CHAMELEON BEHAVIOUR OF α -SYNUCLEIN

*BROWNIAN DYNAMICS SIMULATIONS
OF PROTEIN AGGREGATION*

Ioana - Măriuca Ilie

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CHAMELEON BEHAVIOUR OF α -SYNUCLEIN

BROWNIAN DYNAMICS SIMULATIONS OF PROTEIN AGGREGATION

DISSERTATION

to obtain
the degree of doctor at the University of Twente,
on the authority of the rector magnificus,
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on the account of the decision of the graduation committee,
to be publicly defended
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Science cannot solve the ultimate mystery of nature. And that is because, in the last analysis, we ourselves are part of the mystery that we are trying to solve.

Max Planck

To my family.

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1. Introduction

1.1 Protein Folding

Proteins are large molecules, consisting of chains of amino acids, that regulate many processes in the human body. In order to perform their biological functions, proteins undergo a folding process in which a string of amino acids interacts with itself to form a stable three dimensional structure. Proteins fold into their unique native three-dimensional structure under specific physiological conditions and this structure is encoded in their amino acid sequence [204]. In a healthy cell, proteins will follow the correct folding path and perform their natural functions. However, it may happen that a protein is unable to fold in the proper way and will therefore not perform its biological function. These are usually removed and broken apart. A class of proteins that do not have a well defined three dimensional structure but still play an important role in cellular functions are the intrinsically disordered proteins (IDPs) or natively disordered proteins [10, 142, 195]. IDPs have multiple functions within the cell, some of which include regulation of transcription, recognition, signaling, and control [25, 205] as well as synaptic function and plasticity [105].

Proteins can follow numerous folding paths and can have multiple minimum free-energy states corresponding to their folded structures [50]. A schematic representation of the energy landscape, depicted in Fig. 1.1, shows that there are less folded conformations of low energy than unfolded or partly folded structures, which have a higher energy [46]. Protein folding is a complex process consisting of numerous folding events. Therefore, at times, proteins may not fold correctly and can get trapped in a local energy minimum. They can result in toxic behaviour

Figure 1.1: Funnel-shaped energy landscape of protein folding showing many high energy unfolded structures and few low energy folded structures. Adapted from [46].

[49]. A healthy organism detects misfolded proteins and employs two mechanisms of response [27]. In the first case, the quality control systems try to repair the misfolded protein [174]. If this fails the cell will try to dispose of these structures through different waste disposal systems [27, 35]. However, under special circumstances, *e.g.* if the degradation process fails, misfolded proteins may aggregate and self-assemble into bigger supramolecular structures [47].

The inability of a protein to fold properly or to remain correctly folded prohibits it from performing its function. It can then aggregate and give rise to multiple diseases, including various forms of cancer [28, 50] or cardiovascular diseases [205]. Misfolded proteins can accumulate or aggregate and form fibrils, like the neurofilaments which can be found in the brain, heart or spleen of patients [47, 205]. Their accumulation is associated with a number of diseases such as Alzheimer's [31, 39, 75], type-II diabetes [47, 205] and Parkinson's disease [77, 120, 141, 182, 213].

1.2 Parkinson's Disease

Parkinson's disease is one of the most common degenerative disorders of the central nervous system and it affects over 6 million people worldwide, with one in ten being diagnosed before the age of 50 [11]. Parkinson's disease affects over 1 % of the population aged over 65, being

Disease	Protein
Alzheimer's disease	amyloid- β peptide or tau protein
Parkinson's disease	α - synuclein
Huntington's disease	huntingtin
type II diabetes	amylin
cataract	γ - crystallins

Table 1.1: Overview of the most commonly known amyloidogenic diseases

therefore not only the most common movement disorder but, after Alzheimer's, also the second most common neurodegenerative disease.

Parkinson's disease is caused by the death of neuronal cells in the brain, leading to a reduction of the neurotransmitter dopamine. The loss of dopamine leads to movement disorder and the development of key motor symptoms of the disease. These are tremor (about 4 – 5 per second), deficiency in spontaneous movement, also known as *Bradykinesia*, and rigid posture. Patients suffering from Parkinson's disease often also experience depression, anxiety, insomnia, nerve pain and loss of sense of smell.

The cause of death of the dopaminergic neurones in the substantia nigra is still unknown. However, there are a number of pathological features commonly associated with Parkinson's disease. A cure for Parkinson's disease has not been found yet, but efforts are being made in finding a treatment for this disorder. Since the exact trigger of the disease is yet unexplained and the knowledge about the disease at molecular level is still incomplete, it is difficult to develop a drug that targets the triggering or the cause of the disorder rather than the symptoms. Current treatments are based on dopamine medication to diminish the symptoms.

1.3 α -synuclein

α -synuclein (α -syn) is a small 14 kDa acidic protein found mainly in neuronal tissue. Composed of 140 amino acids (AA), it is a natively unfolded protein present at presynaptic terminals, in abundance in the neocortex, hippocampus, striatum, thalamus and cerebellum [90, 134, 161]. α -synuclein is believed to play a role in the regulation of neurotransmitter release [32, 65, 111, 121, 129], synaptic function [138] and plasticity [105] and it has been proven to be involved in the pathogenesis of Parkinson's disease. α -synuclein is a typical intrinsically disordered protein with little or no structure in its native form [205], and which adapts its conformation upon

1	65	95	140
N-terminal	NAC region	C-terminal	
<ul style="list-style-type: none"> - α-helix, disordered - amphipathic (both hydrophilic and lipophilic) 	<ul style="list-style-type: none"> - α-helix, β-sheets, disordered - hydrophobic 	<ul style="list-style-type: none"> - disordered - highly acidic - negatively charged 	

Figure 1.2: Schematic representation of the conformations that α -synuclein segments can adopt and the properties of the individual regions. The numbers on top indicate the approximate residues defining these regions.

interaction with ligands or other proteins [161, 205]. α -synuclein can be subdivided into three main regions: the N-terminal, which consists of the first 65 AA, the non-amyloid component or NAC region (AA 65 to AA 95) and the C-terminal (last 45 AA) which is also known as the terminal acidic tail [161]. These individual regions can adopt multiple conformations depending on the surrounding environment, as shown in Fig. 1.2. The N-terminal is positively charged and amphipathic. It can adopt an α -helical conformation upon binding to membranes [92, 112]. The NAC region plays an important role in the aggregation and formation of amyloid fibrils and oligomeric structures [90, 118]. The C-terminal is unstructured and highly charged. It is believed to aid the formation of oligomers, which are considered to be toxic, and is believed to slow down the fibrillar aggregation [157].

Because of this ability to adapt its secondary structure to the environment, α -synuclein is also known as the ‘protein-chameleon’ [207]. To sum up the properties of this protein, we mention that it adopts a disordered state in a solvent, it can curl into an α -helix near a membrane [45, 218] or a unilamellar lipid vesicle [52] and stretch into β -sheets in fibrils or amyloids [118].

1.4 α -synuclein Aggregation

The accumulation of misfolded proteins gives rise to higher order insoluble aggregates which can lead to neurodegenerative diseases. There are strong links between accumulations of α -synuclein aggregates in the brain and the onset of neurodegenerative diseases [102, 202]. It is believed that different α -synuclein conformations are linked to the pathogenesis of Lewy body diseases¹ [105]. These include oligomers, protofibrils and fibrils localised mainly in the neuronal cells [207]. The

¹Lewy Body Diseases (LBD) are disorders such as Parkinson’s disease, Parkinson’s disease with dementia and dementia with Lewy bodies.

aggregation mechanisms of α -synuclein are not fully understood. Studies have shown that α -syn oligomers secreted from neuronal cells can induce toxicity in neurons [42] and, potentially, glial cells [105]. Oligomers are also believed to interfere with axonal transport of synaptic proteins leading to neurodegeneration and disfunctional synapses [105, 175]. In addition, fibrils and their aggregates are involved in the process of neurodegradation and dopaminergic loss [105, 150]. Even though current research shows a clear connection between α -synuclein and Parkinson's disease, the exact correlation between its different aggregates and the neurodegenerative disorder is yet unknown and open to debate.

1.5 Simulations of Protein Aggregation

Protein aggregation has attracted a lot of attention over the past decades. It has been investigated by both experimental techniques and simulation models. A number of difficulties limit the understanding of protein aggregation at experimental level. These predicaments are due to the limited structural order, insolubility in water and involvement of cell membrane [117]. Computer simulations, however, are a tool that potentially offer insight into the mechanisms of folding and aggregation at microscopic scale where experimental data is insufficient. Computer simulations represent complementary techniques to experimental methods that enable the understanding and analysis of biological processes still unexplained by experiments.

There are multiple types of computational models that help understand the folding and aggregation of proteins. These range from all-atom simulations, where every single detail of the protein is simulated, to coarse-grained methods, in which an amino acid is represented by several particles or even consecutive amino acids are grouped into a single particle. Various simulation approaches are being used to study the properties of these models and to better understand the aggregation mechanisms. Examples include Monte Carlo (MC) techniques, Molecular Dynamics (MD) simulations, Elastic Network Models (ENM), Langevin Dynamics (LD) or Brownian Dynamics (BD) simulation methods. The following subsections will present some of the existing models and methods.

1.5.1 All-atom vs. coarse-grained simulations

All-atom simulations incorporate every single detail of a molecule in the model. These representations contain all the information about the protein, its atomic composition, interaction strengths, and all the atoms of the surrounding solvent [24, 89, 215]. **Coarse-grained (CG)** simulations, however, represent a system through a reduced number of degrees of freedom by

grouping several atoms or consecutive amino acids into single particles [71, 115, 122, 219].

Both models have been intensively used in the study of protein aggregation and protein folding, and each comes with advantages and disadvantages. All-atom simulations contain all the information about the system which makes them computationally very expensive and unable to reach aggregation timescales [53, 109]. All-atom simulations can provide insight into the precise aggregation mechanism for small peptides [109, 187], but they are unable to predict fibrillation pathways [153] and fibril nucleation [225]. Coarse graining eliminates the finer details of the system and thereby reduces the number of degrees of freedom. Larger systems can be simulated over longer times and one may reach the timescales at which aggregation occurs [219].

1.5.2 Simulation Methods

Monte Carlo Simulations

Named after the famous gambling city in Monaco, the Monte Carlo (MC) technique represents a class of algorithms that use random numbers in the simulations. One of the most common methods is the Metropolis sampling algorithm, which consists of accepting or rejecting random trial moves based on their potential energy changes [67, 104]. For a system composed out of N identical particles interacting through a potential Φ , every simulation step consists of moving one single particle at a time. The energy change $\Delta\mathcal{E}$ between the state before and after the move is then calculated. If $\Delta\mathcal{E} \geq 0$ the move is energetically favourable and therefore accepted. In the opposite case a random number between 0 and 1 is generated and compared against the Boltzmann factor $\exp(-\Delta\mathcal{E}/k_B T)$. If the generated random number is smaller than the Boltzmann factor the move is accepted and the total energy of the system is updated. If the number is bigger then the move is rejected, another particle is chosen at random and the entire process is repeated to sample the Boltzmann distribution.

MC techniques are intensively used to study fibril formation [170, 209] and protein aggregation [145, 147]. One of the disadvantages of MC is the fact that aggregates have been observed to freeze. As a solution, cluster moves have been introduced but they are rather complicated. Another disadvantage is the fact that the Monte-Carlo moves are unrelated to the actual dynamics of the system and it is difficult to recover realistic time-scales.

Molecular Dynamics Simulations

Molecular Dynamics (MD) simulations evaluate the time development of a system composed of N particles by numerically integrating Newton's equations of motion,

$$\mathbf{a}_i = \frac{\mathbf{F}_i}{m_i} \quad (1.1)$$

where $\mathbf{a}_i$ the acceleration of particle i arising from the force $\mathbf{F}_i = -\partial\Phi/\mathbf{r}_i$ and m_i the mass. The time evolution of particle i is completely described through the three-dimensional position vector $\mathbf{r}_i$ and the velocity $\mathbf{v}_i$. By simply numerically integrating Newton's equations of motion one can easily determine the positions and velocities of a particle in time. The most common algorithm associated with MD is the Verlet algorithm, mainly because of its simplicity and long time stability. The standard steps of this algorithm are:

$$\begin{aligned}\mathbf{r}_i(t + \Delta t) &= \mathbf{r}_i(t) + \mathbf{v}_i(t) \Delta t + \frac{1}{2} \mathbf{a}_i(t) \Delta t^2 \\ \mathbf{v}_i(t + \Delta t) &= \mathbf{v}_i(t) + \frac{1}{2} [\mathbf{a}_i(t) + \mathbf{a}_i(t + \Delta t)] \Delta t\end{aligned}\quad (1.2)$$

where $\mathbf{a}$ is the acceleration calculated from Eq. (1.1) and Δt the time increment.

Molecular Dynamics simulations can provide insight into the fluctuations and conformational changes of proteins and they are used to analyse the structure and dynamics of biological systems. In contrast to Monte Carlo simulations, MD techniques are able to access the dynamics of the system and recover realistic time-scales.

Langevin and Brownian Dynamics Simulations

Langevin and Brownian Dynamics algorithms have been developed in the early 1900s to describe the dynamics of colloidal particles in a solvent. These methods consist of replacing the explicit solvent molecules by a stochastic force, $\mathbf{R}$ and a friction force. In other words, a particle in a solvent will experience collisions with the solvent molecules which give rise to a systematic force, opposite to the particle's velocity $\mathbf{v}$, and to a stochastic force. The Langevin equation of motion for particle i then reads:

$$m_i \mathbf{a}_i = \mathbf{F}_i - \xi \mathbf{v}_i + \mathbf{R}_i \quad (1.3)$$

where $\mathbf{F}_i$ represents the sum of all conservative forces acting on particle i . The stochastic force is correlated with the friction coefficient ξ through the fluctuation-dissipation theorem. The random force has no correlation between its components (Markovian), zero mean and friction dependent variance

$$\begin{aligned}\langle \mathbf{R}(t) \rangle &= 0 \\ \langle \mathbf{R}(t) \mathbf{R}(t') \rangle &= 2k_B T \xi \delta(t - t') \mathbf{1}\end{aligned}\quad (1.4)$$

where t and t' represent two distinct moments in time and $\mathbf{1}$ the unit matrix.

The main difference between the two methods is that Langevin Dynamics uses second order equations of motion, to simulate the time evolution of the particle positions and their

velocities, whereas Brownian Dynamics uses first order equations of motion, focussing solely on the evolution of the positions of the particles. More details on Brownian Dynamics will be presented throughout this thesis.

1.6 Thesis Outline

This thesis describes the use of coarse grained simulations to investigate the aggregation of proteins into ordered structures. The aim is to first study the aggregation of a simple protein by using Brownian Dynamics to simulate the dynamics of the system. Both the algorithm and the method are further developed throughout the thesis in order to fully describe the aggregation of the complex α -synuclein protein. The thesis is organised as follows.

In **Chapter 2** we simulate the aggregation of clathrin, a coat protein involved in endocytosis, into polyhedral cages. We represent a single protein as a rigid particle with interaction patches on its surface and apply an already existing Rotational Brownian Dynamics algorithm for anisotropic particles to study its aggregation on realistic timescales.

Since the algorithm in Chapter 2 proves to be fairly complex, we introduce a novel elementary Brownian Dynamics algorithm in **Chapter 3**. This algorithm uses quaternions to describe the rotation and orientation of anisotropic particles and removes all complications usually associated with Rotational Brownian Dynamics algorithms. The theory and its validation are presented throughout this chapter.

In **Chapter 4** we proceed by combining the algorithm introduced in Chapter 3 with a patchy particle approach to represent α -synuclein. Here we model α -synuclein as a single particle with variable properties, reflecting the chameleonic behaviour of this protein, and study its aggregation behaviour. We investigate aggregation pathways and compare them against existing theories.

In **Chapter 5** we extend the model to represent a protein as a chain of particles able to adapt their conformation. Here we investigate the attachment of a protein to a matured fibril and study their structures.

At the end of this thesis the results are summarised both in English and Dutch.

2. Clathrin Cage Formation

The self-assembly of nearly rigid proteins into ordered aggregates is well suited for modelling by the patchy particle approach. Patchy particles are traditionally simulated using Monte Carlo methods, to study the phase diagram, while Brownian Dynamics simulations would reveal insights into the assembly dynamics. However, Brownian Dynamics of rotating anisotropic particles gives rise to a number of complications not encountered in translational Brownian Dynamics. We thoroughly test the Rotational Brownian Dynamics scheme proposed by Naess and Elsgaeter, confirming its validity. We then apply the algorithm to simulate a patchy particle model of clathrin, a three-legged protein involved in vesicle production from lipid membranes during endocytosis. Using this algorithm we recover time-scales for cage assembly comparable to those from experiments. We also briefly discuss the undulatory dynamics of the polyhedral cage ¹.

2.1 Introduction

Patchy particles are rigid nanoscopic bodies, subjected to Brownian motion, with attractive and/or repulsive interaction sites on their surfaces. These features enable patchy particles to self-assemble into a wide variety of well ordered structures. Patchy particles have recently started to attract a great deal of interest because of their wide potential application spectrum and the developments in production techniques of patchy particles with tailored properties. Patchy particles are also being used as model systems to study the self-assembly of biological, physical

¹This chapter has been published as **I.M. Ilie**, W.K. den Otter and W.J. Briels, 'Rotational Brownian Dynamics simulations of clathrin cage formation', *J. Chem. Phys.*, **141**, 065101 (2014)

and chemical composites into well-ordered aggregates. We refer the interested reader to a number of recent reviews for more applications [73, 119, 220].

The self-assembly of patchy particles into aggregates is also being studied by computer simulations. Examples include crystal formation [18, 226], fibril formation [209], virus capsids [78, 217] and clathrin cages [125, 145, 147]. Almost all these studies use the Monte Carlo (MC) simulation technique of accepting or rejecting random trial moves based on the potential energy changes in these moves [67, 104]. The Monte Carlo method is relatively straightforward to implement, can handle both smooth and hard potentials, and is known to sample the canonical distribution correctly, making it an attractive technique to study the phase behaviour of patchy particles. Growing aggregates have been observed to freeze when using single particle trial moves; this issue has been solved by the development of cluster moves [128, 217]. A disadvantage of Monte Carlo is the interpretation of the simulated assembly process, as the MC moves are unrelated to the actual dynamics of the simulated particles.

Brownian Dynamics (BD) and Langevin Dynamics (LD) were specifically developed to simulate the dynamics of colloidal particles in solution. In these methods, to save computer time, the effects of the fluid on the particles dynamics – like friction and random Brownian noise – and on the particle-particle interactions – like screening of Coulombic interactions and in some simulations hydrodynamic interactions – are all included in the equations of motion of the particles and the fluid itself is not simulated. Langevin Dynamics uses second order equations of motion, to simulate the time evolution of the particle positions and their velocities. The integration timestep is much larger than the typical time scale in the collision of a solvent molecule against a colloid, but must be small enough for the inter-particle forces and the particle velocities to not have changed appreciably over the course of a single step. Brownian Dynamics uses first order equations of motion, focussing solely on the evolution of the positions of the particles. The upper limit to the integration time step is now determined by the condition that the inter-particle forces should change little over the interval. The combination of BD with patchy particles – both were developed from similar ideas on sacrificing details to gain access to longer time and length scales – offers a promising approach to study the dynamics of self-assembly processes.

Langevin and Brownian Dynamics are routinely used to solve the collective translational dynamics of spherical particles [186, 189, 192], using for example the algorithm of Ermak and McCammon [54]. Brownian dynamics of translating and rotating spherocylinders [114], ignoring rotations around the rods's long axes, have been used to study liquid crystals [151] and the

collective tumbling of liquid crystals in a shear flow [190]. Rotational Brownian dynamics (RBD) of rigid anisotropic bodies rotating around all three axes is less easily simulated, and a widely accepted algorithm for this generic case appears to be missing in the literature. A complicating factor arises from the degeneracies innate to any set of three coordinates describing the three-dimensional orientation of an anisotropic body [74]; the resulting diverging singularities when the rotational equations of motions are expressed in Euler angles are well known [3]. Elgsaeter, Naess and co-workers have, in a series of papers, [56, 57, 58, 131] developed and numerically validated an RBD algorithm based on a Cartesian rotation vector. This particular set of coordinates substitutes diverging singularities with well-behaved converging singularities. These authors also addressed two additional non-trivial terms, the spurious drift and mass-metric correction (to be discussed in the next section), that prove crucial to recovering the correct equilibrium distribution and rotational diffusion dynamics in simulations based on generalized coordinates.

Rotational Brownian Dynamics algorithms have rarely been used to simulate the self-assembly process of patchy particles [78, 171], presumably mainly because of the absence of an established RBD algorithm. The few reported RBD studies are based on straightforward extensions of translational Brownian dynamics, while surprising little attention has been paid to validating the resulting algorithm [41, 84, 135, 169]. The development of a proven RBD algorithm by Elgsaeter, Naess and co-workers inspired us to apply their technique to patchy particle simulations. In this chapter, we present additional validation tests of the RBD algorithm, followed by the simulation of a biological self-assembling system. The application to clathrin, a cage-building protein involved in endo- and exocytosis, demonstrates the feasibility of patchy particle studies by RBD simulations as well as yielding a self-assembly time scale that compares favourable with existing *in vitro* experiments on clathrin.

The structure of this chapter is as follows: Sec. 3.2 describes the translational and rotational propagators in a Brownian Dynamics algorithm for anisotropic particles. Numerical examples validating the algorithm, by reproducing the correct equilibrium distribution and rotational diffusion dynamics, are presented in Sec. 3.4. The biological example application, the self-assembly of three-legged clathrin proteins into polyhedral cages, is discussed in Sec. 2.4. The chapter ends with a brief summary of the main conclusions in Sec. 2.5.

2.2 Brownian Dynamics Algorithm

2.2.1 Generalized Equation of Motion

Brownian Dynamics (BD) is used to simulate nanoscopic particles moving through a liquid, where they continuously experience collisions with the solvent molecules. These collisions are translated into a friction term and a random contribution in the equation of motion of the particles, with the first term accounting for the average effect of the collisions and the second for the fluctuations around this average. The Brownian Dynamics equation of motion, expressed in terms of generalized coordinates $\mathbf{q}$, reads as [29]:

$$\begin{aligned} \mathbf{q}(t + \Delta t) - \mathbf{q}(t) = & -\boldsymbol{\mu}^q \frac{\partial \mathcal{A}}{\partial \mathbf{q}} \Delta t \\ & + k_B T \frac{\partial}{\partial \mathbf{q}} \cdot \boldsymbol{\mu}^q \Delta t \\ & + (\boldsymbol{\mu}^q)^{1/2} \boldsymbol{\Theta}(t) \sqrt{2k_B T \Delta t}. \end{aligned} \quad (2.1)$$

The first term on the right hand side represents the displacements over a time step Δt due to the average spatial velocity resulting from the balance between the thermodynamic force acting on the particle, $\mathbf{F} = -\partial \mathcal{A} / \partial \mathbf{q}$, and the opposing solvent friction. Here the free energy of the system $\mathcal{A}$ and the mobility tensor $\boldsymbol{\mu}^q$ are both expressed in terms of the generalised coordinates. The last term on the right hand side describes the random displacement of the particle, where the components of the time-dependent Markovian vector $\boldsymbol{\Theta}$ have zero mean, unit variance, no correlations across the components and no memory. The size of these random displacements is related to the mobility tensor through the fluctuation-dissipation theorem, as incorporated in Eq. (2.1), with k_B the Boltzmann constant, T the absolute temperature and where the square root $\mathbf{m}$ of a matrix $\mathbf{M}$ is defined by $\mathbf{m} \mathbf{m}^T = \mathbf{M}$. For a system with a coordinate-dependent mobility tensor, the BD algorithm based on the two preceding terms is known to yield an equilibrium probability distribution that differs from the canonical distribution, $\mathcal{P}(\mathbf{q}) \propto \exp(-\beta \mathcal{A})$, with $\beta = (k_B T)^{-1}$. The second term in Eq. (2.1) corrects for this ‘spurious drift’ to recover the Boltzmann equilibrium distribution.

Equation (2.1) can be used, in combination with a properly chosen set of generalised coordinate, to reproduce the dynamics of the entire system. For clarity of presentation, we will divide this equation into a set of coupled equations, to yield one equation describing the translational motion of a particle and one equation describing the rotational motion of a particle.

2.2.2 Translational Dynamics

The simplest implementation of the Brownian Dynamics equations of motion is encountered in the study of translation dynamics. We will briefly present the translational motion of the centre of mass $\mathbf{R}$ of a particle characterised by a constant space-fixed (s) translational (t) mobility tensor $\boldsymbol{\mu}^{t,s}$. The translational equation of motion for this particle reads as

$$\begin{aligned} \mathbf{R}(t + \Delta t) - \mathbf{R}(t) = & \boldsymbol{\mu}^{t,s} \mathbf{F} \Delta t \\ & + (\boldsymbol{\mu}^{t,s})^{1/2} \boldsymbol{\Theta}^t(t) \sqrt{2k_B T \Delta t}, \end{aligned} \quad (2.2)$$

where for Cartesian position coordinates the thermodynamic force reduces to the usual Cartesian force, $\mathbf{F} = -\partial\Phi/\partial\mathbf{R}$ with Φ the potential, and where $\boldsymbol{\Theta}^t$ denotes a three-dimensional time-dependent Markovian vector. For a rotating non-spherical particle, the translational mobility tensor in space-fixed Cartesian coordinates varies with the orientation of the particle, following

$$\boldsymbol{\mu}^{t,s} = \mathbf{A} \boldsymbol{\mu}^{t,b} \mathbf{A}^T, \quad (2.3)$$

where $\boldsymbol{\mu}^{t,b}$ denotes the constant body-fixed (b) translational mobility tensor. The rotation matrix $\mathbf{A}$ enables transformations from the body fixed frame to the space fixed frame, with its transposed $\mathbf{A}^T$ facilitating the reverse transformation. Hence, the set of three constant body-fixed coordinates $\mathbf{x}_{ip}$ for the p th patch on particle i is mapped onto the space-fixed coordinates

$$\mathbf{r}_{ip} = \mathbf{A}_i \mathbf{x}_{ip} + \mathbf{R}_i. \quad (2.4)$$

In the translational equation of motion, the spurious drift correction of Eq. (2.1) vanishes as the translational mobility matrix $\boldsymbol{\mu}^{t,s}$ is independent of the particle's translational coordinates $\mathbf{R}$. If the body-fixed frame is conveniently chosen to diagonalise $\boldsymbol{\mu}^{t,b}$, then its square root is obtained by simply taking the square roots of the diagonal elements; the square root appearing in the last term of Eq. (2.2) is then given by $(\boldsymbol{\mu}^{t,s})^{1/2} = \mathbf{A}(\boldsymbol{\mu}^{t,b})^{1/2}$.

2.2.3 Rotational Dynamics

The calculation of three dimensional Brownian rotational motion by a set of three coordinates is hampered by numerical complications – the strong singularities associated with rotational dynamics in Euler angles are well known [3]. As a solution, Naess *et al.* [131, 133] proposed a Brownian Dynamics algorithm that uses the components of a Cartesian rotation vector, $\mathbf{a}$, as the set of generalised coordinates for rotation. In their approach, the rotation of a rigid body about its centre of mass is fully represented by the angle of rotation $a = |\mathbf{a}|$ and the unit vector

$\hat{\mathbf{a}} = \mathbf{a}/a$ specifying the rotation axis. Naess, Elgsaeter and coworkers [132] have shown that this set of coordinates enables the development of a Rotational Brownian Dynamics (RBD) algorithm free of strong singularities. Since this RBD technique is not widely known and since the previous introductory descriptions of the method [55, 56, 57, 131, 132, 133] are not entirely consistent, we here provide a short overview of the method. Moreover, we will present in Sec. 3.4 a number of tests to demonstrate that the algorithm reproduces the correct static and dynamic properties, including the first direct analysis of the rotational diffusion process. In the remainder of this section, the rotational equation of motion is discussed and the role of the metric tensor is explained.

The rotational equation of motion of an anisotropic particle subject to a Brownian motion reads as

$$\begin{aligned} \mathbf{a}(t + \Delta t) - \mathbf{a}(t) = & \boldsymbol{\mu}^{r,a} \mathbf{T} \Delta t \\ & + k_B T \frac{\partial}{\partial \mathbf{a}} \cdot \boldsymbol{\mu}^{r,a} \Delta t \\ & + (\boldsymbol{\mu}^{r,a})^{1/2} \boldsymbol{\Theta}^r(t) \sqrt{2k_B T \Delta t} \end{aligned} \quad (2.5)$$

with $\boldsymbol{\mu}^{r,a}$ the rotational mobility tensor relating to the space-fixed rotation coordinates $\mathbf{a}$, the generalized torque $\mathbf{T} = -\partial \mathcal{A} / \partial \mathbf{a}$, and the three-dimensional Markovian vector $\boldsymbol{\Theta}^r$. It proves advantageous to define the body-fixed frame as the coordinate frame that diagonalises the rotational mobility tensor $\boldsymbol{\mu}^{r,b}$ [56]. We henceforth assume that this body-fixed frame also diagonalises the translational mobility tensor $\boldsymbol{\mu}^{t,b}$; the modifications required when this condition fails are straightforward. Similar to the transformation of the body-fixed translational mobility tensor to the space frame in Eq. (2.3), the transformation of the body-fixed rotational mobility tensor to the laboratory frame reads as $\boldsymbol{\mu}^{r,s} = \mathbf{A} \boldsymbol{\mu}^{r,b} \mathbf{A}^T$. This tensor converts a conventional Cartesian torque $\boldsymbol{\tau}$ in the space frame into an angular velocity $\boldsymbol{\omega}$ in the space frame. Before insertion in Eq. (2.5), these two vectors still need to be related to their respective counterparts in generalised coordinates, i.e. the generalised torque $\mathbf{T}$ and the generalised rotational velocity $\dot{\mathbf{a}}$. This translation is achieved by the transformation matrix $\mathbf{E}$ and its transposed, which convert between infinitesimal rotations around the Cartesian laboratory axes and the corresponding infinitesimal changes of the vector $\mathbf{a}$ [57]. A more detailed explanation of this conversion, as well as explicit expressions for the transformation and rotation matrices, are provided in the Appendix 4.5.1. The rotational mobility tensor in terms of the rotation vector is finally expressed as

$$\boldsymbol{\mu}^{r,a} = \mathbf{E} \mathbf{A} \boldsymbol{\mu}^{r,b} \mathbf{A}^T \mathbf{E}^T. \quad (2.6)$$

This equation first rotates the constant body-fixed rotational mobility tensor $\boldsymbol{\mu}^{\text{r,b}}$ into the laboratory frame, by using the two rotation matrices, and then converts the resulting matrix into generalised coordinates $\mathbf{a}$, by applying the two transformation matrices.

The generalized torque on particle i has a component arising from inter-particle interactions, as well as an additional component $\mathbf{T}^{\text{m}}$ associated with the metric tensor,

$$\mathbf{T}_i = -\frac{\partial \Phi}{\partial \mathbf{a}_i} + \mathbf{T}^{\text{m}}(\mathbf{a}_i). \quad (2.7)$$

The mass-metric tensor $\mathbf{g}$ features in the phase space probability distribution function

$$\mathcal{P}(\mathbf{q}, \mathbf{p}) d\mathbf{q} d\mathbf{p} \propto e^{-\beta \mathcal{H}(\mathbf{q}, \mathbf{p})} d\mathbf{q} d\mathbf{p}, \quad (2.8)$$

where the Hamiltonian of a system, expressed in terms of generalized coordinates $\mathbf{q}$ and their conjugate momenta $\mathbf{p}$, reads as

$$\mathcal{H} = \Phi + \frac{1}{2} \mathbf{p}^{\text{T}} \mathbf{g}^{-1} \mathbf{p}. \quad (2.9)$$

When this distribution is mapped onto a configuration space probability distribution function, by integrating over the momenta, we arrive at

$$\begin{aligned} \mathcal{P}(\mathbf{q}) d\mathbf{q} &\propto \int \mathcal{P}(\mathbf{q}, \mathbf{p}) d\mathbf{p} d\mathbf{q} \\ &\propto e^{-\beta \Phi(\mathbf{q})} \sqrt{\det(\mathbf{g})} d\mathbf{q}. \end{aligned} \quad (2.10)$$

The mass-metric contribution to the latter probability distribution is automatically included correctly in simulations with explicit velocities, but it has to be added explicitly in simulations without velocities in order to recover the correct equilibrium configurational distribution (of course, this is only required if $\mathbf{g}$ proves to be a function of $\mathbf{q}$). Hence, the generalized force in the generalized BD equation of motion, see Eq. (2.1), is not derived from the potential energy but from the free energy, with

$$\mathcal{A}(\mathbf{q}) = -k_B T \ln \mathcal{P}(\mathbf{q}). \quad (2.11)$$

For a rigid body whose orientation is described by the rotation vector $\mathbf{a}$, the metric tensor contribution to the torque reads as

$$\mathbf{T}^{\text{m}}(\mathbf{a}) = \frac{1}{2} k_B T \frac{\partial \ln |\mathbf{g}(\mathbf{a})|}{\partial \mathbf{a}}, \quad (2.12)$$

with the explicit expression derived in the appendix. We note that this correction is independent of the mass distribution within the rigid body.

The random contribution to the rotational motion of a particle is generated by the last term on the right hand side of Eq. (2.5), using time dependent Markovian vectors with three independent Cartesian components of unit variance, zero mean, no memory, no correlation between the particle pairs, and unrelated to the translational random vectors. In the simulations presented below, these stochastic terms have been sampled from a Gaussian distribution [3, 123]. The random contribution is again related to the friction by the fluctuation-dissipation theorem, which is incorporated in the equation of motion. The square root of the rotational mobility tensor is readily calculated as $(\boldsymbol{\mu}^{r,a})^{1/2} = \mathbf{E}\mathbf{A}(\boldsymbol{\mu}^{r,b})^{1/2}$. Because the rotational mobility tensor entering Eq. (2.5) is a function of the rotational coordinates, see Eq. (2.6), the aforementioned spurious drift correction is now included as the second term of the right hand side of the equation of motion, in order to recover the canonical Boltzmann distribution.

2.3 Validation of the algorithm

To assess the static and dynamic properties of the Rotational Brownian Dynamics algorithm, we have simulated several anisotropic particles with distinct eigenvalues down the diagonals of their body-fixed translational and rotational mobility tensors. The simulations were run in internal units, with σ as the length unit, τ as the time unit and ϵ as the unit of energy. For the typical one-particle system presented in this section, we chose the diagonal elements as $\mu_1^t = 5$, $\mu_2^t = 7$ and $\mu_3^t = 9$ in internal units of $\sigma^2/(\tau\epsilon)$ and $\mu_1^r = 1$, $\mu_2^r = 10$ and $\mu_3^r = 20$ in internal units of $(\tau\epsilon)^{-1}$, while the off-diagonal elements are all zero. A time step of $\Delta t = 10^{-5}\tau$ was used to ensure the stability of the algorithm. The simulations typically lasted for 10^8 time steps, with the temperature set at $T = 1\epsilon/k_B$. Below we demonstrate that the algorithm samples the correct rotational probability distributions in the absence and presence of an alignment field. We also analyse the rotational diffusion coefficients, which for this algorithm have – to the best of our knowledge – not previously been investigated in this way.

2.3.1 Static Properties

We first verified that body fixed vectors $\hat{\mathbf{u}}^b$ attached to both isotropic and anisotropic particles sample the correct Boltzmann equilibrium distributions for the polar angle θ and the azimuthal angle ϕ when viewed in the laboratory frame (results not shown). Next, we placed a particle with two electric charges $+q$ and $-q$, diametrically opposed along a body-fixed $\hat{\mathbf{u}}^b$ axis and separated by a distance d , in a uniform electric field of strength E oriented along the z direction, $\mathbf{E} = E\hat{\mathbf{e}}_z$. The total force on an electric dipole $\mathbf{p}$ placed in a uniform electric field is zero, while

Figure 2.1: Probability distribution of the polar angle θ between the dipole moment $\mathbf{p}$ of a particle and a uniform electric field $\mathbf{E}$, for $pE/k_BT = 1.5$

a torque $\boldsymbol{\tau} = \mathbf{p} \times \mathbf{E}$ strives to align the dipole with the direction of the field [76]. In the canonical ensemble, the orientational probability is then expected as

$$P(\theta)d\theta \propto e^{-\beta pE \cos \theta} \sin \theta d\theta, \quad (2.13)$$

where θ is the angle between the field direction and the electric dipole, and $p = qd$. The simulation results in Fig. 2.1 are in good agreement with statistical theory, demonstrating that the algorithm samples the correct rotational probability distribution.

2.3.2 Dynamic Properties

The translational diffusion coefficients calculated from the mean square displacement of the centre of mass of a rotating anisotropic body (in the absence of external fields) were found to be the same for all three space-fixed Cartesian directions and in good agreement with the theoretical prediction $D^t = k_BT \frac{1}{3} \text{Tr}(\boldsymbol{\mu}^{t,b})$, provided the simulation was run long enough to sample the equilibrium orientational distribution.

To study the rotational diffusion behaviour, we calculated the time correlation function of a body-fixed unit vector $\hat{\mathbf{u}}^b$ as seen in the space frame,

$$\langle P_{\hat{\mathbf{u}}^b}(t) \rangle = \frac{3}{2} \langle (\hat{\mathbf{u}}^s(t) \cdot \hat{\mathbf{u}}^s(0))^2 \rangle - \frac{1}{2}, \quad (2.14)$$

where $\hat{\mathbf{u}}^s(t) = \mathbf{A}(t)\hat{\mathbf{u}}^b$. Favro [60] derived that this function decays by (at most) a five-fold exponential relaxation process,

$$\langle P_{\hat{\mathbf{u}}^b}(t) \rangle = \sum_{i=1}^5 a_i e^{-t/\tau_i}. \quad (2.15)$$

i	a_i	τ_i
1	$\frac{3}{4}(A + B)$	$(6D - 2\Delta)^{-1}$
2	$3(\hat{u}_2^b \hat{u}_3^b)^2$	$(3(D + \mu_1^r))^{-1}$
3	$3(\hat{u}_1^b \hat{u}_3^b)^2$	$(3(D + \mu_2^r))^{-1}$
4	$3(\hat{u}_1^b \hat{u}_2^b)^2$	$(3(D + \mu_3^r))^{-1}$
5	$\frac{3}{4}(A - B)$	$(6D + 2\Delta)^{-1}$

Table 2.1: The five rotational relaxation times τ_i and the corresponding amplitudes a_i for a body-fixed vector $\hat{\mathbf{u}}^b$, see Eq. (2.15). The three body-fixed basis vectors $\hat{\mathbf{e}}_\alpha$ are eigenvectors of the rotational mobility matrix, sorted by eigenvalue, $\mu_1^r \leq \mu_2^r \leq \mu_3^r$, and $\hat{u}_\alpha^b$ denotes the component of $\hat{\mathbf{u}}^b$ parallel to $\hat{\mathbf{e}}_\alpha$. Furthermore, $\sum_{i=1}^5 a_i = 1$, $D = \frac{1}{3}(\mu_1^r + \mu_2^r + \mu_3^r)$, $\Delta = \sqrt{(\mu_1^r - \mu_2^r)^2 + (\mu_3^r - \mu_2^r)(\mu_3^r - \mu_1^r)}$, $A = -\frac{1}{3} + \sum_{\alpha=1}^3 (\hat{u}_\alpha^b)^4$ and $B = \Delta^{-1}(-D + \sum_{\alpha=1}^3 \mu_\alpha^r [(\hat{u}_\alpha^b)^4 + 2(\hat{u}_\beta^b \hat{u}_\gamma^b)^2])$, where in the latter summation (α, β, γ) is a cyclic permutation of $(1, 2, 3)$.

The relaxation times τ_i and the amplitudes a_i depend on the rotational mobility matrix of the particle and on the selected body-fixed vector $\hat{\mathbf{u}}^b$; explicit expressions are provided in Table 2.1 [60, 94, 196, 197]. Figure 2.2 shows the simulation results for three $\hat{\mathbf{u}}^b$ -s coinciding with the three body fixed basis vectors that diagonalise the mobility matrix. For these particular choices of $\hat{\mathbf{u}}^b$ three of the terms in Eq. (2.15) vanish identically. Of the two remaining exponentials, mode 5 dominates for short times and mode 1 for long times. The figure also shows the relaxation of the body fixed unit vector parallel to $(5, 4, 1)$, to illustrate a decay with five distinct relaxation times. The decomposition into the five exponentials is shown in Figure 2.3 where modes 4 and 1 are seen to dominate at short and long times respectively. We observe excellent agreement with the theory by Favro, proving that the algorithm produces the correct rotational dynamics. Equally good agreements were found for other body-fixed vectors and for other values of the diagonal elements of the body-fixed rotational mobility matrix.

2.4 Application: Clathrin Cage Formation

As an application illustrating the potential of the Rotational Brownian Dynamics algorithm described in Sec. 3.2, we here present simulations of the self-assembly of clathrin into cages. Firstly, we introduce clathrin and the characteristics of importance in cage formation, followed by a description of a patchy particle model that captures these features. Using this model, den

Figure 2.2: Rotational relaxation in the simulations (open symbols) compared with theoretical predictions (solid lines) for the three body fixed eigenvectors of the rotational mobility matrix and one 'random' vector, specified in the main text.

Otter et al. established that the self-assembly of cages requires the interaction patches to be concentrated at the inside of the legs [147] and that the experimental *in vitro* critical assembly concentration [40] corresponds to an average binding energy of 20-25 $k_B T$ per clathrin [145]. Since these simulations used Monte Carlo simulations, they could not explore the timescale of the self-assembly process. We here present Rotational Brownian Dynamics (RBD) simulations of the assembly of clathrin cages from solution, and briefly discuss the relaxation dynamics of these cages.

2.4.1 Simulation Model

Clathrin is a protein constructed of three identical long curved units, referred to as *legs*, which are conjoined by trimerization domains near their C-terminus at the clathrin's *hub*, see Fig. 2.4 [23, 98, 165, 224]. Each leg consists of a proximal segment, running from the hub to the bend at the *knee*, and a distal segment, from the knee to the *ankle*. In a cage, the knee resides beneath the hub of a neighbouring clathrin, and the ankle below the hub of a next-nearest clathrin [66]. Hence, every edge of the cage is composed of four leg segments. Affixed to the ankles and pointing toward the cage interior are the terminal domains that link clathrin to assisting proteins; since this domain does not appear to contribute to the cage's stability, it is not included in the current simulations.

Den Otter et al. [145, 147] proposed a rigid patchy particle model for clathrin, in which the three proximal legs radiate under a pucker angle χ relative to the particle's three-fold rotational

Figure 2.3: Contributions of the five exponentials in Eq. (2.15) to the total relaxation dynamics of the 'random' body fixed vector of Figure 2.2.

Figure 2.4: (a) Coarse grained simulation model of the clathrin triskelion, with the hub in blue, the knees in grey and the ankles in red. The polarity vectors $\hat{\mathbf{m}}$ of one proximal and one distal segment are indicated. (b) Side view illustrating the curvature of the particle, with pucker angle χ between the proximal leg segments and the normal vector $\hat{\mathbf{n}}_h$ parallel to the rotational symmetry axis. (c) Self-assembled cage of 60 clathrins in the soccer-ball configuration.

symmetry axis. The fixed orientation of the distal legs was selected to maximise the contact between two adjacent clathrin proteins with their hubs located at their neighbour's knee. Both proximal and distal segments have the same length σ . A four-site inter-segmental interaction potential was introduced between every pair of leg-segments, based on the distances between the ends of the two segments and their relative orientation. As an illustrative example, we will here discuss the interaction between the proximal (p) segment of the α pleg of particle i and the distal (d) segment of the β pleg of particle j . This interaction potential is described by the two possible combinations that pair up the four involved segmental end points: $\Phi_{j\beta,ka}^{i\alpha,hk}$ for the hub of the (i, α) leg being close to the knee of the (j, β) leg and simultaneously the knee of the (i, α) leg being close to the ankle of the (j, β) leg, and $\Phi_{j\beta,ak}^{i\alpha,hk}$ for the hub of the (i, α) leg being close to the

ankle of the (j, β) leg and simultaneously the knee of the (i, α) leg being close to the knee of the (j, β) leg. The sub- and superscripts to Φ specify the pairing, with h, k and a referring to the hub, knee and ankle, respectively. The latter of the two potential contributions is specified by

$$\Phi_{j\beta,ak}^{i\alpha,hk} = -\varepsilon_{ak}^{hk} \cdot f(r_{j\beta,ak}^{i\alpha,hk}) \cdot g(\hat{\mathbf{m}}_{\alpha p}^i \cdot \hat{\mathbf{m}}_{\beta d}^j) \quad (2.16)$$

where ε_{ak}^{hk} represents the interaction strength or inter-segmental binding energy (with a positive value for attractions and a negative value for repulsions). For clarity, the discussion of the potential will focus on this particular contribution, as it has the same structure as all other interactions. The numerical values of the force field parameters have been collected in Table 2.2.

The average hub-ankle and knee-knee distance entering Eq. (2.16) is calculated as

$$r_{j\beta,ak}^{i\alpha,hk} = \frac{1}{2} |\mathbf{r}_{\alpha h}^i - \mathbf{r}_{\beta a}^j| + \frac{1}{2} |\mathbf{r}_{\alpha k}^i - \mathbf{r}_{\beta k}^j|, \quad (2.17)$$

where $\mathbf{r}$ denotes the laboratory frame position of the segmental end specified by the indices. The distance dependent part of the potential is given by

$$f(r) = \frac{\tanh[A(r_{\text{cut}}/2 - r)]}{2 \tanh[A r_{\text{cut}}/2]} + \frac{1}{2}, \quad (2.18)$$

where A sets the steepness of the potential and r_{cut} the cut-off distance beyond which there is no interaction between the leg segments. The value of the function f gradually increases from -1 at a distance of zero to 0 at the cut-off distance, with an inflection point at $r_{\text{cut}}/2$. The smooth shape allows for small displacements of the leg segments perpendicular to and along one another, in addition to permitting some inter-segmental misalignment [147]. Asymmetric interactions between the leg segments, generating the orientational ordering between adjacent particles that proved crucial to the self-assembly of cages [147], involves defining body-fixed polarity vectors $\hat{\mathbf{m}}$ perpendicular to the segments, see Fig. 2.4(a). In the laboratory frame, the polarity vector to the proximal segment of the α leg of particle i reads as

$$\hat{\mathbf{m}}_{\alpha p}^i = \mathbf{A}_i \frac{(\mathbf{x}_{\alpha k}^i - \mathbf{x}_{\alpha h}^i) \times \hat{\mathbf{n}}_h^i}{|(\mathbf{x}_{\alpha k}^i - \mathbf{x}_{\alpha h}^i) \times \hat{\mathbf{n}}_h^i|}, \quad (2.19)$$

where $\hat{\mathbf{n}}_h^i$ is a body-fixed normal vector parallel to the particle's rotational symmetry axis, pointing in the outward direction for particles bound in a cage. The polarity vector $\hat{\mathbf{m}}_{\beta d}^j$ to the distal segment of the interacting particle is constructed in a similar manner, by combining the knee and ankle positions with the knee normal vector $\hat{\mathbf{n}}_{\beta k}^j$; the latter makes an angle χ with both segments connected by the knee and points outward in a cage. The polarity-dependent function g in Eq. (2.16) reads for binding interactions as

$$g(x) = \begin{cases} -x & \text{for } x \leq 0 \\ 0 & \text{for } x > 0. \end{cases} \quad (2.20)$$

Attractive	$\varepsilon/k_B T$	A/σ^{-1}	r_{cut}/σ	g
hk – k'h'	8	4	0.4	$\hat{\mathbf{m}}_p \cdot \hat{\mathbf{m}}'_p$
ka – a'k'	8	4	0.4	$\hat{\mathbf{m}}_d \cdot \hat{\mathbf{m}}'_d$
hk – k'a'	4	4	0.4	$-\hat{\mathbf{m}}_p \cdot \hat{\mathbf{m}}'_d$
hk – a'k'	4	4	0.4	$\hat{\mathbf{m}}_p \cdot \hat{\mathbf{m}}'_d$
Repulsive				
hk – h'k'	-80	0.8	0.8	1
ka – k'a'	-80	0.8	0.8	1

Table 2.2: Interaction parameters of the six distinct segmental interactions. In the first and last columns, the primed and unprimed labels refer to sites on two different particles. The elements of the last column represent the arguments of the polarity function $g(x) = -x \cdot \Theta(-x)$ for the attractive segment combinations and the actual values of this function for repulsive segment combinations, with Θ the Heaviside function.

Repulsive interactions, to prevent the accumulation of more than 4 segments along an edge, are radially symmetric: $g = 1$.

There are six possible inter-segmental interactions, depending on the types of segments involved (pp, dd or pd) and their alignment (exchanging the two end points of either segment changes the interaction). Each combination is characterised by a particular combination of inter-segmental binding energy ε , steepness of the potential A , cut-off distance r_{cut} and polarity function g . The expressions for these interactions are of the form given in Eqs. (2.16) through (2.20), with the numerical values of the parameters listed in Table 2.2.

In the Brownian Dynamics algorithm of Sec. 3.2, the generalised forces and torques acting on the particles are used in the equations of motion. The total force on particle i is readily obtained by differentiating the above pairwise potentials with respect to the segmental end positions (i.e. one hub, 3 knees and 3 ankles) and summing the resulting forces on these 7 positions. For rigid bodies, the polarity vectors as seen by a lab-fixed observer remain constant during the differentiation with respect to the particle's centre of mass position, hence only the distance dependent functions f need to be differentiated when evaluating these forces. Calculating the generalised torque, see Eq. (2.7), is slightly more involved. The above forces on the segmental end points are readily converted, by applying Eq. (2.4) and the chain rule, into a contribution to the torque. The polarity vectors rotate with the body and thereby provide an additional

contribution to the generalised torque. Finally, we recall that the metric tensor correction also generates a torque. The sum of these three contributions represents the total generalised torque acting on a particle's centre of mass and enters the equation of motion.

Besides the force field, the Brownian Dynamics algorithm described in Sec. 3.2 also requires translational and rotational mobility tensors. These were determined using the HYDRO++ program [198, 199] designed to calculate the translational and rotational diffusivity matrices, $\mathbf{D} = k_B T \boldsymbol{\mu}$, of rigid objects consisting of multiple beads. We followed the approach taken by Ferguson et al. [61] and Yoshimura et al. [223]: in their evaluation of clathrin's mobility matrices a single clathrin is approximated as an array of 52 spheres inspired by the real dimensions (leg segment length and diameter) and overall shape of the protein. At this point the orientation of the triskelion must be specified: the center of mass is at the origin of the body-fixed coordinate system, the third axis of this orthonormal system runs along the three-fold rotational symmetry axis, the particle's first proximal leg lies in the plane spanned by the first and third axis, and the second axis follows from the cross product of the other two axes. This definition of the orientation has the appealing property that it simultaneously diagonalizes the body-fixed translation and rotational diffusivity tensors. For a triskelion with a pucker angle $\chi = 101^\circ$, dissolved in water at a temperature of 293 K with a viscosity of $\eta = 10^{-3}$ Pa s, HYDRO++ gives the eigenvalues of the Cartesian translational diffusivity tensor as $D_1^t = D_2^t = 1.29 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ and $D_3^t = 1.07 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$, and the diagonal elements of the rotational diffusivity tensor as $D_1^r = D_2^r = 1.64 \times 10^4 \text{ s}^{-1}$ and $D_3^r = 1.02 \times 10^4 \text{ s}^{-1}$. These values agree with the previous calculations [61, 223], and the corresponding average translational diffusion coefficient is in good agreement with experimental data [223].

2.4.2 Results and Discussions

The simulation systems were initialised by placing 200 triskelia at random positions, and with random orientations, in a cubic simulation box with periodic boundary conditions. The concentration of one particle per $10^3 \sigma^3$, with $\sigma \approx 17$ nm the length of a leg segment, corresponds with twice the experimental critical assembly concentration (CAC) of about $50 \mu\text{g/ml}$ [40]. In a previous study, den Otter and Briels [145] established a set of interaction strengths to match the CAC of the model particles with the experimental CAC, arriving at an average energy per clathrin of about $23 k_B T$. They rationalised their findings by means of theoretical models for micelle formation [70]. Muthukumar and Nossal extended this theory with a curvature contribution, thereby arriving at an average binding energy per clathrin of $21.2 k_B T$. [130] Saleem et al.

Figure 2.5: Potential energy plot for a box of 200 clathrin particles as a function of time. Critical nuclei are formed after ~ 0.4 s and ~ 0.6 s, and both grow into complete polyhedral cages of about 60 triskelia in about half a second.

have recently derived a binding energy of $23 k_B T$ per clathrin from the mechanical strength of a coat. [168] The interactions strengths used here, see Table 2.2, are higher by one-third than those used by den Otter and Briels, to enhance the formation of nucleation clusters. Visual inspection of the simulations using VMD[85] clearly shows the formation of clathrin cages by a nucleation and growth process. This is also illustrated by the potential energy plot in Fig. 2.5 and by the snapshots in Fig. 2.6. In this particular system, about 0.4 seconds of Brownian motion suffices to generate a stable nucleus, which in the subsequent half a second grows into a complete cage of about 60 triskelia. A second nucleus emerges after about 0.6 seconds, also requiring approximately half a second to grow into a complete cage. During the growth phase, newly arriving properly oriented particles attach to the rim of the partial cage, leaving no vacancies in the lattice beyond the rim. Almost all cages are defect-free at the end of the simulation. We did not observe any kinetically trapped partial cages for the binding energies used here, but they did occur at higher binding energies due to depletion of free clathrins. The emergence of the first stable nucleus varies considerably between the simulations, while the time elapsed during growth of a nucleus into a full cage is fairly constant between one-half and a full second. Experimental *in vitro* studies on clathrin in slightly acidic solutions report self-assembly time scales of the order of a minute, with a strong influence of pH and salt concentration [91, 136, 155]. For a first attempt at establishing realistic assembly time scales by Brownian dynamics simulations of patchy particles, and in view of the various approximations made – out of necessity – in creating the highly coarse grained patchy particle model, this is a very satisfactory agreement.

Figure 2.6: Magnified snapshots depicting the assembly of a cage during a simulation.

The current RBD approach to clathrin is motivated by the absence of realistic time scales in previous simulations of cage self-assembly. The Monte Carlo simulations by the Briels group[145, 147, 148] and by Matthews and Likos[125] clearly do not provide a time scale. The latter authors report the propensity of MC simulations to develop kinetically trapped aggregates, which appears to be a consequence of the employed MC technique – rather than of the triskelion model – since the kinetic trapping disappeared when using more advanced MC techniques and is also absent in dynamics simulations with explicit solvent[125]. The triskelion model by Schoen et al.[173] features straight legs (resulting in two legs along every cage edge) which can rotate

instantaneously around the hub normal; hence, we expect this model to aggregate quicker than our model. Neither of these alternative dynamical simulations provides a translation from internal time units to experimental time scales. Finally, we verified that the current RBD simulations recover the critical binding energy of $\sim 23k_B T$ per bound clathrin established in previous MC simulations[145] at the same particle concentration: cage nucleation only occurs above this binding energy, and existing cages disintegrate when the binding energy is lowered below this value.

Besides revealing the self-assembly process, the Brownian Dynamics simulations also provide insights into the fluctuation dynamics of assembled cages. Here, we will focus on the radial fluctuations. Since a cage comprises a large number of particles nearly homogeneously distributed over an almost spherical surface, an obvious choice is to describe the collective nature of these fluctuations in terms of the spherical harmonics $Y_{lm}(\theta, \phi)$. The shape of the cage is then expressed by

$$r(\theta, \phi) = \sum_{l=0} \sum_{m=-l}^l \rho_{lm} Y_{lm}(\theta, \phi), \quad (2.21)$$

where the polar angle θ and the azimuthal angle ϕ are measured in the laboratory frame. The amplitudes are calculated as

$$\rho_{lm} = \sum_{i=1}^N r_i(\theta_i, \phi_i) Y_{lm}^*(\theta_i, \phi_i), \quad (2.22)$$

where r_i denotes the distance from the center of mass of the cage $\mathbf{R}_{\text{cage}}$ to the center of mass $\mathbf{R}_i$ of particle i , and the asterisk denotes complex conjugation. As a consequence of this definition of r_i , the $l = 1$ modes have zero amplitude. This is best seen by noting that for $l = 1$ the product $r_i Y_{lm}^*(\theta_i, \phi_i)$ reduces to (a linear combination of) the components of the difference vector connecting the cage center to center of particle i ; since the cage center is calculated as the average of the particle positions, the summation over the difference vectors yields zero. The dynamics of the undulations of the near-spherical $N = 60$ bucky-ball and the slightly prolate $N = 36$ hexagonal barrel were analyzed by calculating the auto-correlations

$$S_{lm}(t) = \langle \Delta \rho_{lm}(\tau) \Delta \rho_{lm}^*(\tau + t) \rangle \quad (2.23)$$

of the deviation from the average amplitude,

$$\Delta \rho_{lm}(t) = \rho_{lm}(t) - \langle \rho_{lm}(\tau) \rangle, \quad (2.24)$$

where the pointy brackets denote an average over time τ .

Figure 2.7: Plot of the normalised undulatory auto correlation functions S_{lm} for the 36 triskelion hexagonal barrel, (a) before and (b) after correcting for rigid-body rotations of the entire cage.

Figure 2.7(a) shows the results for the hexagonal barrel. A curious difference emerges between the modes with even l , whose correlations hardly decay at all, and the modes with odd l , which display the anticipated relaxation behaviour – although there is no indication of the expected dependence on m . Inspection of the modes with even l reveals that $\Delta\rho_{lm}(t)$ combines a quickly fluctuating component with a small amplitude and a slowly fluctuating component with a large amplitude. The latter, which dominates the auto correlation functions, appears to be linked to the rotational dynamics of the entire cage. We therefore performed a second analysis to separate cage rotations from undulations prior to analysing the undulations. The overall rotation matrix $\mathbf{B}$ of a given cage configuration is determined by rigidly rotating the cage and comparing the resulting particle positions with their positions $\mathbf{R}_i^{\text{ref}}$ in a symmetric reference cage. Note that the center of mass position of the cage is subtracted before rotation, and that the reference

structure has its center of mass in the origin of the laboratory frame. In the selected reference configurations, the five-fold symmetry axes of the bucky-ball and the hexagonal barrel were oriented parallel to the laboratory z -axis, i.e. the line $\theta = 0$. The mean square difference

$$f = \sum_i \left\{ \mathbf{B}[\mathbf{R}_i(t) - \mathbf{R}_{\text{cage}}(t)] - \mathbf{R}_i^{\text{ref}} \right\}^2 \quad (2.25)$$

was numerically minimized with respect to $\mathbf{B}$, using Powell's method [154], to recover the overall rotation matrix $\mathbf{B}$ at time t . The rotation-corrected coordinates $\mathbf{R}'_i = \mathbf{B}(\mathbf{R}_i - \mathbf{R}_{\text{cage}})$ were then analyzed in terms of spherical harmonics using the procedure outlined above. Figure 2.7(b) shows that all autocorrelations now decay, with relaxation times that vary with l and m , in agreement with expectations. The relaxation process shows double exponential decay, combining a fast process with a relaxation time constant of 90 ns ($l = 2$) to 20 ns ($l = 4$) and a slow process with a relaxation time constant of 0.5 μs ($l = 2$) to 0.2 μs ($l = 4$). Since the relaxation rates vary with the cage structure, it is – in principle – conceivable that measuring the relaxation time distribution provides access to the structural composition of a mixture of self-assembled cages. The amplitudes and relaxation times of the experimental S_{lm} will most likely also contain information on the mechanical properties of the cage, like its elasticity and bending rigidity², in a convoluted manner. An analysis along these lines, as has been achieved for the undulations of a lipid membrane [176, 179], exceeds the objectives of the current study. To the best of our knowledge, these large scale modes of entire clathrin cages have not been studied previously.

2.5 Conclusions

The combination of patchy particles with rotational Brownian dynamics proves a powerful tool to study aggregation processes of functionalized colloids and near-rigid proteins. The simulations presented suggest that realistic time scale are accessible with properly parameterized models. It is to be expected that this approach can provide novel insights into the dynamics of self-assembly processes.

²In the simulated system, this bending rigidity of the cage results from the interaction potential between the rigid particles. In experiments, the internal flexibility of clathrin will also contribute to the cage's bending rigidity.

2.6 Appendix

2.6.1 Matrices

The position and orientation of a three-dimensional rigid body are fully defined by its center of mass position $\mathbf{R}$ and a rotation matrix $\mathbf{A}$. We assume, for convenience, that the body's center of mass coincides with the origin of the body-fixed coordinate system, and that in the reference orientation of the body, i.e. for $\mathbf{A} = \mathbf{1}$, the three body-fixed Cartesian directions are parallel to their space-fixed counterparts. The body-fixed coordinates $\mathbf{x} = (x_1, x_2, x_3)$ of a fixed point on the body are then mapped onto space-fixed coordinates $\mathbf{r} = (r_x, r_y, r_z)$ by

$$\mathbf{r} = \mathbf{A}\mathbf{x} + \mathbf{R}. \quad (2.26)$$

Starting with the body in its reference orientation, consider the rotation over an angle a around an axis running through the center of mass of the body; the combination of the angle and the unit vector $\hat{\mathbf{a}}$ along the rotation axis is henceforth combined into the space-fixed rotation vector $\mathbf{a} = a\hat{\mathbf{a}}$. This rotation projects the coordinates of the body-fixed point $\mathbf{x}$ onto the space coordinates

$$\mathbf{r} = \mathbf{x}_{\parallel} + \mathbf{x}_{\perp} \cos a + \hat{\mathbf{a}} \times \mathbf{x}_{\perp} \sin a + \mathbf{R}, \quad (2.27)$$

where $\mathbf{x}_{\parallel} = (\mathbf{x} \cdot \hat{\mathbf{a}}) \hat{\mathbf{a}}$ and $\mathbf{x}_{\perp} = \mathbf{x} - \mathbf{x}_{\parallel}$ denote the components of $\mathbf{x}$ parallel and perpendicular, respectively, to the unit rotation axis. The corresponding rotation matrix reads as [55, 57, 131]

$$\begin{aligned} \mathbf{A} = & \frac{1 - \cos a}{a^2} \begin{pmatrix} a_x a_x & a_x a_y & a_x a_z \\ a_y a_x & a_y a_y & a_y a_z \\ a_z a_x & a_z a_y & a_z a_z \end{pmatrix} \\ & + \frac{1}{a} \begin{pmatrix} a \cos a & -a_z \sin a & a_y \sin a \\ a_z \sin a & a \cos a & -a_x \sin a \\ -a_y \sin a & a_x \sin a & a \cos a \end{pmatrix}. \end{aligned} \quad (2.28)$$

The equation of motion for $\mathbf{a}$ is provided in Eq. (2.5). In the simulations, the unit vector $\hat{\mathbf{a}}$ is allowed to drift over the entire surface of the unit sphere, while we chose the rotation angle a to be limited to the interval $[0, \pi)$. To keep a within this range, whenever a surpasses π we replace its value by $a \rightarrow 2\pi - a$ and replace the rotation vector by $\mathbf{a} \rightarrow -\mathbf{a}$; since a is being calculated in the simulations as the length of $\mathbf{a}$, its value will never decrease below zero.

The transformation matrix $\mathbf{E}$ in Eq. (2.6) converts infinitesimal rotations of the body around the Cartesian laboratory axes into the corresponding infinitesimal changes of the rotation vector

$\mathbf{a}$, which is the same as the transformation of a space-fixed angular velocity vector $\boldsymbol{\omega}$ into to the generalized rotational velocity vector $\dot{\mathbf{a}}$,

$$E_{\alpha\beta} = \frac{\partial \dot{a}_\alpha}{\partial \omega_\beta}. \quad (2.29)$$

The lab velocity of the body-fixed point $\mathbf{x}$ can be expressed as the time derivative of Eq. (2.27),

$$\dot{\mathbf{r}} = \dot{\mathbf{A}}\mathbf{x} + \dot{\mathbf{R}}, \quad (2.30)$$

or in the conventional form for a rigid body rotating around its center of mass with a laboratory angular velocity $\boldsymbol{\omega}$,

$$\dot{\mathbf{r}} = \boldsymbol{\omega} \times \mathbf{A}\mathbf{x} + \dot{\mathbf{R}}. \quad (2.31)$$

By comparing these expressions, it follows that

$$\left(\frac{\partial \mathbf{A}}{\partial \mathbf{a}} \cdot \dot{\mathbf{a}} \right) \mathbf{A}^T = \epsilon \boldsymbol{\omega} \quad (2.32)$$

with ϵ the Levi-Civita symbol. Equating the off-diagonal elements on both sides of the equation yields, by elaborate manipulation, the relation

$$\boldsymbol{\omega} = \frac{1}{a^2} \left[\left(1 - \frac{\sin a}{a} \right) \mathbf{a}\mathbf{a}^T + a \sin a \mathbf{1} + (\cos a - 1) \epsilon \mathbf{a} \right] \dot{\mathbf{a}}. \quad (2.33)$$

Inversion yields the desired transformation matrix,

$$\begin{aligned} \mathbf{E} = & \frac{1}{2} \begin{pmatrix} C & a_z & -a_y \\ -a_z & C & a_x \\ a_y & -a_x & C \end{pmatrix} \\ & + \left[\frac{1 - C/2}{a^2} \right] \begin{pmatrix} a_x a_x & a_x a_y & a_x a_z \\ a_y a_x & a_y a_y & a_y a_z \\ a_z a_x & a_z a_y & a_z a_z \end{pmatrix}, \end{aligned} \quad (2.34)$$

with

$$C = \frac{a \sin a}{1 - \cos a}. \quad (2.35)$$

We note that this matrix is transposed with respect to the corresponding matrix of Naess et al [55, 56, 57, 58, 131].

The rotational kinetic energy of the body, as required in Eq. (2.10) to evaluate the mass-metric contribution to the probability distribution, is now readily expressed in terms of the generalized velocities $\dot{\mathbf{a}}$ or their conjugate momenta $\mathbf{p} = \mathbf{g}\dot{\mathbf{a}}$ by

$$\frac{1}{2} \mathbf{p}^T \mathbf{g}^{-1} \mathbf{p} = \frac{1}{2} \dot{\mathbf{a}}^T \mathbf{g} \dot{\mathbf{a}} = \frac{1}{2} \boldsymbol{\omega}^T \mathbf{I} \boldsymbol{\omega}, \quad (2.36)$$

with $\mathbf{I}$ the inertia tensor in the space-fixed frame. Upon inserting Eq. (2.34), the metric tensor emerges as

$$\mathbf{g} = (\mathbf{E}^{-1})^T \mathbf{I} \mathbf{E}^{-1}. \quad (2.37)$$

The evaluation of $\det(\mathbf{g})$ is helped by [55, 56, 57, 58, 131]

$$\det(\mathbf{E}) = \frac{a^2}{2(1 - \cos a)} \quad (2.38)$$

and the observation that $\det(\mathbf{I})$ does not depend on $\mathbf{a}$. Combination with Eq. (2.12) yields the metric tensor contribution to the torque,

$$\mathbf{T}^m(\mathbf{a}) = k_B T \frac{C - 2}{a^2} \mathbf{a}. \quad (2.39)$$

Note that this correction is independent of the mass distribution within the rigid body.

The denominators of the rotation and transformation matrices in Eq. (2.28) and Eq. (2.34), respectively, give rise to numerical instabilities when the rotation angle a approaches zero. The matrices do not diverge, however, as follows from their Taylor expansions upto second order,

$$\mathbf{A} = \frac{1}{2} \begin{pmatrix} \cos a & -a_z & a_y \\ a_z & \cos a & -a_x \\ -a_y & a_x & \cos a \end{pmatrix} + \begin{pmatrix} a_x a_x & a_x a_y & a_x a_z \\ a_y a_x & a_y a_y & a_y a_z \\ a_z a_x & a_z a_y & a_z a_z \end{pmatrix} \quad (2.40)$$

and

$$\mathbf{E} = \frac{1}{2} \begin{pmatrix} 2 & a_z & -a_y \\ -a_z & 2 & a_x \\ a_y & -a_x & 2 \end{pmatrix} + \frac{1}{12} \begin{pmatrix} a_x a_x & a_x a_x & a_x a_z \\ a_x a_x & a_x a_x & a_x a_z \\ a_z a_x & a_z a_x & a_z a_z \end{pmatrix}. \quad (2.41)$$

Likewise, the torque resulting from the metric tensor correction converges to

$$\mathbf{T}^m(\mathbf{a}) = -\frac{1}{6} k_B T \mathbf{a}, \quad (2.42)$$

where the second order term vanishes identically. These three expressions were used in the simulations whenever the value of a dropped below 10^{-6} .

3. RBD by Quaternions

Brownian Dynamics is the designated technique to simulate the collective dynamics of colloidal particles suspended in a solution, e.g. the self-assembly of patchy particles. Simulating the rotational dynamics of anisotropic particles by a first-order Langevin equation, however, gives rise to a number of complications, ranging from singularities when using a set of three rotational coordinates to subtle metric and drift corrections. Here, we derive and numerically validate a Quaternion-based Rotational Brownian Dynamics (QRBD) algorithm that handles these complications in a simple and elegant way. The extension to hydrodynamic interactions is also discussed¹.

3.1 Introduction

Patchy particles are colloidal particles, susceptible to Brownian motion, with interaction sites – patches – distributed over their surfaces. These properties enable them to spontaneously self-assemble into structured clusters. They make excellent model systems to study and analyse the aggregation processes of various systems, such as fibrils [38], virus capsids [203] and crystals [214]. Besides experiments [20, 51, 103, 119, 128, 220], patchy particles are also being studied by computer simulations [18, 86, 124, 125, 145, 147, 209, 217, 226]. Most simulations of patchy particles use Monte Carlo (MC) techniques, which rely on random trial moves in combination with acceptance-rejection rules to sample the Boltzmann probability distribution. The widely observed aggregation into ‘frozen’ structures has been resolved by introducing cluster moves

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[128, 217]. But analysing the dynamics of the self-assembly process is still difficult as the MC trial moves are unrelated to the actual dynamics of the particles.

Brownian Dynamics (BD) and Langevin Dynamics (LD) algorithms have been specifically developed to simulate the motion of rigid colloids in solution[3]. These implicit solvent methods include solvent effects, like viscous friction and thermal noise, in the equations of motion of the particles. Whereas LD algorithms use second order differential equations to simulate the time evolution of the positions and velocities of the particles, BD algorithms use first order differential equations to propagate only the positions in time. The main advantage of BD is that it often enables the use of larger time steps, limited by the condition that the conservative forces acting on the particles vary only slightly over the course of the time step.

The well-known BD algorithm introduced by Ermak and McCammon [54] is widely used to simulate the translational Brownian motion of spherical particles [108, 186, 192]. Rod-like particles have also been investigated by means of Brownian Dynamics simulations, to study liquid crystals [114, 181, 190, 191]. These require an additional Brownian equation of motion for the orientation of the symmetry axis of the rod, while the rotations around this axis are ignored. The rotations of anisotropic colloids, which represent the most generalised class of rigid particles, is more difficult to simulate by the Brownian Dynamics approach and has hardly been explored in the literature [41, 78, 84, 101, 127, 135, 140, 169, 171]. One difficulty arises from the degeneracy, and the resulting strong singularities in the equation of motion, attached to the commonly used rotational coordinate systems, such as the Euler angles [74]. Furthermore, BD simulations in non-Cartesian coordinates give rise to non-trivial additional terms in the equation of motion, namely metric and drift corrections (discussed in the next section), [29, 69] which are easily overlooked when constructing a Rotational Brownian Dynamics (RBD) algorithm by analogy to a translational BD algorithm.

The common approach in Rotational Brownian Dynamics is to evaluate the potential and stochastic torques in the body-fixed frame, and to calculate the angular displacement vector $\Delta\phi^b$. Since angular displacements are not additive, Allison[5] and Fernandes and García de la Torre[62] proposed to directly propagate the orientation matrix by three consecutive rotations over angles $\Delta\phi_\alpha^b$ around the axes of the instantaneous body-fixed frame. A bias, introduced by applying three non-commuting rotation matrices in a specific order, was removed by using a single rotation around $\Delta\phi^b$ over an angle $|\Delta\phi^b|$, by Beard and Schlick[13] and Whittle and Dickinson[216]. In the absence of orientation coordinates, it is not clear what the metric tensor and drift corrections would look like in this approach, but numerical tests by Fernandes and

García de la Torre[62] show that the resulting dynamics is in good agreement with theoretical predictions[60], indicating that both corrections vanish identically. Hagan and Chandler[78] and Sánchez and Rinaldi[169] use quaternions $\mathbf{q}$ as orientation coordinates and propagate them according to $\Delta\mathbf{q} = (\partial\mathbf{q}/\partial\phi^b)\Delta\phi^b$; they do not discuss the correction terms. In a series of papers, Naess, Elgsaeter and co-workers [55, 56, 57, 58, 131] derived an equation of motion for the rotation vector $\mathbf{a}$ and validated the approach by numerical tests. These authors did include metric and drift corrections and found that the propagator differs from the intuitively expected $\Delta\mathbf{a} = (\partial\mathbf{a}/\partial\phi^b)\Delta\phi^b$ [55, 56, 57, 58, 131]. This technique was recently successfully used to simulate the self-assembly of anisotropic particles into well-ordered structures [86], but it is fairly involved and requires multiple Taylor expansions to solve weak singularities.

Quaternions, introduced by Hamilton [79] as an extension to complex numbers, enable a convenient description of the orientation of a rigid body in three dimensions. Whereas quaternions are extensively used in Molecular Dynamics (MD) simulations [3, 159], their proper implementation in a Brownian Dynamics algorithm has, to the best of our knowledge, never been explicitly demonstrated [131]. Here, a Rotational Brownian Dynamics algorithm is introduced using quaternions to describe the orientation of an anisotropic particle. The equations of motion, presented in Eqs. (3.9) and (3.13) and derived in Sec. 3.3, turn out to be remarkably compact and easy to use and can be extended to include hydrodynamic interactions. A numerical validation of the algorithm is presented in Sec. 3.4, by showing that both the static and the dynamic properties of a single anisotropic particle are in excellent agreement with theoretical predictions.

3.2 Brownian Dynamics Algorithm

3.2.1 Generalised equations of motion

Consider a set of colloidal particles moving under the combined effects of a conservative potential Φ , which describes the inter-colloidal interactions and any external field acting on the colloids, and a multitude of interactions with solvent molecules. In BD simulations it is assumed that the average effect of the collisions with the solvent can be represented by a friction while the fluctuations around this average can be accounted for by a stochastic Markovian process. Expressing the system's configuration as a vector of generalised coordinates $\mathbf{q}$, including all particle positions and orientations, the BD displacements during a time step Δt follow from the

Brownian Dynamics equation [29, 69]

$$\begin{aligned}
Q_\alpha(t + \Delta t) - Q_\alpha(t) &= -\mu_{\alpha\beta}^Q \frac{\partial \mathcal{A}^q}{\partial Q_\beta} \Delta t \\
&+ k_B T \frac{\partial \mu_{\alpha\beta}^Q}{\partial Q_\beta} \Delta t \\
&+ (\sqrt{\boldsymbol{\mu}^Q})_{\alpha\beta} \Theta_\beta \sqrt{2k_B T \Delta t}
\end{aligned} \tag{3.1}$$

where the Einstein summation convention is used. In the first term on the r.h.s. , the free energy function is defined as

$$\mathcal{A}^q(\mathbf{q}) = -k_B T \ln P^q(\mathbf{q}), \tag{3.2}$$

where $P^q(\mathbf{q})$ denotes the probability distribution function in terms of the generalised coordinates, with k_B the Boltzmann constant and T the absolute temperature. In generalised coordinates, this distribution acquires the form

$$P(\mathbf{q}) \sim \sqrt{g^Q} \exp(-\beta \Phi(\mathbf{q})), \tag{3.3}$$

where Φ is the potential energy and the Jacobian $J = \sqrt{g^Q}$, with metric g^Q , measures volume elements in $\mathbf{q}$ space (a formal discussion is provided in Appendix 3.6.1). The gradient of this free energy yields the average force driving the system, while the solvent friction, here absorbed in the mobility tensor $\boldsymbol{\mu}^Q$, opposes this motion. Their balance gives rise to a ‘drift’ velocity, which in the first term on the r.h.s. of Eq. (3.1) has been multiplied by Δt to arrive at displacements. The third term of the generalised Brownian Dynamics equation represents the random displacements due to the erratic nature of the colloid-solvent interactions at the microscopic level. Here $\mathbf{R}$ is a time-dependent vector containing random numbers with zero average, unit standard deviation, no correlations between the components and no correlations in time (Markovian). The latter stochastic property causes the random displacements in the Brownian Dynamics equation to scale with the square root of the time step. The friction and random forces are related by the fluctuation-dissipation theorem, which is incorporated in the third term. Here the square root $\sqrt{\mathbf{M}}$ of a matrix $\mathbf{M}$ is defined by

$$(\sqrt{\mathbf{M}})_{\alpha\beta} (\sqrt{\mathbf{M}})_{\gamma\beta} = M_{\alpha\gamma}. \tag{3.4}$$

Several methods exist to calculate this (non-unique) square root[64, 154]; for a diagonal matrix $\mathbf{M}$, it suffices to construct a diagonal matrix with elements $(\sqrt{\mathbf{M}})_{ii} = \sqrt{M_{ii}}$. The second term on the r.h.s. of Eq. (3.1) arises when all terms on the r.h.s. of that equation are evaluated at time t , as in the usual Itô interpretation followed here. It appears automatically when the Langevin

equation is integrated, as in described by Ermak and McCammon or Gardiner [54, 69]. In a more phenomenological treatment based on the Fokker-Planck equation, it occurs when the Boltzmann equilibrium is demanded to give rise to zero fluxes [54, 69].

3.2.2 Translation Dynamics

A well-known application of Eq. (3.1) is the translational motion of a spherical colloidal particle. This motion is best described in terms of the Cartesian coordinates $\mathbf{R}$ of the particle's centre of mass, in which case the generalised force reduces to the traditional Newtonian force, $F_\alpha = -\partial\Phi/\partial R_\alpha$. These vectors, like all other vectors in this work, are column vectors and Greek indices are used to denote the three Cartesian directions. We thus arrive at the familiar BD expression for the translational motion,

$$R_\alpha(t + \Delta t) - R_\alpha(t) = \mu_{\alpha\beta}^{t,s} F_\beta \Delta t + (\sqrt{\boldsymbol{\mu}^{t(s)}})_{\alpha\beta} \Theta_\beta^t \sqrt{2k_B T \Delta t}, \quad (3.5)$$

with $\boldsymbol{\mu}^{t,s}$ the translational mobility tensor in the space-fixed frame and $\boldsymbol{\Theta}^t$ a vector of three uncorrelated Markovian random numbers with zero mean and unit variance. Here, by assuming that the mobility matrix does not depend on the particle position, the drift correction vanishes identically. For a spherical particle the translational mobility tensor is a diagonal matrix of the form $\mu_{\alpha\beta}^{t,s} = \mu^{t,s} \delta_{\alpha\beta}$, with $\delta_{\alpha\beta}$ denoting the elements of the identity matrix.

The above BD equation of motion is readily extended to rigid non-spherical particles, by substituting the isotropic translational mobility matrix with its orientation-dependent non-isotropic counterpart,

$$\mu_{\alpha\beta}^{t,s}(t) = A_{\alpha\gamma}(t) \mu_{\gamma\delta}^{t,b} A_{\beta\delta}(t), \quad (3.6)$$

where $\boldsymbol{\mu}^{t,b}$ is the constant translational mobility tensor in a body fixed Cartesian coordinate system (to be defined in more detail below) and the rotation matrix $\mathbf{A}(t)$ describes the instantaneous orientation of the particle – and hence its body-fixed frame – relative to the laboratory frame. For a rigid colloid, the rotation matrix maps the constant position of any point in the body frame, $\mathbf{x}$, onto the space-fixed position

$$r_\alpha(t) = A_{\alpha\beta}(t) x_\beta + R_\alpha(t). \quad (3.7)$$

The square root of the mobility matrix in the second term on the r.h.s. of Eq. (3.5) is readily calculated from

$$(\sqrt{\boldsymbol{\mu}^{t(s)}})_{\alpha\beta}(t) = A_{\alpha\gamma}(t) (\sqrt{\boldsymbol{\mu}^{t(b)}})_{\gamma\beta}. \quad (3.8)$$

Inserting these intermediate results into the above equation of motion gives

$$R_\alpha(t + \Delta t) - R_\alpha(t) = A_{\alpha\gamma} \mu_{\gamma\delta}^{t,b} A_{\beta\delta} F_\beta \Delta t + A_{\alpha\gamma} (\sqrt{\mu^{t(b)}})_{\gamma\beta} \Theta_\beta^t \sqrt{2k_B T \Delta t}. \quad (3.9)$$

This equation describes the translational part of the Brownian Dynamics algorithm proposed here.

3.2.3 Rotational Dynamics

The equation of motion for the Brownian rotational dynamics of an anisotropic body is a bit more involved than the translational dynamics discussed above. When using the three Euler angles Q_α to describe the 3D orientation of the particle, the coordinates are propagated by the generalised expression provided in Eq. (3.1). A convention followed in the literature[3, 74] is to convert from the laboratory frame to the body frame by rotating counterclockwise over an angle Q_1 around the laboratory z -axis, next rotating counterclockwise over an angle Q_2 around the intermediate 1-axis, and finally rotating counterclockwise over an angle Q_3 around the line of nodes. The conversion from body-fixed coordinates to laboratory coordinates is realised by the inverse matrix;[74] the latter matrix is used throughout this chapter, and will be denoted as $\mathbf{A}$ for notational convenience. The corresponding metric is given by $g^Q \propto \sin^2 Q_2$, where the proportionality constant – which drops out of the equation upon differentiating $\ln g^Q$ – is related to the second moments of the mass distribution[126] as derived in Appendix 3.6.1. The rotational mobility matrix in Euler angles is obtained from

$$\mu_{\alpha\beta}^Q = \frac{\partial Q_\alpha}{\partial \phi_\gamma^b} \frac{\partial Q_\beta}{\partial \phi_\delta^b} \mu_{\gamma\delta}^{r,b} \quad (3.10)$$

where the ϕ_α^b denote infinitesimal rotation angles around the body-fixed coordinate axes and $\mu^{r,b}$ represents the constant rotational mobility matrix in the body-fixed frame.

A well-known disadvantage of Euler angles is the degeneracy occurring at certain orientations of the body, which result in divergencies when solving Euler's equations for rotational motion.[3, 74] These problems disappear when unit quaternions are used to parameterise the orientation of the body, *i.e.* four coordinates q_a with the constraint of unit length,

$$q^2 = \sum_{a=0}^3 q_a^2 = 1. \quad (3.11)$$

A brief comment on the notation: we use Latin indices to run from 0 to 3 and Greek indices to

run from 1 to 3. The rotation matrix, expressed in its homogeneous form, then reads as

$$\mathbf{A} = \begin{pmatrix} q_0^2 + q_1^2 - q_2^2 - q_3^2 & 2q_1q_2 - 2q_0q_3 & 2q_1q_3 + 2q_0q_2 \\ 2q_1q_2 + 2q_0q_3 & q_0^2 - q_1^2 + q_2^2 - q_3^2 & 2q_2q_3 - 2q_0q_1 \\ 2q_1q_3 - 2q_0q_2 & 2q_2q_3 + 2q_0q_1 & q_0^2 - q_1^2 - q_2^2 + q_3^2 \end{pmatrix}. \quad (3.12)$$

It will be shown below that the propagator of the quaternions over a time step Δt is given by

$$q_a(t + \Delta t) - q_a(t) = B_{a\alpha} \mu_{\alpha\beta}^{r,b} A_{\gamma\beta} \mathbf{T}_\gamma^s \Delta t + B_{a\alpha} (\sqrt{\boldsymbol{\mu}^{r,b}})_{\alpha\beta} \Theta_\beta^q \sqrt{2k_B T \Delta t} + \lambda_q q_a, \quad (3.13)$$

where $\mathbf{T}^s$ denotes the space-based Cartesian torque acting on the body, $\mathbf{R}^q$ is a vector of three uncorrelated random Markovian numbers with zero mean and unit variance, and λ_q is a Lagrange multiplier. The full matrix $\mathbf{B}$ is given by

$$\mathbf{B} = \frac{1}{2q^4} \begin{pmatrix} q_0 & -q_1 & -q_2 & -q_3 \\ q_1 & q_0 & -q_3 & q_2 \\ q_2 & q_3 & q_0 & -q_1 \\ q_3 & -q_2 & q_1 & q_0 \end{pmatrix}, \quad (3.14)$$

but only the last three columns of this matrix are needed [note the combination of one Latin and one Greek index to B in Eq. (3.13)] to convert a body-based angular velocity into a quaternion velocity, reflecting the redundancy in using four rotational velocities. By construction, the reorientations generated by the first two terms on the r.h.s. of Eq. (3.13) are perpendicular to $\mathbf{q}$ – since the last three columns of $\mathbf{B}$ are perpendicular to $\mathbf{q}$ – and therefore preserve the unit length of $\mathbf{q}$ over infinitesimal time steps. To guarantee length conservation over finite time steps, a constraint force directed along $\mathbf{q}(t)$ has been included as the last term in Eq. (3.13), where the strength of the force is obtained by solving the Lagrange multiplier λ_q from the condition $q(t + \Delta t) = 1$. Using $\tilde{\mathbf{q}}(t + \Delta t)$ to denote the quaternions after the unconstrained step, the value of λ_q is obtained from the quadratic equation

$$\lambda_q^2 + 2\lambda_q \mathbf{q}(t) \cdot \tilde{\mathbf{q}}(t + \Delta t) + \tilde{\mathbf{q}}^2(t + \Delta t) = 1. \quad (3.15)$$

The constraint term is then readily added to $\tilde{\mathbf{q}}(t + \Delta t)$ to complete the propagation step.

The appealing features of this algorithm are the simplicity of the equation of motion, the absence of singularities associated with rotational dynamics in three coordinates, the fortuitous absence of metric tensor and drift corrections commonly associated with Brownian Dynamics algorithms in non-Cartesian coordinates, and the use of Cartesian torques rather than generalised torques. The algorithm described above will be derived in the following section. Readers less

interested in the physical and mathematical details underlying the algorithm are advised to proceed to Sec. 3.4, where the algorithm is validated by comparing numerical results on simple systems against known analytic solutions.

3.3 Derivation

3.3.1 Four Coordinates

We start our derivation of Eq. (3.13) by noting that the transition from three Euler angles Q_α to four quaternions q_a introduces an additional coordinate as well as a constraint, see Eq. (3.11), that keeps the number of independent coordinates at three. While the generalised Brownian scheme of Eq. (3.1) holds true for the three independent Euler angles, it is not evident what propagator to use for the four mutually related quaternions [55, 131]. The route followed here is to extend the Euler angles to a set of four coordinates, to prove that one may construct a constrained equation of motion that reproduces the conventional rotational dynamics, and to use the covariance of this equation to make the transformation to quaternions.

In the absence of the length constraint $q = 1$, the homogeneous matrix of Eq. (4.21) combines rotation and uniform scaling,

$$\bar{\mathbf{A}}(\mathbf{q}) = q^2 \mathbf{A}(\mathbf{q}/q). \quad (3.16)$$

The bar indicates that a larger set of space transformations is covered than just rotations. The Euler angles are readily extended with a fourth coordinate to cover the same operational range; the extended set will be denoted by $\bar{\mathbf{Q}} = (\bar{Q}_0, \mathbf{Q})$, where $\bar{Q}_0$ is the supplemented coordinate, and the corresponding rotation-dilation matrix is given by

$$\bar{\mathbf{A}}(\bar{\mathbf{Q}}) = \bar{Q}_0 \mathbf{A}(\mathbf{Q}). \quad (3.17)$$

Comparing the latter two relations suggests that $\bar{Q}_0$ relates to the length q and $\mathbf{Q}$ to the direction of $\mathbf{q}$. If it proves possible to construct a constrained equation of motion for $\bar{\mathbf{Q}}$ that reproduces the correct dynamics of $\mathbf{Q}$, then one can carry through the coordinate transformation from the four $\bar{Q}_a$ to the four q_a and thereby arrive at the desired Brownian equation of motion in terms of

the quaternions. Guided by Eq. (3.1), we are led to

$$\begin{aligned}
 \bar{Q}_a(t + \Delta t) - \bar{Q}_a(t) = & -\bar{\mu}_{ab}^Q \frac{\partial (\Phi - \frac{1}{2}k_B T \ln \bar{g}^Q)}{\partial \bar{Q}_b} \Delta t \\
 & + k_B T \frac{\partial \bar{\mu}_{ab}^Q}{\partial \bar{Q}_b} \Delta t \\
 & + (\sqrt{\bar{\mu}^Q})_{ab} \bar{\Theta}_b^Q \sqrt{2k_B T \Delta t} \\
 & + \bar{\mu}_{ab}^Q \bar{\lambda}_q \frac{\partial (\bar{Q}_0 - 1)}{\partial \bar{Q}_b},
 \end{aligned} \tag{3.18}$$

where all parameters pertaining to $\bar{\mathbf{Q}}$ are marked by a bar to distinguish them from their counterparts in $\mathbf{Q}$. The last term is introduced to keep $\bar{Q}_0$ at a constant value of 1, thereby restricting the motion of $\bar{\mathbf{Q}}$ to the hyperplane of purely rotational $\bar{\mathbf{A}}$ matrices. If this constraint on the dynamics is implemented by means of a stiff harmonic potential, it is evident from the first term on the r.h.s. of Eq. (3.18) that the force has to be left-multiplied by the mobility matrix. Since stiff springs tend to reduce the largest permissible time step, we here follow the common practice in Molecular Dynamics simulations of using constraint forces to eliminate the motion of $\bar{Q}_0$. [3, 67] The direction of the force is provided by the gradient of the constrained quantity, the strength is represented by the undetermined Lagrange multiplier $\bar{\lambda}_q$, and their product is left multiplied by the mobility matrix in the equation of motion, see Eq. (3.18).

An element-wise comparison of Eq. (3.18) and Eq. (3.1) reveals that the new equations of motion for $\bar{Q}_\alpha$ will recover the dynamics of Q_α if two conditions are met. First, the mobility matrices must be related by

$$\bar{\mu}^Q = \begin{pmatrix} \bar{\mu}_{00}^Q & \mathbf{0} \\ \mathbf{0} & \mu^Q \end{pmatrix}, \tag{3.19}$$

where $\bar{\mu}_{00}^Q$ may still be a function of $\bar{\mathbf{Q}}$. Second, the metric $\bar{g}^Q$ must be expressible as the product of g^Q and a function that depends on $\bar{Q}_0$ only. It is shown in Appendix 3.6.1 that the metric tensor of the selected coordinates $\bar{\mathbf{Q}}$ is block diagonal, containing a constant element $\bar{g}_{00}^Q$ and $\bar{Q}_0^2 g^Q$ as the two blocks, and hence the second requirement is fulfilled. After selecting a mobility matrix of the block-diagonal form indicated above, the $\bar{Q}_\alpha$ acquire an equation of motion identical to that of Q_α . The additional equation of motion for $\bar{Q}_0$ is similar to the equations of motion for $\bar{Q}_\alpha$, the main difference being the appearance of a constraint force contribution that effectively eliminates all other terms to preserve $\bar{Q}_0 = 1$. Consequently, the motion of $\bar{\mathbf{Q}}$ is independent of $\bar{\mu}_{00}^Q$ and $\bar{g}_{00}^Q$.

Having established that an equation of motion in four coordinates plus a constraint recovers the correct rotational dynamics, we now make the step to four quaternions $\mathbf{q}$. The two sets of

coordinates are related by

$$\mathbf{q}(\bar{\mathbf{Q}}) = \bar{Q}_0^{1/2} \hat{\mathbf{q}}(\mathbf{Q}), \quad (3.20)$$

where $\hat{\mathbf{q}}(\mathbf{Q})$ denotes the conventional relation between unit quaternions and Euler angles,[3, 74] as detailed in Appendix 3.6.1. Since the equation of motion for $\bar{\mathbf{Q}}$ in Eq. (3.18) inherits the invariance of the generalised equation of motion, one directly obtains

$$\begin{aligned} q_a(t + \Delta t) - q_a(t) = & -\mu_{ab}^q \frac{\partial (\Phi - \frac{1}{2}k_B T \ln g^q)}{\partial q_b} \Delta t \\ & + k_B T \frac{\partial \mu_{ab}^q}{\partial q_b} \Delta t \\ & + (\sqrt{\mu^q})_{ab} \Theta_b^q \sqrt{2k_B T \Delta t} \\ & + \mu_{ab}^q \lambda_q \frac{\partial (q^2 - 1)}{\partial q_b}, \end{aligned} \quad (3.21)$$

as may be explicitly verified by Taylor expanding $\mathbf{q}$ up to second order in $\bar{\mathbf{Q}}$ and retaining all terms up to first order in Δt . The q -labeled parameters appearing in this expression are obviously related to their $\bar{Q}$ -labeled counterparts in Eq. (3.18). Since $\bar{\mu}_{00}^Q$ and $\bar{g}_{00}^Q$ were only introduced to enable the expansion from three to four coordinates – recall that these terms do not appear when using Euler angles – they must exactly cancel against λ_q . We now set forth to evaluate μ^q and simplify the terms appearing in Eq. (3.21).

3.3.2 Mobility Matrix

The mobility matrices in the two four-dimensional coordinate sets are related by

$$\mu_{ab}^q = \frac{\partial q_a}{\partial \bar{Q}_c} \bar{\mu}_{cd}^Q \frac{\partial q_b}{\partial \bar{Q}_d} \quad (3.22)$$

$$= \frac{\partial q_a}{\partial \bar{Q}_\alpha} \mu_{\alpha\beta}^Q \frac{\partial q_b}{\partial \bar{Q}_\beta} + \frac{\partial q_a}{\partial \bar{Q}_0} \bar{\mu}_{00}^Q \frac{\partial q_b}{\partial \bar{Q}_0} \quad (3.23)$$

$$= \mu_{ab}^{q,0} + \frac{\bar{\mu}_{00}^Q}{4q^4} q_a q_b \quad (3.24)$$

where in the second step use was made of the block-diagonal structure of $\bar{\mu}^Q$ and in the third step of Eq. (3.20). In the third line, only the second term depends on $\bar{\mu}_{00}^Q$; the other term represents μ^q evaluated at $\bar{\mu}_{00}^Q = 0$ and converts the three-dimensional Cartesian body-fixed rotational mobility matrix $\mu^{\mathbf{r},\mathbf{b}}$ to a four dimensional counterpart,

$$\mu_{ab}^{q,0} = \frac{\partial q_a}{\partial \bar{Q}_\alpha} \frac{\partial Q_\alpha}{\partial \phi_\gamma^{\mathbf{b}}} \mu_{\gamma\delta}^{\mathbf{r},\mathbf{b}} \frac{\partial Q_\beta}{\partial \phi_\delta^{\mathbf{b}}} \frac{\partial q_b}{\partial \bar{Q}_\beta}, \quad (3.25)$$

where the derivatives with respect to $\bar{Q}_\alpha$ are evaluated at constant $\bar{Q}_0 = 1$. Noticing that[3]

$$\frac{\partial q_a}{\partial \bar{Q}_\alpha} \frac{\partial Q_\alpha}{\partial \phi_\gamma^{\mathbf{b}}} = \frac{\partial q_a}{\partial \phi_\gamma^{\mathbf{b}}} = B_{a\gamma}, \quad (3.26)$$

with the elements of $\mathbf{B}$ provided in Eq. (3.14), gives $\mu_{ab}^{q,0} = B_{a\alpha}B_{b\beta}\mu_{\alpha\beta}^{r,b}$, and by defining $\mu_{00}^{r,b} = q^4\bar{\mu}_{00}^Q$ one can rewrite Eq. (3.24) as

$$\mu_{ab}^q = B_{ac}B_{bd} \left[\delta_{c0}\delta_{d0}\mu_{00}^{r,b} + \delta_{c\alpha}\delta_{d\beta}\mu_{\alpha\beta}^{r,b} \right]. \quad (3.27)$$

Through a series of simple calculations one can easily show that

$$(\sqrt{\mu^q})_{ab} (\sqrt{\mu^q})_{cb} = \mu_{ac}^q \quad (3.28)$$

is solved by

$$(\sqrt{\mu^q})_{ab} = B_{ac} \left[\delta_{c0}\delta_{b0} \left(\sqrt{\mu_{00}^{r,b}} \right) + \delta_{c\alpha}\delta_{b\beta} \left(\sqrt{\mu_{\alpha\beta}^{r,b}} \right) \right], \quad (3.29)$$

which is used in the evaluation of the random contribution to Eq. (3.21).

3.3.3 Simplification of Terms

The above structure of μ^q enables simplifications of the various terms appearing in the equation of motion. It proves advantageous to start with the constraint term, as any motion along the direction of the constraint will be removed by the Lagrange multiplier.

1. Inserting Eq. (3.27) in the last term of Eq. (3.21) yields

$$\mu_{ab}^q \lambda_q \frac{\partial(q^2 - 1)}{\partial q_b} = 2\lambda_q B_{a\alpha} \mu_{\alpha\beta}^{r,b} B_{b\beta} q_b + \underline{\frac{1}{2q^2} \lambda_q \bar{\mu}_{00}^Q q_a}, \quad (3.30)$$

where the first term on the r.h.s. vanishes identically because $B_{b\beta} q_b = 0$, see Eq. (3.14), and where the underlined factor in the second term defines the effective Lagrange multiplier λ'_q of the constraint force, which is seen to act along $\mathbf{q}$.

2. Shifting attention to the contribution of the conservative potential to the equation of motion,

$$\mu_{ab}^q \frac{\partial \Phi}{\partial q_b} = \mu_{ab}^{q,0} \frac{\partial \Phi}{\partial q_b} + q_a \frac{1}{4q^4} \bar{\mu}_{00}^Q q_b \frac{\partial \Phi}{\partial q_b}, \quad (3.31)$$

one observes that the last term is parallel to $\mathbf{q}$ and, hence, will be eliminated by the constraint. Replacing Φ by $\ln g^q$ we observe that also in this case the last term is proportional to $\mathbf{q}$, while for the other term, using g^q as derived in Appendix 3.6.1, it follows that

$$\mu_{ab}^{q,0} \frac{\partial \ln g^q}{\partial q_b} = \frac{8}{q^2} B_{a\alpha} \mu_{\alpha\beta}^{r,b} B_{b\beta} q_b = 0 \quad (3.32)$$

because $B_{b\beta} q_b = 0$. Therefore the metric disappears altogether from our description.

3. The drift correction takes the form

$$\frac{\partial \mu_{ab}^q}{\partial q_b} = \frac{\partial \mu_{ab}^{q,0}}{\partial q_b} + \frac{q_a}{4q^4} \left(\bar{\mu}_{00}^Q + q_b \frac{\partial \bar{\mu}_{00}^Q}{\partial q_b} \right), \quad (3.33)$$

where the last term on the r.h.s. is parallel to $\mathbf{q}$ and therefore will be eliminated by the constraint.

4. The random contribution in Eq. (3.21) contains

$$(\sqrt{\boldsymbol{\mu}^q})_{ab} \Theta_b^q = B_{a0} \sqrt{\mu_{00}^{r,b}} \Theta_0^q + (\sqrt{\boldsymbol{\mu}^q})_{a\alpha} \Theta_\alpha^q. \quad (3.34)$$

Since B_{a0} in the first term on the r.h.s. is proportional to $\mathbf{q}$, see Eq. (3.14), the displacement resulting from this zeroth element will be cancelled by the constraint. The three remaining elements of $\mathbf{R}^q$ produce displacements that are perpendicular to $\mathbf{q}$ and independent of $\bar{\mu}_{00}^Q$.

5. Collecting terms, the equation of motion has thus been reduced to

$$\begin{aligned} q_a(t + \Delta t) - q_a(t) = & -\mu_{ab}^{q,0} \frac{\partial \Phi}{\partial q_b} \Delta t \\ & + k_B T \frac{\partial \mu_{ab}^{q,0}}{\partial q_b} \Delta t \\ & + (\sqrt{\boldsymbol{\mu}^{q,0}})_{a\alpha} \Theta_\alpha^q \sqrt{2k_B T \Delta t} \\ & + \lambda'_q q_a, \end{aligned} \quad (3.35)$$

where all references to $\bar{\mu}_{00}^Q$ have disappeared.

6. The divergence in the drift correction term can be worked out to yield, after the laborious manipulations outlined in Appendix 3.6.2, the surprisingly simple result

$$\frac{\partial \mu_{ab}^{q,0}}{\partial q_b} = -\frac{1}{4} \text{Tr}(\boldsymbol{\mu}^{r,b}) q_a \quad (3.36)$$

which also holds true for non-diagonal $\boldsymbol{\mu}^{r,b}$. Since this vector is proportional to $\mathbf{q}$, its contribution to the displacement cancels against the constraint force.

7. Finally, we note that for conservative potentials in Cartesian coordinates,

$$\mu_{ab}^{q,0} \frac{\partial \Phi}{\partial q_b} = B_{a\alpha} \mu_{\alpha\beta}^{r,b} B_{b\beta} \frac{\partial \phi_\gamma^s}{\partial q_b} \frac{\partial \Phi}{\partial \phi_\gamma^s}, \quad (3.37)$$

where the ϕ_γ^s denote infinitesimal rotation angles around the laboratory-fixed coordinate axes. The transformations to the right of $\boldsymbol{\mu}^{r,b}$ have the nett effect of converting a torque in the space frame into a torque in the body frame; the same result is more conveniently achieved by a single rotation matrix,

$$\mu_{ab}^{q,0} \frac{\partial \Phi}{\partial q_b} = B_{a\alpha} \mu_{\alpha\beta}^{r,b} A_{\gamma\beta} \frac{\partial \Phi}{\partial \phi_\gamma^s}. \quad (3.38)$$

Substitution of Eqs. (3.36) and (3.38) in Eq. (3.35) completes the derivation of Eq. (3.13). The reader interested in including hydrodynamic interactions is referred to Appendix 3.6.3.

3.4 Algorithm Validation

To test the validity and performance of the Rotational Brownian Dynamics algorithm proposed in the previous sections, we have performed tests on single anisotropic particles and analysed their static and dynamic properties. The simulations were carried out in internal units with τ the time unit, ϵ the energy unit and σ the length unit. The typical results presented here were obtained for an anisotropic particle whose body-fixed translational and rotational mobility tensors were both diagonal, with eigenvalues $(5, 7, 9) \sigma^2(\tau\epsilon)^{-1}$ and $(0.5, 4, 10) (\tau\epsilon)^{-1}$, respectively. A time step of $\Delta t = 10^{-4} \tau$ was used and the simulations were run for 10^7 steps. The temperature was set at $T = 1\epsilon/k_B$, the random numbers were sampled from a Gaussian distribution [3]. The equilibrium angle probability distributions in the presence and absence of a uniform electric field, as well as the translational and rotational diffusion coefficients, have been analysed to show that all these quantities are in agreement with theoretical predictions.

For a particle of given shape, the body-fixed mobility tensors can be calculated by *e.g.* the HYDRO++ package; [198, 199] the orientation used in this calculation corresponds to $\mathbf{A} = \mathbf{1}$ and sets the reference point for all subsequent rotations. The coordinates are advanced in time by evaluating all elements on the right-hand sides of Eqs. (3.9) and (3.13) at time t , where the constraint contribution is temporarily ignored, *i.e.* $\lambda_q = 0$. The resulting quaternions $\tilde{\mathbf{q}}(t + \Delta t)$ are next inserted in Eq. (3.15) to solve for λ_q , and the time step is completed by adding the constraint contribution, *i.e.* $q_a(t + \Delta t) = \tilde{q}_a(t + \Delta t) + \lambda_q q_a(t)$. Because of the fortuitous cancellations derived in Sec. 3.3, the integration step for the quaternions is very similar to the corresponding step in quaternion-based molecular dynamics simulations, where $\mathbf{B}$ is used to convert body-based angular velocities into quaternion velocities.

3.4.1 Static Properties

To investigate the static properties we first tested whether body-fixed unit vectors $\hat{\mathbf{u}}^b$, as seen from the space frame, $\hat{\mathbf{u}}^s(t) = \mathbf{A}(t)\hat{\mathbf{u}}^b(t)$, sample the correct Boltzmann distributions for the polar θ and azimuthal ϕ angles in the laboratory frame. In an isotropic environment the equilibrium probability distributions are

$$\begin{aligned} P(\cos \theta) &= \frac{1}{2} \\ P(\phi) &= \frac{1}{2\pi} \end{aligned} \tag{3.39}$$

where $\cos \theta \in [-1; 1]$ and $\phi \in [0; 2\pi]$. Figure 3.1(a) and (b) show simulation results for the set of three mutually perpendicular body-fixed eigenvectors of the rotational mobility matrix. The

Figure 3.1: Laboratory-based polar (a) and azimuthal (b) probability distributions for three basis vectors fixed to an anisotropic particle.

histograms are in excellent agreement with the theoretical predictions in Eq. (3.39), demonstrating that the algorithm of Eq. (3.13) samples the correct rotational probability distribution and confirming the theory presented in the previous section regarding the absence of drift and metric tensor effects.

Next, the particle acquired a dipole moment by placing two charges, $\pm q$, a distance d apart at opposite body-fixed locations relative to the centre of mass, $\pm \frac{1}{2}d\hat{\mathbf{u}}^b$. In a uniform electric field of strength ε , aligned along the laboratory z-axis, the forces acting on the charges of the dipole are equal in moduli and opposite in signs; therefore the particle will not experience a net force. The particle will, however, experience a torque which tends to align the dipole with the direction of the electric field. The angle θ between the field direction and the dipole moment $\mathbf{p} = qd\hat{\mathbf{u}}^b$, will

Figure 3.2: Probability distribution of the polar angle θ between the dipole moment of an anisotropic particle and a uniform electric field, for $p\beta\varepsilon = 5$.

follow the Boltzmann distribution $P(\theta) = \frac{1}{Z} e^{-\beta\varepsilon p \cos \theta} \sin \theta$, with Z the normalisation constant and $p = |\mathbf{p}|$. Figure 3.2 shows that the simulation results (markers) are in good agreement with the theoretical prediction (line), demonstrating that the algorithm correctly includes the conservative force and the equipartition theorem, *i.e.* the temperature, as well as reconfirming (especially at small $p\beta\varepsilon$, not shown) the absence of drift and metric tensor effects.

3.4.2 Dynamic Properties

The translational diffusion coefficient of the anisotropic body were obtained by analysing the mean squared displacement of its centre of mass. For a particle subject to Rotational Brownian Dynamics, the translational diffusion coefficients along the three Cartesian axis of the laboratory frame correspond to the average translational diffusion coefficient in the body fixed frame, $D^{t(s)} = k_B T \frac{1}{3} \text{Tr}(\boldsymbol{\mu}^{t,b})$, because a randomly rotating body moves on average the same along every space fixed direction. This behaviour is indeed recovered by the simulations, as seen in Fig. 3.3(a). Anisotropic diffusion was observed in the simulations of the dipolar particle in an electric field.

The rotational dynamics was analysed by means of the time correlation function of a body-fixed unit vector $\hat{\mathbf{u}}^b$ as viewed from the laboratory frame,

$$P_{\hat{\mathbf{u}}^b}(t) = \frac{3}{2} \langle (\hat{\mathbf{u}}^s(t) \hat{\mathbf{u}}^s(0))^2 \rangle - \frac{1}{2}, \quad (3.40)$$

where $\hat{\mathbf{u}}^s(t) = \mathbf{A}(t) \hat{\mathbf{u}}^b$. Theory [60, 94, 197] predicts multi-exponential decay, where the (at most) five relaxation times τ_i and the corresponding amplitudes a_i depend on both the rotational

mobility matrix and the chosen body-fixed unit vector $\hat{\mathbf{u}}^b$,

$$P = \sum_{i=1}^5 a_i e^{-t/\tau_i}. \quad (3.41)$$

For a body-fixed frame that diagonalises the rotational mobility matrix $\boldsymbol{\mu}^{r,b}$, with the eigenvalues sorted in ascending order down the diagonal, $\mu_1^{r,b} \leq \mu_2^{r,b} \leq \mu_3^{r,b}$, the relaxation times are given by

$$\begin{aligned} \tau_1 &= (6\bar{D} - 2\Delta)^{-1} \\ \tau_2 &= (3(\bar{D} + \mu_1^{r,b}))^{-1} \\ \tau_3 &= (3(\bar{D} + \mu_2^{r,b}))^{-1} \\ \tau_4 &= (3(\bar{D} + \mu_3^{r,b}))^{-1} \\ \tau_5 &= (6\bar{D} + 2\Delta)^{-1} \end{aligned} \quad (3.42)$$

where

$$\Delta = \sqrt{(\mu_1^{r,b} - \mu_2^{r,b})^2 + (\mu_3^{r,b} - \mu_2^{r,b})(\mu_3^{r,b} - \mu_1^{r,b})} \quad (3.43)$$

and $\bar{D} = \frac{1}{3} \sum_{i=1}^3 \mu_i^{r,b}$. The corresponding amplitudes read as

$$\begin{aligned} a_1 &= \frac{3}{4}(A + B) \\ a_2 &= 3(\hat{u}_2^b \hat{u}_3^b)^2 \\ a_3 &= 3(\hat{u}_1^b \hat{u}_3^b)^2 \\ a_4 &= 3(\hat{u}_1^b \hat{u}_2^b)^2 \\ a_5 &= \frac{3}{4}(A - B). \end{aligned} \quad (3.44)$$

where

$$\begin{aligned} A &= -\frac{1}{3} + \sum_{\alpha=1}^3 (\hat{u}_\alpha^b)^4 \\ B &= \frac{1}{\Delta} \left\{ -\bar{D} + \sum_{\alpha=1}^3 \mu_\alpha^{r,b} \left[(\hat{u}_\alpha^b)^4 + 2(\hat{u}_\beta^b \hat{u}_\gamma^b)^2 \right] \right\}, \end{aligned} \quad (3.45)$$

with (α, β, γ) cyclic permutations of $(1, 2, 3)$. The selected rotational mobility matrix matches the aforementioned conditions. It is then straightforward to show that three of the five amplitudes vanish identically when the selected vector $\hat{\mathbf{u}}^b$ is identical to a body-fixed basis vector, and that, of the remaining two exponentials, mode 1 relaxes slower than mode 5. This double exponential decay is recognisable in Fig. 3.3(b). For completeness a ‘random’ body-fixed unit vector parallel

to $(10, 8, 1)$ is also included in Fig. 3.3(b), to demonstrate the existence of five relaxation times. Excellent agreement is observed between the simulation results and the theoretical predictions, also for a set of other non-basis vectors (not shown), demonstrating that the algorithm produces the correct rotational dynamics. Note that the derivation in Sec. 3.3 does not require the rotational mobility matrix to be diagonal in the body-fixed frame. Additional tests (not shown) confirm the validity of the algorithm for non-diagonal $\boldsymbol{\mu}^{r,b}$.

3.5 Conclusions

A new elementary Brownian Dynamics algorithm to simulate Rotational Brownian Dynamics of anisotropic particles has been introduced, see Eqs. (3.9) and (3.13). The theoretical foundation of the algorithm has been validated by simulations of single anisotropic particles, recovering static and dynamic properties in excellent agreement with theoretical predictions. The algorithm is well suited for simulations of patchy-particles, such as functionalised colloids or proteins that act as rigid units, thereby opening routes to study the dynamics of their self-assembly into ordered aggregates [86]. Bonds between the particles are readily included in the interaction potential, thereby creating patchy-particle ‘molecules’. The inclusion of constraints between these particles requires further study. The proposed technique is also readily applicable when hydrodynamic interactions are included, see Appendix 3.6.3. A particularly interesting situation arises when spherical particles – with efficient schemes available to evaluate the complex mobility matrices[22] – are endowed with interaction patches.

3.6 Appendix

3.6.1 Mass-metric tensor

The phase space probability distribution in generalised coordinates $\mathbf{Q}$ and conjugate momenta $\mathbf{p}^Q$ has the invariant form

$$P(\mathbf{Q}, \mathbf{p}^Q) d\mathbf{Q} d\mathbf{p}^Q \propto \exp[-\beta(\Phi + K)] d\mathbf{Q} d\mathbf{p}^Q, \quad (3.46)$$

where the expression for the normalising proportionality constant has been omitted as this does not affect the calculation below. In this Appendix, it is assumed for notational convenience that the centre of mass of the rotating body is fixed. For a rigid body with body-fixed inertia tensor $\mathbf{I}^b$, the kinetic energy expressed in generalised velocities reads as

$$K = \frac{1}{2} \omega_\alpha^b I_{\alpha\beta}^b \omega_\beta^b = \frac{1}{2} \dot{Q}_\alpha g_{\alpha\beta}^Q \dot{Q}_\beta, \quad (3.47)$$

with ω^b the angular velocity in the body-fixed frame and where the mass-metric tensor is given by

$$g_{\alpha\beta}^Q = \frac{\partial \omega_\kappa^b}{\partial \dot{Q}_\alpha} I_{\kappa\lambda}^b \frac{\partial \omega_\lambda^b}{\partial \dot{Q}_\beta}. \quad (3.48)$$

The conjugate momenta then read as $p_\alpha^Q = g_{\alpha\beta}^Q \dot{Q}_\beta$, and integration of Eq. (3.46) over the momenta yields the coordinate space probability distribution

$$P(\mathbf{Q}) d\mathbf{Q} \propto \exp(-\beta\Phi) \sqrt{g^Q} d\mathbf{Q}, \quad (3.49)$$

with the metric $g^Q = \det(\mathbf{g}^Q)$ and where the non-consequential normalizing proportionality constant has again been omitted for notational convenience. Using the known relation between ω^b and the Euler angular velocities $\dot{\mathbf{Q}}$, [74] one readily arrives at [126]

$$g^Q = |\mathbf{I}^b| \sin^2 Q_2. \quad (3.50)$$

We now proceed to calculating the metric $\bar{g}^Q$. To this end we write the kinetic energy as

$$K = \frac{1}{2} \int \rho(\mathbf{r}) \dot{\mathbf{r}} \cdot \dot{\mathbf{r}} d^3\mathbf{r} \quad (3.51)$$

with $\rho(\mathbf{r})$ the mass distribution in the space-fixed frame. Using

$$r_\alpha = \bar{A}_{\alpha\beta} x_\beta + R_\alpha = \bar{Q}_0 A_{\alpha\beta} x_\beta + R_\alpha, \quad (3.52)$$

with R_α being constant, and the fact that $\rho(\mathbf{r}) d^3\mathbf{r} = \rho(\mathbf{x}) d^3\mathbf{x}$, we obtain $K = \frac{1}{2} \dot{Q}_a \bar{g}_{ab}^Q \dot{Q}_b$ with the metric tensor

$$\bar{g}_{ab}^Q = \int \rho(\mathbf{x}) \frac{\partial \bar{\mathbf{A}}\mathbf{x}}{\partial \bar{Q}_a} \cdot \frac{\partial \bar{\mathbf{A}}\mathbf{x}}{\partial \bar{Q}_b} d\mathbf{x}. \quad (3.53)$$

Noticing that

$$\frac{\partial \bar{\mathbf{A}}\mathbf{x}}{\partial \bar{Q}_0} \cdot \frac{\partial \bar{\mathbf{A}}\mathbf{x}}{\partial \bar{Q}_0} = \mathbf{A}\mathbf{x} \cdot \mathbf{A}\mathbf{x}, \quad (3.54a)$$

$$\frac{\partial \bar{\mathbf{A}}\mathbf{x}}{\partial \bar{Q}_\alpha} \cdot \frac{\partial \bar{\mathbf{A}}\mathbf{x}}{\partial \bar{Q}_0} = \bar{Q}_0 \frac{\partial \mathbf{A}\mathbf{x}}{\partial Q_\alpha} \cdot \mathbf{A}\mathbf{x} = 0, \quad (3.54b)$$

$$\frac{\partial \bar{\mathbf{A}}\mathbf{x}}{\partial \bar{Q}_\alpha} \cdot \frac{\partial \bar{\mathbf{A}}\mathbf{x}}{\partial \bar{Q}_\beta} = \bar{Q}_0^2 \frac{\partial \mathbf{A}\mathbf{x}}{\partial Q_\alpha} \cdot \frac{\partial \mathbf{A}\mathbf{x}}{\partial Q_\beta}, \quad (3.54c)$$

we conclude that the metric tensor $\bar{\mathbf{g}}^Q$ is block diagonal, comprising the constant $\bar{g}_{00}^Q = \frac{1}{2}\text{Tr}(\mathbf{I}^b)$ and the block $\bar{Q}_0^2 \mathbf{g}^Q$, and therefore $\det(\bar{\mathbf{g}}^Q) \propto \bar{Q}_0^6 \sin^2 \bar{Q}_2$.

Upon comparing the coordinate probability distributions for two sets of generalised coordinates, say $\bar{\mathbf{Q}}$ and $\mathbf{q}$, it becomes apparent that the square root of the ratio of the two involved metrics plays the same role as the Jacobian of the coordinate transformation,

$$\sqrt{\frac{\partial \bar{g}^Q}{\partial g^q}} = \left| \frac{\partial \mathbf{q}}{\partial \bar{\mathbf{Q}}} \right|. \quad (3.55)$$

The conversion between the extended Euler angles and the quaternions is realised by[3]

$$q_0 = \bar{Q}_0^{1/2} \cos \frac{1}{2} \bar{Q}_2 \cos \frac{1}{2} (\bar{Q}_1 + \bar{Q}_3) \quad (3.56a)$$

$$q_1 = \bar{Q}_0^{1/2} \sin \frac{1}{2} \bar{Q}_2 \cos \frac{1}{2} (\bar{Q}_1 - \bar{Q}_3) \quad (3.56b)$$

$$q_2 = \bar{Q}_0^{1/2} \sin \frac{1}{2} \bar{Q}_2 \sin \frac{1}{2} (\bar{Q}_1 - \bar{Q}_3) \quad (3.56c)$$

$$q_3 = \bar{Q}_0^{1/2} \cos \frac{1}{2} \bar{Q}_2 \sin \frac{1}{2} (\bar{Q}_1 + \bar{Q}_3), \quad (3.56d)$$

yielding

$$\left| \frac{\partial \mathbf{q}}{\partial \bar{\mathbf{Q}}} \right| = \frac{\bar{Q}_0}{16} \sin \bar{Q}_2. \quad (3.57)$$

Combining the preceding results yields

$$g^q = 2^7 \det(\mathbf{I}^b) \text{Tr}(\mathbf{I}^b) \bar{Q}_0^4 \propto q^8 \quad (3.58)$$

for the metric of the quaternions.

3.6.2 Drift correction

Consider a rigid body with a constant, possibly non-diagonal, body-fixed rotational mobility matrix $\mu^{r,b}$. The drift correction entering the equation of motion is calculated as

$$\frac{\partial \mu_{ab}^{q,0}}{\partial q_b} = \frac{\partial B_{a\alpha}}{\partial q_b} \mu_{\alpha\beta}^{r,b} B_{b\beta} + B_{a\alpha} \mu_{\alpha\beta}^{r,b} \frac{\partial B_{b\beta}}{\partial q_b}. \quad (3.59)$$

The second term on the r.h.s. is equal to zero because

$$\frac{\partial B_{b\beta}}{\partial q_b} = -\frac{4}{q^2} q_b B_{b\beta} + \frac{1}{q^4} \frac{\partial(q^4 B_{b\beta})}{\partial q_b} = 0, \quad (3.60)$$

where the first term on the r.h.s. equals zero for the usual reason, and the differentiation in the second term vanishes because Eq. (3.14) yields that $q^4 B_{b\beta} = \pm \frac{1}{2} q_c$ with $c \neq b$. The first term on the r.h.s. of Eq. (3.59) can be rewritten likewise, obtaining

$$\frac{\partial B_{a\alpha}}{\partial q_b} B_{b\beta} = -\frac{4}{q^2} q_b B_{a\alpha} B_{b\beta} + \frac{1}{q^4} \frac{\partial(q^4 B_{a\alpha})}{\partial q_b} B_{b\beta}. \quad (3.61)$$

Here again, the first term on the r.h.s. cancels because $q_b B_{b\beta} = 0$. Evaluation of the second term yields

$$\frac{\partial(q^4 B_{a\alpha})}{\partial q_b} B_{b\beta} = -\frac{1}{4q^4} (q_a \delta_{\alpha\beta} + M_{\alpha\beta}^a), \quad (3.62)$$

where the four matrices M^a are anti-symmetric. Inserting this result in Eq. (3.59) gives

$$\frac{\partial \mu_{ab}^{q,0}}{\partial q_b} = -\frac{1}{4q^8} q_a \text{Tr}(\boldsymbol{\mu}^{r,b}), \quad (3.63)$$

since the product of the symmetric $\boldsymbol{\mu}^{r,b}$ with an anti-symmetric M^a yields zero.

Note that the trace in the final expression is independent of the orientation of the body fixed frame. Hence, the displacement generated by this correction bears no relation to the actual spatial orientation of the body and should consequently not contribute to the simulated dynamics. As will be shown in the main text, the drift correction is cancelled by the constraint force.

3.6.3 Hydrodynamic interactions

The motions of N particles suspended in a fluid are coupled by hydrodynamic interactions [44, 97]. These interactions can be included in Brownian Dynamics simulations by extending the mobility matrix to a $(6N \times 6N)$ matrix $\boldsymbol{\mu}^{r(s),t}$. Because the components of this mobility matrix are typically evaluated in the laboratory frame, and unlike the mobilities in Sec. 3.3 do not rotate rigidly with the particles, we will use the space-based mobility matrix in this appendix, as denoted by the superscript (s). The equation of motion of particle i will be solved in terms of the coordinates $\mathbf{q}_i = (\mathbf{q}_i^r, \mathbf{q}_i^t)$, combining four quaternions describing the orientation, $\mathbf{q}_i^r = (q_{i,0}, q_{i,1}, q_{i,2}, q_{i,3})$, and three coordinates for the center of mass position, $\mathbf{q}_i^t = (q_{i,4}, q_{i,5}, q_{i,6})$. Care must again be taken to ensure that the introduction of additional coordinates and constraints, one of each for every particle, does not alter the motion. The conditions turn out to be identical to those in Sections 3.3.1. In particular, every (6×6) mobility

matrix $\mu_{ij}^{r(s),t}$ coupling particles i and j is augmented with a freely chosen parameter $\mu_{ij,00}^{r(s),t}$, and upon coordinate transformation yields the (7×7) mobility matrix

$$\mu_{ij,ab}^q = \mu_{ij,ab}^{q,0} + \frac{1}{4} \mu_{ij,00}^{r(s),t} \frac{q_{i,c} \delta_{ac}^r}{|\mathbf{q}_i^r|^4} \frac{q_{j,d} \delta_{bd}^r}{|\mathbf{q}_j^r|^4} \quad (3.64)$$

$$= \mathcal{C}_{i,ac} \mathcal{C}_{j,bd} \left[\delta_{c0} \delta_{d0} \mu_{ij,00}^{r(s),t} + \delta_{c\alpha} \delta_{d\beta} \mu_{ij,\alpha\beta}^{r(s),t} \right], \quad (3.65)$$

where particle indices are excluded from the Einstein summation convention, the ranges of the lower case coordinate indices have been extended to run from 0 (Latin) or 1 (Greek) to 6, and in the first line use was made of the notation

$$\delta_{ab}^r = \begin{cases} \delta_{ab} & \text{for } 0 \leq a \leq 3 \\ 0 & \text{for } 4 \leq a \leq 6 \end{cases} \quad (3.66)$$

to extract the rotational elements of $\mathbf{q}_i$. The two preceding expressions for μ_{ij}^q are generalizations of Eq. (3.22) and (3.27), respectively, with the elements of the transformation matrix $\mathcal{C}$ obtained from

$$\mathcal{C}_{i,ab} = \begin{cases} \frac{1}{2} |\mathbf{q}_i^r|^{-4} q_{i,c} \delta_{ac}^r & \text{for } b = 0 \\ \partial q_{i,a} / \partial \phi_{i,b}^s & \text{for } 1 \leq b \leq 3 \\ \partial q_{i,a} / \partial R_{i,b-3} & \text{for } 4 \leq b \leq 6, \end{cases} \quad (3.67)$$

where the vectors ϕ^s and $\mathbf{R}$ introduced in the main text have acquired a particle index i . Evaluation of these derivatives yields that $\mathcal{C}$ is block diagonal,

$$\mathcal{C}_{i,ab} = C_{i,cd} \delta_{ac}^r \delta_{bd}^r + (\delta_{ab} - \delta_{ab}^r) \quad (3.68)$$

with $\mathbf{C}_i = \mathbf{C}(\mathbf{q}_i^r)$ and

$$\mathbf{C}(\mathbf{q}^r) = \frac{1}{2 |\mathbf{q}^r|^4} \begin{pmatrix} q_0^r & -q_1^r & -q_2^r & -q_3^r \\ q_1^r & q_0^r & q_3^r & -q_2^r \\ q_2^r & -q_3^r & q_0^r & q_1^r \\ q_3^r & q_2^r & -q_1^r & q_0^r \end{pmatrix}. \quad (3.69)$$

One readily verifies that the latter matrix is related to the $\mathbf{B}$ matrix in the main text by

$$\begin{aligned} C_{D0}(\mathbf{q}^r) &= B_{D0}(\mathbf{q}^r) \\ C_{D\Delta}(\mathbf{q}^r) &= B_{D\Gamma}(\mathbf{q}^r) A_{\Delta\Gamma}(\mathbf{q}^r), \end{aligned} \quad (3.70)$$

where the upper case coordinate indices $D \in [0, 3]$ and $\{\Delta, \Gamma\} \in [1, 3]$. From the square root of the mobility matrix, the (7×7) segment pertaining to particles i and j reads as

$$(\sqrt{\mu^q})_{ij,ab} = \mathcal{C}_{i,ac} \left[\delta_{c0} \delta_{b0} (\sqrt{\nu^{00}})_{ij} + \delta_{c\alpha} \delta_{b\beta} (\sqrt{\mu^{r(s),t}})_{ij,\alpha\beta} \right], \quad (3.71)$$

where the $(N \times N)$ matrix ν^{00} collects the auxiliary mobility matrix elements, $\nu_{ij}^{00} = \mu_{ij,00}^{r(s),t}$, and

$$\sum_j (\sqrt{\nu^{00}})_{ij} (\sqrt{\nu^{00}})_{kj} = \nu_{ik}^{00}. \quad (3.72)$$

The coupling introduced by the mobility matrix will, in general, convert a force acting on one particle into displacements of all particles; the Brownian equation of motion for particle i therefore reads as [22, 29, 69]

$$\begin{aligned} q_{i,a}(t + \Delta t) - q_{i,a}(t) = & - \sum_j \mu_{ij,ab}^q \frac{\partial (\Phi - \frac{1}{2} k_B T \ln g^q)}{\partial q_{j,b}} \Delta t \\ & + \sum_j k_B T \frac{\partial \mu_{ij,ab}^q}{\partial q_{j,b}} \Delta t \\ & + \sum_j (\sqrt{\mu^q})_{ij,ab} \Theta_{j,b}^q \sqrt{2k_B T \Delta t} \\ & + \sum_j \mu_{ij,ab}^q \frac{\partial}{\partial q_{j,b}} \sum_k \lambda_k^q (|\mathbf{q}_k^r|^2 - 1), \end{aligned} \quad (3.73)$$

where the summations run over all particles, including i , and where the N Lagrange multipliers λ_k^q are to be solved from the N conditions $|\mathbf{q}_i^r(t + \Delta t)| = 1$. The objective in the remainder of this appendix is to show that the equation of motion reduces to

$$\begin{aligned} q_{i,a}(t + \Delta t) - q_{i,a}(t) = & - \sum_j \mathcal{C}_{i,a\alpha} \mu_{ij,\alpha\beta}^{r(s),t} \mathcal{C}_{j,b\beta} \frac{\partial \Phi}{\partial q_{j,b}} \Delta t \\ & + \sum_j k_B T \mathcal{C}_{i,a\alpha} \mathcal{C}_{j,b\beta} \frac{\partial \mu_{ij,\alpha\beta}^{r(s),t}}{\partial q_{j,b}} \Delta t \\ & + \sum_j \mathcal{C}_{i,a\alpha} \left(\sqrt{\mu^{r(s),t}} \right)_{ij,\alpha\beta} \Theta_{j,\beta}^q \sqrt{2k_B T \Delta t} \\ & + q_{i,b} \delta_{ab}^r \tilde{\lambda}_i^q, \end{aligned} \quad (3.74)$$

where the N Lagrange multipliers $\tilde{\lambda}_i^q$ are still to be determined from $|\mathbf{q}_i^r(t + \Delta t)| = 1$. Note that the divergence term in the second term on the r.h.s. results from the hydrodynamic interactions, and is unrelated to the use of quaternions. The evaluation of the two underlined factors on the r.h.s. is simplified by noticing, using Eq. (3.67), that for any function f ,

$$\mathcal{C}_{j,b\beta} \frac{\partial f}{\partial q_{j,b}} = \delta_{\beta\gamma}^r \frac{\partial f}{\partial \phi_{j,\gamma}^s} + (\delta_{\beta\gamma} - \delta_{\beta\gamma}^r) \frac{\partial f}{\partial R_{j,\gamma-3}}, \quad (3.75)$$

thereby eliminating the need to differentiate with respect to quaternions. Consequently, the usual potential derivatives and mobility matrix divergences in the Cartesian laboratory frame can be employed in a simulation. The equations of motion derived in the main text, Eqs. (3.9) and

(3.13), emerge as a special case of Eq. (3.74) upon inserting the appropriate mobility matrix and using the relations provided in Eq. (3.70).

In the analysis of the various contributions to the equation of motion, Eq. (3.73), it proves useful to start with the displacement of particle i due to the constraint, $\Delta \mathbf{q}_i^c$. By using Eqs. (3.65) and (3.69), and the orthogonality property

$$\mathcal{C}_{j,ab} q_{j,c} \delta_{ac}^r = \frac{1}{2 \left| \mathbf{q}_j^r \right|^2} \delta_{b0}, \quad (3.76)$$

the last term of Eq. (3.73) can be rewritten as

$$\Delta q_{i,a}^c = q_{i,b} \delta_{ab}^r \sum_j \frac{\mu_{ij,00}^{r(s),t}}{2 (q_i^r)^4 (q_j^r)^2} \lambda_j^q. \quad (3.77)$$

This displacement is parallel to the orientation $\mathbf{q}_i^r$ of particle i and does not affect its position. Because the displacement is linear in the Lagrange multipliers λ_j^q , the constraints can be decoupled by substituting the above expression with

$$\Delta q_{i,a}^c = q_{i,b} \delta_{ab}^r \tilde{\lambda}_i^q, \quad (3.78)$$

with $\tilde{\lambda}_i^q$ the effective Lagrange multiplier acting on particle i only.

The displacement of particle i due to the potential forces on particle j can be recast, by using Eq. (3.64), as

$$\mu_{ij,ab}^q \frac{\partial \Phi}{\partial q_{j,b}} = \mu_{ij,ab}^{q,0} \frac{\partial \Phi}{\partial q_{j,b}} + \mathcal{C}_{i,a0} \mu_{ij,00}^{r(s),t} \mathcal{C}_{j,b0} \frac{\partial \Phi}{\partial q_{j,b}}, \quad (3.79)$$

where the last term is proportional to $\Delta \mathbf{q}_i^c$ and therefore can be ignored. The metric tensor of the system is given by the product

$$g^q = \prod_{j=1}^N g_j^q, \quad (3.80)$$

with g_j^q denoting the one-particle metric of particle j , as derived in Eq. (3.58). One readily sees that Eq. (3.79) still holds true after replacing Φ by $\ln g^q$, and that

$$\mu_{ij,ab}^{q,0} \frac{\partial \ln g^q}{\partial q_{j,b}} = 8 \mathcal{C}_{i,a\alpha} \mu_{ij,\alpha\beta}^{r(s),t} \mathcal{C}_{j,b\beta} \frac{q_{j,c}}{\left| \mathbf{q}_j^r \right|^2} \delta_{bc}^r = 0, \quad (3.81)$$

where Eq. (3.76) has been used in the last step.

The term being summed in the drift correction can be rewritten, using Eq. (3.64), as

$$\frac{\partial \mu_{ij,ab}^q}{\partial q_{j,b}} = \frac{\partial \mu_{ij,ab}^{q,0}}{\partial q_{j,b}} + \frac{\delta_{ac}^r \delta_{bd}^r}{4} \frac{\partial}{\partial q_{j,b}} \left(\mu_{ij,00}^{r(s),t} \frac{q_{i,c}}{\left| \mathbf{q}_i^r \right|^4} \frac{q_{j,d}}{\left| \mathbf{q}_j^r \right|^4} \right). \quad (3.82)$$

Observing that, for any function f ,

$$\delta_{ac}^r \delta_{bd}^r \frac{\partial}{\partial q_{j,b}} (f q_{i,c} q_{j,d}) = \delta_{ac}^r q_{i,c} \left[f + \delta_{bd}^r \frac{\partial}{\partial q_{j,b}} (f q_{j,d}) \right], \quad (3.83)$$

it follows that the second term on the r.h.s. of Eq. (3.82) is proportional to $\Delta \mathbf{q}_i^c$ and can therefore be ignored. Evaluating the first term yields

$$\frac{\partial \mu_{ij,ab}^{q,0}}{\partial q_{j,b}} = \frac{\partial \mathcal{C}_{i,a\alpha}}{\partial q_{j,b}} \mu_{ij,\alpha\beta}^{r(s),t} \mathcal{C}_{j,b\beta} + \mathcal{C}_{i,a\alpha} \frac{\partial \mu_{ij,\alpha\beta}^{r(s),t}}{\partial q_{j,b}} \mathcal{C}_{j,b\beta} + \mathcal{C}_{i,a\alpha} \mu_{ij,\alpha\beta}^{r(s),t} \frac{\partial \mathcal{C}_{j,b\beta}}{\partial q_{j,b}}. \quad (3.84)$$

The derivatives of $\mathcal{C}$ with respect to a position coordinate yield zero, while those with respect to an orientation coordinate reduce to derivatives of $\mathbf{C}$. One then readily shows, by arguments similar to those used in Eq. (3.60), that the derivative in the last term on the r.h.s. vanishes identically. The derivative in the first term only contributes if $i = j$, in which case an argumentation similar to that followed in Eqs. (3.61) through (3.63) yields that the term cancels against the constraint if the (3×3) rotational part to $\mu_{ii}^{r(s),t}$ is symmetric. Hence the drift correction reduces to the central term on the r.h.s. of Eq. (3.84); this term cancelled in the derivation in the main text, where the mobility matrix rotated rigidly with the body, but this is no longer the case under full hydrodynamics.

Finally, in the random contributions to the displacement of particle i ,

$$(\sqrt{\mu^q})_{ij,ab} \Theta_{j,b}^q = \mathcal{C}_{i,a0} (\sqrt{\nu^{00}})_{ij} \Theta_{j,0}^q + \mathcal{C}_{i,a\alpha} \left(\sqrt{\mu^{r(s),t}} \right)_{ij,\alpha\beta} \Theta_{j,\beta}^q, \quad (3.85)$$

where the first term on the r.h.s. cancels against the constraint. Substitution of the preceding steps into Eq. (3.73) yields the equation of motion in Eq. (3.74), where the metric and all references to $\mu_{ij,00}^{r(s),t}$ have vanished and where the remaining drift correction is entirely of hydrodynamic origin.

Figure 3.3: Simulation results (symbols) for the mean squared displacements (a) of the particle along the three space fixed axes, and rotational relaxations (b) for the three body-fixed eigenvectors of the rotational mobility matrix and a ‘random’ vector detailed in the main text, compared against the theoretical predictions (lines).

4. α -synuclein as a patchy particle

*A number of amyloidogenic proteins, including α -synuclein in Parkinson's disease and amyloid- β in Alzheimer's disease, are intrinsically disordered in solution but form highly ordered cross- β -sheets in fibrils. The early aggregation stages of these proteins show complex behaviour, with the coexistence of solvated monomers, small oligomers and proto-fibrils. We propose a highly coarse-grained model to simulate the aggregation processes of α -synuclein. The protein is modelled as a patchy particle which – like the protein being studied, but unlike a particle in conventional particle-based simulations – is able to change its internal state, and thereby its shape and interaction parameters, in response to its environment. Translational and rotational motion, as well as the evolution of the internal state, are simulated by Brownian Dynamics. We present simulation results showing the formation of oligomers and fibrils by a direct nucleation-and-growth mechanism, by two-step-nucleation through the conversion of an oligomer into a fiber or vice versa, and by fibril-enhanced conversion of oligomers into fibrils.*¹

4.1 Introduction

The self-assembly of proteins into amyloids has been strongly linked to a number of neurodegenerative disorders such as Parkinson's disease (PD) [33, 77, 120, 141, 182, 213] and Alzheimer's disease (AD) [31, 39, 75]. The two proteins associated with these diseases are α -synuclein (α syn) and amyloid- β ($A\beta$), respectively [99]. They are known to aggregate and give rise to numerous structures such as oligomers [15, 185, 207], fibrils [157] and Lewy bodies [106], localised mainly

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in brain cells. Some of these aggregates are believed to be toxic [14, 15, 37, 212] and lead to neuronal death [106, 121, 150, 175]. Both proteins are intrinsically disordered, meaning that they have no prevalent secondary structure in their native solvated state, but they can adapt their internal state in response to the surrounding environment. Upon changing structure they can interact with other proteins or membranes and exhibit biological functions [194, 205]. Both proteins are known to form cross- β -sheets in fibrils or amyloids [118] and α helices against membranes [193]. Several other proteins such as insulin, amylin and huntingtin [99, 184] show similar characteristics, suggesting generic behaviour.

Fibrils and oligomers are aggregates formed from initially unstructured proteins undergoing conformational changes. These structural transitions as well as the nature of the aggregates have been studied intensively over the past decades. Single-molecule photobleaching experiments of α -synuclein oligomers showed the presence of a single well defined species composed of 31 monomers per oligomer [228]. Single-molecule fluorescence studies on A β oligomers show that membrane exposure may catalyse the formation of larger oligomers [93]. Small angle X-ray scattering (SAXS) showed the presence of wreath shaped oligomer accumulations during α -synuclein fibrillation, suggesting that the oligomers are the direct precursor of fibrils (on-pathway mechanism) [72]. In addition, electron microscopy (EM) studies suggest that protofibrils may be transient assembly intermediates on the fibril formation pathway [107]. To gain insight into the polymorphic behaviour of α -synuclein and its associated aggregates quantitative atomic force microscopy (AFM) has been intensively used [157, 158, 177]. Fibrils form a characteristic cross- β secondary structure and have a width of about 4-10 nm [172]. Deeper insight into the arrangement of the β -strands in the amyloidogenic structure has been gained via hydrogen/deuterium (H/D) exchange measured by solution-state nuclear magnetic resonance (NMR) [212].

Computer simulations present a powerful tool to investigate the various aggregates that a protein can form [162]. All-atom simulations provide insight into the precise aggregation mechanism for small peptides [12, 109, 115, 187]. The typical parallel and in-register arrangement of α syn in a fiber has been studied by Molecular Dynamics (MD) simulations [222]. Other studies show that upon interaction with a membrane the protein will change conformation from an initial broken-helix to a semi-extended one [211]. The excessive computational demands of all-atom simulations make it difficult to predict pathways of fibril formation or to analyse the higher order aggregates which are believed to be crucial to the triggering of neurodegenerative diseases. By sacrificing the finer details of the system, so-called coarse-grained (CG) models aim to reach

larger time and length scales at which aggregation occurs [219]. CG lattice models predicted that nucleation cannot occur without inter-sheet interactions [225]. Dynamic Monte Carlo (DMC) simulations have been used to predict the existence of a critical nucleus of 4 monomers to start fibril formation [19]. Growth, however, proceeds through monomer addition at both fibril ends [153]. Recent Monte Carlo (MC) simulations on the mechanism of fibril formation suggest the existence of two pathways, *i.e.* one step nucleation (1SN) at high protein concentrations and two step nucleation (2SN) at low peptide concentrations [170].

Here we introduce a CG model that represents a protein as a single particle able to adjust its internal state, reflecting the ability of intrinsically disordered proteins (IDPs) to adapt their secondary structure on the surrounding environment. The disordered state is represented by a single soft sphere and the folded β -sheet state by a hard spherocylinder with attractive patches on its surface, see Fig. 4.1. The state, and hence the shape of the particle, continuously evolves in time in response to internal and inter-particle interactions. To investigate the dynamics of the system, a novel Brownian Dynamics algorithm [87] is used to simulate the translational and rotational motions of the particles as well as their internal dynamics.

This chapter is structured as follows: Sec. 5.2 introduces the coarse grained model, the interaction potentials, and describes the Brownian Dynamics algorithm. The simulation results on the self-assembly of the particles into oligomers and fibrils is discussed in Sec. 5.4 and the chapter ends with a summary and discussion of the main results in Sec. 5.5.

4.2 Model and Methods

4.2.1 Polymorphism

The model introduced here is a highly coarse grained generic representation of amyloid forming proteins. Whenever numerical values are required we will refer to α -synuclein, a small 14 kDa intrinsically disordered protein, composed of 140 aminoacids (AA), found mainly in neuronal tissue and commonly associated with Parkinson's disease. Also known as the 'protein chameleon', α -synuclein adopts a disordered state in a solvent, curls up into an alpha-helix against a membrane [45, 218] or vesicle [52], and folds into cross β sheets in fibrils or amyloids [118, 212]. In this chapter, we will focus on the disordered and the β -rich conformations. The radius of gyration of α -synuclein in the disordered conformation, $R_g \approx 40 \text{ \AA}$, is a bit smaller than that of a 140 AA random coil, with $R_g \approx 52 \text{ \AA}$ [194] but significantly bigger than that of a 140 AA folded globular protein, $R_g \approx 15 \text{ \AA}$ [16], suggesting that α syn is slightly more compact than the classic random coil [208]. In fibrils, the approximately 60 residues between

positions ~ 30 and ~ 90 adopt a condensed structure that enables this segment to bind strongly to identically folded neighbouring segments by numerous hydrogen bonds [99, 212]. The N and C-terminal tails flanking this segment are solvent exposed and appear not involved in the hydrogen bonding in the core of the fiber [95, 113, 212]. Experiments show that fibrillation proceeds quicker after shorting the N-terminal [95] or neutralising the large negative charge on the C-terminal [82], which suggests that the two tails may play a role in preventing amyloid accumulation. With these considerations in mind, and with an eye on the excessive computational demands of simulating fibril nucleation by a highly detailed model, we set forth to create a coarse-grained patchy particle representing the central ~ 60 amino acids of α -synuclein.

Figure 4.1: Cartoons of the coarse grained representation of the amyloid protein in (a) the disordered state and (b) in the compactly folded state, with colours indicating surfaces with distinct interaction properties. The unit vector $\hat{\mathbf{u}}$ along the long axis of the particle, in combination with the perpendicular unit vector $\hat{\mathbf{n}}$, define the orientation of the particle relative to the laboratory coordinate system.

An essential feature of the protein, and hence of the model particle, is its ability to switch between two internal states, *i.e.* a disordered state and a folded β -sheet-rich state. This is realised in the simulations by introducing a parameter λ_i to describe the internal state of the i^{th} particle. In line with experimental findings, the probability distribution of this coordinate must be constructed in such a way that for unbound particles the disordered state is favoured over the ordered state, with only few particles in an intermediate transitory state. Hence, the internal potential $\Phi_\lambda(\lambda)$ should be of the form shown in Fig. 4.2(a), where an activation barrier separates the disordered state ($\lambda < 0$) from the ordered state ($\lambda > 0$). For mathematical convenience, we construct this potential by combining two third-order polynomials,

$$\Phi_\lambda(\lambda) = \begin{cases} -\varepsilon_\lambda^S \frac{27}{4} (\lambda^2 + \lambda^3) & \text{for } \lambda < 0 \\ -\varepsilon_\lambda^R \frac{27}{4} (\lambda^2 - \lambda^3) & \text{for } \lambda \geq 0 \end{cases} \quad (4.1)$$

Figure 4.2: (a) The internal potential plotted against the internal coordinate for $\epsilon_\lambda^S = 3$ and $\epsilon_\lambda^R = 2$, see Eq. (5.1). (b) Length and diameter as functions of λ , see Eq. (5.3), for the parameter values employed in the simulation. Note that both dimensions decrease as the protein evolves from the low density disordered state to the compactly folded ordered state.

that reach local minima of $-\epsilon_\lambda^S$ and $-\epsilon_\lambda^R$ for $\lambda = \pm \frac{2}{3}$, and both have a local maximum for $\lambda = 0$. Although the folded state is unfavourable in solution, it is the stable state for proteins in a fiber. This shift indicates that the stability gained in fibrils arises from attractive interactions between proteins, which are modelled by the attractive potential discussed below.

The shape of the protein is strongly linked to its internal state, hence the shape of the particle should depend on the parameter λ . We idealize the disordered state as a sphere with soft isotropic interactions and the folded state as a spherocylinder with hard anisotropic interactions, see Fig. 4.1. This selection of shapes allows for continuous morphing as a function of the internal coordinate. To restrict the shape changes to the barrier region, we introduce the smoothed

step-function

$$\mu(\lambda) = \begin{cases} 0 & \text{for } \lambda < -1 \\ \frac{\tanh(A_\mu \lambda)}{2 \tanh(A_\mu)} + \frac{1}{2} & \text{for } -1 \leq \lambda \leq 1 \\ 1 & \text{for } \lambda > 1 \end{cases} \quad (4.2)$$

where A_μ determines the width of the transition region. The tip-to-tip length and the diameter of the particle are then defined following

$$\begin{aligned} L(\lambda) &= L_S + \mu(\lambda)(L_R - L_S), \\ D(\lambda) &= D_S + \mu(\lambda)(D_R - D_S), \end{aligned} \quad (4.3)$$

where the spherical and sphero-cylindrical extremes of the morphing process are marked by a subscript S and R, respectively, and where $D_S = L_S$ for a sphere.

4.2.2 The Repulsive Potential

Having defined the shape and size of the particle, we next focus on the interactions between the particles. Because of the polymorphism of the particles, the potential energy is coupled to the internal states of the interacting particles. Overlap of particles i and j is avoided by a repulsive potential based on the minimum distance $d_{\min}$ between their long axes [210],

$$d_{\min}(\mathbf{r}_i, \mathbf{r}_j, \hat{\mathbf{u}}_i, \hat{\mathbf{u}}_j, \lambda_i, \lambda_j) = \min_{\alpha_i, \alpha_j} |(\mathbf{r}_i + \alpha_i \hat{\mathbf{u}}_i) - (\mathbf{r}_j + \alpha_j \hat{\mathbf{u}}_j)|, \quad (4.4)$$

with $\mathbf{r}_i$ and $\mathbf{r}_j$ the centre of mass positions of the two particles, and $\hat{\mathbf{u}}_i$ and $\hat{\mathbf{u}}_j$ denoting unit vectors pointing along the long axes of the particles. For two infinitely long cylinders, the analytic expressions for the minimisation parameters α_i and α_j are readily solved from two coupled linear equations. By restricting their values to the λ -dependent ranges $|\alpha_i| \leq \frac{1}{2}(L_i - D_i)$ and $|\alpha_j| \leq \frac{1}{2}(L_j - D_j)$, the points of closest approximation are guaranteed to lie either within the cylindrical segment of a spherocylindrical particle or at the centre of a spherical particle. Substitution of these restricted values into Eq. (5.5) yields the requested minimum distance. When two rod-like particles are nearly parallel, a small change of their relative orientations can cause a major change in the points of closest approximation; these points may even jump from one end of the cylindrical segment to the opposite end. Hence, for $(\hat{\mathbf{u}}_i \cdot \hat{\mathbf{u}}_j)^2 \geq 0.99$ the centre to centre vector $\mathbf{r}_{ij} = \mathbf{r}_i - \mathbf{r}_j$ is decomposed into components parallel $r_{\parallel}$ and perpendicular $r_{\perp}$ to the average direction $\hat{\mathbf{u}}_{\text{av}} = (\hat{\mathbf{u}}_i \pm \hat{\mathbf{u}}_j)/|\hat{\mathbf{u}}_i \pm \hat{\mathbf{u}}_j|$, where the plus (minus) sign applies when the orientation vectors point in the same (opposite) direction. The minimum distance is then

Figure 4.3: Plot of the distance dependence of the total interaction energy of two particles, see Eq. (5.8). Two particles in the disordered state (black) have a longer-ranged attraction and a softer repulsion than two aligned particles in the folded state (red), while the interaction in a mixed pair is intermediate (green). Here $C_{SS} = 3$, $\mathcal{F}_{RR} = 20$ and $C_{SR} = 1$; the employed particle dimensions are discussed in Sec. 4.3.1.

calculated as

$$d_{\min} = \begin{cases} r_{\perp} & \text{for } |r_{\parallel}| \leq (\bar{L} - \bar{D}) \\ \sqrt{r_{\perp}^2 + [r_{\parallel} - (\bar{L} - \bar{D})]^2} & \text{for } |r_{\parallel}| > (\bar{L} - \bar{D}), \end{cases} \quad (4.5)$$

with the first line the perpendicular distance between two cylindrical segments and the second line the distance between two end caps.

The repulsive potential should become relevant, *i.e.* increase above the thermal energy $k_B T$ with k_B Boltzmann's constant and T the temperature, when the minimum distance between two particles reduces below the average of their radii, $\bar{D} = \frac{1}{2}(D_i + D_j)$. Due to the marked difference in amino acid packing densities, the repulsion between two disordered proteins will be much softer than that between two compactly folded proteins. This is accounted for by the potential

$$\frac{\Phi_{\text{rep}}^{\lambda}}{k_B T} = \begin{cases} \left(\frac{2\bar{D}}{d_{\min}} - 1 \right)^n & \text{for } d_{\min} < 2\bar{D} \\ 0 & \text{for } d_{\min} > 2\bar{D}, \end{cases} \quad (4.6)$$

where the exponent, *i.e.* the hardness of the potential, was selected to vary from 4 to 6 following $n = 4 + (\mu_i + \mu_j)$.

4.2.3 The Attractive Potential

The aggregation of amyloid proteins into oligomers and fibrils is driven by attractive interactions between the amino acids: van der Waals interactions, hydrophobic interactions and hydrogen bonding. The number of those interactions and their overall strength depend on the internal state of the protein; disordered proteins have larger radii of gyration and hence larger interaction ranges than densely folded proteins; the interactions between two random coils gradually change with distance, while hydrogen bonds between two compacted proteins are short-ranged. Furthermore, the disordered state is in good approximation isotropic, while the folded state will experience anisotropic interactions. For two particles i and j , we propose the attractive potential

$$\begin{aligned} \frac{\Phi_{\text{attr}}^\lambda}{k_B T} = \eta(d_{\min}) & \left\{ \mathcal{C}_{\text{SS}} (1 - \mu_i) (1 - \mu_j) \right. \\ & + \mathcal{F}_{\text{RR}} \mu_i \mu_j \\ & \left. + \mathcal{C}_{\text{SR}} [\mu_i (1 - \mu_j) + \mu_j (1 - \mu_i)] \right\}, \end{aligned} \quad (4.7)$$

with $d_{\min}$ the previously introduced minimum distance. The distance dependence of the potential is given by

$$\eta(d_{\min}) = \begin{cases} \frac{\tanh(A_{\text{attr}}(d_{\min} - 2\bar{D}))}{\tanh(2A_{\text{attr}}\bar{D})} & \text{for } d_{\min} < 2\bar{D} \\ 0 & \text{for } d_{\min} \geq 2\bar{D} \end{cases} \quad (4.8)$$

where $A_{\text{attr}} = a_{\text{attr}}(\mu_i + \mu_j) + b_{\text{attr}}$, with $a_{\text{attr}} > 0$ and $b_{\text{attr}} > 0$, determines the steepness of the transition between $\eta(0) = -1$ and $\eta(2\bar{D}) = 0$. The resulting binding energy between two particles in the spherical state, $\mu_i \simeq \mu_j \simeq 0$, depends on their center-to-center distance $d_{\min}$ only, and decays smoothly with decreasing distance, from zero at the cut-off distance to a minimum of $-\mathcal{C}_{\text{SS}}$. For two spherocylindrical particles, $\mu_i \simeq \mu_j \simeq 1$, the function $\eta(d_{\min})$ is both shorter-ranged due to the smaller $\bar{D}$ and its decay with decreasing distance to a minimum of $-\mathcal{F}_{\text{RR}}$ is steeper, *i.e.* A_{attr} is larger, reflecting a tightly packed protein. The interaction between a spherical particle, $\mu_i \simeq 0$, and a spherocylindrical particle, $\mu_j \simeq 1$, is accounted for by the last term in the r.h.s. of Eq. (5.8), with a decay rate intermediate between those of the two states mentioned before and a minimum at $-\mathcal{C}_{\text{SR}}$. All three cases are illustrated in Fig. 4.3.

The interaction between two non-isotropic proteins, *i.e.* the sum of the contacts between atoms on their surfaces, varies with the relative orientations of the proteins. This complex dependence is qualitatively accounted for in Eq. (5.8) by the rod-rod contribution $\mathcal{F}_{\text{RR}} \mu_i \mu_j$, with

$$\mathcal{F}_{\text{RR}} = [\mathcal{C}_{\text{RR}}^w + g_{ij} \mathcal{C}_{\text{RR}}^s] f_{ij}, \quad (4.9)$$

where

$$f_{ij} = (\hat{\mathbf{u}}_i \cdot \hat{\mathbf{u}}_j)^2 h(\hat{\mathbf{u}}_i \cdot \hat{\mathbf{r}}_{ij}) h(\hat{\mathbf{u}}_j \cdot \hat{\mathbf{r}}_{ij}) \quad (4.10)$$

and $0 \leq f_{ij} \leq 1$. In f_{ij} , the first factor promotes, at given $d_{\min}$ and $\mathbf{r}_{ij}$, the alignment of the long axes $\hat{\mathbf{u}}$ of the spherocylinders. The second and third factors, with

$$h(x) = \begin{cases} 1 - ax^2 & \text{if } x^2 < 1/a \\ 0 & \text{if } x^2 > 1/a, \end{cases} \quad (4.11)$$

reflect energy variations when a rod is transported along its long axis, at fixed orientation and $d_{\min}$, with the lowest interaction energy reached when the connecting vector $\mathbf{r}_{ij}$ is perpendicular to both rods, *i.e.* for $\alpha_i = \alpha_j = 0$ and $|\mathbf{r}_{ij}| = d_{\min}$. The parameter $\mathcal{C}_{\text{RR}}^{\text{w}}$ in Eq. (4.9) represents the interaction strength of the weak van der Waals and hydrophobic interactions between two proteins. Folded proteins in fibers are known to form an array of hydrogen bonds with both neighbours along the direction of the fiber, creating a cross β -sheet [99, 212]. This indicates that the interactions of spherocylindrical particles should be non-uniform under rotations along their long axes, as illustrated in Fig. 4.4. Every particle is therefore endowed with a second orientational body-fixed unit vector $\hat{\mathbf{n}}_i$, perpendicular to the long axis $\hat{\mathbf{u}}_i$, see Fig. 4.1(b), and $\mathcal{F}_{\text{RR}}$ contains a contribution proportional to

$$g_{ij} = (\hat{\mathbf{r}}_{ij} \cdot \hat{\mathbf{n}}_i)^p (\hat{\mathbf{r}}_{ij} \cdot \hat{\mathbf{n}}_j)^p \quad (4.12)$$

that favours alignment of the orientation vectors $\hat{\mathbf{n}}$ parallel to the center-to-center direction $\hat{\mathbf{r}}_{ij}$, see Fig. 4.4. Here p is an even integer, $0 \leq g_{ij} \leq 1$, and the parameter $\mathcal{C}_{\text{RR}}^{\text{s}}$ in Eq. (4.9) represents the strength of the hydrogen bonds.

4.2.4 Brownian Dynamics Algorithm

The evolution of the collection of polymorph particles is simulated by Brownian Dynamics. In this section, we provide the overall equation of motion of the system, which is subsequently split into equations of motion for translation, rotation and internal dynamics.

Brownian Dynamics algorithms have been developed to simulate the motion of colloidal particles in a solvent, where the particles interact with each other as well as continuously experience multiple collisions with the solvent molecules. The generalised equation of motion

Figure 4.4: Cartoons of two aligned folded proteins, with their long axes perpendicular to the projection plane. (a) The strongest binding is achieved when the surfaces capable of forming hydrogen bonds, coloured in red, are facing each other. (b) Weaker affinities apply between the remaining surface areas, in blue, and between unlike surfaces.

[29, 69] is expressed as

$$\begin{aligned}
 \mathbf{Q}(t + \Delta t) - \mathbf{Q}(t) = & -\boldsymbol{\mu}^Q \frac{\partial \mathcal{A}}{\partial \mathbf{Q}} \Delta t \\
 & + k_B T \frac{\partial}{\partial \mathbf{Q}} \cdot \boldsymbol{\mu}^Q \Delta t \\
 & + (\boldsymbol{\mu}^Q)^{1/2} \boldsymbol{\Theta}^Q(t) \sqrt{2k_B T \Delta t},
 \end{aligned} \tag{4.13}$$

where $\mathbf{Q}$ represents the full set of generalised coordinates, *i.e.* positions $\mathbf{r}$, orientations $\mathbf{q}$ and internal coordinates λ for all particles. The first contribution on the r.h.s. gives the displacements over a time-step Δt due to the balance between the thermodynamic force $\mathcal{F} = -\partial \mathcal{A} / \partial \mathbf{Q}$, with $\mathcal{A}$ the free energy as a function of $\mathbf{Q}$, and the opposing solvent friction, whose inverse is the mobility matrix $\boldsymbol{\mu}^Q$. The second contribution arises in the Itô interpretation, *i.e.* all terms on the r.h.s. are evaluated at time t , due to the inhomogeneity of the mobility tensor $\boldsymbol{\mu}^Q$. The last contribution represents the erratic Brownian displacements of the coordinates, where the components of the time-dependent vector $\boldsymbol{\Theta}^Q$ have zero mean, unit variance and are not correlated to each other nor to previous values (Markovian). The size of these random displacements is connected to the mobility through the fluctuation-dissipation theorem, which is incorporated in the last term of Eq. (5.15), where the square root $\sqrt{\mathbf{M}}$ of a matrix $\mathbf{M}$ has the property $\sqrt{\mathbf{M}}_{\alpha\beta} \sqrt{\mathbf{M}}_{\gamma\beta} = M_{\alpha\gamma}$.

The simplest application of the Brownian Dynamics algorithm is the translational motion of a particle in a solvent. In this case, the coordinates denote the centre of mass position of the particle, $\mathbf{R}$, which combined with the translational mobility tensor in the space-frame $\boldsymbol{\mu}^{\text{t},\text{s}}$ yields

the equation of motion

$$\begin{aligned} \mathbf{R}(t + \Delta t) - \mathbf{R}(t) &= \boldsymbol{\mu}^{t,s} \mathbf{F} \Delta t \\ &+ \sqrt{\boldsymbol{\mu}^{t,s}} \boldsymbol{\Theta}^t \sqrt{2k_B T \Delta t}, \end{aligned} \quad (4.14)$$

where the conservative force is given by $\mathbf{F} = -\partial\Phi/\partial\mathbf{R}$, and $\boldsymbol{\Theta}^t$ is a three-dimensional Markovian vector. For non-spherical rotating particles, the translational mobility in the space-frame varies with the orientation and is given by

$$\boldsymbol{\mu}^{t,s} = \mathbf{A} \boldsymbol{\mu}^{t,s} \mathbf{A}^T \quad (4.15)$$

where $\boldsymbol{\mu}^{t,s}$ is the constant body-fixed translational mobility tensor and $\mathbf{A}$ represents the rotation matrix from the body frame to the laboratory frame, see Appendix 4.5.1. The square root of the laboratory mobility tensor is readily calculated from its constant body-fixed counterpart by

$$\sqrt{\boldsymbol{\mu}^{t,s}} = \mathbf{A} \sqrt{\boldsymbol{\mu}^{t,s}}. \quad (4.16)$$

For a body-fixed coordinate system that diagonalises $\boldsymbol{\mu}^{t,s}$, the square root of $\boldsymbol{\mu}^{t,s}$ is simply obtained by taking the square roots of its diagonal elements.

The rotational Brownian motion is simulated by a recently introduced algorithm [87] using quaternions $\mathbf{q}$ as the rotational coordinates. This approach proved to be very accessible - it avoids the singularities, metric tensor correction and Itô correction that complicate BD simulations in other rotational coordinates [55, 56, 57, 58, 131] - making it the ideal candidate for the current study. The rotational equation of motion reads as

$$\begin{aligned} \mathbf{q}(t + \Delta t) - \mathbf{q}(t) &= \mathbf{B} \boldsymbol{\mu}^{r,b} \mathbf{A}^T \mathbf{T} \Delta t \\ &+ \mathbf{B} \sqrt{\boldsymbol{\mu}^{r,b}} \boldsymbol{\Theta}^r \sqrt{2k_B T \Delta t} \\ &+ \Lambda \mathbf{q} \end{aligned} \quad (4.17)$$

where $\boldsymbol{\mu}^{r,b}$ represents the rotational mobility matrix in the body-fixed frame. The first term on the r.h.s. represents the rotational displacement that arises from the balance between the torque $\mathbf{T} = -\partial\Phi/\partial\boldsymbol{\phi}^s$ and the rotational friction, where $\boldsymbol{\phi}^s$ denotes infinitesimal rotations around the laboratory coordinate axes. The 4×3 transformation matrix $\mathbf{B}$ converts angular displacements in the body-frame into quaternion velocities.; the employed expressions for $\mathbf{A}$ and $\mathbf{B}$ in terms of $\mathbf{q}$ are given in Appendix 4.5.1. The second term on the r.h.s. represents the Brownian contribution, where the components of the random vector $\boldsymbol{\Theta}^r$ are again chosen such that they have zero mean, unit variance, no memory and are unrelated to the translational random contributions. The last term introduces a constraint on the length of $\mathbf{q}$, as for finitely sized time steps the length

of the quaternion vector - which must be unity in order for $\mathbf{A}(\mathbf{q})$ to be a rotation matrix - is only approximately conserved. A simple rescaling, $\mathbf{q} \rightarrow \mathbf{q}/|\mathbf{q}|$ will change the sampled phase space distribution. The constraint calculation applied here follows the method of undetermined Lagrange multipliers, as also used in the seminal SHAKE algorithm [166]. The strength of the constraint force is determined by the Lagrange multiplier Λ , which is readily solved from the quadratic equation

$$|\mathbf{q}(t + \Delta t)|^2 = |\mathbf{q}^{\text{uncons}}(t + \Delta t) + \Lambda \mathbf{q}(t)|^2 = 1, \quad (4.18)$$

where $\mathbf{q}^{\text{uncons}}(t + \Delta t)$ denotes the unconstrained propagated coordinates in the absence of the constraint force.

The evolution of the internal coordinate λ , characterising the internal state of a particle, is also simulated by Brownian Dynamics. The equation of motion reads as

$$\begin{aligned} \lambda(t + \Delta t) - \lambda(t) &= \mu^\lambda F^\lambda \Delta t \\ &+ \sqrt{\mu^\lambda} \Theta^\lambda \sqrt{2k_B T \Delta t}, \end{aligned} \quad (4.19)$$

where μ^λ denotes the mobility of the internal coordinate, $F^\lambda = -\partial\Phi/\partial\lambda$ the force on the internal coordinate arising from internal and external potentials, and Θ^λ a Markovian random number of zero average and unit variance.

4.3 Results

4.3.1 Simulation Details

The variable dimensions of the particles were chosen to match experimental data. Small angle X-ray scattering on monomeric α -synuclein in solution yielded a radius of gyration, R_g , of 4.0 nm [208]. For the 60 AA segment, assuming the scaling behaviour of a Gaussian chain, this converts into $R_g^{60\text{AA}} = 2.61$ nm. The diameter of the particle in the disordered state was selected by demanding that the repulsive potential between two particles reaches $1 k_B T$ at a distance $2R_g$, hence $D_S = L_S = 5.2$ nm. For the folded state, we based the dimensions on the structural data on fibrils from the solid state hydrogen/deuterium (H/D) exchange NMR measurements by Vilar *et al.* [212]. The length of the rod-like particle, $L_R = 3.5$ nm, matches the average length of the five β strands per protein. The diameter was set at $D_R = 2.5$ nm, which is the average of the collective width of the five β -strands and the distance between two proteins along the fibril direction. In the conversion from λ to μ , see Eq. (5.2), the transition is confined to a small region by $A_\mu = 10$. For the steepness of the attractive potential, see Eq. (4.8), we selected $A_{\text{attr}} = 0.4(\mu_i + \mu_j) + 0.2$, with A_{attr} in units of nm^{-1} .

Simulation systems were generated by placing 200 particles, with random orientations and $\lambda = -2/3$, at random positions in a cubic simulation box with periodic boundary conditions. The concentration was fixed at one particle per $3 \cdot 10^{-5} \text{ nm}^3$, corresponding to an experimental concentration of $50 \mu\text{M}$, which lies close to the critical assembly concentration of wild-type α -synuclein. The temperature was set at body temperature, 310 K, and the viscosity equalled that of water, $\eta = 7 \cdot 10^{-4} \text{ Pa s}$. The translational and rotational mobility tensors were calculated using the equations presented in Appendix 4.5.2. By simulating a pre-assembled fibril at a range of time steps, we established that the system permits a time step $\Delta t = 1.7 \times 10^{-13} \text{ s}$. The mobility of the internal parameter μ^λ , represents the ‘internal friction’ of the protein supplemented with a solvent contribution. We settled on a value of $\mu^\lambda = 1.4 \times 10^{30} (\text{J s})^{-1}$, which made internal transitions rare events yet numerous transitions occurred during the entire length of a simulation. For instance, for a non-interacting particle with the well depths $\varepsilon_\lambda^S = 7$ and $\varepsilon_\lambda^R = 5$, the rate constants of the forward and reverse reactions were measured as once per 1.4×10^5 steps and once per 1.9×10^4 steps, respectively. To test whether the proposed combination of Brownian propagators correctly samples the internal coordinate, the sampled probability distribution along λ was compared against the theoretical prediction $P(\lambda) \propto \exp(-\beta\Phi_\lambda)$. Good agreement was obtained, demonstrating that the sampling of λ was not influenced by the shape-dependent mobilities of the particle (in the absence of interactions). Note that this agreement does not extend to second order Newtonian equations of motion, where the λ -dependence of the inertia tensor will affect the sampled distribution.

In preparation for the simulations with polymorph particles, we first studied the aggregation behaviour of the disordered and folded particles separately. The stability limits of oligomers were established by simulating systems containing pre-constructed oligomers of various sizes, surrounded by disordered particles, while freezing all internal coordinates at $\lambda = -2/3$. All oligomers were observed to disintegrate when $C_{\text{SS}} < 2.4$. For $C_{\text{SS}} = 3$, oligomers of less than about six particles tended to dissolve while larger oligomers showed a propensity to grow through monomer attachment and occasional fusions with other aggregates, suggesting a critical aggregate size of roughly 6 particles. Because particles in the disordered state have isotropic interactions, there is no inherent restriction on the maximum size of the agglomerates. The stability limits of fibrils were assessed in a similar fashion, from the fate of pre-constructed fibrils in simulations with all internal coordinates fixed at $\lambda = 2/3$. A series of simulations at various values of the directional interaction parameter yielded a critical binding strength $C_{\text{RR}}^S \approx 17$. Fibrils were observed to stick together, forming multi-stranded bundles, when

Figure 4.5: Phase diagram of the aggregation behaviour of the polymorph particles, plotted against the internal stabilities ε_λ^S and ε_λ^R of the disordered and folded states of the particle, respectively. The lines highlight approximate locations of phase boundaries. The inter-particle interactions are $C_{SS} = 3$, $C_{SR} = 1$, $C_{RR}^s = 20$ and $C_{RR}^w = 0$.

the weak non-directional interactions were turned on, $C_{RR}^w > 0$, and/or when the range of the directional interaction was too wide. For the simulations discussed below, the non-directional interactions have been turned off and to narrow the range of the directional potential the power p in Eq. (4.12) was set to a value of 6. In the production runs, the attractive interactions between like particles were set slightly above their respective critical values for aggregation: the sphere-sphere interaction was set at $C_{SS} = 3$, and the directional rod-rod interaction at $C_{RR}^s = 20$; these were also the values used in Fig. 4.3. A small attraction was introduced between unlike particles, by setting the sphere-rod interaction at $C_{SR} = 1$. The depths of the two wells in the internal potential was systematically varied, with $\varepsilon_\lambda^S \geq \varepsilon_\lambda^R$.

4.3.2 Polymorph aggregation

The polymorph particles can form both oligomers of particles in the disordered state and fibers of particles in the folded state. Figure 4.5 shows a phase diagram of the aggregation behaviour as a function of the two well depths ε_λ^S and ε_λ^R of the internal potential, see also Fig. 4.2(a). Each point represents three to five independent simulations, initiated with all particles in the disordered state, with most of the runs lasting up to the order of $2 \cdot 10^9$ steps, *i.e.* over 0.3 ms. During this time interval, a non-interacting spherical particle diffuses over $\sim 5 \times 10^2$ nm, equivalent to almost three box lengths, while the smaller rod-shaped particle travels even further. The aggregate species present at the ends of the simulations, as determined by visual inspection in

Figure 4.6: Snapshots of aggregates that formed spontaneously in simulations started from random configurations of particles in the spherical state (a). All runs share identical interaction parameters, as specified in the main text and in the caption to Fig. 4.5. Oligomers are formed for $\varepsilon_{\lambda}^S = 7$ and $\varepsilon_{\lambda}^R = 3$ (b), fibrils at $\varepsilon_{\lambda}^S = 7$ and $\varepsilon_{\lambda}^R = 6$ (c), and both are present at $\varepsilon_{\lambda}^S = 5$ and $\varepsilon_{\lambda}^R = 3$ (d).

VMD [85], see Fig. 4.6, are marked in the phase diagram with squares for fibrils and circles for oligomers. In the bottom-right corner, below the blue drawn line, only oligomers are formed because the internal potential strongly favours the disordered state. Both oligomers and fibrils are observed between this stability boundary and the red dashed line. Because there typically are only a few aggregates present at the end of a run, some boxes contain aggregates of one type only while others contain both types. Above the second phase boundary mainly fibrillar aggregates are present at the end of the simulations, indicating that the directional intermolecular interactions between rod-like particles are dominant in determining the final state.

4.3.3 Aggregation mechanisms

Visualisation of the simulations in VMD showed that the aggregates arise and evolve by the nucleation-and-growth mechanism. Randomly diffusing particles meet each other and – depending on their internal states and relative orientations – can stick together. For two rod-like particles the result will be a fibril nucleus, for two spherical particles an oligomer results. Note that the

Figure 4.7: Cartoon illustrating the major pathways to fibril formation: direct aggregation (top) of particles in the folded state and two step nucleation (bottom) via an oligomer.

latter dimer is smaller than the critical oligomer cluster, estimated at roughly 6 particles under the prevailing conditions, while there is no critical nucleus for fibrillar aggregates. We regularly observed that the two particles in a dimer transited, in rapid succession, from one internal state to the other. Eventually, the dimers either fell apart due to the perpetual Brownian motion or grew into long-lived aggregates by binding additional particles. Particles attaching to an aggregate were observed to quickly conform to the dominant internal state in that aggregate.

An exception to the above nucleation-and-growth mechanism is found for the combinations of well depths marked by checkered squares in the phase diagram of Fig. 4.5. In these systems, small oligomers formed as usual. Next, the particles in the oligomer changed in rapid succession from the disordered state to the folded state, forming a fibril in the process. This two step nucleation mechanism (2SN) is highlighted in Fig. 4.7. Above the dashed red line in the phase diagram, this mechanism converts almost all spontaneously formed oligomers into fibrils, explaining the absence of oligomers at the end of those simulations. Two step nucleation is suppressed upon reducing the stability of rods over spheres, *i.e.* by enlarging $\varepsilon_{\lambda}^S - \varepsilon_{\lambda}^R$, as is clear from the phase diagram. Two step nucleation is also suppressed by the growth of the oligomer: because the interactions between particles in the disordered state are soft and wide-ranged, the potential energy of a small oligomer increases supra-linearly with the number of particles, while

for a fibril the energy increases linearly. Whether or not there is a critical size to the oligomeric intermediate in two step nucleation will depend on the details of the interactions between and within the proteins. For the simulated particles, we estimate a critical size of roughly 6 particles. Oligomers exceeding this maximum size only transformed into short fibrils when attached to a large stable fibril. This process, observed in a number of simulations, suggests that the primary fibril acts as a catalyst in the conversion of oligomers into secondary fibrils. Two step nucleation is not limited to the formation of fibrils: in several simulations we observed the assembly of rod-shaped particles into a fibrillar intermediate that subsequently disordered into an oligomer.

Oligomers readily fused when they came into contact, while fibrils only merged when their ends met in a properly aligned orientation. In encounters of oligomers with fibrils, large oligomers tended to break down the fibril and absorb the released particles while small oligomers located at the end of a fibril were typically absorbed by the fibril. The reduction in the number of sphere-sphere interactions during the latter process made it relatively easy for particles to escape from the oligomer, hence the fibril often grew by less than the original oligomer size.

4.4 Discussion

To simulate the aggregation behaviour of the chameleon protein α -synuclein, we developed a coarse-grained simulation model in which the ~ 60 AA core of α -synuclein is represented by a single particle with the same versatility as the core of the real protein. This was achieved by creating a particle that smoothly morphs between a spherical shape with weak isotropic interactions, representing the disordered state of the protein in solution and in oligomers, and a spherocylindrical shape with strong directional interactions, representing the folded state of the protein in a fibril. The particles aggregate into oligomers of the disordered state and fibrils of the ordered state. Aggregation proceeds by a nucleation-and-growth mechanism, as well as by a two-step mechanism in which an aggregate transforms from one type into the other. These observations confirm experimental and theoretical studies on the formation of fibrils [9, 21, 59, 116, 152]. In the simulations, the conversion of oligomers into fibrils appears to be enhanced by the presence of fibrils, what has been proposed as a mechanism to explain the exponential growth phase of the amyloid fraction in aggregation experiments [99]

Developing a highly coarse grained particle representing a protein involves judicious choices on the protein features to be included in the model and the less relevant properties to be omitted. By sacrificing detail, it becomes possible to study the complex behaviour of the ‘simple’ model – a study that is computationally excessively demanding for a model with fine-grained details on

the level of the atoms or residues. A property of wild-type α -synuclein that was intentionally omitted in the model, by focussing on the central ~ 60 residues that form the core of oligomers and fibrils, are the flexible N and C-terminal tails. Without the tails, especially the hydrophilic C-terminal tails, the protein will probably not be able to assemble into the micellar-like oligomers that have been reported in a number of studies [6, 83, 96, 164, 228] ; instead, the truncated protein is more likely to form droplet-like oligomers of unspecified sizes, as observed for our model. It is well-conceivable that the size and number of oligomers are important in determining the rates of fibril nucleation and growth. We note that the highly coarse-grained model by Saric *et al.* [170], who employed a particle of fixed spherocylindrical shape with Monte Carlo moves to instantaneously swap the single attractive patch from the endcap (the disordered state) to the cylindrical segment (the ordered state), by construction has the ability to form oligomers of limited maximum size. The oligomers that transform into fibrils are about the same size in both their and our simulations.

The proposed CG model offers an interesting platform for future studies. By simulating amyloidogenic proteins as chains of polymorph particles, with each particle representing a small section of the wild-type protein, we expect to gain further insights in the aggregation dynamics.

4.5 Appendix

4.5.1 Matrices

The position and orientation of a rigid body in the laboratory frame can be fully described by the coordinates of its centre of mass $\mathbf{R}$ and by its rotation matrix $\mathbf{A}$. The coordinate in the space frame of any point on this particle, given its body-fixed coordinates $\mathbf{x}$, are obtained by:

$$\mathbf{r} = \mathbf{A}\mathbf{x} + \mathbf{R}. \quad (4.20)$$

The rotation matrix is expressed in terms of quaternions $\mathbf{q}$ of unit length, $q = |\mathbf{q}| = 1$, by the following expression:

$$\mathbf{A} = \begin{pmatrix} q_0^2 + q_1^2 - 2(q_2^2 + q_3^2) & 2(q_1q_2 - q_0q_3) & 2(q_1q_3 + q_0q_2) \\ 2(q_1q_2 + q_0q_3) & q_0^2 + q_2^2 - 2(q_1^2 + q_3^2) & 2(q_2q_3 - q_0q_1) \\ 2(q_1q_3 - q_0q_2) & 2(q_2q_3 + q_0q_1) & q_0^2 + q_3^2 - 2(q_1^2 + q_2^2) \end{pmatrix}. \quad (4.21)$$

Differentiation yields that the angular velocity $\boldsymbol{\omega}^b$ in the body-frame is related to the quaternion velocity by $\dot{\mathbf{q}} = \mathbf{B}\boldsymbol{\omega}^b$, with

$$\mathbf{B} = \frac{1}{2} \begin{pmatrix} -q_1 & -q_2 & -q_3 \\ q_0 & -q_3 & q_2 \\ q_3 & q_0 & -q_1 \\ -q_2 & q_1 & q_0 \end{pmatrix}. \quad (4.22)$$

This transformation matrix $\mathbf{B}$ features in the equation of motion, Eq. (4.17).

4.5.2 Mobility Tensors

The translational and rotational mobility tensors in Eq. (5.16) and (4.17) are determined by the geometry of the particles, which is coupled to the particle's internal state. The mobility tensors are updated every timestep, following the expressions of Aragon and Flamik [7] for the translational and rotational diffusion matrices for spherocylindrical particles in the body-fixed frame. The translational mobilities perpendicular and parallel to the long axis, at an aspect ratio

$p = L/D$, are given by

$$\begin{aligned}\boldsymbol{\mu}^{\text{t,s}}_{\perp} &= \frac{1}{4\pi\eta L} \left[\ln(p) + 0.902952 - \frac{0.311044}{\sqrt[4]{p}} \right. \\ &\quad \left. + \frac{0.18085}{\sqrt{p}} - \frac{0.344085}{p^2} - \frac{0.190732 \ln(p)}{p^2} \right] \\ \boldsymbol{\mu}^{\text{t,s}}_{\parallel} &= \frac{1}{4\pi\eta L} \left[2\ln(p) - 0.113192 - \frac{1.30429}{\sqrt[4]{p}} + \frac{1.19032}{\sqrt{p}} \right. \\ &\quad \left. + \frac{3.12756}{p} - \frac{1.56699}{p^2} - \frac{0.930791 \ln(p)}{p^2} \right]\end{aligned}\tag{4.23}$$

with η the viscosity of the solvent. These mobilities appear twice and once, respectively, along the diagonal of the body-fixed mobility matrix $\boldsymbol{\mu}^{\text{t,s}}$ in Eq. (4.15) and (4.16). Similarly, the perpendicular and parallel rotational mobility components are calculated from:

$$\begin{aligned}\boldsymbol{\mu}^{\text{r,b}}_{\perp} &= \frac{3}{\pi\eta L^3} \left[\ln(p) - 0.372093 - \frac{0.95622}{\sqrt[4]{p}} + \frac{1.24792}{\sqrt{p}} \right. \\ &\quad \left. + \frac{1.23085}{p} - \frac{1.99498}{p^2} + \frac{1.84201}{p^3} - \frac{0.664147}{p^4} \right] \\ \boldsymbol{\mu}^{\text{r,b}}_{\parallel} &= \frac{p^2}{\pi\eta L^3} \left[1 - 0.00158688 \ln(p) \right. \\ &\quad + \frac{0.0369518 \ln(p)}{\sqrt{p}} - \frac{0.239276 \ln(p)}{p} \\ &\quad \left. + \frac{0.610134 \ln(p)}{p^2} - \frac{0.808254 \ln(p)^3}{p^3} \right],\end{aligned}\tag{4.24}$$

and together yield the rotational mobility matrix $\boldsymbol{\mu}^{\text{r,b}}$ in Eq. (4.17). Equations (4.23) and (4.24) reduce to the well known Stokes drag expressions [100] $\boldsymbol{\mu}^{\text{t,s}} = (3\pi\eta D)^{-1}$ and $\boldsymbol{\mu}^{\text{r,b}} = (\pi\eta D^3)^{-1}$ for spherical particles ($p = 1$).

*We present simulations of α -synuclein, the amyloidogenic protein causing Parkinson's disease, as a short chain of coarse-grained particles. Each particle represents a sequence of about a dozen amino acids. The fluctuating secondary structure of this intrinsically disordered protein is modelled by dynamic variations of the shape and interaction characteristics of the patchy particles, ranging from spherical with weak isotropic attractions for the disordered state to spherocylindrical with strong directional interactions for a β -sheet. Flexible linkers between the particles enable sampling of the tertiary structure. The model is applied here to simulate the growth of an amyloid fibril, by calculating the free energy profile of a protein attaching to the end of the fibril.*¹

5.1 Introduction

Intrinsically disordered proteins (IDPs) represent a class of proteins that lack a stable three dimensional structure in solution but which are able to fold in suitable environments and perform various functions within the cell. Their misfolding and aggregation are strongly linked to a number of neurodegenerative disorders such as Parkinson's (PD) or Alzheimer's disease (AD) [48]. The proteins linked to these disorders are α -synuclein (α syn) and amyloid- β ($A\beta$), respectively. They are known to aggregate and form ordered fibrillar or amorphous structures [163] that may be toxic [30], leading to dopaminergic neural loss and both result in neurodegradation [105, 150].

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α -synuclein can change its secondary structure and engage in the formation of various aggregates which are known to accumulate in specific brain areas leading to progressive dopaminergic neurodegeneration [160]. Depending on the physiological conditions, the protein can aggregate into oligomers of various sizes and geometries, into fibrils of distinct polymorphism [17, 183] or into higher order aggregates such as Lewy bodies.

The prevailing ordered structures commonly associated with neurodegradation are amyloids, which are β -sheet-rich protein aggregates [200]. Amyloids are characterised by a cross- β architecture [8, 178] in which the β -strands of distinct peptides are running perpendicular and intermolecular hydrogen bonds are running parallel to the long axis of the fibril [200]. Over the past decades more insight has been gained on how folding occurs in regular, ordered proteins. However, the folding mechanism of intrinsically disordered proteins is still poorly understood despite being explored by both experiments and simulations.

To explore the folding pathways and the structure of α -synuclein within a fiber, various experimental techniques have been used. By folding and aggregating, α -synuclein undergoes conformational changes from a random coil to the amyloidogenic cross- β structure. This transition has been observed by circular dichroism (CD) [178]. Site directed spin labelling (SDSL) and electron paramagnetic resonance (EPR) spectroscopy showed that the building blocks of amyloid fibers are the central 70 amino acids of α -synuclein, from residue 34 to 101 [43]. This has been confirmed by Magic Angle Spinning (MAS) and solid-state nuclear magnetic resonance spectroscopy (NMR), identifying the amyloid core comprising AA 34 to 95 [80]. It has been shown that the central region forms cross- β sheets packed in a highly ordered fashion, parallel and in-register [43, 80, 212]. The core of the fibrils is formed of 5 strands, consisting of about 10-12 residues [212]. Upon aggregating into fibers, the N-terminal of the protein is structurally more heterogeneous than the core and the C-terminal remains unfolded [43].

Computer simulations provide insights into the folding process at length- and timescales that are difficult to access by experiments. All-atom simulations of fragments of α -synuclein showed that the secondary structure strongly depends on the solvent conditions [89, 221]. Atomistic simulations provide detailed information on the folding of proteins, but they are computationally expensive and unable to reach aggregation timescales. Instead, coarse grained simulations reduce the numbers of configurational degrees of freedom to enable access to higher timescales and to make accurate predictions of the folding mechanisms [36, 68, 201]. Transitions between the conformations of various IDPs have been intensively studied by Molecular Dynamics (MD) simulations [110, 156, 227]. Molecular docking and MD simulations on α syn showed that

β -sheet-rich oligomers are less stable than α -helical oligomers [167]. Studies showed that β -sheet-rich oligomers are more compact than helical oligomers suggesting that they could be the precursors of fibrils [167]. The most intensively used coarse grain models for IDPs are lattice models, in which each residue is represented by a single particle occupying one site on a cubic lattice, while all unoccupied sites are considered as solvent [1]. Recent studies on small peptides predict that the self-assembly into fibers occurs either through random aggregation or via a templated-folding process, the latter suggesting strong correlations between folding and assembly [139]. Moreover, simulations show that H-bonds are formed both intramolecularly in β -sheet-rich folded proteins as well as in intermolecular cross- β structures [2]. To study the aggregation of IDPs into higher order aggregates, coarse grained models have been used, in which a protein is represented by a single polymorph patchy particle [19, 88, 170]. Studies showed that fibrillization can occur either through one step nucleation, by a nucleation-and-growth mechanism, or via two step nucleation, by oligomers transforming into fibers [88, 170].

The current study uses a coarse grained model to study the folding and aggregation of α -synuclein. To describe the protein's nature of adapting its conformation to the surrounding environment, a single protein is represented as a chain of polymorph patchy particles that are responsive to their surroundings [88], see Fig. 5.1. The disordered state is modelled as a random chain of spherical particles. In the most ordered state, the protein segments have formed β -sheets, which are modelled as spherocylinders. Both the translational and rotational as well as the internal dynamics of the system are described by a recently introduced Brownian Dynamics algorithm [87, 88].

This chapter first introduces the coarse grained model in Sec. 5.2. It then continues with the basics of constrained Brownian Dynamics in Sec. 5.3. Sec. 5.4 presents results on the behaviour of α -synuclein upon aggregation and the paper ends with conclusions in Sec. 5.5.

5.2 The simulation model

5.2.1 Polymorph particles

α -synuclein is a 14 kDa 140 amino acid protein with variable secondary structure: it is mainly disordered in a solvent, it forms β structural species in fibrils or amyloids and it adopts an α helical conformation near membranes [206]. The focus of this chapter is on the disordered and the β -rich states. Starting from the protein's geometrical features we coarse grain the central fragment of α -synuclein (60 amino acids) into a chain of 5 particles that can adapt their conformations depending on the surrounding environment [88]. The motivation for the

Figure 5.1: Cartoons of a protein in a disordered state (a) and in the most ordered state (b).

choice of the central 60 AA is the fact that they are the building block of amyloid fibers [164]. Furthermore, both N and C-terminal regions are believed to slow down the aggregation process but do not have any other major influence on the fibrillation [82].

Upon engaging in fibril formation α -synuclein adopts a β -rich conformation composed of 5 β -strands that are able to form intermolecular hydrogen bonds with the neighbouring proteins, *i.e.* to engage in the cross- β -sheet conformation characteristic for this protein [188]. The lengths of the β strands vary from 2 to 3.5 nm and the measured inter-sheet distance within a fiber is 1 nm [212]. With this in mind we represent the protein as a chain of 5 particles connected by 4 springs. Each particle represents about 12 amino acids which can vary from disordered (sphere) to β -sheet (spherocylinders). The dimensions have been chosen to match experimental findings. The spherocylinder is defined by a tip to tip distance of $L_R = 3.5$ nm and a diameter $D_R = 1$ nm. The measured radius of gyration of α -syn in the disordered state is about 40 Å. The protein is known to be more closely packed than a random coil [208] but less closely packed than a globular protein of the same contour length [16]. Therefore, the calculated radius of gyration of a single sphere of 12 amino acids is $R_g = 1.17$ nm, corresponding to $D_S = L_S = 2.34$ nm.

To model the ability of α -synuclein to change its conformation depending on the environment, we follow a model previously introduced for polymorph particles [88]. A parameter λ_i is introduced to describe the internal state of the i^{th} particle. Since experiments have shown that α -synuclein has little structure in solution, the potential that governs the distribution of λ_i favours

the disordered state over the ordered one. This potential is given by a combination of two third order polynomials

$$\Phi_\lambda(\lambda) = \begin{cases} -6.75 \varepsilon_\lambda^S (\lambda^2 + \lambda^3) & \text{for } \lambda < 0 \\ -6.75 \varepsilon_\lambda^R (\lambda^2 - \lambda^3) & \text{for } \lambda \geq 0 \end{cases} \quad (5.1)$$

with their minima at $\lambda = \pm 2/3$, as shown in Fig. 5.2(a). Their depths are $-\varepsilon_\lambda^S$ and $-\varepsilon_\lambda^R$ and correspond to the disordered (spherical) and ordered (spherocylindrical) states, respectively. To describe the connection to the geometrical features of the particle, the smoothed step function $\mu(\lambda)$ is introduced:

$$\mu(\lambda) = \begin{cases} 0 & \text{for } \lambda < -1 \\ \frac{\tanh(A\lambda)}{2 \tanh(A)} + \frac{1}{2} & \text{for } -1 \leq \lambda \leq 1 \\ 1 & \text{for } \lambda > 1, \end{cases} \quad (5.2)$$

where $A = 10$ determines the width of the transition region. Length and diameter of a particle are given by

$$\begin{aligned} L &= L_S + \mu(\lambda)(L_R - L_S) \\ D &= D_S + \mu(\lambda)(D_R - D_S), \end{aligned} \quad (5.3)$$

where L_R corresponds to the end to end distance of a spherocylinder shown in Fig. 5.2(b), while for a sphere L_S equals D_S .

5.2.2 The repulsive potential

The repulsive component of the potential avoids strong overlap between particles. The repulsive potential, as a function of the distance $d_{\min}$ (to be defined below), between particles i and j is given by

$$\frac{\Phi_{\text{rep}}^\lambda}{k_B T} = \begin{cases} \left(\frac{2\bar{D}}{d_{\min}} - 1 \right)^n & \text{for } d_{\min} < 2\bar{D} \\ 0 & \text{for } d_{\min} > 2\bar{D}, \end{cases} \quad (5.4)$$

and extends up to twice the average diameter $\bar{D} = 0.5(D_i + D_j)$ of the particles. The hardness of the potential is designed to vary with the internal states of the interacting particles, $n = 4 + (\mu_i + \mu_j)$. This is motivated by the physical properties of the protein segments: disordered sections are more loosely packed and hence softer than the ordered β -strands, which are more compact and robust. The minimum distance $d_{\min}$ between the long axes of two particles depends

Figure 5.2: (a) The internal potential as a function of the internal parameter λ , for $\epsilon_\lambda^S = 4$ and $\epsilon_\lambda^R = 3$. (b) Diameter (red line) and length (blue line) as functions of λ .

on their centres of mass positions $\mathbf{r}_i, \mathbf{r}_j$ and orientations $\hat{\mathbf{u}}_i, \hat{\mathbf{u}}_j$ of the long axes, and is determined by [210]

$$d_{\min}(\mathbf{r}_i, \mathbf{r}_j, \hat{\mathbf{u}}_i, \hat{\mathbf{u}}_j, \lambda_i, \lambda_j) = \min_{\alpha_i, \alpha_j} |(\mathbf{r}_i + \alpha_i \hat{\mathbf{u}}_i) - (\mathbf{r}_j + \alpha_j \hat{\mathbf{u}}_j)|. \quad (5.5)$$

This equation accounts for the internal state of the particles by restricting the two minimisation parameters α_i and α_j to $|\alpha_i| \leq \frac{1}{2}(L_i - D_i)$ and $|\alpha_j| \leq \frac{1}{2}(L_j - D_j)$, respectively. For two spherical particles this distance reduces to the distance between their centres. If two rods are nearly aligned, differentiation of Eq. (5.5) may become singular. The minimum distance calculation then requires the determination of the lengths of the projections of the centre to centre vector $\mathbf{r}_{ij} = \mathbf{r}_i - \mathbf{r}_j$, perpendicular, $r_\perp$, and parallel, $r_\parallel$, to the average direction $\hat{\mathbf{u}}_{\text{av}}$

$$\hat{\mathbf{u}}_{\text{av}} = \begin{cases} \frac{\hat{\mathbf{u}}_i + \hat{\mathbf{u}}_j}{|\hat{\mathbf{u}}_i + \hat{\mathbf{u}}_j|} & \text{for } \hat{\mathbf{u}}_i \simeq \hat{\mathbf{u}}_j \\ \frac{\hat{\mathbf{u}}_i - \hat{\mathbf{u}}_j}{|\hat{\mathbf{u}}_i - \hat{\mathbf{u}}_j|} & \text{for } \hat{\mathbf{u}}_i \simeq -\hat{\mathbf{u}}_j, \end{cases} \quad (5.6)$$

for both parallel and antiparallel rods respectively. The minimum distance is then calculated as [4]

$$d_{\min} = \begin{cases} r_{\perp} & \text{for } r_{\parallel} < \bar{L} - \bar{D} \\ \sqrt{(r_{\perp})^2 + (r_{\parallel} - (\bar{L} - \bar{D}))^2} & \text{for } r_{\parallel} \geq \bar{L} - \bar{D}, \end{cases} \quad (5.7)$$

where $\bar{L} = 0.5(L_i + L_j)$ is the average length of the two particles.

5.2.3 The attractive potential

The formation of amyloidogenic aggregates is the result of hydrophobic, Van der Waals and H-bonds both within and between proteins [63]. The relative strengths are determined by the internal states of the particles. Disordered proteins are mainly governed by hydrophobic and Van der Waals interactions, with the flexibility of the chain permitting close contacts up to approximately $2\bar{D}$. On the other hand, in fibrils proteins adopt a more compact structure and are governed by strong short ranged inter-molecular H-bonds and weak intra-molecular interactions. The potential introduced here captures these features of the protein. The pair potential is given by

$$\begin{aligned} \frac{\Phi_{\text{attr}}^{\lambda}}{k_B T} = \eta(d_{\min}) & \left\{ \mathcal{C}_{\text{SS}} (1 - \mu_i) (1 - \mu_j) \right. \\ & + (m_{ij} \mathcal{C}_{\text{RR}}^{\text{w}} + g_{ij} \mathcal{C}_{\text{RR}}^{\text{s}}) \mu_i \mu_j f_{ij} \\ & \left. + \mathcal{C}_{\text{SR}} [\mu_i (1 - \mu_j) + \mu_j (1 - \mu_i)] \right\}, \end{aligned} \quad (5.8)$$

which has been designed to convey directionality between spherocylindrical particles, to mimic hydrogen bonding, and to be non-directional between spheres, to resemble anisotropic protein segments. This potential applies to all the particles pairs in the system.

The first factor in Eq. (5.8) describes the distance dependence of the attraction energy between particles i and j ,

$$\eta(d_{\min}) = \frac{\tanh(A_{\text{attr}}(d_{\min} - 2\bar{D}))}{\tanh(2A_{\text{attr}}\bar{D})} \quad \text{for } d_{\min} < 2\bar{D}. \quad (5.9)$$

It smoothly increases from -1 to 0 with increasing distance, with a steepness set by the λ dependent parameter $A_{\text{attr}} = a_{\text{attr}}(\mu_i + \mu_j) + b_{\text{attr}}$. Here $a_{\text{attr}} = 1.25 \text{ nm}^{-1}$ and $b_{\text{attr}} = 1.5 \text{ nm}^{-1}$ are two positive parameters which ensure that the steepness of the attractive potential varies between 1.5, for two interacting spherocylinders, and 4 for two interacting spheres.

For two spheres the sum between the curly brackets of Eq. (5.8) reduces to the sphere-sphere interaction strength $\mathcal{C}_{\text{SS}}$. The last term between the curly brackets introduces an interaction

Figure 5.3: Schematic representation of the orientation vectors $\hat{\mathbf{l}}_i$ and $\hat{\mathbf{n}}_i$ associated with the Van der Waals (light blue surface) and the H-bonds (red surface), respectively in a cross section of a rod. The long axis $\hat{\mathbf{u}}_i$ is into this plane.

between a spherical, $\mu(\lambda) \simeq 0$, and a spherocylindrical particle, $\mu(\lambda) \simeq 1$. This introduces a non-directional component in the potential and has an interaction strength C_{SR} . The second term between the curly brackets defines the interactions between two spherocylindrical particles and accounts for both weak, Van der Waals and hydrophobic, and strong, H-bonds, interactions. The orientation of the rods plays an important role as the interactions are directional. Figure 5.3 shows the strong intermolecular H-bonds, in red, and the weak intramolecular bonds, in light blue. The strengths of the specific interactions are given by the constants C_{RR}^{s} for the strong bonds and C_{RR}^{w} for the weak interactions. The orientational dependence of the interaction between two spherocylinders in Eq. (5.8) is described by the function

$$f_{ij} = (\hat{\mathbf{u}}_i \cdot \hat{\mathbf{u}}_j)^2 h(\hat{\mathbf{u}}_i \cdot \hat{\mathbf{r}}_{ij}) h(\hat{\mathbf{u}}_j \cdot \hat{\mathbf{r}}_{ij}), \quad (5.10)$$

which varies from 0 to 1. The maximum contact between the surfaces of two spherocylinders is reached when they are parallel, accounted for in the first factor in Eq. (5.10), and stand in line, accounted for in the last two factors of the same equation where $\hat{\mathbf{r}}_{ij}$ is a unit vector along $\mathbf{r}_{ij}$ and

$$h(x) = \begin{cases} 1 - ax^2 & \text{if } x^2 < 1/a \\ 0 & \text{if } x^2 > 1/a. \end{cases} \quad (5.11)$$

Here $a = 1.5$ is a parameter that penalises longitudinal displacement along the spherocylinders' long axes. The anisotropy of the interactions between two spherocylindrical particles under rotations around their long axes is described by the factor $(g_{ij}C_{\text{RR}}^{\text{s}} + m_{ij}C_{\text{RR}}^{\text{w}})$, with m_{ij} and g_{ij}

Figure 5.4: Front view of two aligned folded proteins showing the strong (red-red) intermolecular hydrogen bonds and the weak (light blue-light blue) intramolecular affinities. The crosses and the dots show the arrangement of the sheets within a fiber, *i.e.* out of $\odot$ and into $\otimes$ the plane of the paper.

defined as

$$\begin{aligned} g_{ij} &= (\hat{\mathbf{r}}_{ij} \cdot \hat{\mathbf{n}}_i)^p (\hat{\mathbf{r}}_{ij} \cdot \hat{\mathbf{n}}_j)^p \\ m_{ij} &= (\hat{\mathbf{r}}_{ij} \cdot \hat{\mathbf{l}}_i)^p (\hat{\mathbf{r}}_{ij} \cdot \hat{\mathbf{l}}_j)^p. \end{aligned} \quad (5.12)$$

The vectors $\hat{\mathbf{n}}_i$ and $\hat{\mathbf{l}}_i$ are two perpendicular unit vectors, both perpendicular to $\hat{\mathbf{u}}_i$, see Fig. 5.3. They are associated with the regions on the surface involved in van der Waals attraction and hydrogen bonding, respectively. Note that the hydrogen bonds are highly directional, as is accounted for by the power $p = 6$ for the g_{ij} component in Eq. (5.12). To mimic the flatness of a β -sheet, the same value for p has been chosen in m_{ij} . In addition, this power limits the width of the potential and thereby favours square ordering over hexagonal ordering. Summarising, $\eta C_{\text{RR}}^s g_{ij} f_{ij}$ represents the strong hydrogen bonds and $\eta C_{\text{RR}}^w m_{ij} f_{ij}$ the weak Van der Waals and hydrophobic interactions between spherocylinders.

5.2.4 The FENE potential

To model the connectivity between adjacent beads within the chain we use the finitely extensible nonlinear elastic (FENE) potential. The expression for the FENE potential reads

$$\Phi_{\text{FENE}} = -\frac{1}{2} k R_0^2 \log \left[1 - \left(\frac{db}{R_0} \right)^2 \right] \quad \text{for } 0 < db < R_0 \quad (5.13)$$

where $db = b_{i,i+1} - 1.1\bar{D}$ the distance between the end caps minus a margin of $0.1\bar{D}$, where the particles don't interact, and $b_{i,i+1}$ the end to end distance between the ends of the cylindrical

Figure 5.5: Cartoon of two aligned folded proteins showing the strong (red-red) intermolecular interactions and the weak (light blue-light blue) intramolecular affinities.

segments of two consecutive particles, see Fig. 5.5. R_0 is the limit to the bond extension which is calculated based on the number of amino acids in a connection. We start from the assumption that the length of one amino acid is about 0.37 nm [81]. This implies a maximum distance between the centres of mass of two 12 amino acids chameleon particles of $R'_0 = 12 \times 0.37 = 4.44$ nm. The limit to the bond extension is adjusted according to the dimensions of the particles, *i.e.* $R_0 = R'_0 - (\bar{L} - \bar{D}) - 1.1\bar{D}$. It reduces to $R_0 = 1.86$ nm for the maximum distance between the surfaces of two spheres and to $R_0 = 0.8$ nm for the maximum extensibility between the surfaces of two spherocylinders end caps. To estimate the stiffness k of the spring a persistence length of $l_p = 0.3$ nm has been used, matching experimental and computational findings for disordered α -synuclein at this temperature [137]. For a chain of 12 AA, the estimated spring constant for disordered proteins is about $k = 3k_B T / (2R_0 l_p) = 1 k_B T / \text{nm}^2$. For a chain of 4 AA between two spherocylindrical particles $k = 3 k_B T / \text{nm}^2$. Since these linker segments are rather short to represent a real Gaussian chain we use $k = 10 k_B T / \text{nm}^2$ in the simulations. To avoid computational problems we linearised the FENE potential beyond $0.9R_0$.

5.2.5 The torsion potential

The previously introduced attractive potential accounts for the weak interactions between all spherocylindrical particles in the system. In a β -pleated-sheet the hairpin, the connection between the rods, limits the freedom of the particles to rotate around their long axes. This prevents the particles to form internal hydrogen bonds and in order to achieve this we added a torsion potential on the particles that correspond to the same molecule. This potential acts only on the consecutive particles i and $i + 1$ and reads as

$$\Phi_{\text{TORS}} = \varepsilon_{\text{TORS}} (\hat{\mathbf{n}}_i \cdot \hat{\mathbf{r}}_{i,i+1})^6 \cdot (\hat{\mathbf{n}}_{i+1} \cdot \hat{\mathbf{r}}_{i,i+1})^6; \quad (5.14)$$

with $\varepsilon_{\text{TORS}}$ the strength of alignment.

5.3 Constrained Brownian Dynamics

5.3.1 Equation of Motion

To describe the dynamics of the particles in the system we use Brownian Dynamics simulations. This method has been developed to efficiently simulate the motion of colloidal particles in a solvent by including the collisions with the (non-simulated) solvent molecules in the equations of motion. We use Brownian Dynamics equations of motion to describe the translational and rotational motions of anisotropic particles as well as to characterise the evolution of the internal coordinate λ . Here we present only the generalised equation of motion, which can be then adapted by following the steps provided in more detail in Chapters 3 and 4. Next we focus on free-energy calculations from the simulations.

The generalised Brownian Dynamics equation of motion [29, 69] reads as

$$\begin{aligned} \mathbf{Q}(t + \Delta t) - \mathbf{Q}(t) = & -\boldsymbol{\mu}^Q \frac{\partial \mathcal{A}}{\partial \mathbf{Q}} \Delta t \\ & + k_B T \frac{\partial}{\partial \mathbf{Q}} \cdot \boldsymbol{\mu}^Q \Delta t \\ & + (\boldsymbol{\mu}^Q)^{1/2} \boldsymbol{\Theta}^Q(t) \sqrt{2k_B T \Delta t}, \end{aligned} \quad (5.15)$$

with $\mathbf{Q}$ the full set of generalised coordinates. Since the equations of motion are sufficiently decoupled, $\mathbf{Q}$ may be replaced by the position of a particle, $\mathbf{R}$, the quaternions $\mathbf{q}$ representing the orientation of a particle or the internal coordinate λ , depending on the type of motion to which Eq. (5.15) is being applied to. The first term on the r.h.s. describes the displacement of a particle which arises from the balance between a conservative force $\mathcal{F} = -\partial \mathcal{A} / \partial \mathbf{Q}$ and the opposing friction from collisions with the solvent molecules, where $\mathcal{A}$ represents the free energy as a function of the generalised coordinate. The second term on the r.h.s. represents a drift term accounting for the inhomogeneity of the mobility tensor, which is required in the Itô representation to recover the correct Boltzmann probability distribution. The last contribution on the r.h.s. represents the stochastic displacement that replaces the random kicks from explicit solvent molecules. Here $\boldsymbol{\Theta}^Q$ is a time-dependent Markovian vector and its elements have zero mean, unit variance, no correlations across them and no memory. The random displacements are related to the mobility via the fluctuation-dissipation theorem as given by the last term with k_B the Boltzmann constant, T the temperature and Δt the time step.

To study the attachment of a protein to a fiber we simulate a system in which a stable fibril

of 10 proteins interacts with a peptide isolated from the aggregate. To analyse the attachment process we fix the distance between the centres of mass of the fiber, $\mathbf{R}_{\mathcal{F}}$, and the protein, $\mathbf{R}_{\mathcal{P}}$, at $d = |\mathbf{R}_{\mathcal{P}} - \mathbf{R}_{\mathcal{F}}|$. Here we define the generic centre of mass calculation as $\mathbf{R} = \sum_{i=1}^N \mathbf{r}_i / N$ with N the number of particles in an aggregate, *i.e.* 50 for a fiber and 5 for the isolated peptide. This distance restriction is realised by a constraint on the translational equation of motion and has no direct effect on the rotational or the internal dynamics of the particles.

The unconstrained positions of the particles after a BD step are given by [87]

$$\begin{aligned} \mathbf{R}_i^u(t + \Delta t) = & \mathbf{R}_i^c(t) + \boldsymbol{\mu}_i^{t,s}(t) \mathbf{F}_i(t) \Delta t \\ & + \sqrt{\boldsymbol{\mu}_i^{t,s}(t)} \boldsymbol{\Theta}_i^t(t) \sqrt{2k_B T \Delta t}, \end{aligned} \quad (5.16)$$

with $\mathbf{R}^c$ the constrained position at the previous time step, $\boldsymbol{\mu}^{t,s}$ the translational mobility matrix in the space frame, $\mathbf{F}_i$ the conservative force acting on the particle and $\boldsymbol{\Theta}_i^t$ the time dependent Markovian vector. Imposing the constraint on the distance, the positions change into

$$\mathbf{R}_i^c(t + \Delta t) = \mathbf{R}_i^u(t + \Delta t) + \Lambda(t) \boldsymbol{\mu}_i^{t,s}(t) \nabla_i d(t) \quad (5.17)$$

with Λ a Lagrange multiplier and $\nabla_i d$ providing the direction of the constraint force, the latter two being evaluated at the previous timestep. Its strength is obtained by solving the Lagrange multiplier from the condition $|\mathbf{R}_{\mathcal{P}}^c(t + \Delta t) - \mathbf{R}_{\mathcal{F}}^c(t + \Delta t)| = d$. Using $\mathbf{d}^u = \mathbf{R}_{\mathcal{P}}^u(t + \Delta t) - \mathbf{R}_{\mathcal{F}}^u(t + \Delta t)$ as the vector connecting the positions of the unconstrained centres of mass of the fiber and the isolated molecule at time $t + \Delta t$, and $\mathbf{d}^c = \mathbf{R}_{\mathcal{P}}^c(t) - \mathbf{R}_{\mathcal{F}}^c(t)$ the vector after the previous constrained timestep, the value of Λ is determined from the quadratic equation

$$|\mathbf{d}^u(t + \Delta t)|^2 + 2\Lambda \mathbf{d}^u(t + \Delta t) \cdot \nabla \mathbf{d}^c(t) + \Lambda^2(t) |\nabla \mathbf{d}^c(t)|^2 = d^2. \quad (5.18)$$

The constraint term is then introduced in Eq. (5.17) to determine the updated positions of all particles.

5.3.2 Free Energy

In simulations of reacting systems, the determination of the equilibrium constant and the reaction rate is usually achieved by calculating the free energy as a function of the reaction coordinate. This has been intensively applied in Molecular Dynamics simulations and the expression for the potential of mean constraint force (PMCF) for systems with one or multiple constraints is known for these type of simulations [67, 143, 144, 149]. The free energy determines the probability that a system will adopt a given state and indicates how the process might evolve [34]. The transition

region between reactants and products is often so highly elevated in free energy that it can only be sampled efficiently in strongly biased simulations, *e.g.* by means of constraints. In these cases simulations can be used to determine the free energy of the system as a function of a reaction coordinate [67, 143, 144, 149]. The free energy along d is defined as

$$\mathcal{A}(d) = -k_B T \ln Q(d) \quad (5.19)$$

where the partition function $Q(d)$ is proportional to the integral of the Boltzmann factor over a hyperplane defined by the reaction coordinate [149],

$$Q(\bar{d}) \propto \int \exp[-\beta\Phi(\mathbf{Q})] \delta[d(\mathbf{Q}) - \bar{d}] d\mathbf{Q}. \quad (5.20)$$

Evaluating $\mathcal{A}(d)$ directly is impossible, but one may recover $\mathcal{A}(d)$, up to an inconsequential constant, by integrating its derivative [143],

$$\frac{d\mathcal{A}(\bar{d})}{d\bar{d}} = \left\langle g_d^{-1} \sum_{i=1}^N \nabla_i d \cdot \nabla_i \Phi - k_B T g_d^{-1} \sum_{i=1}^N \nabla_i^2 d + k_B T g_d^{-2} \sum_{i=1}^N \nabla_i d \cdot \nabla_i g_d \right\rangle_{\bar{d}} \quad (5.21)$$

with $g_d = \sum_{i=1}^N \nabla_i d \cdot \nabla_i d$ an element of the metric tensor, Φ the interaction potential, and where $\langle \dots \rangle_{\bar{d}}$ represents the canonical average over configurations with $d(\mathbf{Q}) = \bar{d}$ irrespective of their velocity, *i.e.* irrespective of

$$\Delta d = \sum \frac{\partial d}{\partial \mathbf{R}_i} \cdot [\mathbf{R}_i(t + \Delta t) - \mathbf{R}_i(t)]. \quad (5.22)$$

To enhance the sampling efficiency, one may constrain the coordinate d to the fixed value $\bar{d}$, in which case the conditional average of a function f is given by

$$\langle f \rangle_{\bar{d}} = \frac{\langle f g_d^{-1/2} \rangle_{\bar{d}}^c}{\langle g_d^{-1/2} \rangle_{\bar{d}}^c} \quad (5.23)$$

where $\langle \dots \rangle_{\bar{d}}^c$ denotes the average over a constrained BD simulation and the factor $g_d^{-1/2}$ corrects for the bias introduced by $\dot{d} = 0$. It is known that in Molecular Dynamics simulations the free-energy can be derived from the average constraint force, including some corrections related to the mass-metric [143, 144, 149]. Free-energy and constraint force are likewise related in Brownian Dynamics simulations,

$$\frac{d\mathcal{A}(\bar{d})}{d\bar{d}} \approx \frac{\langle \Lambda g_d^{-1/2} \rangle_{\bar{d}}^c}{\langle g_d^{-1/2} \rangle_{\bar{d}}^c}, \quad (5.24)$$

as will be proven elsewhere [146].

Figure 5.6: Representation of the system setup.

5.4 Results

5.4.1 Simulation Setup

All simulation systems were initialised by placing a fiber consisting of 10 ordered proteins in the centre of the box and a single isolated molecule at distance d from the filament's centre of mass, see Fig. 5.6. For ease of presentation we refer to the central 60 amino acids of α -synuclein, that have the ability to form cross- β -sheets and form the core of the amyloid fiber, as 'the protein'. We performed simulations in which the centre of mass of an initially ready formed sheet was constrained at distances between 4.3 and 11 nm from the centre of mass of the fiber.

All simulations were performed with water as the implicit solvent, having a viscosity of $7 \cdot 10^{-4}$ Pa s, at a temperature of 37 °C, corresponding to the approximate body temperature. The timestep was fixed at $\Delta t = 1.7 \cdot 10^{-15}$ s, as this proved to be the maximum timestep permitted by the potential. Following existing theory on the diffusion coefficients of spherocylindrical particles, the translational and rotational mobility tensors could easily be calculated and be used in the equations of motion [7, 88]. Note that the mobilities reduce to the well-known Stokes-Debye-Einstein relations for a sphere [100]. The mobility of the internal coordinate was set at $\mu^\lambda = 1.4 \cdot 10^{32}$ (J s) $^{-1}$ which ensures in combination with Φ_λ that the internal transitions are rare events. The simulation parameters for the attractive potential were chosen such that they stabilise an unconstrained fiber consisting of 10 proteins. This was achieved by choosing the constants in the attractive potential according to $C_{RR}^s = 20$, $C_{RR}^w = 10$ and $C_{SR} = 0.5$. The well depths for the spherical and spherocylindrical particles were chosen such that the spherical state is favoured over the spherocylindrical state: $\varepsilon_\lambda^S = 2$ and $\varepsilon_\lambda^R = 1$. With these parameters

Figure 5.7: The free-energy profile calculated from Eq. (5.21) (red line) and Eq. (5.24) (black line) for a system with $C_{RR}^s = 20$, $C_{RR}^w = 10$, $C_{SR} = 0.5$, $k = 10 k_B T / \text{nm}^2$ and $\varepsilon_{\text{TORS}} = 0$. The snapshots show the behaviour of the system at the indicated distances.

and $\varepsilon_{\text{TORS}} = 0$ a single isolated protein relaxes into a sheet with four internal hydrogen bonds. Besides these simulations we performed simulations with $\varepsilon_{\text{TORS}} \neq 0$ thereby preventing the formation of internal hydrogen bonds in the isolated protein.

5.4.2 Free Energy Profile

In this section we present results on the free-energy profile for the aforementioned systems in the presence and absence of a torsion potential. Fig. 5.7 shows the free-energy profile as a function of the constrained distance for systems with no torsion potential. This plot shows two curves obtained by integrating either Eq. (5.24), in black, or Eq. (5.21), in red. The plot shows excellent agreement between the two methods. The smallest distance at which the protein ceases to interact with the fiber, d_0 , appears as a maximum in the free energy; beyond this distance the free energy gradually decreases following $\mathcal{A}(d) = \mathcal{A}(d_0) - k_B T \ln(d^2/d_0^2)$. The minimum near $d = 4.9 \text{ nm}$ has been set equal to zero.

The free energy in Fig. 5.7, for the model without hinge-torsion between consecutive particles, shows a minimum at a distance of $\sim 4.9 \text{ nm}$. This is also the equilibrium distance between the protein and the fiber in simulations without distance constraint. Near this distance, all five

particles of the protein are in the spherocylindrical state and they are folded nicely with respect to each other to form the 11th layer of the fiber. With decreasing distance, the free energy increases as the protein is effectively being ‘pushed’ into the fiber. A transition occurs for distances below 4.5 nm: in order to obey the distance constraint, while simultaneously keeping as many particles as possible near their preferred locations at the top of the fiber, two terminal particles of the protein dislodge from the top and adopt positions along the flank of the fiber, thereby stretching the internal bonds by which the particles are attached to their neighbours. For distances exceeding the equilibrium distances, when the protein is pulled away from the fiber, a similar competition arises between contact with the fiber and intramolecular interactions. With increasing distance, first the outer particles move upwards to form a partial 12th layer so they can still bind by strong red-red interactions to the particles remaining in the 11th layer. To further improve the interactions, the particles of the protein and of the top of the fiber are tilted. Next, the particles of the protein are one by one ‘peeled’ from the fiber, and transform into the random state. From the snapshot at $d = 7.5$ nm, it is clear that the fiber is being deformed by the protein pulling at the top, but also by elongation at the bottom to maintain the desired distance between the constraints. The last contact between the protein and the fiber terminates at $d_0 \approx 10$ nm. Beyond this distance, the particles of the unbound protein adopt the spherocylindrical state and fold to form intramolecular red-red interactions, while the constraint causes the protein to diffuse over a spherical surface.

In the second protein model, the limited flexibility of the hairpins connecting the β -strands is taken into account by a torsion potential, see Eq. (5.14). The free energy profile calculated for this model is shown in Fig. 5.8. The most stable structure in this case again occurs at $d = 4.9$ nm and is virtually identical to that in the previous case. In particular, the contributions of the torsion potentials are almost zero. Therefore we have, also in this case, put the zero in the free-energy profile for this structure equal to zero. The overall picture of the free-energy profile is very similar to that calculated in the absence of this torsion potential, with a couple of notable differences. First, the maximum of the curve has increased by about $20 k_B T$. The reason for this is that the torsion potential has largely removed the possibility to form internal hydrogen bonds in the isolated molecule. In fact the remaining weak van der Waals bonds are not strong enough to prevent part of the beads from turning spherical as indicated in Fig. 5.8 at $d = 10$ nm. Second, for distances larger than 5 nm, initially, the protein retains its planar structure, until its constituent beads are again peeled off one by one. Moreover, the added stability provided by the torsion makes the fiber retain its structure much better than in the case with no torsion

Figure 5.8: The free-energy profile calculated from Eq. (5.21) (red line) and Eq. (5.24) (black line) for a system with $C_{RR}^s = 20$, $C_{RR}^w = 10$, $C_{SR} = 0.5$, $k = 10 k_B T / \text{nm}^2$ and $\varepsilon_{\text{TORS}} = 10 k_B T$. The snapshots show the behaviour of the system at the indicated distances.

potential, as no structural modifications in the the fiber have been observed, see Fig. 5.8. Finally, at large distances, the detached protein largely disorders. It has been observed to form 2 to 3 internal bonds while the remaining segments adopt a disordered conformation. To further test the influence of the torsion potential we performed simulations for $\varepsilon_{\text{TORS}} = 20$ (plot not shown). Compared against the case when $\varepsilon_{\text{TORS}} = 10$ no major differences in the free-energy profiles between the two cases have been registered.

The free energy curves indicate that proteins within a certain distance from the fiber will be attracted to the fiber and attach. To test this conclusion, we released neatly folded and disordered proteins from several distances above the top of the fiber. These proteins gradually drifted to the fiber, adopted the spherocylindrical state and folded in the correct way, forming the next layer of the fiber.

5.5 Conclusions

Highly ordered filaments of α -synuclein are known to form intracellular inclusions in the brains of patients with Parkinson's disease. The mechanisms by which these filaments nucleate and grow are not fully understood. To study fibril growth, *i.e.* proteins in solutions that attach to

the end of a fiber, we introduced a coarse-grained model representing α -synuclein as a chain of polymorph patchy particles. The simulations show that this type of model is computationally feasible and captures the transitions in secondary and tertiary structures induced by interactions between pairs of proteins. Having proven these features, the logical next step will be to derive a systematic set of force field parameters from simulations at the atomistic level. This involves extracting the average internal energy and interaction energies of short peptides comprising a dozen residues each, from relatively short molecular dynamics simulations.

The monotonic decrease of the free-energy profile between the partially ordered protein in solution, $d \geq 9.5$ nm, and the ordered state in a fibril, $d \approx 5$ nm, would suggest that attachment to the fibril is a diffusion limited process in the current model. If we analyse the experimental growth rate of one full protein per 15 seconds per fiber in a solution of 50 μ molar, the sink in the monomer diffusion equation turns out to have a radius of the order of 10^{-15} m, which is clearly unrealistic [26, 180]. Hence, the experimental growth process will involve a rate-limiting barrier that the current naively parameterised model misses, probably due to the fact that it only considers the central 60 amino acids of the protein. For this latter case the growth rate of a fiber is known to be much higher than with the full protein, in which the C-terminal is inhibiting the fibrillation process. It is to be expected that a model building on parameters extracted from atomic level simulations can properly address the free energy profile and thereby provide further details on the growth process.

The model with a torsion potential accounting for the limited flexibility of the hair pins performs better than the model without this potential.

6. Conclusions, Summary and Outlook

6.1 Conclusions and Summary

Over the past decades a large number of studies have been carried out in order to determine the physiological function of α -synuclein and its implication in Parkinson's disease. A complementary tool to experiments are computer simulations, which are intensively used for problems for which experiments can not yet provide a clear answer. In this thesis, we focused on studying the aggregation of α -synuclein by using coarse grained representations of the protein. We introduced a polymorph patchy particle model to describe α -synuclein and have combined it with Brownian Dynamics simulations to describe the translational and rotational dynamics. In addition, to describe the chameleonic nature of the protein, we used Brownian Dynamics to describe the internal dynamics of the particles. The internal state is coupled to the interactions between the particles.

The work carried out here started with using an existing Rotational Brownian Dynamics algorithm for anisotropic particles. This is discussed in **Chapter 2** together with an application on the aggregation of clathrin, a coat protein involved in endocytosis, into polyhedral cages. We modelled a clathrin protein as a single rigid particle with attractive patches on its surface, and obtained aggregation timescales comparable to experimental results. In addition, we recovered the critical binding energies previously predicted by other types of simulations.

The Brownian Dynamics algorithm used in Chapter 2 produces correct results but is fairly complex. Therefore we developed a new Brownian Dynamics algorithm which uses quaternions to describe the rotational motion of an anisotropic particle. Its derivation and validation are

fully presented in **Chapter 3**, where the final equation of motion proves to be much simpler and clearer than the previously used algorithm. The novel Rotational Brownian Dynamics algorithm is elementary and well suited for the simulations of patchy-particles. The main advantages of this algorithm are the absence of metric tensor and drift corrections, commonly associated with Brownian Dynamics algorithms in non-Cartesian coordinates, and the absence of singularities commonly associated with rotational motion.

A new coarse grained approach towards the study of α -synuclein aggregation is proposed in **Chapter 4**. Here we modelled a protein as a patchy particle that is able to change its internal state, and thereby its shape and interaction parameters, in response to its environment. A protein in the disordered state is modelled as a single sphere with a soft repulsive core and non-directional longer-ranged attractive interactions, to mimic the Van der Waals, hydrophobic, and transient hydrogen bond interactions the real protein experiences. A protein in the ordered state, *i.e.* a (mis)folded protein in a fiber, has a much more rigid and compact structure and is therefore represented by a single spherocylinder with a hard core and shorter-ranged attractive directional patches on its surface. We used Brownian Dynamics simulations to describe the translational, rotational as well as the internal motions of the particles. The simulation results showed the formation of oligomers and fibrils by a direct nucleation-and-growth mechanism, by two-step-nucleation through the conversion of an oligomer into a fiber or vice versa, and by fibril-enhanced conversion of oligomers into fibrils.

To gain further insights into the aggregation process, we continued with the development of a less coarse representation of α -synuclein in **Chapter 5**. Here we coarse grained a protein into a chain of 5 particles connected through springs. Each bead in the chain of particles represents about 12 amino acids of the protein's real sequence. A disordered protein was modelled as a random chain of spheres. By transformations of the particles into spherocylinders and by their rearrangement into a planar configuration, the protein adopts the ordered state capable of forming fibrils. To study the growth of a fibril, we performed constrained Brownian Dynamics simulations. The centre of mass of a single isolated protein was kept at a fixed distance from the centre of mass of a fibril consisting of 10 proteins to calculate the free-energy as a function of this distance. We obtained a free-energy difference of 80 to 100 $k_B T$ between a solvated protein and a protein in a fiber, with the free-energy decreasing monotonically in going from the former to the latter.

6.2 Outlook

The formation mechanism of highly ordered α -synuclein filaments that accumulate into intracellular inclusions in the brains of patients with Parkinson's disease is only partly being understood by current research. The present thesis represents a small step towards improving this understanding. The methodology introduced here can definitely be further developed to gain more insights into the formation of fibrillar and oligomeric structures of amyloidogenic proteins, as well as to study their interactions with membranes or lipid bilayers.

A worthwhile next step is to perform fine grained simulations to determine realistic values for the parameters used in the coarse grained model. The properties of ~ 12 amino acid coarse grained particles can be derived from all atom molecular dynamics simulations of fragments of wild type α -synuclein. Clearly, when using all atom models, the simulation of fragments rather than entire proteins is attractive from a computational point of view. The conformational distribution of a fragment relates to the internal potential of the corresponding coarse grained particle, the conformational transition rate to the mobility coefficient of the internal coordinate. Molecular Dynamics simulations also provide binding affinities between fragments in predetermined conformations, either by sampling equilibrium states or by means of free-energy calculations, that relate to the state-dependent interactions parameters of the coarse-grained particles. The accordingly re-parameterized coarse grained model is expected to yield an accurate description of the formation of fibrils and oligomers. In addition, the configurations obtained from the coarse grained simulations can be used as starting point for fine grained simulations by re-instating the atoms in conformity with the internal states of the particles. This enables one to study in more detail the most interesting aspects of the aggregation process as predicted by the coarse grained model.

The simulations presented throughout this study refer to the central 60 amino acids of the protein that constitute the building block of fibrillar structures. One could include the full N- and C-terminal domains as additional beads in order to study their involvement in the aggregation process. They are known to slow down the fibrillar growth and may contribute to the stability of fibers. They are also involved in the formation of micelle-like oligomeric aggregates, where the hydrophilic tails are likely to form the outer layer.

Further development of the model could include the interaction with a membrane, as illustrated in Fig. 6.1(a). This would require an extension of the internal dynamics with a third state to account for an additional fold of the protein, see Fig. 6.1(b). These simulations provide insight into membrane leakage, which is believed to have an important role in the pathology of

Figure 6.1: (a) Coarse grained representations of α -synuclein interacting with a membrane. (b) Internal potential describing three states: disordered, ordered (in fibril) and bound (upon interaction with the membrane) and its effect on the dimensions of the particles.

Parkinson's disease.

6.3 Samenvatting

In de afgelopen decennia werden een groot aantal studies uitgevoerd om de fysiologische functie van α -synuclein en de betrokkenheid bij de ziekte van Parkinson te bepalen. Een aanvullend instrument voor deze experimenten zijn computersimulaties. Zij zijn intensief gebruikt voor de problemen waarvoor de experimenten nog geen duidelijk antwoord kunnen bieden. In dit proefschrift hebben we ons gericht op het bestuderen van de aggregatie van α -synuclein door een grofkorrelige representatie van het eiwit. We hebben een polymorf deeltjes model geïntroduceerd om α -synuclein te beschrijven en hebben het met Brownse dynamica simulaties voor de translationele en rotationele dynamica gecombineerd. Bovendien, om de kameleontische aard van de eiwitten weer te geven, hebben we Brownse dynamica voor de interne dynamiek van de deeltjes gebruikt. De interne toestand is gekoppeld met de interacties tussen de deeltjes.

Het getoonde werk is een voortzetting van een bestaande Rotationale Brownse Dynamica algoritme voor anisotrope deeltjes. Dit wordt in **Hoofdstuk 2** samen met een applicatie op de aggregatie van clathrine, een ‘coat’ (mantel) eiwit betrokken bij endocytose, besproken. We hebben een clathrine-eiwit als één stijf deeltje met aantrekking posities op het oppervlak gemodelleerd, en verkregen aggregatie tijdslimieten die vergelijkbaar zijn met experimentele resultaten. Daarnaast hebben we de kritische bindingsenergieën, die eerder door andere simulaties voorspeld waren, bevestigd gekregen.

De Brownse dynamica algoritme gebruikt in hoofdstuk 2 levert de juiste resultaten, maar is erg complex. Daarom ontwikkelden we een nieuwe Brownse dynamica algoritme dat quaternionen gebruikt om de roterende beweging van een anisotrope deeltje te beschrijven. Het algoritme zijn afleiding en validatie zijn volledig in **Hoofdstuk 3** beschreven. De uiteindelijke vergelijking van de beweging blijkt veel eenvoudiger en duidelijker dan de eerder gebruikte algoritme. Daarnaast is de nieuwe rotationale Brownse dynamica algoritme elementair en goed geschikt voor de simulaties van anisotrope deeltjes. De belangrijkste voordelen van dit algoritme zijn de afwezigheid van metrische tensor en drift correcties vaak geassocieerd met Brownse Dynamica algoritmen in niet-cartesiaanse coördinaten, en de afwezigheid van singulariteiten geassocieerd met een roterende beweging.

Een nieuwe ‘coarse-grained’ methode van de studie van α -synuclein aggregatie wordt in **Hoofdstuk 4** uiteen gezet. Hier hebben we een eiwit als een anisotrope deeltje dat hun interne status, en daarmee zijn vorm en interactie parameters in reactie op de omgeving, kan veranderen. Een eiwit in de ongeordende toestand wordt als een bol met een zachte kern en niet-directionele lange, variërende aantrekking posities gemodelleerd. De interacties beschrijven de Van der

Waals, hydrofobe, en voorbijgaande waterstofbruggen die een echt eiwit ervaart. Een eiwit in de geordende toestand, *i.e.* een (mis) gevouwen eiwit in een fiber, heeft een veel stijvere en compactere structuur en wordt daarom voorgesteld door een spherocilinder met een harde kern en directionele korte, variërende aantrekking posities op het oppervlak. We gebruikten Brownse dynamica simulaties om de translatie, rotatie en de interne dynamica van de deeltjes te beschrijven. De simulatieresultaten toonden de vorming van oligomeren en fibrillen door een directe nucleatie-en-groei-mechanisme, door twee-stap-nucleatie, door omzetting van een oligomeer tot een fiber, of vice versa, en fibril-versterkte omzetting van oligomeren in fibrillen.

Om meer inzicht in het aggregatie proces te krijgen leggen we de ontwikkeling uit met een minder grove representatie van α -synuclein in **Hoofdstuk 5**. Hier hebben we een eiwit in een keten van 5 deeltjes, verbonden door middel van veren, uiteen gezet. Elke deeltje in de keten vertegenwoordigt ongeveer 12 aminozuren van de echte sequentie van het eiwit. Een ongeregeld eiwit wordt gemodelleerd als een ongeordende keten van bollen. Door veranderingen van de deeltjes in spherocilinders en de omlegging in een vlakke configuratie, wordt het eiwit geordend en kan fibrillen vormen. Om de groei van een fibril te onderzoeken, voerden wij gedwongen Brownse dynamica simulaties te gebruiken. Het zwaartepunt van een enkele geïsoleerde eiwit wordt op een vaste afstand van de zwaartepunt van een fibril, dat uit 10 eiwitten bestaat, gehouden. Daarna wordt de vrije energie als functie van de afstand berekend. We kregen een vrije-energie verschil van $80\text{--}100\ k_B T$ tussen een opgeloste eiwit en een eiwit in een vezel.

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Cu drag,
Dr. *Nana*

. Scientific Output

List of publications

1. I.M. Ilie, W.K. den Otter and W.J. Briels, *Rotational Brownian Dynamics of Clathrin Cage Formation*, J. Chem. Phys. - featured article, **141**, 065101, 2014
2. I.M. Ilie, W.J. Briels and W.K. den Otter, *An Elementary Singularity-free Rotational Brownian Dynamics Algorithm for Anisotropic Particles*, J. Chem. Phys., **142**, 114103, 2015
3. I.M. Ilie, W.K. den Otter and W.J. Briels, *A Coarse Grained Model for Proteins with Internal Degrees of Freedom. Application to α -synuclein*, J. Chem. Phys., submitted, 2015
4. I.M. Ilie, W.K. den Otter and W.J. Briels, *The Attachment of α -synuclein to a Fiber. A Coarse Grained Approach*, in preparation
5. W.K. den Otter, I.M. Ilie and W.J. Briels, *Free-energy Calculations from Constrained Brownian Dynamics Simulations*, in preparation

Conference proceedings

1. I.M. Ilie, W.K. den Otter and W.J. Briels, *Developing a Brownian Dynamics Algorithm to Simulate Protein Aggregation*, Biophys. J., **104**, Supplement 1, p390a, 2013
2. I.M. Ilie, W.K. den Otter and W.J. Briels, *Clathrin Aggregation by Rotational Brownian Dynamics*, Biophys. J., **106**, Supplement 1, p31a, 2014
3. I.M. Ilie, W.K. den Otter and W.J. Briels, *Adaptive Particle Simulations of Alpha-Synuclein Fibril Formation*, Biophys. J., **108**, Supplement 1, p227a, 2015

Presentations

1. I.M. Ilie, W.K. den Otter and W.J. Briels, *Developing a Brownian Dynamics Algorithm to Simulate Protein Aggregation*, 13th Dutch Soft Matter Meeting, Wageningen, the Netherlands (November 2012)
2. I.M. Ilie, W.K. den Otter and W.J. Briels, *Brownian Dynamics Simulations for Protein Aggregation*, 3rd Dutch Molecular Dynamics Day, Eindhoven, The Netherlands (March 2013)
3. I.M. Ilie, W.K. den Otter and W.J. Briels, *Brownian Dynamics Simulations of Protein Aggregation*, Scientific seminar - invited speaker, Cluj-Napoca, Romania (April 2013)
4. I.M. Ilie, W.K. den Otter and W.J. Briels, *Clathrin Aggregation by Rotational Brownian Dynamics*, Chemistry in Relation to Biology and Medical Research, Veldhoven, the Netherlands (December 2013)
5. I.M. Ilie, W.K. den Otter and W.J. Briels, *Rotational Brownian Dynamics: simulating patchy particles and protein aggregation*, Physics@FOM, Veldhoven, the Netherlands (January 2014)
6. I.M. Ilie, W.K. den Otter and W.J. Briels, *Clathrin Aggregation by Rotational Brownian Dynamics*, 58th Annual Biophysical Society Meeting, San Francisco, United States of America (February 2014)
7. I.M. Ilie, W.K. den Otter and W.J. Briels, *Clathrin Aggregation by Rotational Brownian Dynamics*, The Physics of Soft and Biological Matter, Homerton College, Cambridge, United Kingdom (April 2014)
8. I.M. Ilie, W.K. den Otter and W.J. Briels, *Rotational Brownian Dynamics: Simulating Patchy Particles*, SoftComp/ESMI Annual Meeting, Heraklion, Greece (May 2014)
9. I.M. Ilie, W.K. den Otter and W.J. Briels, *A New Rotational Brownian Dynamics Approach for the Simulation of Biological Processes*, Dutch Biophysics Conference, Veldhoven, the Netherlands (September 2014)
10. I.M. Ilie, W.K. den Otter and W.J. Briels, *Simulating Patchy Particles by Rotational Brownian Dynamics*, Jülich Soft Matter Days, Bad Honnef, Germany (November 2014)
11. I.M. Ilie, W.K. den Otter and W.J. Briels, *Simulating Patchy Particles by Rotational Brownian Dynamics*, Chains 2014 - Dutch Chemistry Conference, Veldhoven, the Netherlands (November 2014)
12. I.M. Ilie, W.K. den Otter and W.J. Briels, *Fibril Formation by Rotational Brownian Dynamics*, Physics@FOM, Veldhoven, the Netherlands (January 2015)

13. I.M. Ilie, W.K. den Otter and W.J. Briels, *α -synuclein as a Patchy Particle*, Amyloid Aggregation - Single Molecule Approaches to a Many Molecule Problem, Leiden, the Netherlands (April 2015)
14. I.M. Ilie, W.K. den Otter and W.J. Briels, *selected talk – Aggregation of rigid and polymorph proteins by Brownian Dynamics simulations*, The Physics of Protein Self-Assembly, Lausanne, Switzerland (June 2015)

Posters

1. I.M. Ilie, W.K. den Otter and W.J. Briels, *Developing a Simulation Method towards Protein Aggregation*, Pierre-Gilles de Gennes Advanced School on Cellular Biophysics, Hyeres, France (September 2012)
2. I.M. Ilie, W.K. den Otter and W.J. Briels, *Developing a Simulation Method towards Protein Aggregation*, EMBO Conference Physics of Cell: From Soft to Living Matter, Hyeres, France (September 2012)
3. I.M. Ilie, W.K. den Otter and W.J. Briels, *Developing a Simulation Method towards Protein Aggregation*, MESA+ annual internal Symposium, Enschede, the Netherlands (September 2012)
4. I.M. Ilie, W.K. den Otter and W.J. Briels, *Developing a Simulation Method towards Protein Aggregation*, Annual Meeting of the German Biophysical Society, Gottingen, Germany (September 2012)
5. I.M. Ilie, W.K. den Otter and W.J. Briels, *Developing a Simulation Method towards Protein Aggregation*, Dutch meeting on Molecular and Cellular Biophysics, Veldhoven, the Netherlands (October 2012)
6. I.M. Ilie, W.K. den Otter and W.J. Briels, *Developing a Simulation Method: Protein Aggregation*, Study Group Meeting: Chemistry in Relation to Biology and Medical Sciences, Veldhoven, the Netherlands (December 2012)
7. I.M. Ilie, W.K. den Otter and W.J. Briels, *Developing a Brownian Dynamics Algorithm to Simulate Protein Aggregation*, 57th Annual Biophysical Society Meeting, Philadelphia, United States of America (February 2013)
8. I.M. Ilie, W.K. den Otter and W.J. Briels, *Brownian Dynamics simulation of Clathrin Cage Formation*, 9th European Biophysical Congress, Lisbon, Portugal (July 2013)
9. I.M. Ilie, I.S. Santos de Oliveira, G. Villalobos Camargo, W.K. den Otter and W.J. Briels, *MESOScopic Simulations at MESA+*, MESA+ annual internal Symposium, Enschede, the

Netherlands (September 2013)

10. M. Giani, I.M. Ilie, W.K. den Otter and W.J. Briels, *Simulating the self-assembly of clathrin cages*, EMBO Conference: System Dynamics in Endocytosis, Villars-sur-Ollon, Switzerland (September 2013)
11. I.M. Ilie, W.K. den Otter and W.J. Briels, *Brownian Dynamics Simulation of Clathrin Cage Formation*, Dutch meeting on Molecular and Cellular Biophysics, Veldhoven, the Netherlands (October 2013)
12. I.M. Ilie, W.K. den Otter and W.J. Briels, *Brownian Dynamics Simulation of Clathrin Cage formation*, Chemistry in Relation to Biology and Medical Research, Veldhoven, the Netherlands (December 2013)
13. I.M. Ilie, I.S. Santos de Oliveira, G. Villalobos Camargo, W.K. den Otter and W.J. Briels, *MESOScopic Simulations at MESA+*, MESA+ annual internal Symposium, Enschede, the Netherlands (September 2014)
14. I.M. Ilie, W.K. den Otter and W.J. Briels, *α -synuclein as a Patchy Particle*, Amyloid Aggregation - Single Molecule Approaches to a Many Molecule Problem, Leiden, the Netherlands (April 2015)
15. I.M. Ilie, W.K. den Otter and W.J. Briels, *Aggregation of rigid and polymorph proteins by Brownian Dynamics simulations*, The Physics of Protein Self-Assembly, Lausanne, Switzerland (June 2015)