeert hoe het mechanisme of reactiesnelheidsconstante van aggregatie verandert met het veranderen van de interactie tussen deeltjes of de dynamica van de deeltjes zelf.

In hoofdstuk 3 presenteren we een raamwerk om het aggregatiegedrag te begrijpen van colloïdale halters in een binair oplosmiddel die aggregeren wanneer het oplosmiddel dicht bij het kritisch punt wordt gebracht waar het mengsel begint te ontmengen, de zogenaamde kritische Casimir krachten. Dit raamwerk laat zien hoe een simpel aggregatiemodel geoptimaliseerd kan worden met behulp van Monte Carlo simulaties. We laten zien dat het geoptimaliseerde model verassend goed het aggregatiegedrag van dit soort anisotrope deeltjes kan beschrijven. Niet alleen worden de correcte morfologieën gevonden, ook het punt wanneer aggregatie optreedt wordt correct voorspeld door dit model.

In het tweede deel van dit thesis bestuderen we de afhankelijkheid van het mechanisme van binden en de reactiesnelheidsconstante van simpele zelforganiserende systemen op de dynamica en interactie van anisotrope deeltjes. Hiervoor gebruiken we de geavanceerde padsimulatie methode Single Replica Transition Interface Sampling om deze constanten en mechanismen uit te rekenen.

In hoofdstuk 4 bestuderen we hoe de rotationele diffusie constante van deeltjes het mechanisme van zelforganisatie kan veranderen. We zien dat wanneer de rotationele diffusie constante verlaagd wordt, dat er minder gefrustreerde structuren gevormd worden in de complexe structuurvorming van een tetrahe. De rotatie van nanodeeltjes zoals eiwitten en colloïden speelt dus een bepalende rol bij de zelfassemblage van deze deeltjes tot complexen en clusters.

In hoofdstuk 5 wordt het effect van multivalentie van deeltjes, de mogelijkheid om meerdere bindingen te maken, bestudeerd. We vinden dat deeltjes met een hogere multivalentie meer kans hebben om gefrustreerd te raken wanneer we het associatieproces vergelijken bij gelijke bindingsenergie. Wanneer we vergelijken bij een gelijke totale energie, dan is de reactiesnelheidsconstante van associatie/dissociatie min of meer onafhankelijk van de multivalentie van deeltjes.
Wat wel sterk kan veranderen met multivalentie, is het mechanisme van dissociatie of associatie. Dit is sterk afhankelijk van de aard van de intermediaire toestanden dichtbij de grondtoestand. Deeltjes met een hoge multivalentie hebben meer de neiging om gefrustreerde toestanden te vermijden dan deeltjes met een lage multivalentie.

In hoofdstuk 6 wordt het effect van een extra anisotrope bindingsplek naast de hoofdbinding bestudeerd op de reactiesnelheidsconstante van associatie of dissociatie van twee deeltjes. Het blijkt dat zo’n extra binding altijd de reactiesnelheidsconstante verlaagt, ongeacht de positie of sterkte van deze binding. Dit komt doordat het proces van wederom ontbinden startend van deze extra binding altijd waarschijnlijker is dan van de extra binding naar de hoofdbinding te springen. In tegenstelling hierop verhoogt een extra isotrope attractie tussen de twee deeltjes de reactiesnelheidsconstante. In dit geval is er een stuk sterkere afhankelijkheid van de positie van de extra anisotrope binding wat vooral aanwezig is bij een sterkere extra anisotrope binding. Wanneer we de geometrie van één van de deeltjes veranderen naar een multicomponent deeltje, dan wordt de reactiesnelheidsconstante van associatie eerst verhoogd met isotrope attractie, maar weer verlaagd wanneer de isotrope interactie te sterk wordt. Dit principe kan belangrijk zijn voor eiwitassociatie waar vaak de geometrie van deeltjes verschilt van een bol.
Resúmen

Bo por mira outo organisashon, e formashon spontáneo di òrdū struktural, na difer-
ente nivel, di molekül amfifil (manera habon) ku ta forma misèl (aglomerashon) te na strea ku ta organisá nan mes den sistema. Un asuntu ta e struktura di viro: kon nan kaska ta hinká den otro. E ta remarkabel ku nan kaska por tin struktura komplecho, miéntras nan te eksistí di solamente di algun proteina. Esaki natural-
mente tin bentaha pasobra e informashon di e kaska ta keda determiná dor di e DNA (i RNA) relativamente chikitu di e viro. Na un banda ta bon pa tin mas tiki informashon pa kodifiká, pero lo keda e pregunta kon struktura kompliká por keda formá dor di solamente algun piesanan. E kontesta lo ta ku e proteinanan ta forma dor di konekshonnan spontáneo e kaska di e viro. En bes ku kada komponente ta keda koutelósamente poné dor di mekanismo eksterno, e ta manera pa asamblea di e struktura molekular aki ta imprimí den e komponentenan mes i dor di esei e komponentenan mes por forma un struktura kompliká. Manera ta tira e piesanan pa un outo den laira i kada piesa sa unda e tin ku bai. Ku e konsepto aki e ta kla ku hopi struktura den naturalesa a forma spontáneamente te sierto nivel. (misèl, strea, hende)

E tesis aki ta toka e outo organisashon di mikro partikula òf partikulan nan koloidal. Partikula koloidal ta sirbi mas bia komo sistema modelo pa preguntanan teorétiko. Esaki pa motibu ku por mira partíkula koloidal ainda dor di mikroskop, miéntras nan tambe ta tene nan mes na leinan di naturalesa ku ta importante pa outo organisashon manera e formashon spontáneo di konekshon. E kombinashon aki ta pèkurá ku pronóstiko teorétiko tokante formashon struktural di partikula mikroskópiko por keda di tèst eksperimentalmente.

Kontrali na molekül unda su struktura por keda determiná dor di análisis retrosintétiko (di kada parti di e molekül), e prinsipionan di diseño pa e asamblea di struktura na nivel di mikrometer ta limitá. Na otro banda no tin falta di piesanan mikroskópiko. E último dekadanan tatin hopi èksito den e síntesis di partikula koloidal, Yen di forma di partikula ta posibel: bolobouchi, palitu, kubus, forma di strea ets. Tambe ta posibel pa kambia solamente un parti di e superfísie di partíkula parcialmente (hasi nan anisotrópiko). E tipo di partikulan nan eì ta yama patchy particles. E pèchnan ei por sòru ku dos partíkula por konektá solamente via e pèchnan aki. E analògia ku molekúlnan ta opvio i dor di esei ta parse ku patchy particles lo ta bon kandidado pa traha materialnan nobo. Tambe patchy
particles por fungi komo sistema modelo pa proteina globular ku hopi bia tin superfisienan hidrófobo ku ta sòru pa konekshonnan eksakto.

Pasobra e posibilidatnan ta parse sin fin, ta usa simulashon ku kòmpiuter den e tesis aki pa haña un mihó idea kon di usa patchy particles pa sistema di outo organisashon. Aki nos ta studia tema mayó. E promé parti ta toka kon nos por modelá e forsa prinsipal tras di e asamblea di partkula anisotrópiko for di data ku a haña eksperimentalmente. Aki nos ta usa e asina yamá forsa Casimir kritiko pa laga e partikulánan agregá. E di dos parti ta studia kon e mekanismo òf e rapides di reakshon di e agregashon ta kambia ku kambio di e interakshon entre partikula òf e dinámika di e partikulanan mes.
Acknowledgements

Four years have passed and the thesis is done. It has been more difficult than I thought it would be, but luckily I have many people to thank for helping me reach this goal.

First of all, I would like to thank my promotor, Peter Bolhuis. Ik was bezig met een stage op Amolf toen ik jou vroeg als tweede beoordelaar voor mijn project. Jij liet weten dat er ook projecten waren in jouw groep, als ik geïnteresseerd was in theorie en simulaties. Terugkijkend weet ik niet zeker of je een studentenproject bedoelde of een PhD-project. Ik heb in ieder geval zeker geen spijt dat ik kort daarna begon aan mijn PhD in jouw groep. Het was een ongelooflijke ervaring, en ik heb veel van je geleerd over het doen van onderzoek, van het stellen van de juiste onderzoeksvraag tot het schrijven van het artikel. Wat me vooral bij blijft en ik erg waarder en je bevlogenheid bij het uitleggen van bijvoorbeeld TIS details of colloidale modellen tijdens een path-meeting wanneer ik nog een 'klein technisch vraagje’ had, waardoor soms de meeting nog lang door ging. Al kunnen sommige meetings er heftig aan toe gaan, het is altijd vanuit je wil om tot de bodem van de probleem te komen, wat jou naar mijn mening een goede onderzoeker/begeleider maakt. I would also like to thank my co-promotor, Peter Schall. Right from the start we collaborated on the critical Casimir project. Although we encountered many problems, I am glad that we managed to in the end solve a few big ones which lead to chapter 3. I value the meetings we had where you always tried to solve the problem from a new angle.

I would also like to thank the members of my committee. Willem, meerdere keren zijn Peter en ik naar Utrecht gekomen op visite om met jou en Jan nieuwe projecten te bespreken. Deze momenten waren altijd net voor kerst, een ideale timing, omdat ik door je enthousiasme (en die van Jan) weer fris het nieuwe jaar in kon met nieuwe ideeën. Pieter-Reijn, met jou en Adithya zijn Peter en ik ook een aantal keer samengekomen, om “the most natural potential” en “the crux of the problem” van het modelleren van de patchy particles te bespreken. Je manier van onderzoek doen en vragen stellen waardeer ik erg. Marjolein, jouw groep was min of meer tegelijkertijd begonnen aan het Casimir probleem. Ik was daarom ook altijd bij conferenties benieuwd naar alle praatjes en posters van jou en je groep, en ik ben daarom ook blij dat je in mijn commissie zit. Ook wil ik de leden vanuit de UvA bedanken voor het lezen van mijn thesis, Fred, Daniël en Evert-Jan.
There are two other persons important during my defense, but even more important before and after, are my two paranimphs. Ariana, Corazon!, you were the first person I got to know from the group. We quickly grew from colleagues to friends, not needing more than a look to start laughing because we were immediately on the same wavelength. I don’t think I could have wished for a better office with you around and I will (and already do) miss everything about it wholeheartedly. I will keep on making brownies, if it means more corridor dances and local jokes with you.

I would also like to thank the people from the Computational Chemistry group for all the discussions and social events. David (there is still ample opportunity for more chicken related lunches, thanks for all your help with Carbon), Bernd (I will help with MolSim 2017 in exchange for your lasagne, best in town), Jocelyne (thanks for all the path meetings and help with students), Christopher (unfortunately you could not be there much in the end, but I have always enjoyed your lectures as a student and your advice after research talks), the Indian connection: Rakesh, Nitish, Arjun and Ambuj, the Iedema connection: Ivan, Yulia and Varenna. You guys definitely bring new energy to the group, all the best. Phaedon, you and I started and will defend around the same time, you have been a great presence in the group, you handsome grown-up you. Jurn, met jou hield ik de Hollandse eer hoog, deelde grappen die het daglicht niet mogen zien en natuurlijk de avonturen van vier jongens in Zuiderpark. David, thanks for allowing me to barge into your office from time to time to ask about TIS and such, and of course for all the drinks, outside and in your office. I would also like to thank the former members of the group. Jurriaan (thanks for all the code snippets in the beginning, it helped heaps), Eva (met jou werden dooie uurtjes altijd weer levendig), Ran, Kush, Aatish, Rosanne, Dapeng, Andri, Nazila and Aurora. Wagner, with you and Ariana we had a great office together, I will remember the moments of sharing administrative passwords, “it is raining, man” and of course your salty cheesy Brazilian balls. Our group also had our share of guests. Nick, after so many visits you really were Bound 2 the office, hope that you will have more Amsterdam visits soon. Kristof, Pimperella!, ik zal alle la Bamba avonden niet vergeten. Thierry, misschien binnenkort een Star Wars marathon voor/na een avondje Roots in Den Haag? I would also like to thank the people from the Soft Condensed Matter group I collaborated with. Anh, we did not always understand each other fully I think, but despite that I am glad we managed to get some cool results together. Simon, I wish you much success with continuing the Casimir project. Ook wil ik graag de studenten bedanken die ik heb begeleid. Jorramienses (memcrop4 hangt nog steeds!), Jorien, met jou had ik een goede klik van Grieks en Latijn tot tandartsgrappen, veel succes in London! Rameses, jij kwam zelfs weer terug voor een bachelorproject, bedankt voor je werk ook aan hoofdstuk 6. Yujokishua, Michelle en Joshua bedankt voor het weer in leven blazen van het dumbbell project. Samia, bedankt voor alle werk aan mijn pet project, mobiele valentie, helaas door tijdgebrek niet in mijn thesis gekomen. Veel succes met je PhD. Ook de andere studenten bedankt voor jullie enthousiasme in de groep: Sander, Mascha, Florent, Janneke, Hannah.
I also want to thank my ever changing neighbors across the corridor, from Louis, Ron to Paula to Tanja. Ik wil graag ook de mensen aan de overkant bedanken voor al het werk tijdens MolSim, voor studentenprojecten, etc: Ineke, Petra, Maureen, Renate. Ook Paul en Rob bedankt voor alle hulp met Macs. Marcel en Gerda ook bedankt. Due to proximity, coffee machine sharing, and my student project history, the Molecular Photonics group, old and new, has also been important. Wybren-Jan, Sander, Matthijs (bedankt voor het begeleiden van zo wat al mijn studentprojecten), Chris (thanks for looking up that weird obscure Python/Inkscape/LateX issue..but mostly for all the dinners, drinks and Delirious references), Tibert (de Haagse connectie, bedankt voor alle discussies), Steven (bedankt voor het omdopen van dinsdag tot voetbaldag), Heleen (bedankt voor de avocado lunches en goede tijden), Adriana, Sergio, Ben, Roberto, Saeed, Tomislav (co-founder PhD council), Elena, Tatu, Dongdong, Michiel, Bert, Paul (Red hand).

There are also many people from the E-building that deserve thanks for all the official Hims meetings in the Oerknal and the Polder. HomKat and MolInc definitely brought memorable moments. Esther S, Sandra (een reünie van cohort 2006 softbaltoernooi?), Fenna, Sandra N, Rene (Marcel), Monalisa (paranimf veteran), Danny, Tessel (zo thug als een duif), Lukas, Anne Marie, Bart, SanderOmypony, Martin, Stefan, Julia (no dolphins!), Sander, Zof, Soraya (telkens als ik jou zie lopen...), penvriendin Vlien, Joen (voor de fascinatie voor taal: \LaTeX, Gnuplot, Mathematica...).

One of the nice things about a PhD is that you have the opportunity to attend conferences, workshops and summer schools. Thomas, Niall and Ireney, thanks for the great two weeks in Manchester. Pau, at first I was hesitant about sharing a room, but you and me ended up having an amazing week at Varenna. After the Nyenrode workshop, extended lunches with Ingrid, Wilbert, Jeroen, Carien and Andrea were great, but also Frank, Hilde, Auke, Aernout (Firefly, Serenity?) and Wessel thanks for all the support during these workshops.

I would also like to thank Adithya en Joris for all the shockwaves through the community that we caused during our lunches. All the people from Utrecht, Giulia, Guido, Simone, Ernesto, Chris, it was nice meeting you at all the soft matter conferences and summer schools. Rens, Nick en Mitra, al doen we het niet vaak genoeg, het is altijd leuk om met jullie af te spreken.

Natuurlijk ook speciale dank aan het hardloopgroepje. Vincent, vriend van het eerste uur. Ik vind het mooi om terug te denken dat al vanaf de introductieweek tot nu, we elkaar nog altijd vinden. Ik ben trots dat ik je paranimf mocht zijn, mogen er nog meer mooie momenten volgen. Linda, vriendin van het eerste uur. Op een of andere manier herinner ook ik mij het eerste 'ezelenmoment', niet dat het zo speciaal was, maar wel doordat het het begin was van mooie avonden koken, hardlopen, dansen, watergevechten op de achterbank, vlammetjes xxl, lachen en vriendschap. Laten we er gewoon nog eens tien jaar mee doorgaan. Ruben, “als je alles altijd maar kan controleren, doe je toch alleen maar de dingen die je wilt”, en natuurlijk ook voor de BBQ avonden, volgens mij hebben we nog een paar keer nodig om te perfectioneren. Freek, een van de weinige personen waar ik het kan hebben over zowel lasers als voetbal. Guido (mede highfive masochist en Dalmuti)
en Rutger (eerste kerstdiner koningskoppel), hopelijk zijn we binnenkort buren en kan ik mezelf weer meer kapot lachen! Niels, ik hoop dat je binnenkort kan beginnen aan je promotie.

De andere cohort 2006 leden natuurlijk ook bedankt voor de mooie oude tijden, Sander, Lianne, Bart, Jeroen, Carien en Kasper. Maud en Pien (en Bas en Wendy) wil ik ook bedanken dat ik vaak langs mocht komen. Jullie blije gezichten deden heel goed en ik hoop dat ik snel die onzichtbaarheidsmantel voor jullie kan kopen. Michelle, Chris en Joog, we zien elkaar niet zo vaak meer, mede door drukke levens en grote stukken water die in de weg zitten, maar ik vind het altijd nog leuk om met jullie af te spreken. De familie en vrienden uit Zeeland, Stoffelen, Kees, Mieke, Inan, Twan, Kiki, bedankt voor alle steun, en natuurlijk alle CAS-gangers voor de leuke tijden.

Ten slotte wil ik mijn familie bedanken. Ineke en Bert, Carla en Willem, Margie, beste peetooms John en Hans, bedankt voor de steun afgelopen vier jaar. Michael en Peter, jullie zijn de grote broers waar ik altijd op kan bouwen en terug kan vallen. Michael, jouw passie voor eten en koken is zeker op mij over geslagen. Nederland heeft gefaald als Eigenwijz over een paar jaar niet bovenaan alle lijstjes van beste restaurants staat! Peter, door jou ben ik geïnteresseerd geraakt in programmeren wat een groot gedeelte van mijn PhD heeft betekend. Jij en Ridien bedankt voor Collin en Preci, liefste kinderen van de Noordoostpassaat. Papa, bedankt voor alles, jouw verhalen hebben mij laten kiezen voor natuurwetenschappen. Ik denk ook dat het feit dat je me de volle maan en zijn kraters hebt laten zien toen ik drie minuten oud was, mij ook onderbewust heeft doen kiezen voor patchy particles. Mama, bedankt voor alles. Een van je beslissende oudermomenten was dat ik toch gewoon N&T profiel moest kiezen, ondanks dat ik toen vond dat ik dat misschien niet aan kon. Sorry dat ik je even liet schrikken toen alles even in elkaar leek te vallen. Ik hou heel veel van je en hoop dat je nu kan genieten.

Lieve Esther, al bijna negen jaar samen, daar ben ik heel trots op. Ik heb je verschrikkelijk lief. Ik hoop dat je me nu voortaan alleen maar hoort zingen vanuit de keuken terwijl ik pannenkoeken voor je maak. Terwijl ik dit schrijf is mijn toekomst nog niet zeker, maar ik kijk er naar uit met jou. Alle liefs.

Arthur
December 2016