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Molecular Dynamics of Bioinspired Nanotubes

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Molecular Dynamics of Bioinspired Nanotubes

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"I was supposed to be having the time of my life."

- Sylvia Plath, *The Bell Jar*, 1932-1963

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Chapter 1

Introduction

"Given for one instant an intelligence which could comprehend all the forces by which nature is animated and the respective positions of the beings which compose it, if moreover this intelligence were vast enough to submit these data to analysis, it would embrace in the same formula both the movements of the largest bodies in the universe and those of the lightest atom; to it nothing would be uncertain, and the future as the past would be present to its eye."

- Pierre-Simon Laplace, 1749-1827

The nature of our interaction with the physical world is abstract. Our perception is restricted to our senses and our senses are limited to specific stimuli. Thus, we can never obtain a complete knowledge of the natural world and its properties. For example, mixing unknown chemicals might trigger a reaction. We might be able to tell whether or not the reaction took place by observing changes in the colour of the solution, feeling the heat excluded from the mixture, witnessing an explosion, etc., but this is more or less the maximum information we can obtain with our senses (touch, sight, etc.). Along the history of humankind, there have been plentiful theories to interpret the physical world and give explanations beyond the limitations of our senses. Philosophy and religion were the first attempts, but most of them were not quite successful. More than 2000 years ago, Aristoteles suggested that matter is constructed by five classical elements: fire, water, air, earth and aether. Today, it is known that things are "slightly" different. Around the same time, Democritus proposed that matter is constituted by tiny, invisible and inseparable units, that he

called atoms (meaning indivisible in Ancient Greek). Today, it is known that atoms are not the smallest elements of matter, but there are extremely small fractions of it. Democritus' theory was quite accurate considering the time it was formulated. It makes sense that matter cannot be divided eternally, so there has to be a tiny unit regardless of it being called atom, quark or something else. In the end, any theory may sound realistic (or not), but, certainly, neither our senses nor logic is proof for the validity of the theory.

The scientific method together with experiments and mathematics revolutionized the way the physical world is perceived. Theories were formulated, tested and, when the theories did not match the experiments, they were reformulated or gave up their place to more complex theories that were in better agreement with experimental observations. After many years of theory formulation and experimentation, scientists obtained a better picture on matter and its properties. Today, scientists are able to drive particles through matter and study its structure in tiny scales or use magnetic fields to determine their chemical structure. Despite the great successes in theory and techniques, there are still limits in the information that can be extracted through experimental techniques, mostly in terms of resolution and/or dynamics.

When experiments fail to procure answers, models can be used to shed light on these dark spots. Models are simplifications of reality that allow us to describe natural phenomena in a consistent and controlled manner. They are constructed based on our interpretations of these phenomena, and they are fitted to reproduce specific properties. At this point, it needs to be made clear that models are not experiments and should not be treated as such. Instead, they are elaborate tools helping us in our quest of understanding the world.

1.1 Self-assembly

Self-assembly describes the process of small building blocks organizing into large supramolecular structures. Under certain conditions, self-assembly occurs spontaneously driving the system towards favourable states in terms of free energy. The chemical nature and structure of the building blocks determine the conditions under which this process takes place and the type of the final aggregate, Figure 1.1. Non-covalent interactions are dominant in this process and play a crucial role in determining the properties and functionality of the self-assembled structures.

The past decades, different molecules able to form ordered suprastructures with unique properties have been synthesized and extensively studied [1, 2]. Despite the success in developing such molecules, the principles of self-assembly are not fully understood and developing such molecules is mainly based on trial and error approaches. The limited predictive power in determining the final aggregate types and their properties and the lack of information on the arrangement of the building blocks at normal conditions, due to experimental limitations, are major obstacles in achieving full control over the final properties and opening the way to rational design of molecules with specific traits. Consequently, methods that tackle these issues are greatly valued.

Figure 1.1: Schematic representation of building blocks forming different types of aggregates. The chemical and structural properties of the building blocks, together with the conditions of the system, such as type of solvent, solute/salt concentration or temperature, determine the morphology and characteristics of the aggregate. Modifying any of these parameters may have drastic effects on the final product.

1.2 Molecular Dynamics

Molecular dynamics (MD) simulations comprise an invaluable computational tool to study molecular systems in atomistic detail. Their applications cover a wide range of scientific fields, such as Chemistry, Biology, or Material Science, since they combine two features that cannot be captured by experimental methods at the same time, information on atomistic resolution and dynamic behaviour of the system under study. In MD simulations, atoms are treated as single points with specific properties, such as mass, size, charge, etc., that can interact and affect the environment around them. The position, velocity and acceleration of atoms are calculated by numerically solving the Newtonian equations of motion (Eq. 1.1) and obtaining the forces that act upon every atom.

$$F_i = m_i a_i = m_i \frac{\delta^2 r_i}{\delta t^2}, i = 1 \dots N \quad (1.1)$$

F_i corresponds to the force that acts upon each atom, m_i to the atom's mass and a_i to its acceleration, which is the second derivative of the position r_i as a function of time, t . N is the total number of atoms in a system.

The time-dependent integration of the classical equations of motion provides trajectories of atomic and molecular coordinates. Kinetic theory and statistical mechanics are employed to extract thermodynamic properties for the systems under study and to obtain macroscopic data from the microscopic world.

The atomic forces and their potential energies are calculated by using mathematical models (force fields) that describe the potential energy of each system as a

1.2. Molecular Dynamics

function of position. The force (F) that acts on each atom can be calculated from the negative derivative of the potential energy (U), (Eq. 1.2).

$$F = -\nabla U \quad (1.2)$$

A simple functional form of a force field includes two types of terms that describe the interactions between atoms that are covalently bonded (bond lengths, angles and dihedral terms), on one hand, and the interactions between non-bonded atoms (van der Waals forces and electrostatics), on the other hand, (Eq. 1.3).

$$\begin{aligned}
 U = & \\
 & U_{bonds} + U_{non-bonds} = \\
 & U_{bonds}(r_{ij}) + U_{angles}(\theta_{ijk}) + U_{dihedrals}(d_{ijkl}) + U_{LJ}(r_{ij}) + U_{Coul}(r_{ij}) = \\
 & \sum_{bonds} \frac{1}{2} k_{ij}^b (r_{ij} - r_{ij}^0)^2 + \sum_{angles} \frac{1}{2} k_{ijk}^\theta (\theta_{ijk} - \theta_{ijk}^0)^2 + \\
 & \sum_{impropers} \frac{1}{2} k_{ijkl}^d (d_{ijkl} - d_{ijkl}^0)^2 + \sum_{torsions} k_{ijkl}^{d'} (1 + \cos(n d_{ijkl} - d_{ijkl}^0)) + \\
 & \sum_{LJ} \left(\frac{C_{ij}^{(12)}}{r_{ij}^{12}} - \frac{C_{ij}^{(6)}}{r_{ij}^6} \right) + \sum_{Coulomb} \frac{1}{4\pi\epsilon_0} \frac{q_i q_j}{e_r r_{ij}}
 \end{aligned} \quad (1.3)$$

In this case, bonds, angles and dihedrals are treated as harmonic oscillators with specific force constants, k_{ij}^b , k_{ijk}^θ and k_{ijkl}^d , respectively. r_{ij}^0 , θ_{ijk}^0 and d_{ijkl}^0 are the equilibrium values for the respective bonded potential. Dihedral angles could be represented either as harmonic oscillators, or as periodic functions with specific multiplicity, n , and force constant, $k_{ijkl}^{d'}$. Interatomic interactions are modelled with Lennard-Jones (LJ) potentials and electrostatic forces with Coulomb potentials. In the LJ potentials, $C_{ij}^{(12)}$ is the repulsive term that describes the repulsion at close distances due to the overlap of the electron orbitals and $C_{ij}^{(6)}$ is the attractive term at longer distances modelling the van der Waals interactions. Regarding Coulomb potentials, q_i and q_j are the atomic partial charges, ϵ_0 is the vacuum electric permittivity and e_r the relative permittivity constant of the material. Bonded and non-bonded interactions are summarized in Figure 1.2.

Several force fields have been developed over the past decades, AMBER [3], CHARMM [4], GROMOS [5], OPLS [6], etc. Their functional forms carry small variations depending on the simulation program and distribution, but the basic idea is the same. The small differences between force fields arise from the way in which their parameters have been generated and the aspects of the physical systems that their developers wanted to reproduce. More specifically, the bonded parameters

Figure 1.2: Schematic representation of the bonded and non-bonded interactions in an MD simulation.

have been tuned based on theory using quantum mechanical calculations or on experimental data from X-ray or electron diffraction, Nuclear Magnetic Resonance, etc., and the non-bonded parameters to reproduce free energy of solvation, heat of vaporization or other thermodynamic properties. The parameters of each force field are usually specific for discrete types of systems (proteins, lipids, nucleic acids, etc.), but there are also general versions for broader applications.

Major limitations in MD simulations are related to the system size and the time scale. Systems, especially in the field of nanomaterials, may require hundreds of thousands of atoms to be simulated at the same time and the computational costs of calculations increases with the size of the system. A common way to reduce the computational cost is decreasing the level of complexity of a system. Specifically, there are force fields that treat specific atoms differently and could be classified into the following categories:

- **All-atom (AA) force fields:** the interactions for every atom in the system are calculated
- **United-atom (UA) force fields:** non-polar hydrogens (of methyl or methylene bridge) are implicitly treated
- **Coarse-grained (CG) force fields:** groups of atoms are represented as large beads

Regarding time, specific events (protein folding, ligand binding, etc.) may require several microseconds or milliseconds in order to be sampled by MD, whereas the time step of integration in MD is on the scale of femtoseconds. Simulating rare or long events is computationally expensive and time consuming, especially, if the possibility of being trapped in a specific conformation (local minimum) is taken into account. Several methods have been developed to address these issues and improve the performance and conformational sampling of MD simulations.

1.3 The Martini force field

The MARTINI force field is the most popular CG force field used to study different biomolecular systems. Initially, it was developed to perform MD simulations of lipid

1.3. The Martini force field

systems [7], but in time, the area of applications was expanded to other types of systems, e.g. proteins, carbohydrates or other bio-materials [8].

MARTINI was parametrized to reproduce thermodynamic data, such as specifically partitioning free energies of solutes in polar and non-polar solvents. It uses a chemical building block approach in which different chemical groups (groups of atoms) are represented by specific beads based on their physical and chemical properties. Traditionally, MARTINI used a four-to-one mapping scheme, where each group of four heavy atoms (not hydrogen) corresponded to one bead, but due to the need to describe aromatic rings and/or smaller chemical moieties better, newer versions of MARTINI included three-to-one and two-to-one mapping schemes [9, 10]. Consequently, there are three MARTINI bead sizes, normal (N), small (S) and tiny (T), Figure 1.3. Additionally, there are four main types of beads: polar (P), non-polar (N), apolar (C) and charged (Q). Each bead type is further divided into subtypes/flavours, in order to represent better specific properties of the chemical groups, e.g., the ability to act as hydrogen donor/acceptor, the degree of polarity, etc. The bonded parameters, which include bonds, angles and dihedrals, are tuned to reproduce the distributions of the mapped atoms that constitute each bead. The non-bonded interactions are modelled by using a Lennard-Jones potential, where the strength of the interactions and the position of the minimum depend on the bead types and sizes. The electrostatic interactions between charged beads are calculated by using a Coulombic energy function. The mapping scheme and the parametrization procedure are the main features that make the MARTINI force field highly efficient, flexible and transferable for use in different systems.

Figure 1.3: Summary of the MARTINI bead types and sizes. Molecules are presented in different resolution (UA and CG). Subtypes and flavours are not shown for simplicity. In the MARTINI water model, four atomistic water molecules are assigned to one bead.

CG methods are usually a good solution when dealing with enormously large systems. However, there are issues related to the concept of coarse-graining that should be always taken into account when using CG methods. Specifically, reducing the level of detail of a molecular system affects the spatial and chemical resolution.

For example, the directionality of specific interactions, such as hydrogen bonds, is lost when the atoms involved (hydrogen bond donor/acceptors) are grouped with other atoms that do not participate in the interaction. Another issue is the treatment of partial charges of distinct atoms inside the apolar beads. A few solutions have been proposed to deal with these problems [11, 12].

1.4 Enhanced Sampling

The second major limitation of MD simulations is related with time scales and sampling of the available conformational space of a system. In other words, if the energy landscape of a process, such as the folding of a protein, is rugged with many local energy minima, the system will spend most of the simulated time trapped far away from the global minimum. Similar issues occur when studying transitions between conformations separated by high energy barriers.

There are several approaches to improve the sampling of MD simulations and usually they are referred to as enhanced sampling methods. Nowadays, the most commonly used methods are Replica-Exchange MD (REMD) simulations [13] and Metadynamics [14, 15], but many more are being used based on the nature of the system and the underlying problems, namely Transition Path Sampling [16], Steered Molecular Dynamics [17], etc.

In REMD simulations, the sampling can be improved by running parallel simulations (replicas) of the same system at different temperatures (t-REMD) and allow each system to exchange coordinates with other replicas according to an energy based scoring function. Effectively, replicas with lower energy interact with higher energy replicas and escape from potential local minima. Temperature is the most commonly used variable parameter in REMD, but there can be variations, such as Hamiltonian-REMD [18], where the energy terms of the force field are modified in each replica instead of the temperature.

Metadynamics belongs to a group of methods, such as Conformational Flooding [19] or Local Elevation [20], that use history-dependent potentials to drive a system away from sampled states. In more detail, instead of biasing one general variable of the system, a good collective variable (CV) that describes the process of interest should be defined. Then, positive Gaussian potentials are added on the energy landscape described by the defined CV, in order to prevent the system from visiting conformations that have already been sampled. The added potentials act as a memory dependent factor that biases the system towards exploring all the available conformation described by the defined CV. At the end, the deposited Gaussians can be recollected and be used to obtain the real energy landscape of the process. Even though the basic idea between these methods is practically the same, metadynamics offers a integrated framework for performing enhanced sampling simulations with different CVs, monitor the deposited bias potential and reconstruct the free energy landscapes [21]. Metadynamics has been simply and ingeniously described as "filling the free energy wells with computational sand" [22].

Enhanced sampling methods significantly boost the performance and capabilities of MD simulations. Detailed overviews of different enhanced sampling methods and their applications can be found in the literature [22, 23].

1.5 Preassembled structures

Even if the "quiver" of computational methods is equipped with many tools to overcome intrinsic limitations and bottlenecks of MD simulations, the task of obtaining realistic structures in reasonable timescales via brute force or enhanced sampling simulations is far from trivial. Especially in supramolecular self-assembly, where the relatively weak non-covalent interactions are dominant, the simulated systems might be kinetically trapped in thermodynamically stable states for a long duration of time, until the native state is reached. Consequently, experimental constraints, such as overall morphology, diameter in case of nanowires/nanotubes, pitch length in fibrils, are commonly used to create starting configurations by packing molecules inside predetermined shapes (e.g., bilayers, tubes, fibrils) at the correct density, without creating unphysical overlap between molecules. The procedure might not always be straightforward, but crystal structures or theoretical models are usually good starting points to obtain the initial configurations. It has to be noted, that the precise level of disorder is not known, as this is difficult to assess experimentally, and idealized structures often used to illustrate the packing of molecules might be unrealistic or overly ordered. The lack of knowledge about the packing details of the supramolecular aggregates in solution is one of the main reasons to resort to MD simulations.

The basic idea is that, starting from idealized structures, simulations can be employed to study the validity of the initial conformations. In practice, simulations provide a more relaxed and dynamic picture, and can help discriminating between alternative structural models. The evaluation between such models is mostly done by using the overall stability and relative order of the system as criterion. Examples in the literature are plenty [24, 25]. In case more than one model proves stable in the simulations, further analysis and comparison to experiments is needed. Assuming a realistic structure, MD simulations can be further used to explore the effect of state conditions (temperature, pH or ionic strength) on the properties of the aggregates [26, 27], or to explore the effect of substituents or additives [28, 29].

1.6 Thesis outline

In this thesis, the results from modelling different bio-inspired nanomaterials that self-assemble into different types of aggregates will be presented. Additionally, we will compare the convergence of different force fields in calculating the binding energy of small aromatic molecules.

As you probably noticed, **Chapter 1** is a general discussion on life, science, and the topics this thesis is dealing with.

Chapter 2 includes MD simulations of a small cyanine dye (C8S3) that forms double-walled nanotubes. Our main goal was to study structural features of pre-assembled C8S3 aggregates, such as the arrangement of the monomers or the dimensions of the aggregates, since their structural details were poorly understood at atomistic resolution. Revealing the structural features of C8S3 aggregates is crucial for modelling and understanding their design principles.

Chapter 3 describes the CG model that was developed for C8S3 and similar cyanine dyes. We resorted to CG simulations to alleviate the burdens of atomistic simulations in self-assembly simulations and used the available experimental results

from different cyanine dyes of the same family to improve parametrization of the CG models. The basic dynamics of self-assembly and the parameters that affect the aggregate morphologies are discussed.

Chapter 4 is a study of a naphthalene based oligopeptide (2NapFF) that self-assembles into nanotubes. 2NapFF aggregates have been studied with different experimental techniques, but there is little information for the arrangement of the monomers when the aggregates are formed. The setup of the simulations includes the driven formation of a nanotube according to experimental results and the interpretation of the naphthalene and side chains position and orientation. The simulations provide insights on the way that the 2NapFF molecules organize.

In **Chapter 5**, we compare the results of different force-fields when calculating the free energy of dimerization of small aromatic molecules. Interactions between aromatic molecules are extremely important for self-assembly and the resulting functional and structural properties of the formed aggregates. Enhanced sampling methods are employed to explore the available conformational space and reduce the statistical error of the free energy calculations.

Topologies, starting and final structures of the reported systems, as well as input files are available at <https://osf.io/5f72c/>.

Chapter 2

Insights on the structure of C8S3 nanotubes

"Eppur si muove."

- Galileo Galilei, 1564-1642

2.1 Abstract

Self-assembled nanostructures arise when building blocks spontaneously organize into ordered aggregates. The chemical nature of the building blocks determines the type of these suprastructures that exhibit different properties compared to the disorganized building blocks. In this study, we were interested in an amphiphilic cyanine dye (C8S3) that spontaneously self-assembles into nanotubes with different optical properties. Both experimental and theoretical studies to date lack an explicit atomistic description of the monomer position and arrangement. To study the structural features of the nanotubes, we performed atomistic MD simulations of preformed bilayers and double-walled nanotubes, since computational studies are able to resolve in high resolution the details that cannot be captured directly by experiments or theory. Our results reveal that different arrangements of the dyes lead to stable nanotubes and the nanotube wall thickness, which is directly accessible from the MD simulations, is much smaller than the experimentally derived thickness from cryo-TEM (computational: ~ 2.5 nm versus experimental: ~ 3.5 -4 nm). The lower thickness calculated from simulations is compatible with SAXS measurements and theoretical calculations for the optical properties of the nanotubes.

2.2 Introduction

Cyanine dyes are molecules with extraordinary optical and structural properties, including ultrafast emission, sharp absorption and fast electronic transfer [30, 31, 32]. These properties make cyanine dyes excellent model systems for fundamental research on exciton transfer and energy transport at molecular level and have caused a surge of interest in these systems and their applications in fields such as photography [33, 34], labelling [35, 36], nonlinear optics [37], etc.

Over the past decades, several cyanine dyes that incorporate the chromophore 5,5',6,6'-tetrachloro-benzimida-carbocyanine and different substituents have been synthesized and studied [38, 39]. Most of these dyes have the ability to aggregate and form highly ordered supramolecular structures of various shapes and sizes that exhibit different optical properties [40, 41]. An interesting example is the amphiphilic cyanine dye 3,3'-bis(2-sulfopropyl)-5,5',6,6'-tetrachloro-1,1'-dioctyl-benzimida-carbocyanine (C8S3). The chemical structure of a single C8S3 monomer can be divided into three parts, the hydrophilic heads, the cyanine dye chromophore (aromatic core) and the hydrophobic tails (Figure 2.1a). When solvated in polar solvents, C8S3 monomers spontaneously self-assemble into long double-walled tubular aggregates (nanotubes) [41] (Figure 2.1b).

Figure 2.1: a) Chemical structure of C8S3 monomer. b) Graphical representation of a C8S3 nanotube (centre). Cryo-TEM image of C8S3 nanotubes (top left). Slice from the top view of a proposed arrangement model (top right), slice from the side (bottom right) and C8S3 monomers arranged in the Herringbone formation (bottom left). Images have been prepared in VMD [42].

The driving force for the formation of the C8S3 nanotubes is a combination of intermolecular interactions, where entropic and enthalpic contributions are carefully balanced. The hydrophobic effect determines the orientation of the polar groups toward the solvent on the inside and outside of the nanotube and non-polar groups toward the interior of the nanotube, whereas electrostatics and pi-pi stacking create a network of interactions that regulates the position and arrangement of the aromatic

cores with respect to each other. The high polarizability of the conjugated system generates strong van der Waals forces (induced dipole interactions) and consequently dense packing of the C8S3 monomers.

Despite the various experimental data, mainly from cryo-Transmission Electron Microscopy (cryo-TEM) [41, 43, 44, 45, 46], the resolution of the current techniques is not sufficient to reveal the atomistic structural details of the C8S3 nanotubes. On the other hand, theoretical models for the molecular arrangement of the C8S3 monomers present good agreement with transition dipole moments of the experimental spectra, but they lack an explicit atomistic description [45, 47, 48, 49].

Computer simulations comprise an invaluable tool to obtain information not accessible by experimental methods and fill the gaps between theory and experiments [1, 50, 51]. Previous computational studies on the C8S3 nanotubes or other members of the cyanine dye family presented good agreement with experimental results [52]. However, the simulated structures were either not stable or were simulated for short time scales. There are several issues that render the tasks of constructing and performing MD simulations of such systems far from trivial. The first group of issues is related to the dimensions of the aggregates and the arrangement of the monomers. Experimental data provide a vague idea of the dimensions of the aggregates when compared to atomistic resolution. Consequently, it is extremely difficult to estimate accurately the number of C8S3 molecules in the inner and outer wall and construct structures that maintain their designed formation, if the initial number of molecules is not optimal. Since experimental evidence for the packing of C8S3 is not yet available, the packing motifs (e.g. brickwork, herringbone, staircase) are extrapolated from the experimental results based on similar molecules or theoretical models [53, 54, 25]. Furthermore, experimental or theoretical based approaches to construct models provide ideal structures that diminish the presence of disorder and do not necessarily produce either correct or stable structures. The second group of issues is related to the differences between the available force fields in MD simulations that can ultimately produce results that do not converge. Last, the feasible simulated time scales for such large systems are usually inadequate to sample different packing rearrangements and reach the most favourable energy states, especially, if the systems are kinetically trapped in intermediate low energy states.

In this work, we employed Molecular Dynamics (MD) simulations to shed light on the structural properties of C8S3 nanotubes and on the preferred arrangement of the C8S3 monomers inside the aggregates. We optimized specific parameters of the standard GROMOS force field by using Quantum Mechanical (QM) methods to obtain a better description for C8S3 monomers and performed simulations of different systems (bilayers and nanotubes) to understand the behaviour of these molecules in different formations. To verify our findings, we conducted Small Angle X-ray Scattering (SAXS) experiments and compared the profiles with simulated SAXS spectra from C8S3 nanotube simulations.

Furthermore, simulated C8S3 nanotubes were used as templates for the calculation of optical spectra, which were compared with experimental absorption spectra. Our results provide insights in the structure of the C8S3 nanotubes and reveal features that have not been resolved by experimental methods before.

2.3 Methods

2.3.1 MD simulations

C8S3 bilayer and nanotube simulations were performed with the GROMACS 5.1.4 simulation package [55, 56]. The GROMOS force field version G53a6 [57] was used and systems with this force field were solvated in cubic or rectangular boxes with explicit SPC water molecules [58]. C8S3 simulations were also performed with the General Amber Force Field [59] (GAFF) to compare the results between G53a6 and GAFF. In GAFF simulations, the TIP3P water model [60] was used instead of SPC. Counter ions (Na^+) were introduced to neutralize all systems. The temperature was kept constant at 300 K by using the V-rescale algorithm with a time constant of 0.1 ps [61]. The Berendsen barostat [62] was used to maintain the pressure constant at 1 bar with a time constant of 1 ps and compressibility of $4.5 \times 10^{-5} \text{ bar}^{-1}$. Semi-isotropic pressure coupling was used for most systems to allow the systems to equilibrate separately in the z-dimension, which coincides with the bilayer normal, and in the bilayer plane. For the solvated nanotubes in cubic boxes, an isotropic pressure bath was used. The cut-off for electrostatic and van der Waals interactions was set to 1.4 nm and the Verlet update scheme [63] was used for the short range non-bonded interactions with a buffer tolerance of $0.005 \text{ kJ mol}^{-1} \text{ ps}^{-1}$. Long range interactions were calculated with the Reaction Field method [64]. The LINCS algorithm was employed for constraining the bond lengths [65].

All bilayer and short nanotube systems were minimized for 10^3 steps by using the Steepest Descent algorithm and equilibrated in two phases, in the NVT and NPT conditions, each for 10 ps with a 1 fs time step. The production phase was performed in the NPT ensemble, and the time step for integration was set to 2 fs.

It has to be noted that some systems (long nanotubes) required special treatment before starting the production phase. The main issue was the imbalance of ions inside and outside of the tube, when the ions were randomly placed inside the solvent. To ensure optimal concentration of Na^+ around these aggregates, the ions were placed inside and outside of the nanotubes close to 1/1 ratio with the C8S3 monomers in each wall of the nanotube, inner and outer. Simulations with randomly placed ions resulted in a depleted concentration of ions inside the nanotube along the trajectories and instabilities that deformed the cylindrical structures and did not heal during the simulated time scales. We speculate that the imbalance of positive and negative charge around the C8S3 molecules of the inner wall was the main cause of the instabilities. After solvation, the systems were minimized with the Steepest Descent algorithm for 10^3 steps and equilibrated in the NVT and NPT ensembles, for 10 ps and 5 ns with 1 fs time step, respectively. During the equilibration, the aromatic core of the C8S3 monomers was restrained using harmonic potentials with 1000 kJ/mol force constant. In this way, the side chains and the solvent were able to relax before the production phase. For the actual MD simulation, the time step for integration of the equations of motion was set to 2 fs. To prevent the aggregates from crossing the periodic boundary conditions, the systems were translated and rotated to remove the centre of mass motion of the nanotubes every 100 steps.

Bilayer trajectories were 500 or 250 ns long, and each short nanotube trajectory was 100 ns long. The long nanotube simulations lasted 10-100 ns. The last 100 ns of each trajectory were used for the analysis of the bilayers, the last 50 ns and last 5-10 ns for the analysis of the short and long nanotubes, respectively.

2.3.2 C8S3 Parametrization

QM calculations were performed by using the hybrid density functional B3LYP [66, 67, 68, 69] with the 6-31G* basis set, and Dipole preserving analysis [70] (DPA) was used to determine the partial charges of the molecule. QM calculations were performed in the GAMESS-UK package [71]. Due to the size of the conjugated system and its high polarizability, the partial charges of the C8S3 molecule and the dihedral angles of the polymethine bridge were optimized based on a simpler system, 5,5',6,6'-tetrachloro-1,1',3,3'-tetramethylbenzimidacarbocyanine (C1C1, Figure 2.4a). C1C1 has the same aromatic core as C8S3, but the tails have been substituted with methyl groups. The partial charges of C1C1 and C8S3 were calculated at the optimum geometry, and the raw values resulting from the DPA analysis were rounded to the second decimal to maintain symmetry and simplicity. Some values were adapted to maintain integer net charges. We assume that the distribution of the partial charges is more accurate for the less complex system, so C8S3 charges were rounded according to the C1C1 charges. The partial charge of the atoms belonging to the aliphatic tails was set to 0 in accordance with standard G53a6 charges for united-atom aliphatic chains.

Furthermore, the description for the dihedrals of the linker between the two aromatic rings was examined by performing potential energy scans of the dihedrals of the atoms that constitute the polymethine bridge (C5-C3-C2-C4 and N9-C5-C3-C2, Figure 2.4a) by rotating in steps of 10° around the torsional angles and measuring the energy in each conformation. The QM curve represents the energy profile calculated from the QM calculation, and the MM profile represents the final energy profile after including the calculated dihedral parameters. At each value of the dihedral angle of interest, all other degrees of freedom were fully relaxed during the minimization procedure.

2.3.3 C8S3 bilayers

Small systems, where the monomers were organized in preformed bilayers, were constructed (Figure 2.5) to study the arrangement of C8S3 molecules and calculate structural properties (such as bilayer thickness, molecular arrangement, tail order parameter, etc.) of small slices representative of the C8S3 nanotubes. The box size of these systems was $\sim 10 \times 7 \times 10$ nm, and each box contained ~ 200 C8S3 molecules. The crystal structures of molecules [53, 54] with the same aromatic core as C8S3 and previous models for the arrangement of the cyanine dyes or C8S3 monomers [41, 25] were used as templates for the preparation of the initial coordinates of each system. Bilayers with three different arrangements were prepared (brickwork, herringbone and staircase formation). A list of all simulated systems is included in the Appendix, Table 6.1.

The initial thickness of the bilayers was ~ 3.5 nm, close to the reported values for the thickness of the C8S3 nanotube in several experimental studies [44, 45, 46]. To avoid overlaps and atom clashes during the construction of the bilayers, the atoms of the C8S3 tails were stretched to their optimal bond distance and then translated in order to be at the same plane as the aromatic core. In other words, the structure of C8S3 in the initial unit cell resembled its chemical structure in Figure 2.1a and 2.1b, which is flat along the plane of this thesis page. Consequently, since the tails were stretched in the initial configurations, the effective thickness of all aggregates would

be ~ 0.5 nm smaller, when the tails adopt their natural conformation in water.

Apart from the force field comparison (G53a6 and GAFF), the effect of the temperature and the solvent on the bilayers were tested. Specifically, an extra set of bilayer simulations with the G53a6 force field was performed at 288 K, the temperature at which AFM [72] experiments were conducted. C8S3 aggregates can self-assemble through a direct route (pure water) or an alcoholic route (water and methanol solution), and the final aggregates differ in size [44]. The effect of methanol was measured by substituting a portion of water with methanol (20% v/v) to resemble the experimental conditions [44, 45, 46]. Parameters for methanol were obtained from Automated Topology Builder [73] (<https://atb.uq.edu.au/>, ATB Molecule ID: 15607). The duration of these simulations was 250 ns, and for the methanol systems the final frames of the initial G53a6 simulations were used as initial coordinates.

The bilayer thickness was calculated by monitoring the distance between the centre of mass of the SO_3^- groups in each leaflet, analogous to a typical measure to calculate the thickness of the common phospho-lipid bilayers based on the position of the PO_4^- group. Electron density profiles across the bilayer were generated and the distance between the highest peak of each leaflet was measured as an alternative measure of the thickness, which is in principle accessible through electron scattering. We keep in mind that the centre of mass distance and electron density calculations could be affected by undulations of bilayers and can produce unrealistic results when these undulations are large. In order to avoid overestimation of the bilayer dimensions, we also measured the thickness of small leaflet patches (~ 10 nm²) by monitoring the distance between oxygens of the hydrophilic tails.

In the bilayer simulations, the ordering of the aromatic cores with respect to the initial formation was measured by defining a second-rank order parameter, P_2 (Eq. 2.1) for the angle theta (θ) between the vector pointing from the start to the end of the polymethine bridge (C5-C6 atoms) and the average orientation of the molecules at the start of the simulation. The same order parameter was used to calculate the angle between the bilayer normal and the vectors defined by each bond of the hydrophobic tails.

$$P_2 = \frac{1}{2} (3 \cos^2(\theta) - 1) \quad (2.1)$$

2.3.4 C8S3 nanotubes

MD simulations of preformed C8S3 nanotubes were conducted to evaluate the ability of C8S3 molecules to maintain a tubular formation. The initial coordinates were generated by creating 2D lattices from single unit cells (herringbone and brickwork formation) and rolling them into cylinders with specific rolling angles (α , Eq. 2.2) and radii (R , Eq. 2.3). The rolling angles and radii depend on the unit cell size and the imposed pitch providing discrete values for both parameters. This procedure used was inspired by previous methods to study similar systems [41, 25] and is summarized in Figure 2.2.

$$\sin(\alpha) = \frac{p * z}{(n * x)^2 + (p * z)^2} \quad (2.2)$$

$$R = \frac{n * x}{\sin(\alpha)} \quad (2.3)$$

The rolling angle (α) is a function of the imposed pitch, which is proportional to the dimension of the unit cell in the z-axis (p^*z), and the number of unit cells in the x-axis (n^*x). The z-axis represents the height (h) of the lattice and the x-axis the width (w). The radius (R) of the folded cylinder is a function of the rolling angle and the width of the lattice.

Figure 2.2: Schematic representation of the nanotube preparation procedure. Two different unit cells with different arrangement of C8S3 molecules (top left) were used to prepare 2D lattices (top right). Rolling angles for the lattices were determined based on the size of the unit cells and the imposed pitch (bottom left), and the lattices were rolled into nanotubes with specific radius, R (bottom right).

Again, the C8S3 monomers in the initial unit cells were flattened to avoid atom overlaps during the rolling procedure. Additionally, the bond distance of the alkyl tails was reduced to prevent clashes of the inner and outer wall molecules. These changes to the C8S3 molecular structure did not lead to unnatural tube structures, since the natural orientation of the C8S3 atoms was restored during the minimization and equilibration steps.

Short nanotube simulations (~ 1200 C8S3 molecules) with a tube length of ~ 15 nm were generated. According to cryo-TEM measurements, the inner and outer radii are ~ 3.0 and ~ 6.5 nm [74], respectively. The rolling angle of the lattice was set to $\sim 30^\circ$. The constructed C8S3 nanotubes were solvated in cubic boxes with water. The size of the periodic boxes was $25 \times 25 \times 25$ nm to allow ~ 5 nm between nanotube and the periodic boundary conditions. Furthermore, simulations of solvated nanotubes with ~ 5000 - 8000 C8S3 molecules, referred as long nanotubes with length ~ 75 - 100 nm were prepared. The radius of the inner and outer wall was set to ~ 3.0 and ~ 6.5 nm and different rolling angles of the lattices were tested, ~ 20 - 50° . The box size was $\sim 18 \times 18 \times 100$ - 120 nm, depending on the length of the nanotube.

The extremely large length of the box (z-axis) compelled us to make compromises in the x- and y-dimensions of the simulation box, in order to save computational resources. More details for the initial configurations of the simulated systems are included in the Appendix, Table 6.2 and 6.3.

Regarding structural features of the nanotubes, the thickness of the double-wall was measured by using the electron density of the system. For estimating the dimensions of the C8S3 nanotubes based on the electron density profiles, the thickness of the double-walled object was measured by calculating the distance between the highest peaks of the SO_3^- electron density in the inner and outer wall. To diminish the effect of disordered regions, we calculated the electron density profiles from different angles perpendicular to the long axis (z-axis) of nanotube by making scans every 45° (Figure 2.3). The eight profiles from each angle were averaged, and the mean value for the nanotube thickness along the trajectory was calculated.

Figure 2.3: Graphical representation of the electron density profile scans along the z-axis. The electron densities are obtained from different angles and the average distance between the highest peaks is calculated. The inner and outer SO_3^- groups are coloured in red and orange, respectively, for clarity. The thickness is calculated by measuring the distance between the position of the highest peak of the inner and outer wall.

2.4 Results

2.4.1 C8S3 Parametrization

Molecules of this family of cyanine dyes have not been previously parametrized for the GROMOS force fields, so we resorted to higher levels of theory (QM methods) to improve the C8S3 parameters. In general, during the refinement of force fields for new classes of molecules, optimization is mainly directed towards dihedral angles and partial charges. The G53a6 [57] force field mainly focuses on small molecules, and such large conjugated systems are not necessarily well described by the standard

parameters. In order to study the properties of the aromatic core and optimize the dihedral angles of the polymethine bridge of the C8S3 molecule, we prepared and used C1C1 as a reference for QM calculations (Figure 2.4a). Reducing the size and complexity of the system allows us to concentrate on the native conformations and dynamics of the core. Furthermore, smaller systems tend to be more accurate and less expensive for calculations at the QM level. The dihedral profiles for torsions around the bonds C5-C3-C2-C4 and N9-C5-C3-C2, respectively, are presented in Figure 2.4b and the force constants for the fitted dihedrals in Table 2.1. A proper dihedral description was used and its functional form is shown in Eq. 2.4. The dihedral definition was splitted among different atoms involved in the rotation around those bonds. The phase and the multiplicity of all torsional angles was 180° and 2, respectively.

$$U_{dihedral}(\phi_{ijkl}) = k_\phi(1 + \cos(n\phi - \phi_s)) \quad (2.4)$$

k_ϕ is the force constant, n is the multiplicity and ϕ_s is the phase.

Figure 2.4: a) Chemical structure of C1C1 monomer. The atoms involved in the dihedral angle definitions are highlighted on the bottom panel. b) Potential energy profiles for dihedrals C5-C3-C2-C4 (top) and N9-C5-C3-C2 (bottom).

The MM profiles are in good agreement with the QM profile. We were able to reproduce the width of the well near the energy minimum. The reason for not being able to capture the height of the curves is the type of dihedrals that we decided to use for these descriptions. Modelling the dihedral potentials with more complex descriptions, rather than simple cosine functions, could give better agreement with the QM energy profiles, but was beyond the scope of this study. The systems in

Dihedral	Force constant (kJ/mol)
H1-C2-C3-H7	6.44
C5-C2-C3-C4	25.74
N9-C5-C3-C2	19.30
N9-C5-C3-H7	4.82

Table 2.1: Dihedral angles for the definition of the polymethine bridge

the QM calculations for the C5-C3-C2-C4 became unstable for dihedral angles lower than 50° and higher than 310° probably due to steric clashes. This part of the energy profile was not calculated and was excluded from the plot.

Additionally, the partial charges of C1C1 and C8S3 were calculated at QM level. The procedure is described in the Methods section and the results reported in the Appendix, Table 6.4 and 6.11.

2.4.2 C8S3 bilayers

Several bilayers were simulated to measure structural features of C8S3 molecules in small systems representative of vertical slices of the double-walled C8S3 nanotube. We were mainly interested in the thickness and the packing arrangement of these formations, since they can be compared to the available experimental results from AFM [72] and X-ray crystallography [53, 54], respectively. The packing arrangements tested were a brickwork, a herringbone and a staircase formation illustrated in Figure 2.5b. Additionally, we tested different conditions, such as solvent types, temperature and force field parameters. Initial and final frames of simulations are presented in Figure 2.5a and 2.5b, which show side views and top views of the simulated systems, respectively.

While constructing each system, the thickness of each bilayer was set to ~ 3.5 nm, close to the experimental values from AFM [72] and cryo-TEM [41, 43, 44, 45, 46] for the C8S3 aggregate thickness. In all cases the bilayers collapse from the separated leaflet formation to a partially interdigitated one with smaller thickness and interdigitated tails (Figure 2.6a). Furthermore, the bilayers become slightly curved in all simulations. Regarding the lateral arrangement, the brickwork formation was the most stable arrangement, since it remained almost intact throughout the simulation, Figure 2.5b. The herringbone formation looks in general disordered, but locally small regions maintained the initial arrangement, whereas the staircase formation was completely lost.

In general, the simulated values for thickness (2.48 ± 0.12 nm - mean value $\pm$ standard deviation of the mean values for each system), as measured from the distance between the centre of mass of the SO_3^- groups in the upper and lower leaflets, are smaller than the reported experimental values (3.00 ± 0.15 nm) from AFM [72], Figure 2.6a. AFM experiments were performed at 288 K, whereas the MD simulations were conducted at 300 K. Small changes in the temperature can affect the thickness and the organization of the lipid tails in the vicinity of the phase transition. However, if such an effect is present, it was not captured by our simulations with G53a6 force field at 288 K. Looking at the raw height histogram in the AFM experiments suggests that the thickness of the bilayer is most likely

Figure 2.5: Snapshots from C8S3 bilayers with G53a6 before and after the MD simulation for each arrangement. a) Side view of the bilayer. Water is represented as transparent surface and Na⁺ cations as magenta spheres. b) Top view of one bilayer leaflet, From left to right, brickwork, herringbone and staircase arrangement. Solvent and side chains have been removed for clarity. The red arrows indicate the average direction of the core.

smaller than 3.0 nm and larger than 2.5 nm. Methanol does not seem to affect the thickness or the order of the monomers with the G53a6 force field, whereas in the GAFF simulations, the thickness and the order are slightly higher. Furthermore, the method of calculating the bilayer thickness does not dramatically change the results since the deviations vary from 0.1 to 0.3 nm. The comparison of the measurements is included in the Appendix, Table 6.6.

As reported in the Appendix, Table 6.7, for all simulated systems, the aliphatic tails are not parallel to the bilayer normal (Tail order parameter: 0.17 ± 0.06), the area per molecule does not change significantly among simulations (area per molecule: 0.86 ± 0.04 nm²) and the C8S3 molecules have diffusion coefficients indicative of low lateral mobility (Diffusion coefficient: 7.80 ± 4.06 μm²/s). It has to be noted that among the three arrangements, the brickwork formation presents the highest order in the aliphatic tails and the lowest values for diffusion. Even though the differences are subtle, they confirm the observations from the visual inspection of the bilayers, Figure 2.5b.

Figure 2.6: a) Comparison between experimental bilayer thickness measured by AFM and the thickness calculated with MD simulations. b) Order parameter (P_2) for the orientation of the polymethine bridge with respect to average direction in the initial structure. P_2 ranges from -0.5 to 1, where values close to -0.5 indicate that the aromatic core vector and the average direction are perpendicular, where values close to 1 indicate that the vectors are parallel. Values close to 0 indicate either a lack of preference (random orientation) or orientation at the magic angle (54°). The error bars correspond to standard deviation.

2.4.3 C8S3 nanotubes

C8S3 nanotubes of different dimensions were built to test the ability of the constructed models to maintain a tubular formation. Then, structural properties, such as inner/outer wall radii and nanotube thickness, were measured and compared with experimental observations.

Regarding the short C8S3 nanotube simulations, we report the results from four 100 ns simulations, two with the brickwork and two with the herringbone formation. Initial inner and outer wall radii, as well as rolling angles for each wall are reported in Table 6.2. The thickness of the nanotubes at the beginning of the simulations was ~ 3 nm (effectively ~ 2.5 nm when the tails adopt a more natural configuration). All simulated systems maintained the tubular structure during the simulations. The nanotube thickness converged to an average value of 2.35 ± 0.04 nm (Figure 2.7), which is lower compared to most of the experimental measurements, $3.5\text{-}4 \pm 0.5$ nm [41, 43, 44, 45, 46]. The final dimensions of each nanotube are reported in the Appendix, Table 6.8.

The performance of our models was also tested in long C8S3 nanotubes that maintained the tubular formation for 20 ns. Regardless of the force field, the results

Figure 2.7: Comparison between experimental C8S3 nanotube thickness measured by cryo-TEM and the C8S3 nanotube thickness calculated with MD simulations.

Figure 2.8: Snapshots from the 100 nm C8S3 nanotube after 20 ns of simulation. a) Side view along the x-axis (atomistic resolution). b) Side view along the y-axis (atomistic resolution). c) Side view along the x-axis (transparent isosurface). d) Top view (atomistic resolution).

are roughly the same and suggest a thickness of 2.55 ± 0.05 nm. Snapshots from the final frame of a long nanotube simulation show how the tubular formation was maintained at the end of the trajectory, Figure 2.8. The final dimensions of the long nanotubes are reported in the Appendix, Table 6.8.

2.4.4 MD simulations and cryo-TEM

In order to resolve the apparent discrepancy between the thickness of the simulated nanotubes and the experimentally measured ones, we directly compare the electron density profiles from the simulation with long nanotubes to the contrast signal from cryo-TEM results [46], Figure 2.9. Both profiles are actually very similar. The superimposition helps us interpret the results from the cryo-TEM experiments, since the separation of the inner and outer wall signal is feasible in the MD simulations. Indeed, the diameter of the object is fairly well estimated from the contrast profile, but the boundaries of the inner wall do not correspond to the highest electron density point of the inner wall or the beginning of its signal. This deviation could be attributed to the special shape of the aggregate or the curvature on the surface of the nanotube. The hollow cylinder structure makes the definition of the inner wall signal extremely difficult from only the cryo-TEM signal. The problem in the end lies in converting and interpreting the structural features of a complex 3D-object based on one dimensional signals, especially when structural features (diameter and thickness) are at the same order of magnitude and close the maximum resolution of the experimental technique.

Figure 2.9: Superimposition of the electron density profile from MD simulations and a cryo-TEM profile (top graph). Electron density profiles of each MD system and the corresponding SO_3^- group (bottom graph). Black dashed lines indicate the positions of the cryo-TEM boundaries for the definition of the inner and outer wall and the red dashed lines highlight the positions for measuring the nanotube thickness in the MD simulations.

2.4.5 MD simulations and SAXS

To validate further our simulation results, we conducted SAXS experiments to characterize the structural features of C8S3 nanotubes. The obtained SAXS spectra were compared to MD simulated spectra [75] from short C8S3 nanotubes with different dimensions and are reported in Table 6.8. The experimental spectrum was also compared to a number of simple models for the electron density distribution in tubular assemblies, Figure 2.10.

Figure 2.10: Comparison of SAXS spectra, MD simulated spectra and analytical models. a) SAXS profile for the C8S3 nanotubes in water-methanol solution together with the simulated curves using an analytical model for a long hollow nanotube with three concentric shells (black line) and the scattering intensity from the MD simulated structure (red line, Herringbone 1). b) Guinier analysis to determine the nanotube cross-sectional radius R_c . c) Schematic radial electron density profile from the nanotube centre 0 until the outer R_{out} wall. The relative electron densities in the inner and outer wall and in the aliphatic region are indicated by the heights of the blue and red bars overlaying the atomistic structure. d) Comparison between the SAXS experimental data and the simulated intensity from a three concentric shell cylindrical model with three different total shell thicknesses, 2.0 nm, 2.5 nm and 3.0 nm, but same outer radius $R_{out} = 5.8 \text{ nm}$.

The scattering curve of the C8S3 nanotubes prepared via the alcoholic route [76] shows a decaying trend with the scattering vector q , characterized by several oscillations characteristic for the size of self-assembled objects. The decay of the intensity in the low q region ($q < 0.5 \text{ nm}^{-1}$) is typical for long anisotropic objects, in agreement with the long nanotubes observed by cryo-TEM [41, 43, 44, 45, 46], Figure 2.10a. In order to first measure the radius of the nanotubes, we have estimated the cross-sectional radius using the Guinier analysis [77]. The cross-sectional Guinier plot for the C8S3 nanotubes is plotted in Figure 2.10b. The cross-sectional radius of gyration R_c is found to be 5.8 nm.

The region for $q < 1 \text{ nm}^{-1}$ can be well described by the scattering intensity from a cylinder with three concentric shells of high-low-high electron density (black curve in Figure 2.10a), with parameters $R1 = R_{in} = 3.3 \text{ nm}$, $R2 = 3.7 \text{ nm}$, $R3 = 5.4 \text{ nm}$ and $R4 = R_{out} = 5.8 \text{ nm}$ (see Figure 2.10c). The discrepancy between the experimentally measured scattering intensity and the model for $q > 1 \text{ nm}^{-1}$ can be ascribed to several factors. Size polydispersity, structural disorder and limited instrumental resolution can smear the intensity oscillations. Moreover, this portion of the curve is expected to also contain contributions from the intermolecular distances among the molecules of the nanotube walls that are not considered in the analytical model.

Notably, the low- q region can be used to verify the validity of the structural parameters from MD calculations. Figure 2.10d shows the comparison between the experimental data and the simulated curves with different shell thickness but same nanotube outer radius (5.8 nm). It can be seen that the shape and first minimum position of the experimental data can be well reproduced by the model with total shell thickness 2.5 nm.

These SAXS results indicate that the optimal thickness calculated from MD is highly probable. Moreover, the simulated scattering spectra from the MD nanotube structure (red curve in Figure 2.10a) is in excellent agreement with the experimentally measured SAXS data.

2.4.6 MD simulations and optical spectra

The optical properties of C8S3 nanotubes were simulated by converting structures from the MD simulations of long nanotubes to Frenkel exciton Hamiltonian [48, 49]. In this type of calculations, the chromophores of each molecule are represented as vectors (transition dipoles) with specific magnitude and orientation and the basic form of the Hamiltonian usually includes two parameters, one for the electronic excitation of one monomer and another one for the dipole-dipole interactions (excitonic coupling) of different molecules. The optical spectra are then obtained by numerically diagonalizing the Hamiltonians.

Several long nanotubes have been constructed to test the effect of structural parameters (lattice density, radii and rolling angles) on the MD simulated nanotubes and the simulated spectra. Combining MD and absorption spectra simulations allows us to: 1) evaluate the stability of the constructed structures, unreasonable initial conformations will be severely deformed during the simulations, 2) introduce disorder, ideal models lack disorder that should naturally be present inside such systems, and 3) distinguish between those structures that reproduce the experimental absorption spectra and those that do not. The initial and final positions of the transition dipoles for the simulated systems are included in the Appendix, Table

6.9.

By iterating through nanotubes with different structural parameters, we have found optimal values (System 4*) that present excellent agreement with the experimental absorption spectra of the C8S3, Figure 2.11a, and capture the different contributions of the inner and outer wall to the total spectra as reported through experiments [74], Figure 2.11b.

Figure 2.11: a) Total absorption spectrum of System 4* compared to the experimental one. b) Contributions to the absorption spectrum from the inner and outer walls of System 4*: dashed lines correspond to the spectra of the ideal structure before the MD simulations.

2.4.7 Other systems

Continuous C8S3 nanotubes along the periodic boundary conditions (thereby modelling an infinitely long tube) have been also constructed, but they were severely deformed during the production phase. In all nanotube simulations, the double-wall collapsed generating a lack of available water molecules inside the nanotubes. In the solvated nanotubes, water from the surroundings migrated inside the nanotube, but in the periodic ones the increased pressure inside the aggregate forced the structures to contract. A snapshot from a deformed periodic nanotube is presented in Figure 2.12a and 2.12b.

2.5 Conclusions

To summarize, we performed atomistic simulation of C8S3 molecules in bilayers and nanotubes. Most simulated systems resulted in structures close to the initial formations indicating both Brickwork and Herringbone arrangements to be plausible and with Brickwork arrangement being more stable. Furthermore, we were able to construct stable nanotubes of different sizes that maintained their tubular formation in contrast to previous attempts to simulate these systems.

MD simulations suggest that the thickness of the C8S3 aggregates is ~ 2.3 - 2.5 nm. This observation is also supported experimental results from SAXS, indicating a thickness of ~ 2.5 nm for the C8S3 nanotubes, and theoretical spectra simulations, showing almost perfect agreement with experimental absorption spectra.

Figure 2.12: Snapshots from the periodic C8S3 nanotube after 10 ns of simulation. Side view from different perspectives a) and top view b). The nanotube lost the tubular formation and adopted a ellipsoid shape.

Even though, the electron density profiles from simulated C8S3 nanotubes are similar with the contrast profiles from cryo-TEM, the measurements for the thickness are quite different. This difference could be attributed to the special shape and/or the size of the aggregate that make the definition of the inner wall extremely difficult to obtain from only cryo-TEM signal alone. Consequently, we argue that the actual thickness of the C8S3 nanotubes is lower than the value of $\sim 3.5\text{-}4.0$ nm suggested by cryo-TEM measurements [41, 43, 44, 45, 46].

There are additional data that support our measurement for the nanotube thickness and are summarized in Figure 2.13. In more detail, if two C8S3 molecules are placed stretched and flat next to each other, the maximum distance between the SO_3^- groups would be ~ 4 nm, Figure 2.13a. However, this arrangement suggests there are gaps between the aliphatic tails. At room temperature, the area occupied by the aliphatic tails is ~ 0.2 nm² in lipid bilayers [78], and in the C8S3 bilayers the area per molecule is ~ 0.86 nm², Table 6.7 in the Appendix. Consequently, in the bilayer formation for every C8S3 molecule approximately four aliphatic tails are required to fill the available space suggesting a strongly interdigitated model, Figure 2.13b. The thickness of the C8S3 bilayer in this formation is ~ 3 nm. However, to reach this thickness, the aliphatic tails would have to be in the gel phase close to room temperature, but the length of the tails does not suggest so. Looking at a slightly different type of system, but nevertheless well-studied, such as lipids, the temperature for the transition to the gel phase is correlated with the length of their alkyl tails. In other words, as their length decreases, so does the temperature at which they enter the gel phase. For example, DLPC and DMPC lipids have twelve and fourteen carbons in their aliphatic tails, respectively, and their transition temperature to the gel phase is 277 and 297 K, respectively. Even though DLPC and DMPC lipids are different from C8S3 molecules, all of them are amphiphiles that have hydrophylic head groups and long hydrophobic tails and self-organize in specific type of structures. In C8S3, the size of the aliphatic tails is eight carbons suggesting that the transition temperature to the gel phase is significantly lower than 277 K. It is quite unlikely that the molecules are actually stretched to that extent at room temperature. This argument is also supported by the values of the aliphatic tail order parameter during the simulations, Table 6.7 in the Appendix. To summarize, the most realistic representation for the arrangement of the C8S3 molecules would be when the tails are disordered (melted) and interdigitate to fully occupy the inter-

leaflet space (Figure 2.13c). The thickness of the bilayer in this formation is ~ 2.5 nm.

Figure 2.13: Graphical representation of C8S3 molecules position in a bilayer. a) Suggested arrangement of monomers in the nanotube. b) Arrangement based on the AFM measurements. c) Snapshot from a bilayer simulation. The aliphatic tails are splayed and strongly interdigitated.

With all this information in mind, we consider that the real values for the thickness of the C8S3 nanotubes is closer to the size of the C8S3 bilayers as measured by AFM and SAXS, and it should be taken into account when modelling these system for studying their optical and/or structural properties.

Chapter 3

Coarse-graining cyanine dyes

"The saddest aspect of life right now is that science gathers knowledge faster than society gathers wisdom."

- Isaac Asimov, 1920-1992

3.1 Abstract

Self-assembly is a ubiquitous process spanning from biomolecular aggregates, such as proteins and lipids, to nanomaterials. Even though, the results of building blocks aggregating into ordered structures are visible through experimental techniques, the principles and mechanisms of self-assembly are yet poorly understood. This limits the predictive power in designing building blocks with specific final properties. At the same time, modelling the self-assembly with computational methods is not trivial, due to the lack of optimized parameters for different building blocks and the time scales of self-assembly that are usually beyond the limits of classical simulations. In this chapter, we describe the procedure of constructing a CG model for a class of cyanine dyes. We optimized the bonded parameters based on different levels of theory, performed random self-assembly simulations and tested the CG model on preformed aggregates, where structures were available. The aggregates were studied in terms of local arrangement. Our results provide guidelines on obtaining optimal parameters for CG simulations.

3.2 Introduction

Cyanine dyes are a family of molecules that contains a polymethine bridge, a chain with an odd number of methine groups ($=\text{CH}-$) connected via alternating single and double bonds, with nitrogen atoms on both ends, Figure 3.1a. The conjugated system created by the pi-orbitals of the polymethine bridge provides a chromophore responsible for the optical properties of each cyanine dye. The number of methine groups affects the position and strength of the absorption band, whereas the nature of the side groups attached to the nitrogen atoms influence not only the optical properties, but also the way in which the monomers organize when they form aggregates.

Figure 3.1: Examples of different cyanine dyes. a) Generic cyanine dye chemical type, b) 1,1'-Diethyl-2,2'-cyanine, Pseudo-isocyanine (PIC), c) 5,5',6,6'-tetrachloro-benzimida-carbocyanine ($R=\text{H}$; C0C0). The R-tails can be substitute with various chemical groups giving rise to different optical and structural properties.

In different solvents, cyanine dyes form organized structures that exhibit different optical properties compared to the solvated monomers. The special optical properties were first reported in 1936, when Jelley [79] and Scheibe [80] independently observed significant changes in the absorption spectrum of the dye pseudo-isocyanine (PIC), Figure 3.1b, in aqueous solution at increased dye concentrations. More specifically, the broad absorption band of PIC vanished and a narrow absorption band was observed at lower energies. Later, it was suggested that the strong coupling of the individual molecules which gave rise to special electronic excitations was responsible for the spectral changes [81]. At that time, the concept special excitation states (excitons) had already been described by Frenkel in insulator lattices [82]. Frenkel excitons constitute extended electronic excitations coherently shared by a number of molecules, while electrons remain localized on the same molecule. Excitons can be treated as chargeless particles able to transport energy, without transporting net charge, and they are usually observed in materials with a small dielectric constant, such as molecular crystals, polymers, etc.

In general, changes in the absorption bands with respect to the monomer is a common trait of molecular aggregates. Aggregates whose absorption band shifts to higher spectrum (red-shifted) are called J-aggregates or Scheibe aggregates after Jelley and Scheibe, who observed this phenomenon, whereas aggregates whose absorption bands shifts to higher absorption energies and lower spectrum (blue-shifted) are called H-aggregates (hypsochromic shift). The spectral changes depend on the structure of the aggregates and relative positions among the monomers. Understanding the driving forces that govern the formation of specific structures and the relationship with the monomer arrangement is of major importance in order to design molecules with specific chemical groups and, ultimately, predict the supramolecular structures and optical properties.

One of the cyanine dyes that has been extensively studied the past decades is 5,5',6,6'-tetrachloro-benzimida-carbocyanine [38, 39], Figure 3.1c. Several molecules with this specific core and different substituents have been designed and synthesized, and their structural and optical properties have been examined [40, 41]. Interestingly, different types of aggregates were formed ranging from bilayers and ribbons, to wires and nanotubes, depending on the chemical nature of the substituents and their size. In this study, we were interested into three different cyanine dyes that have the same aromatic core, but different side chains, Figure 3.2:

- 1,1',3,3'-tetraethyl-5,5',6,6'-tetrachloro-benzimida-carbocyanine (TTBC/C2C2)
- 1,1'-diethyl-3,3'-bis(4-sulfobutyl)-5,5',6,6'-tetrachloro-benzimida-carbocyanine (TDBC/C2S4)
- 1,1'-dioctyl-3,3'-bis(2-sulfopropyl)-5,5',6,6'-tetrachloro-benzimida-carbocyanine (C8S3)

Figure 3.2: Chemical structure of a) C2C2, b) C2S4 and c) C8S3.

Based on experimental results, mainly from cryo-TEM, C2C2 forms hollow one-layer nanotubes [83, 25], C2S4 forms monolayers or nanotubes [40, 84] and C8S3 forms double-walled nanotubes [41, 43, 44, 45, 46], Figure 3.3. The structure of C2C2 and C8S3 aggregates have been previously studied with MD simulation by testing the stability of different types of aggregates and monomer arrangements [25, 52]. However, no small or large scale self-assembly simulation has been reported for any of these molecules.

Figure 3.3: Images of cyanine dye aggregates from different experimental techniques. a) Cryo-TEM image of 0.54 mM C2C2 in water-methanol solution [25], b) Cryo-TEM image of 0.28 mM C2S4 in water [43]. The darker regions indicate the presence of a sheet-like structure. c) High Resolution Scanning Electron Microscope (XHR-SEM) image of 1.0 mM C2S4 in water [84]. d) Cryo-TEM image of 0.33 mM C8S3 in water-methanol solution [74]. Suggested models for the arrangement of the monomers are shown. All images were obtained and adapted from the original publications.

Intrinsic pitfalls of atomistic MD simulations render the task of obtaining ordered aggregates from random self-assembly simulations extremely difficult or even beyond the realm of feasibility, at least by using brute force atomistic MD simulations [1]. The main issues are the time scales, at which self-assembly occurs, and the free energy landscape of the procedure, that is rugged with many local minima in which the systems get trapped. Coarse-grained (CG) approaches are a common solution to alleviate these problems. The simplified representation of the atomistic coordinates and the smaller number of interactions that need to be calculated in CG simulations offer significant speed-ups and smoothen the free energy landscape, facilitating the formation of self-assembled aggregates [85, 86, 87].

Our goal was to design a CG model able to accurately represent the spatial

and dynamic properties of the common aromatic core and use it as a template to build each cyanine dye by changing the side chains, respectively. To achieve this, we optimized the atomistic parameters for the aromatic core based QM calculations and tested their performance on available crystal structures of cyanine dyes. The optimized atomistic parameters were used as a reference for tuning the bonded parameters of a CG model with the latest version of the Martini force field. Its fastidious parametrization procedure, the flexible mapping scheme and the transferability of the building block approach allowed us to construct an accurate representation of the cyanine dye aromatic core and its side chains. The final CG models were tested in unbiased small and large scale self-assembly simulations. Additionally, simulations of preformed structures were attempted for C8S3, whose structure was extensively studied in Chapter 2. The workflow for constructing the CG model is described in the following sections.

3.3 Creating the CG model for cyanine dyes

3.3.1 QM calculations

QM methods were used to obtain different sets of parameters, whose performance was then evaluated in atomistic simulations. After optimizing the geometry of the C0C0 (Figure 3.1c, where R represents hydrogen atoms) and C1C1 (Figure 3.1c, where R represents methyl groups) molecules with the Hartree-Fock method and 6-31G* basis set, point charges that are used in MD simulations were generated in two different ways: Dipole Preserving Analysis (DPA) [70] using the GAMESS-UK software [71], and Restricted Electrostatic Potential (RESP) [88] method using the Gaussian16 software [89]. Partial charges for cyanine molecules are reported in the Appendix, Table 6.10 and Table 6.11.

As discussed in Chapter 2, optimization was required in the dihedral angles of the polymethine bridge to ensure that the large conjugated system of the cyanine dyes is adequately described. Again, the torsional potentials to be added to the dihedral between atoms C2-C3-C5-N9 and C4-C2-C3-C5 have been determined based on the energy difference between the QM and the MD dihedral profiles for C0C0 and C1C1. The atom names of the aromatic core are the same as for the C8S3 core and they are shown in the Appendix, Table 6.4. QM dihedral profiles were obtained by performing a relaxed scan with angle increments of 5 degrees using the Gaussian16 software. For these scans, ω B97xD functional with 6-311G(d,p) basis set was used. MD dihedral profiles were obtained by performing similar relaxed dihedral scans, where the existing dihedral potentials on the dihedrals of interest were removed. Then, the difference between the QM and MD profiles was fitted to a Ryckaert-Bellemans (RB) type of function, Eq. 3.1. The force constants for optimized MD parameters are reported in Table 3.1.

$$V_{RB} = \sum_{n=0}^5 C_n (\cos(\theta - 180))^n \quad (3.1)$$

n is the number of added potentials, C_n is the RB coefficient for each potential and θ is the dihedral angle.

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Molecule	Method	Dihedral	C1 (kJ/mol)	C2 (kJ/mol)	C3 (kJ/mol)	C4 (kJ/mol)	C5 (kJ/mol)	C6 (kJ/mol)
C0C0	HF-DPA	C5-C3-C2-C4	103.0725	35.0723	-131.2985	-26.7065	48.1962	10.6666
		N9-C5-C3-C2	57.7083	5.2693	-79.2583	-9.3074	23.1247	5.1088
C0C0	HF-RESP	C5-C3-C2-C4	96.3923	23.1281	-134.1660	-28.4803	47.4333	13.6272
		N9-C5-C3-C2	62.1772	1.8762	-89.1708	-3.8494	27.5176	2.0697
C1C1	HF-RESP	C5-C3-C2-C4	99.6354	26.5038	-136.8010	-37.0012	52.2696	22.8211
		N9-C5-C3-C2	59.4377	4.6123	-85.0340	-11.1293	27.2898	6.3321

Table 3.1: Force constant coefficients for RB potential functions for C0C0 and C1C1 with different methods.

It is evident that the choice of QM method affects the results and the values for the force constant coefficients show variations. However, the values are not dramatically different. Even for C0C0 and C1C1, the force constant coefficients are pretty similar. The R squared for all fitted profiles was ~ 0.99 , suggesting the MD profiles are almost identical to the QM ones. An overlap of the energy profiles between the QM and MD methods for C0C0 is shown in Figure 3.4.

Figure 3.4: Potential energy profiles for dihedrals of C0C0 for the HF-RESP method, a) C5-C3-C2-C4 and b) N9-C5-C3-C2.

Since the values for the RB coefficients of the dihedral angles are similar between C0C0 and C1C1, we could claim that the contribution of the substituents does not

significantly affect the potentials for the rotation around the bonds of the polymethine bridge. To maintain simplicity, the respective C0C0 force constant coefficients (DPA/RESP) were used for the simulated cyanine dyes.

3.3.2 MD simulations - Atomistic

After obtaining different sets of optimal parameters at the QM level, we conducted different types of atomistic simulations to test their ability to reproduce experimental results and use the best candidate as a reference for the CG model. All simulations were performed with the GROMACS 2018 simulation package [55, 56]. For the atomistic simulations, an extended version of the GROMOS force field version G54a7 [90] was used that incorporates recent parameters for molecules from the ATB database [73] and can be found on the ATB website (<https://atb.uq.edu.au/>). In this version, there is an additional atom type for carbons in aromatic rings (CAro) that was used for representing the carbons of the cyanine core. Furthermore, parameters for iodine (ATB Molecule ID:337757), dimethyl-sulfoxide (DMSO, ATB Molecule ID:1202) and methanol (ATB Molecule ID:15607), that were present in the crystal structures of the cyanines dyes, were also downloaded from the ATB database. Simulations with solvated cyanines dyes in water were used to obtain distributions for the bonded terms of the CG model. In those cases, explicit SPC water molecules [58] were used to model water and counter ions, either Cl^- or Na^+ , were introduced to neutralize the systems.

Regarding pressure coupling, the Berendsen barostat [62] was used to maintain the pressure constant at 1 bar with a time constant of 1 ps and compressibility of $4.5 \times 10^{-5} \text{ bar}^{-1}$. Isotropic pressure coupling was used for most systems. On the contrary, the crystal simulations were performed in anisotropic boxes, where the pressure of each dimension of the box was decoupled from the other dimensions. In all systems, the temperature was kept constant at 300 K by using the V-rescale algorithm with a time constant of 0.1 ps [61]. The cut-off for electrostatic and van der Waals interactions was set to 1.4 nm and the Verlet update scheme [63] was used for the short range non-bonded interactions with a buffer tolerance of 0.005 $\text{kJ mol}^{-1} \text{ ps}^{-1}$. Long range interactions were calculated with the Reaction Field method [64] for maximum performance during the production phase. The LINCS algorithm was employed for constraining the bond lengths [65].

All systems were minimized for 1000 steps using the Steepest Descent algorithm. The production phase of the crystal simulations started immediately after the minimization and it lasted 200 ns. In contrast, the systems with solvated cyanines in water were equilibrated first in NVT and then in NPT conditions, each for 10 ps with a 1 fs time step. Their production phase was performed in the NPT ensemble and its duration was 100 ns. The time step for integration was set to 2 fs for the production phase of all atomistic simulations.

3.3.3 Crystal simulations

The crystal structures of two cyanines dyes with the 5,5',6,6'-tetrachloro-benzimidacarbocyanine core and different substituents, C2C2 [53] and C8O3 [54], were simulated to evaluate the performance of the QM generated parameters. The unit cell of each crystal is presented in Figure 3.5. To prepare the initial structure for the

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simulations, the unit cells for C2C2 and C8O3 were replicated along each dimension by a factor of 3 resulting in supercells consisting of 27 unit cells and 108 cyanine dye molecules. C2C2 crystal structures also included 108 iodine molecules to neutralize the charge and 216 methanol molecules, whereas C8O3 crystal structures included 54 DMSO molecules and no counter-ions.

Three sets of parameters for the C2C2 and C8O3 molecules were used in atomistic crystal simulations:

- Model 1: DPA charges from QM calculations (B3LYP/6-31G*) on C1C1 and cosinoid dihedral definitions (the parameters were Chapter 2, Table 2.1)
- Model 2: DPA charges (HF/6-31G*) from QM calculations (ω B97xD/6-311G(d,p)) and Ryckaert-Bellemans dihedral definitions
- Model 3: RESP charges (HF/6-31G*) from QM calculations (ω B97xD/6-311G(d,p)) and Ryckaert-Bellemans dihedral definitions

Our goal was to find the best set of parameters that is able to maintain the experimental determined structures and, then, use it as reference for the CG model. The atomistic parameters were evaluated based on their ability to maintain structural features of the initial unit cells, such as box dimensions and density. Additionally, the root-mean-square deviation (RMSD) from the initial conformation and the position of the first peak in Radial Distribution Function (RDF) calculations are reported. The results from the crystal simulations are reported in Table 3.2.

Molecule	Analysis	Reference	Model 1		Model 2		Model 3	
			Average	Error	Average	Error	Average	Error
C2C2	X (nm)	4.012	4.218	0.001	3.989	0.001	4.003	0.003
	Y (nm)	3.310	3.444	0.001	3.480	0.001	3.426	0.001
	Z (nm)	6.451	6.015	0.001	6.127	0.001	6.160	0.001
	Density (kg/m ³)	1442	1470.09	0.17	1510.11	0.41	1520.69	0.21
	RMSD (nm)		0.311		0.187		0.118	
	RDF (nm)	0.868	0.807		0.883		0.875	
C8O3	X (nm)	4.974	5.326	0.004	4.925	0.001	4.999	0.006
	Y (nm)	4.682	5.184	0.008	4.765	0.001	4.695	0.003
	Z (nm)	5.201	4.422	0.005	5.131	0.002	5.141	0.008
	Density (kg/m ³)	1254	1245.19	0.56	1262.47	0.23	1260.11	0.50
	RMSD (nm)		1.081		0.877		0.885	
	RDF (nm)	0.420	0.487		0.453		0.418	

Table 3.2: Summary of crystal simulations analysis. Different structural features of the simulated systems are compared with the initial values of the crystal structure [53, 54]. The RMSD is calculated for every atom of the cyanine dyes. The RDF value refers to the position of the first peak for the distribution of the central atom of the cyanine core (C2). The error is calculated based on block averages over 5 blocks. The cells of the table are coloured based on the value of the simulated feature compared to the initial value. Green colour indicates that the reported value is $\pm 1\text{-}2\%$ of the initial value. Yellow represents $\pm 2\text{-}5\%$, orange $\pm 5\text{-}10\%$ and red $< 10\%$.

In general, even though the differences are quite small, the models with HF-RESP charges performed better in terms of maintaining the structural properties of

Figure 3.5: Crystal structure unit cells of cyanine dyes from different perspectives, a-c) C2C2 d-f) C8O3. Cyanine dyes are shown in licorice representation, methanol and DMSO in CPK and iodine as large spheres.

the cyanine crystal structures. Model 1 performed worse than the others, especially in the C8O3 crystal. Such behaviour was also observed in Chapter 2 and the bilayer simulations with the staircase formation, Figure 2.5. The staircase formation was generated based on the structure of the C8O3 crystal. The results for Model 2 and Model 3 are quite similar, but Model 3 reproduced slightly better the position of the first peak in the RDF calculation. The parameters of Model 3 were used for the atomistic simulations of cyanine monomers in water.

3.3.4 MD simulations - CG

For all CG simulations, the Martini 3 force field [10] was used. Cyanine dye molecules were solvated in Martini 3 water beads (W, representing 4 H₂O molecules), and either Cl⁻ (TQ5 bead with -1 charge) or Na⁺ (TQ5 bead with +1 charge) were used to neutralize the charge of each system. Methanol was also modelled with standard Martini 3 parameters (SP2r, representing 2 CH₄O molecules).

In all systems, the Berendsen barostat [62] was used to maintain the pressure constant at 1 bar with a time constant of 1 ps and compressibility of 3×10^{-4} bar⁻¹. The temperature was kept constant at 300 K by using the V-rescale algorithm with a time constant of 0.1 ps [61]. Van der Waals and electrostatic interactions were calculated using the Verlet list scheme [63] with the cut-off set at 1.1 nm and a buffer tolerance of 0.01 kJ mol⁻¹ ps⁻¹. Long range interactions were treated using the Reaction Field method [64] and the bond lengths were constrained by using the LINCS method [65].

Each system was minimized for 1000 steps using the Steepest Descent algorithm. Then, a short equilibration step followed in the NPT ensemble for 100 ps with a time step of 10 fs. The production phase for solvated molecules in water was 100 ns, whereas the self-assembly simulations lasted 10 μ s. Last, two nanotubes with length $\sim$ 50 nm ($\sim$ 3650 molecules) were prepared, one with the brickwork and one with the herringbone formation. The protocol for preparing the initial coordinates and carefully equilibrating the ions before the production phase is described in Chapter 2. The inner and outer wall dipole radii were 3.72 and 5.49 nm, respectively, and the rolling angles were 31.35° and 30.96°. The nanotube simulations were 1000 and 500 ns long, respectively. The time step during the production phase was 20 fs.

3.3.5 CG model preparation

In this section, the structural and chemical properties of the cyanine dye core and the side chains of each molecule will be discussed, and how these properties were tuned to create an accurate CG model for each cyanine dye. In more detail, we will focus on mapping, bead choices, surface area and the bonded distributions. The next paragraphs offer general guidelines on improving the bonded parameters of novel molecules at CG level.

1. Mapping: The first step of the CG model preparation was selecting a mapping for the aromatic core that would be rigid enough to maintain the flat surface of the benzimidazole rings and, at the same time, flexible enough to adopt different orientations of the side chains. The initial mapping of the cyanine core is presented in Figure 3.6a.

The benzimidazole rings are mapped with 4 real beads and 1 virtual site at their

Figure 3.6: Chemical structure of cyanine dyes superimposed with the CG model. a) The initial mapping of the aromatic core. The axis for the rotation of benzimidazole ring is presented as a dashed line. Each bead is coloured based on its type (C:gray and N:blue). The partial charge of the charged beads is reported. b) Bonds and constraints of the aromatic core are presented as green and black lines, respectively. c) Angles of the aromatic core. d) Dihedrals of the aromatic core. e) Bonds (green) and angles (orange) of the side chains for cyanine dyes.

centre of geometry. Virtual sites have no mass, so the mass of the respective bead has been evenly distributed to the 4 normal beads. In Martini 3, the mapping of aromatic moieties is based on centre of geometry of all atoms (heavy and hydrogen

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atoms) that participate in each chemical group. However, this rule is slightly flexible, since parametrization aims at optimizing the surface area of the molecules, and different definitions can be used to maintain the overall shape. Additionally, there is a dummy particle (red sphere) that allows the definition of the axis (dashed line) around which the benzimidazole ring is able to rotate, Figure 3.6a. In contrast to virtual sites, dummy particles do not interact via non-bonded interactions with any bead, and only act as supportive particles to allow specific conformations.

Constraints hold together the aromatic rings, whereas bonds connect the polymethine bridge with the benzimidazoles, Figure 3.6b. The angles and dihedrals that control the orientation of the aromatic core are presented in Figure 3.6c and 3.6d. There were no angles between the side chains and the central bead to allow free rotation around the defined axis. No dihedral definitions were used between the core and side chains, since the angle definitions were sufficient to describe the movement of the side chains. The aromatic core was the same for C2C2, C2S4 and C8S3, and the side chains were added as extensions to the core. The bonds and angles for the modelled side chains are shown in Figure 3.6e. Finally, the aromatic core of these cyanine dyes is positively charged. The extra charge was assigned to the beads that represent atoms of the polymethine chain, Figure 3.6a.

2. Bead types: Martini 3 offers a wide variety of bead types and sizes covering a vast number of chemical groups. The selection of the bead types for the aromatic core of the cyanine dyes and each substituent is shown in Figure 3.7. Default Martini 3 bead types were used for each chemical group.

Figure 3.7: Bead types for C2C2, C2S4 and C8S3. Each bead is coloured based on its type (C:gray, N:blue and Q:green). The size of the spheres is proportional to the size of the Martini 3 beads.

3. Bonded distributions: The bonded distributions of the CG model were optimized to reproduce the atomistic distributions of the respective chemical groups. To obtain the atomistic distributions, monomers of each cyanine dye of interest (C2C2, C2S4 and C8S3) were solvated in water boxes together with a counter ion and simulated for a short time. Then, monomers of the CG models were simulated in the same way and their bonded parameters were tuned by adapting their reference values and/or their force constants until there was sufficient overlap. The comparison of the bonded distributions between the atomistic and the CG model are included in the Appendix, Figure 6.1 to 6.4. The aromatic core parameters for all molecules was chosen based on C2C2 and the side chains of C2S4 and C8S3 were optimized for each molecule, separately.

Figure 3.8: Comparison of angle distributions for a C2C2 monomer in solution with default and stiff dihedrals for the polymethine bridge. a-c) The angle distributions of the flat molecule were used as reference for the CG model. d) Bead names of the C2C2 CG model.

It is important to note that the distributions of the bonded terms are affected by the surroundings of the molecule. For example, the aromatic core of the cyanine dyes adopts slightly different conformations in water and inside the aggregates. This

effect is evident in the bilayer simulations, Figure 2.5, as well as, in the C2C2 and C8O3 crystal structures, Figure 3.5, where the molecules are stacked and relatively flat. In these cases, the dihedrals of the polymethine bridge and benzimidazole rings are close to 180° . Since we want to obtain the final structures of the aggregates, the bonded parameters should be tuned in such a way that they reproduce the correct conformation in the final product. Otherwise, the CG models will be biased towards the conformations in water. To alleviate such effects, we tuned the angles that control the orientation of the benzimidazole rings based on an atomistic simulation where the dihedral angles for the polymethine bridge (C5-C3-C2-C4 and N9-C5-C3-C2) were stiff and kept the molecule flat, Figure 3.8.

4. Surface area: Maintaining the surface area of the atomistic models has become significantly important in Martini 3, especially with the new small bead sizes that are able to describe aromatic rings with high detail. After building the initial CG models, it is always a good idea to check the volume of the CG model and compare it with the atomistic values. If the surface area is different, alternative mappings or different bond lengths could give a better description for the shape of the molecule. In this case, the solvent accessible surface area (SASA) was calculated for the atomistic and the CG model, Figure 3.9. The initial mapping underestimated the volume of the molecule. Overlapping the volume of the atomistic and CG models revealed that the area around the chlorines was slightly smaller in the initial CG models (Model 1). The mapping was modified to increase the overlap of the two surfaces. Instead of mapping the SX3 bead on the centre of geometry of the CHCCl group (Model 1), the bead was placed at the centre of the C-Cl bond (Model 2). The surface of the C0C0 with the new mapping is almost identical to the atomistic value, Figure 3.9a.

The SASA was also calculated for C2C2 that has bulkier side chains, Figure 3.9b. In this case, the position of the beads that represent the ethyl groups was modified to match the atomistic surface area values. Specifically, instead of mapping the bead between the two carbons of the ethyl groups (Model 2), the bead was placed on the most distant carbon of the side chain (Model 3).

5. Other: Important details that should be always taken into account when creating CG models for novel molecules are:

- Minimum bond lengths. The bond lengths should not be smaller than the radius of the beads participating in the bond. Shorter bond lengths will result in stronger interactions, while making molecules more hydrophilic [91]. In that case, the initial mapping should change.
- Exclusions in aromatic/conjugated moieties. Close interactions of beads inside conjugated systems might affect the general morphology. Non-bonded interaction between these atoms should be excluded. Reasonable bonded terms should keep the intended structure in place.
- Collinear dihedral angles. Dihedral angles in which the angles between three consecutive atoms adopt values close to 180° should be avoided, since they make simulations extremely unstable [92].

Figure 3.9: Solvent accessible surface area for a) C0C0 and b) C2C2 models. The histograms represent the SASA for one monomer in solution for a trajectory of 100 ns. The number of dots for SASA in the histogram analysis was set to 10000, whereas for the surface representation the number of dots were 50.

3.4 Results of CG simulations

3.4.1 Self-assembly

The CG models for C2C2, C2S4 and C8S3 were used in self-assembly simulations, where random molecules were solvated in cubic boxes filled with water. Two setups were prepared for each system. First, a small scale self-assembly system with 100 monomers in a 10x10x10 nm box and, second, a large-scale simulation with 1000 monomers in a 25x25x25 nm box. Cl^- or Na^+ were placed randomly in each system to neutralize the total charge. In C2C2 systems, methanol was also added at $\sim 20\%$

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to represent experimental conditions. The small scale systems were preliminary simulations to study the early steps of nucleation and the morphology of small clusters, whereas the large systems were large enough to allow the formation of experimentally observed aggregates or partially formed structures. For the simulated time scales, no native formation similar to the experimental determined structures, Figure 3.3, was observed during the 10 μ s of each simulation. Snapshots from the last frames are presented in Figure 3.10.

Figure 3.10: Snapshots from large scale self-assembly simulations, a) C2C2, b) C2S4 and c) C8S3. The top panel corresponds to the small scale self-assembly simulation. The bonds and constraints are shown with lines. Each monomer is coloured differently. The bottom panel corresponds to the large scale self-assembly simulation. The aromatic core is coloured in blue, the hydrophilic tails in red and the hydrophobic tails in green. In C2C2, CA/CB beads are coloured in red and C1A/C1B beads in green, Figure 6.4.

All systems were analysed in terms of aggregate formation and local arrangement of the monomers with respect to each other. Specifically, the number of clusters in each system was calculated by measuring the number of molecules within a certain cut-off. If the central beads (C3) of the monomers were within 1.5 nm, the molecules were accounted to be in the same cluster. Cluster size gives an estimate of how fast the monomers aggregate and whether the clusters merge to form larger aggregates. However, it does not provide information on the orientation of the molecules and the dynamics inside the aggregates. In small systems, a cluster is rapidly formed and is maintained along the trajectory, Figure 3.11. A similar behaviour was also observed in the large systems, but for the simulated timescales, a single aggregate was only observed for C2C2.

Figure 3.11: Cluster size of monomers for self-assembly simulations. Small (left) and large (right) scale simulation. Monomers are considered to be in the same cluster if their central bead (C3) is within 1.5 nm of any other monomer.

To study the local arrangement inside the aggregates, the RDF of the central beads and the orientation of the polymethine bridges were measured at different time steps of the trajectories. For the orientation of the monomers, the P_2 order parameter (Eq. 2.1) for the angle theta (θ) between the normal of the plane formed by the NNA-C3-NNB beads was used, Figure 3.12a. Additionally, the relative position of each monomer was presented as a function of distance and angles between different vectors, Figure 3.12b.

Figure 3.12: Graphical representation of the monomer arrangement and the defined angles for the analysis of the local arrangement. a) P_2 was calculated based on the angle θ . b) Vector angle 1 (orange) and Vector angle 2 (cyan) were used to describe the orientation of the molecules with respect to each other.

The order inside the aggregates increases during the simulations, since characteristic peaks in the graphs become more and more evident. Additionally, the angles for the relative orientation of the monomers show that there is internal flexibility that is not captured by the previous analysis, and the monomers reorganize inside the aggregates. The results for the large scale C8S3 self-assembly simulation are

3.4. Results of CG simulations

presented, Figure 3.13 and 3.14. The RDF and P2 profiles from the analysis of all simulated systems are included in the Appendix, Figure 6.5 to 6.10.

Figure 3.13: Analysis of the C8S3 large scale self-assembly simulation at different time periods. RDF of the C3 bead (left). P_2 parameter for angle θ (right).

Figure 3.14: Relative position of monomers in the C8S3 system as a function of distance and angles at 0.0-1.0 (top), 4.5-5.5 (middle) and 9.0-10.0 (bottom) μ s.

3.4.2 Preformed aggregates

Interpreting the profiles for the local arrangement is not trivial, unless there is a reference pattern to compare with. For this reason, the same analysis was performed in one of the short atomistic C8S3 nanotubes with the brickwork arrangement from Chapter 2. Furthermore, the CG model for C8S3 was used to simulate preformed C8S3 nanotube structures and study the local arrangement during the simulation. The results are presented in Figure 3.15 and 3.16.

Figure 3.15: Analysis of a C8S3 preformed nanotubes with atomistic resolution. RDF of the central atom of the core (C2/C3) (left). P_2 parameter for angle θ (right). The analyses were performed for the last 10 ns for the atomistic simulation and for the last 100 ns for the CG simulation. For the brickwork simulation, the inner and outer wall profiles are shown separately to highlight the order of the outer wall. In the atomistic simulation, the profiles for the inner and wall were almost identical.

In the atomistic simulation, the structure is ordered generating well-defined and distinctive patterns. For the CG simulation with the brickwork arrangement the inner and the outer wall are shown separately. The CG nanotube with brickwork outer wall presents the highest level of order. In fact, the profiles from the outer wall are more ordered than the atomistic simulation. The sharper peaks could be attributed to the nature of the CG models that, in principle, make the free energy landscapes for the binding/interaction of the molecules flatter with more discrete energy minima. The order is diminished in the brickwork inner wall and is almost completely lost in the herringbone simulation, where the profiles for the RDF and the P_2 parameter resemble the profiles from the random self-assembly simulations Figure 3.13.

Snapshots from the final frame of each CG nanotube simulation are presented in Figure 3.17. The nanotube with the brickwork arrangement maintained the tubular formation. The outer wall was almost intact, while the inner wall presented periodic defects. On the other hand, the system with the herringbone formation was severely deformed and holes appeared along the nanotube.

3.4. Results of CG simulations

Figure 3.16: Relative position of monomers as a function of distance and angles. a) Atomistic simulation. b) Brickwork simulation, inner (top) and outer (bottom) wall. c) Local arrangement for the herringbone simulation. The analyses were performed for the last 10 ns for the atomistic simulation and for the last 100 ns for the CG simulation.

Figure 3.17: Snapshots from C8S3 nanotube simulations with the CG model, a) Brickwork and b) Herringbone Formation. In the brickwork formation the inner (left) and outer (right) wall are shown separately. The aromatic core is coloured in blue, the hydrophilic tails in red and the hydrophobic tails in green.

3.5 Discussion and conclusions

A bottom-up approach was followed to create a CG model for cyanine dyes. During this procedure different aspects of the atomistic and CG features were optimized to ensure that the final models represent the structural and chemical properties of the cyanine dyes. Initially, QM methods were used to obtain optimal parameters for the description of the polymethine bridge dihedrals and different sets of partial charges. Then, the obtained parameters were tested on available crystal structures for cyanine dyes with the same aromatic core. The best set of parameters was used for atomistic simulations that provided the reference point for constructing the cyanine dye CG model. The CG model was further optimized to reproduce better the structural features of the monomer conformation when aggregates are formed. The final CG model represented with great detail structural/spatial properties of the cyanine dyes. Our results provide guidelines and best practices for constructing CG models for simple or complex nanomaterials.

Three different cyanine dyes were prepared by changing the side chains of the aromatic core, namely, C2C2, C2S4 and C8S3. The CG models of these cyanine dyes were used in self-assembly simulations. Even though the assembled structures were not similar to the experimental determined structures, each dye formed different aggregates. The mismatch between the experimental and the computational structures could be attributed to the system sizes, the monomer concentration, the limited simulation time scales and/or the bead types of the CG model. Potential solutions are changing the system sizes, extending the length of the trajectories, run enhanced sampling simulations and/or try driven self-assembly approaches by using conformational constraints based on the experimental results, as described in the protocol of Chapter 4. The presence of many fully or partially charged beads might also require a different treatment for the electrostatics and charges of the system. Apart from modifying the system setup and the MD protocols, the performance of the CG model could be improved by changing the initial choice of bead types for the side chains. The polar aromatic core and side chains most likely affect the chemical

3.5. Discussion and conclusions

nature of the adjacent atoms justifying changes in the default bead types of the side chains.

The consistency of the CG model with suggested supramolecular structures of cyanine dyes was tested by performing simulations of preformed C8S3 nanotubes and comparing the results with an atomistic reference structure. The tubular double-wall formation was partially maintained in one simulation, as well as, the initial local arrangement of the monomers in the outer wall, suggesting that the CG model is compatible with the nanotube structure. In the same simulation, the inner wall was partially deformed. A possible explanation is the close contacts of highly polar and highly hydrophobic beads in the side chains of the C8S3 molecules due to the inward curvature of the nanotube. Modifications in the initial packing and/or the bead types could improve the stability of the CG C8S3 nanotubes.

Chapter 4

Directed self-assembly of a 2NapFF nanotube

"The secret to getting ahead is getting started."

- Mark Twain, 1835-1910

4.1 Abstract

This chapter outlines a protocol for the preparation of aggregates for MD simulations and for studying structural features of the respective building blocks (monomers) without prior information on their arrangement, but only knowledge of the aggregate dimensions. The protocol was used to study a small amphiphile, 2NapFF, that forms tubular structures at high pH. Once the aggregate was formed, the internal molecular structure of the monomers was evaluated. Our results show that the inferred structure from experimental studies is stable in MD simulations, verifying the experimental observation from a computational point of view. Furthermore, they suggest the lack of crystalline order and propose a relatively flexible arrangement of the monomers, which are typical features of molecules forming low molecular weight gels, such as 2NapFF.

4.2 Introduction

Low molecular weight gels (LMWG) are a class of materials that are formed by the self-assembly of small molecules into anisotropic structures [93, 94, 95]. The past years, they have attracted attention due to their potential applications in several areas, such as tissue engineering and drug delivery [96], cancer therapy [97], and many other applications [98].

In contrast to polymer gels that create covalently cross-linked networks, LMWGs entangle via reversible non-covalent interactions, such as hydrophobic effect, hydrogen bonds and pi-pi stacking, that can be more easily manipulated, creating unique controllable properties and functionalities. A careful balance between these interactions determines the shape and properties of the final product. The type of aggregates vary from bilayers and micelles to tubes and wires, but the most common structures encountered are fibres. Apart from the chemical structure of the molecules, there are several other parameters that determine the formation of gels and the type of structure. The temperature [99], the type of solvent [100], the presence of ions [101] or additives [102] are important parameters among them. Consequently, it is extremely complex to predict which molecules will form gels or not, estimate the optimal conditions for the formation of the supramolecular structures and, ultimately, foresee the type and properties of the final product.

Since the predictive power of estimating the structure and properties of LMWG is limited, the study of these systems is mostly based on experience and trial-and-error attempts. Synthesizing different molecules, testing their ability to self-assemble into different aggregates and measuring their properties with different experimental methods is the most commonly followed route.

An interesting example of LMWGs is 2-naphthalene-diphenylalanine (2NapFF) molecules (Figure 4.1). 2NapFF is a naphthalene-protected dipeptide that forms double-walled cylindrical aggregates (nanotubes) at high pH ($\text{pH} > 10$) [103, 104]. The slightly hydrophobic nature of the naphthalene rings generates a hydrophobic band, around which the aromatic moieties organize, while the carboxylic end is exposed to the solvent.

Figure 4.1: Chemical structure of 2NapFF monomer. At high pH, the carboxylic group is deprotonated, as shown. Both phenylalanines are D-amino acids.

There are various experimental techniques that can be used to study the structural properties of LMWG, but there are always limitations. For example, X-ray diffraction can be used to probe the relative arrangement of the monomers in crys-

tals, but it has been shown that the orientation of the monomers might be different between the crystal and the gel phase [105]. Additionally, drying the sample during the preparation of the crystal might also affect the structure of the LMWG [106]. Microscopy methods are commonly used to study the structure of the self-assembled aggregates, but there are usually issues with the interpretation of one- or two-dimensional data in terms of 3D objects [106]. The problem of dimensionality could be removed by using confocal microscopy, but it comes at the price of lower resolution. Small-angle X-ray or neutron scattering provide high quality data on the morphology of the samples, but the results need to be inferred by fitting to analytical models [104] that might not be very accurate. To summarize, LMWGs have been studied with plentiful experimental techniques, but each one of them only reveals a different part of the puzzle. As a result, different experimental methods should be combined to obtain a complete picture and meaningful results that represent the real structure at normal conditions.

The structure of the 2NapFF nanotubes has been extensively studied and confirmed by different experimental techniques (Figure 4.2) [107, 108], that suggest the presence of a hollow cylinder with inner radius ~ 1.7 nm and outer radius ~ 3.8 nm. More specifically, the fibrillar nature has been clearly pictured in cryo-TEM images, and the cylindrical morphology and its dimensions was inferred from analysing Small-Angle X-ray Scattering (SAXS) and Small-Angle Neutron Scattering (SANS) data. Based on cryo-TEM profiles, the diameter of the nanotube was estimated to be 6.9 ± 1.0 nm. From SAXS experiments, the outer radius was measured to be 4.3 ± 0.1 nm, while the inner radius could not be resolved due to the lack of contrast. According to SANS data, the thickness of the cylinder was 2.10 ± 0.01 nm and the inner radius 1.65 ± 0.01 nm, resulting in an outer radius ~ 3.75 nm. The discrepancy between the SAXS and SANS results could be attributed to limitations of the experiments at short distances, but comparison with the cryo-TEM results suggests that the SANS measurements are closer to the real values.

Even though, there are several studies on the 2NapFF system, the information they provide on the molecular packing of the monomers is limited. According to data on the arrangement of similar naphthalene dipeptides in crystals [109], a network of hydrogen bonds between the backbone of the peptide stabilizes the system, when the amino acids are glycines, but the network is disrupted when bulkier side chains are introduced. The size of the phenylalanine side chains suggest a similar effect in present in 2NapFF aggregates, but to our knowledge, it has not been experimentally confirmed so far. A recent study showed that the chirality of the peptide amino acids affects the structure of the aggregates [108], indicating a relationship between the orientation of the backbone and aggregate structure.

Our goal was to study the supramolecular structure and the molecular packing of 2NapFF monomers inside nanotubes. We conducted MD simulations of 2NapFF molecules of a short cylindrical aggregate and measured different structural properties. Our results are compatible with the experimental measurements for the nanotube size and they shed light on the arrangement of 2NapFF, when the nanotubes are formed.

Figure 4.2: a) Cryo-TEM images of the solutions of 2NapFF at high pH. b) Cartoon of structures formed by 2NapFF end on, with colour coding for the different sections of 2NapFF (red = naphthalene ring, light blue = phenylalanine next to naphthalene (Phe1), dark blue = terminal phenylalanine (Phe2)). c) SAXS data and fit (red line) for 2NapFF. The structure is modelled as a cylinder with radius 4.3 ± 0.1 nm. d) SANS data and fit (blue line) for 2NapFF. The structure is modelled as a hollow cylinder with inner radius 1.65 ± 0.01 nm and thickness 2.10 ± 0.01 nm. Experimental data were provided by D. J. Adams.

4.3 Methods

4.3.1 MD simulations

MD simulations were performed using the GROMACS 2016.3 [55, 56] package with the CGenFF force field [110]. The topology for 2Napff was generated with the CGenFF web server (<https://cgenff.umaryland.edu/>). Based on the Henderson-Hasselbalch equation, since the pK_a of the carboxylic group is ~ 4 -5 and the pH of solvent is ~ 10 -11, all carboxylic groups were considered to be deprotonated. One system with 300 2NapFF molecules in random positions in a box of size $10 \times 10 \times 10$ nm was generated. The system was solvated using the SPC water model [58] and Na^+ ions were used to neutralize the charge of the system. Energy minimization

followed for 1000 steps using the Steepest Descent algorithm to remove unfavourable interactions and odd contacts. Equilibration was performed in two steps, first in the NVT and then in the NPT ensemble, for 100 ps each. The temperature during the production phase was kept constant at 298 K using the Velocity Rescaling thermostat [61] with a time constant of 0.1 ps and the pressure at 1.0 bar along the x-y plane and z-axis using the Parrinello-Rahman barostat [111] and a semi-isotropic pressure coupling with a time constant of 2 ps and compressibility set to $4.5 \times 10^{-5} \text{ bar}^{-1}$. Van der Waals and electrostatic interactions were calculated by using the Verlet list scheme with the cut-off set at 0.96 nm. Long-range electrostatic interaction were treated using Particle mesh Ewald (PME) method [112]. The bond lengths were constrained by using the LINCS method [65]. The time step during the production phase was set to 0.002 ps.

4.3.2 Driven self-assembly

In order to accelerate the formation of the nanotubes and focus on the arrangement of the 2NapFF molecules, when the aggregates are formed, cylindrical restraints were introduced to the system after the equilibration step based on the experimental measurements from SANS on the size of the aggregates. By using cylindrical restraints, specific atoms are free to move along the axis and the circumference of a hollow tube, while they are forced to stay within the designated areas. The bias force was added by using Plumed 2.4 [113] in the form of flat-bottom potentials with force constant of 50 kJ/mol in two steps. First, cylindrical biases were applied to restrain the centre of mass of the naphthalene rings in a cylinder with inner radius of 2.5 nm and outer radius of 3.1 nm, while the carbon of the carboxylate group was restrained in cylinders of radii 1.7-2.0 nm for the inner wall and with radii 3.6-4.0 nm for the outer wall. This step lasted 50 ns. The number of molecules in the inner and outer cylinder was not equal and it was calculated by using simple geometrical formulas, Eq. 4.1 and 4.2.

$$c = 2 * r * \sin\left(\frac{\theta}{2}\right) \quad (4.1)$$

$$N = \frac{2 * \pi}{\theta} \quad (4.2)$$

c is the chord of circle with a specific radius, r , for a designated angle, θ . N is the maximum number of chords that fit in a circle for a specific angle, θ .

We assumed that 2NapFF are oriented in a similar way in the inner and outer wall, so the area they occupied should be roughly the same, as well as the length of their chords. Since the angle of the chord is inversely proportional to the radius and the chords are the same, the ratio of molecules in the inner and outer should only be a function of the respective radii. For an outer wall radius of ~ 3.8 nm and an inner wall radius of ~ 1.8 nm, the ratio of 2NapFF should be ~ 2.1 . In the reported system, a ratio 2.0 was used, meaning that 200 and 100 2NapFF were initially restrained in the outer and inner wall, respectively.

In the next step, the bias on the carboxylate groups was removed and only the naphthalene rings were restrained. During this step that lasted 50 ns, temperature

annealing from 300 K to 400 K and back to 300 K was performed to relax the system and enable it to escape potential local minima. Finally, the entire biasing force was removed starting the production phase of the preformed nanotube for 100 ns. The final MD run was performed to confirm the stability of the tubular structure and obtain information on the structure of the aggregate.

4.4 Results

4.4.1 2NapFF nanotube structure

In this study, we used MD simulations to study the spatial arrangement of 2NapFF molecules, when they aggregate into nanotubes. We prepared a system with 2NapFF molecules in solution and drove them into a tubular formation with biasing potentials, Figure 4.3. After the nanotube was formed and relaxed, the bias was released and a production phase of 100 ns started. The tubular formation was maintained along the trajectory, Figure 6.11a and 6.11c.

Figure 4.3: Snapshots from the 2NapFF simulation before and after the biasing potentials were introduced. 2NaphFF molecules are coloured with standard colour code and the naphthalene rings are highlighted in red. Water molecules and ions are not presented for clarity. a-c) Side view of the forming nanotube. d-f) Top view of the forming nanotube.

Figure 4.4: Snapshots from the simulation of the 2NapFF nanotube. a) Side view of the 2NapFF nanotube after the production phase. 2NapFF molecules and Na^+ ions are presented with all molecular detail and water molecules are presented as an isosurface. Na^+ ions are represented as blue beads and water with a cyan isosurface. The 2NapFF nanotube is replicated along the periodic boundary conditions. b) Top view of the 2NapFF nanotube simulation at 0 ns and 100ns.

The semi-isotropic pressure coupling allowed the box to shrink along the z-axis and expand in the x- and y-axis creating a periodic nanotube, where the length of the final aggregate was ~ 5 nm. The structure was uniform at this length indicating the concentration of the solvent inside and outside of the tube was optimal. Imbalances in the amount of water would have deformed the structure, as reported for C8S3 nanotubes when the concentration of the solvent was not optimal inside the nanotube (Chapter 2). There was no exchange of molecules between the inner and the outer wall suggesting that the initial estimate for the number of molecules in each wall was quite accurate. A quick look at the structure shows a well-ordered arrangement of the naphthalene rings, before removing the cylindrical restraints, that was not maintained until the end of the trajectory (Figure 6.11c). Nevertheless, the general morphology was maintained and the band of the naphthalene rings is clearly visible. The phenylalanine side chains form a layer around the naphthalenes, but also penetrate the inner band. The Na^+ are located at the edges of the inner and the outer wall stabilizing the structure. Simulating systems without bias potentials resulted in random aggregates for the simulated time scales, as shown in the Appendix, Figure 6.11.

4.4.2 2NapFF molecular arrangement

To obtain a better view on the molecular arrangement of the 2NapFF molecules inside the tube, the radial distribution functions (RDF) among the centres of the aromatic groups, Naph, Phe1 and Phe2, (Figure 4.1), and the P_2 order parameter (Eq. 2.1) for the orientation of the aromatic rings were computed along the trajectory of the production phase (Figure 4.5a and 4.5b). For P_2 , the angle between the normal of the planes of the respective aromatic rings was used. Combining these two measurements gives a qualitative impression for the relative position of the aromatic groups and quantitative measure of the order among each group.

As can be seen from the RDFs, the first interaction shell among all the aromatic rings, Naph, Phe1 and Phe2, ranges from 0.5 to 0.8 nm (Figure 4.5a and 4.5b). Within this distance, the aromatic rings are mostly randomly oriented. The only exception are the naphthalene rings that are partially ordered at ~ 0.45 nm, where a small peak is observed in the RDF and the P_2 is ~ 0.4 . Considering the size and dimension of naphthalene, we conclude that a slided and slightly tilted arrangement is mostly adopted (Figure 4.5c). The P_2 peaks at distances smaller than 0.4 nm belong to really small populations of partially ordered stacks.

4.4.3 2NapFF density profiles

Partial mass density plots provide a different perspective for the position of the aromatic rings in the simulated 2NapFF nanotube (Figure 4.6a and 4.6b). It is clear that naphthalene or phenylalanine rings tend to be in the middle and inner/outer layer, respectively. At the same time, the phenylalanine rings adopt various conformations and visit the middle layer by folding and penetrating the naphthalene ring stacks, as suggested also by the RDF plots (Figure 4.5a). Furthermore, the boundaries of the inner and outer phenylalanine layers are located at radii of ~ 2 and ~ 4 nm from the centre of the nanotube, respectively, which are in good agreement with the suggested model of a hollow cylinder with inner radius ~ 1.8 nm and outer radius ~ 3.8 nm.

4.4.4 2NapFF hydrogen bonds

To evaluate the presence of hydrogen bonds in the simulated structure, we have computed the total amount of hydrogen bonds among all possible donors and acceptors at each frame. The cutoff for defining a hydrogen bond was set to 0.35 nm for the donor-acceptor distance and to 30° for the hydrogen-donor-acceptor angle. The average number of hydrogen bonds between the amides of 2NapFF per frame was ~ 48 , whereas the number of hydrogen bonds between the amides of 2NapFF and water was ~ 155 . Considering that the system contained 300 2NapFF molecules and the fact that the peptide backbone has at least two donors/acceptors, the maximum number of hydrogen bonds formed would be 600 in a periodically connected nanotube, where every amide participates in two hydrogen bonds. We conclude that the hydrogen bond network is disrupted by the presence of the phenylalanine side chains and the hydrogen bond donors and acceptors of 2NapFF are partially exposed to the solvent.

Figure 4.5: a) RDF and P_2 order parameters for Naph-Naph and Naph-Phe1/2 aromatic rings. b) RDF and P_2 order parameters for Phe1/2-Phe1/2 aromatic rings. c) Snapshot from the last frame of the trajectory. The arrangement of naphthalenes is highlighted.

Figure 4.6: a) Partial mass density map of naphthalene rings in the tube structures. b) Density map of phenylalanine rings. The remaining atoms were excluded from the calculations for the sake of clarity.

4.5 Conclusions

LMWGs, such as 2NapFF, are interesting and complex systems to study. The nature of their interactions and their physical properties make it extremely difficult to predict the final structures and resolve their arrangement in normal conditions. We have conducted MD simulations of a 2NapFF nanotube, in order to study the internal molecular structure and in particular the orientation of the aromatic moieties, when the aggregates are formed. Based on the experimental measurements, the 2NapFF molecules were driven to form a nanotube with given dimensions. The simulated structure was stable and maintained the tubular formation for 100 ns. Our results show that the naphthalene rings are partially ordered when at close distances, adopting a slided and slightly tilted arrangement, but the orientation of the other aromatic rings is relatively flexible. The naphthalene rings are mainly located in a band in the centre of the hollow cylinder and the phenylalanine side chains are located around it, but also inside it. The phenylalanine rings disrupt the hydrogen bonds that could potentially be formed among the amides of the peptide backbones. Such disruptive effects have been observed in crystal structures of molecules similar to 2NapFF but with less bulky side chains. These observations are compatible with the lack of crystalline order in LMWGs and support the hollow tube model suggested by experimental results. Further study should focus on the effect of L- and D-amino acid configurations of the 2NapFF backbones on the molecular structure of the nanotubes. According to recent experimental results, the type of enantiomers affects the aggregate formation. Potential differences could be explored by using a similar protocol to the one described in this chapter.

Chapter 5

Dimerization free energies of small aromatic rings

"Life is really simple, but we insist on making it complicated."

- Confucius, 551-479 BC

5.1 Abstract

Dimerization free energies are fundamental quantities that describe the interaction strength of different molecules. Obtaining accurate experimental values for small molecules and disentangling the conformations that contribute most to the binding is not trivial, due to the size of the systems and the small energy differences, that are extremely difficult to be measured. Consequently, one has to resort to computational methods to calculate such properties. In this chapter, we measured the convergence of different force fields, when calculating the free energy of dimerization of small aromatic rings. At the same time, we compared the generated parameters from popular online servers for atomistic MD simulations. We show that, regardless of the force field, the profiles for the dimerization free energy of small molecules converge at specific distances and values. However, significant care needs to be taken when calculating binding free energies for larger aromatic molecules.

5.2 Introduction

Determining the binding energies between molecules is of major importance in fields such as drug design, nanotechnology, etc. The strength and dynamics of the interactions will determine whether a specific binding will take place or not, and how long it will last, e.g. a drug binding to its target. Experimental techniques, such as Nuclear Magnetic Resonance [114, 115, 116] or Isothermal Titration Calorimetry [117, 118], can be used to calculate binding energies between macromolecules, but are limited by the nature and the size of the molecules. Additionally, experimental techniques measure an ensemble average without detailed knowledge for the contributions of the conformations averaged over. Consequently, experimental data are sparse and only available for large systems, such as large aromatic compounds or proteins, where the differences in energy can be measured.

Computational approaches comprise a powerful tool to measure the interaction strength and binding modes between small molecules, and they are usually employed to support experimental measurements and/or deal with experimental pitfalls. The level of detail and the computational complexity varies, and many different methods have been developed that are more or less suitable for different applications. In more detail, while Quantum Mechanical (QM) calculations in vacuum provide an accurate description for the favourable binding modes, the size of the systems and the need for treating the solvent explicitly render the use of QM methods prohibitively expensive in systems aiming to represent realistic environments [119, 120]. On the other hand, docking simulations are a fast option, but simplifications in scoring functions and limited information on the dynamics of the interactions restrict their use to protein-ligand binding [121, 122, 123]. Molecular Dynamics (MD) simulations is an ideal option to study the binding energies, since they combine high level of accuracy and reasonable computational costs [124, 125, 126, 127, 128].

In this study, we compared the performance of commonly used MD force fields (CHARMM [4], GROMOS [5] and OPLS [6]) and measured their convergence in calculating the free energy surface (FES) for the dimerization of small aromatic molecules, Figure 5.1. These molecules are used in different MD simulations and most of them are usually encountered as solvents or as building blocks in large biomolecules or nanomaterials. Previous MD studies show that different force fields produce consistent results in terms of structural and energetic properties, and they are usually compatible with QM or experimental studies, when available [119, 129, 130]. This study focuses on small aromatic molecules shows that, regardless of the force field, the free energy of binding and the binding modes are roughly the same. However, the results for larger aromatic molecules should be interpreted with care. Increasing the size of the molecules leads to significant deviations.

5.3 Methods

5.3.1 Molecule parameters

We conducted MD simulations to study the FES for the dimerization of small aromatic molecules and compare the obtained FES from different force fields. The parameters for all the simulated molecules were obtained from different online servers that generate topology and parameter files for each force field. Specifically, the Au-

Figure 5.1: List of the molecules for which the FES of dimerization was calculated. The black vectors represent the dihedral angle and the red vector the normal of the plane for each molecule.

tomated Topology Builder (ATB) server [73] (<https://atb.uq.edu.au/>) was used for the G54a7 [90] simulations. ATB includes a large data base of topologies for different molecules. In the cases when optimized parameters for the molecules of interest were available, those parameters were chosen instead. The entries in ATB are reported in the Appendix, Table 6.12. The CGenFF server (<https://cgenff.umaryland.edu/>) was used to generate the CHARMM36/CGenFF [110] parameters. For the OPLS [6] parameters, the LigParGen server [131, 132, 133] (<http://zarbi.chem.yale.edu/ligpargen/>) was used.

5.3.2 MD simulations

All simulations and analysis were performed with the Gromacs.2016 [55, 56] package patched with PLUMED 2.4 [113]. Each simulation was performed in a cubic box with dimensions 5x5x5 nm. The temperature was kept constant at 300 K with the V-rescale algorithm and a time constant of 0.1 ps [61]. For the pressure coupling, the Berendsen barostat [62] was used to maintain the pressure constant at 1 bar in an isotropic pressure bath with a time constant of 1 ps and compressibility of $4.5 \times 10^{-5} \text{ bar}^{-1}$. The cut-off for electrostatic and van der Waals interactions was set to 1.4 nm and the Verlet scheme was used for the short range non-bonded interactions with buffer tolerance of $0.005 \text{ kJ mol}^{-1} \text{ ps}^{-1}$. Long range interactions were calculated with the Reaction Field method [64] and ϵ_r set to 54. The LINCS algorithm was employed for constraining the bond lengths [65]. All systems were minimized for 10^3 steps by using the Steepest Descent algorithm and equilibrated in the NVT and NPT conditions, each for 10 ps with a 2 fs time step. The production phase lasted 100-150 ns depending on the system, until the average errors from the block analysis converged. More system details are included in the Appendix, Table 6.12.

Regarding the water models, each force field has specific water model parameters based on which it was optimized. Consequently, G54a7 systems were solvated with explicit SPC water molecules [58], whereas CHARMM36 and OPLS systems were solvated in TIP3P water model [60].

Apart from the water models, there are additional parameters that guarantee optimal performance when using each force field. In this case, we used the same cut-offs and parameters regardless of the force field to maintain simplicity and be consistent in the way the simulations were performed. This setup will be referred to as general parameters. At the same time, we performed test simulations with the default parameters of each force field to evaluate the sensitivity of the results to the two setups. In the default G54a7 parameters, the Group cut-off scheme was used with a twin range cut-off for the non-bonded interactions, where the short-range neighbour cut-off distance was set to 0.8 nm and the long range cut-off to 1.4 nm. In the CHARMM36 simulations, the LINCS algorithm was applied only to hydrogen atoms, the Verlet scheme was used with 1.2 nm distance cut-off and the long range electrostatic interaction were calculate with PME method [112]. As for the OPLS systems, the Verlet scheme was used with 1.0 nm distance cut-off and for the long range electrostatic interaction the PME method was used.

Finally, to stay updated with the latest halogen parametrization and compare potential effects in the FES, the chlorobenzene for the CHARMM36 force field was modelled with a drude oscillator around the chlorine to model the effect of the "sigma hole" [134, 135], a small area opposite to the bond that has positive electrostatic

potential. The charge of the drude particle was set 0.05 e and its mass to 0.1 u. The bond length between the drude particle and the chlorine was 0.164 nm and the force constant 2508 kJ mol⁻¹ nm⁻². The equilibrium value for the angle formed by the carbon of the benzene ring, the chlorine and the drude particle was set to 180° and the force constant to 836 kJ mol⁻¹ rad⁻².

5.3.3 Metadynamics

The free-energy surface (FES) profiles for the dimerization of each molecule were obtained by metadynamics simulations [136, 137] with the well-tempered [15] adaptation. In Metadynamics positive Gaussian potentials are added on the system as a memory dependent factor that drives the system towards unexplored regions of the available conformational space. After the simulation, the deposited Gaussians are summed and they are used to calculate the unbiased free energy landscape as a function of the collective variables (CV) of interest. In the well-tempered adaptation, the height of the deposited Gaussian is smoothly decreased leading to converged FES profiles. The CVs on which the bias was added were the distance between the centre of geometry (COG) of the aromatic rings and the torsional angle between atoms of the rings and their COGs, Figure 5.1. The angle between the normal of the ring planes was used as a third CV to project the FES by using a reweighting algorithm [138]. The height of the deposited Gaussians was set to 1.0 kJ/mol and the width to 0.05 nm and 0.2 rad for the distance and torsion, respectively. Gaussian were deposited every 500 steps and the bias factor was set to 5. A wall, in the form of a harmonic potential with force constant 200 kJ/mol, was added at distance beyond 2 nm to prevent the molecules from exploring conformations at distances beyond the second interaction shell. All profiles were translated in order to be zero at distances where the profiles were almost flat (~1.7 nm). Block analysis was used to estimate the error from the free-energy calculations.

The contribution of entropic term in the FES has been taken into account when calculating and presenting of the profiles, Eq. 5.1.

$$\Delta V_{FES}(r) = (n - 1) R T \log(r) \quad (5.1)$$

where $\Delta V_{FES}(r)$ is the entropic free energy as a function of distance (r), n is the number of dimensions, R is the gas constant and T is the temperature of the system.

5.4 Results

5.4.1 Comparison of parameter setups

Benzene, the simplest aromatic compound, was used as a reference system for comparing the results between different force fields and the effect of using default cut-off parameters or the general setup in the FES of dimerization. The results for the FES as a function of the distance between the COG of two benzenes and the dihedral

angle describing the relative orientation are presented in Figure 5.2. In Figure 5.3, the FES as a function of the distance between the COG and the angle between the normal of each benzene ring is presented. The average error of all points of the FES as a function of the block size is presented in Figure 5.4.

Regardless of the simulation setup, the binding energy and modes in the first interaction shell (~ 0.4 - 0.7 nm) are almost identical. The only significant difference is seen in the G54a7 case, where the well in the second interaction shell (~ 0.8 - 1.1 nm) is deeper ~ 2 kJ/mol in the general setup compared to the simulation with the default parameters. This effect could be attributed to the larger cut-offs for the non-bonded interaction. However, it did not have any significant impact on the FES for CHARMM36 or OPLS.

Small differences in the FES can affect the measurement of the binding energy. In theory, when comparing computational and experimental results, we can use the FES to obtain information on the association constants of dimers, K_a [129], Eq. 5.2. The computed association constants from MD can then be compared with the experimental values, if available.

$$K_a = \frac{4\pi R^3 \int_0^{r_c} r^2 g(r) dr}{3v^\circ \int_{r_c}^R r^2 g(r) dr} \quad (5.2)$$

where R is the maximum distance are the molecule that we take into consideration, r_c is cut-off for determining when two molecules are associated, r is the distance between two molecules, $g(r)$ is the radial distribution function and v° is the (molecular) standard volume (1.66 nm^3).

The main issue when calculating the association constant, K_a , from FES is choosing the cut-off, r_c , for determining the distance at which two molecules are still associated. For example, a small cut-off will result in a strict definition for interaction and small values for the association constant, whereas a large cut-off will have the opposite effect. Experimental values for the association constant could be used as guidelines to the set this cut-off and decide whether or not the computed values are within reasonable margins. Without them, the definition of the cut-off is quite arbitrary. In the end, the computed values only make sense under the scope of the experimental results.

Unfortunately, accurate experimental results for binding energies are sparse, most computational studies for the interactions of aromatic rings are obtained in the gas phase, and computational results are dependent on the method of use [119, 139, 140], presenting deviations ~ 2 - 4 kJ/mol for small aromatic molecules. Consequently, it is extremely difficult to obtain converged results, when there are so many independent variables. Comparisons are mostly meaningful within specific methods, e.g. QM or MD, where the number of variables is limited and deviations from the trends are evident. In this study, we will focus on the position and the depth of the well in the first interaction shell. Both features are consistent in all force fields and simulation setups in our reference system, benzene.

Figure 5.2: FES for the dimerization of benzene as a function of the distance and the torsional angle. The results for each force field are presented in each row. The plots on the left correspond to the FES based on the general setup and the plots on the right to the default parameters of each force field.

Figure 5.3: FES for the dimerization of benzene as a function of the distance and the angle between the normal of benzene planes. The results for each force field are presented in each row. The plots on the left correspond to the FES based on the general setup and the plots on the right to the default parameters of each force field.

Figure 5.4: Statistical error of the FES calculations as a function of the block size. The dashed lines correspond to the error of the reweighted profiles.

5.4.2 Comparison of force fields

Figure 5.2 and 5.3 show that the FES for the dimerization of benzene is roughly the same in all simulations. The average error of the FES calculation does not change as a function of the block size for the error analysis, indicating that the simulations were converged, Figure 5.4.

As expected, there is no preference in the orientation of benzenes with respect to the dihedral angle, since benzene is symmetrical. However, conformations with angles $\sim 90^\circ$ (T-shaped) are more favourable than stacked conformations. When the FES is projected on the distance and the dihedral angle, the energy minimum in the first interaction shell is ~ 3 kJ/mol, whereas the depth of the well is ~ 5 kJ/mol, when the angle between the normal of the planes is used. In the first case, conformations with low and high energy minimum are grouped together resulting in a lower value for the energy, whereas in the second case, the main variable that discriminates between the two conformations is effectively dissociated. This effect is only visible when the proper CVs are used for projecting the FES. Nevertheless, our results for the FES of benzene are close to the reported values from previous Monte Carlo [141] and MD simulations [141], where the depth of the well was ~ 6 kJ/mol and ~ 1.5 kJ/mol, respectively, and the minimum of the FES was located at distance ~ 0.55 nm in both studies.

Regardless of the force field, similar results are obtained for most of the aromatic rings regarding the depth of the minimum energy in the first interaction shell, Figure 5.5. The energy differences among them are on the order of thermal fluctuations ($< k_b T$), suggesting that their energy states are almost indistinguishable.

Figure 5.5: Binding energy at the lowest point of each molecule's dimerization FES for different force fields. All simulations were performed with the general setup. The error bars correspond to the average error calculated from block analysis.

The FES profiles for each molecule and the standard errors are included in the Appendix, Figure 6.12 to 6.19. T-shaped conformations are mainly preferred by most of the aromatic rings. At the same time, conformations close to the stacked arrangement are more populated in some molecules, e.g. chlorobenzene as shown in Figure 6.13. Surprisingly, molecules with polar groups do not show significant differences in the orientation of the binding modes with respect to the dihedral angle that could be attributed to the presence of the charged groups. For example, phenol has a polar hydroxyl group that should favour conformations where the oxygens would be diametrically opposed. However, this polar effect of the hydroxyl group was not observed in any FES, Figure 6.14. Such behaviour might have been observed by using a polarizable force field. Effects in the orientation of the molecules with respect to the dihedral angle become slightly visible in para-cresol, Figure 6.16, and more evident as the size of the ring increases.

Even though the results for the small aromatic molecules are roughly the same, as the size of the aromatic moiety increases so do the deviations between each force field, Figure 5.5. The most extreme differences are observed in the FES of tetracene, Figure 5.6. G54a7 predicts that parallel and closer arrangements are more favourable, whereas according to CHARMM36, parallel arrangements and conformations with angle $\sim 45^\circ$ are similar in terms of energy. The difference in the energy of binding between G54a7 and CHARMM36 is ~ 5 kJ/mol in the sandwich formation. On the other hand, OPLS predicts that conformations with angle $\sim 45^\circ$ are most favourable and the energy of binding is similar to CHARMM36.

5.5 Conclusions

We performed MD simulations of small aromatic molecules to study the FES for their dimerization. Different force fields were used to compare the obtained FES and examine their convergence. The sparse experimental results and computational results in similar environments make comparisons between experiments and theory or different theories extremely difficult. So, performance can be only assessed within the boundaries of MD simulations. Our results show that, regardless of the force field, we obtained free energy profiles with similar binding modes and strength of interactions. This enhances our confidence in using MD simulations to calculate the binding affinity of small compounds. On the other hand, deviations from the general trends were observed in large aromatic molecules, such as pyrene and tetracene, indicating that further optimization of the force fields is needed, before using such computational methods to make accurate quantitative predictions for these systems.

Figure 5.6: FES for the dimerization of tetracene as a function of the distance and the torsional angle (left) and the distance and the angle between the normal of aromatic planes (right). The results for each force field are presented in each row.

Chapter 6

Appendix

”Tell people there’s an invisible man in the sky who created the universe, and the vast majority will believe you. Tell them the paint is wet, and they have to touch it to be sure.”

- George Carlin, 1937-2008

6.1 Insights on the structure of C8S3 nanotubes

System	Force field	Formation	Initial box size (nm)	No. C8S3	No. water	No. MeOH	Temperature (K)	Simulation time (ns)
1	G53a6	Brickwork	10.69x7.05x10.00	200	15491	-	300	500
2	G53a6	Herringbone	8.78x7.72x10.00	198	13354	-	300	500
3	G53a6	Staircase	10.85x8.75x10.00	198	19437	-	300	500
4	G53a6	Brickwork	10.69x7.05x10.00	200	15491	-	288	250
5	G53a6	Herringbone	8.78x7.72x10.00	198	13354	-	288	250
6	G53a6	Staircase	10.85x8.75x10.00	198	19437	-	288	250
7	G53a6	Brickwork	11.33x7.47x8.27	200	10835	1738	300	250
8	G53a6	Herringbone	9.5x8.5x7.95	198	9152	1498	300	250
9	G53a6	Staircase	10.39x8.38x9.33	198	13080	2180	300	250
10	GAFF	Brickwork	10.69x7.05x10.00	200	14624	-	300	250
11	GAFF	Herringbone	8.78x7.72x10.00	198	12825	-	300	250
12	GAFF	Staircase	10.85x8.75x10.00	198	18611	-	300	250

Table 6.1: Summary of simulated C8S3 bilayer systems.

System	Force field	No C8S3	Inner radius SO ₃ ⁻ /PMB (nm)	Inner rolling angle (°)	Outer radius SO ₃ ⁻ /PMB (nm)	Outer rolling angle (°)	Simulation time (ns)	Length (nm)
Brickwork 1	G53a6	1289	3.31/4.00	31.35	6.43/5.73	31.37	100	15
Brickwork 2	G53a6	1098	2.58/3.27	32.40	5.71/5.00	31.75	100	15
Herringbone 1	G53a6	1319	3.33/4.00	31.35	6.43/5.73	31.37	100	15
Herringbone 2	G53a6	1515	4.02/4.73	31.29	7.17/6.46	31.08	100	15
Herringbone Long 1	G53a6/GAFF	8275	3.03/3.73	31.17	6.42/5.72	21.60	10	100

Table 6.2: Initial parameters for the preparation of C8S3 nanotubes simulations. The distance of the SO₃⁻ and polymethine bridge (PMB) from the center of the tube is reported. The construction of the nanotubes was based on the radius of the polymethine bridge, which coincides with the radius of the chromophore and was significant for simulating the absorption spectra of the nanotubes. The rolling angle for each wall, simulation time is also reported.

6.1. Insights on the structure of C8S3 nanotubes

System	No C8S3	Inner radius PMB (nm)	Inner rolling angle (°)	Outer radius PMB (nm)	Outer rolling angle (°)	Simulation time (ns)	Length (nm)
1	5463	3.72	30.96	5.49	31.53	100	75
2	6953	3.54	25.63	5.27	49.74	23	100
3	7035	3.73	31.17	5.44	31.17	20	100
4*	7024	3.72	30.96	5.44	32.73	20	100
5	5442	3.73	24.22	5.45	20.55	23	75
6	5467	3.73	55.21	5.44	54.06	23	75

Table 6.3: Initial parameters for the preparation of C8S3 nanotubes simulations for the optical spectra calculations. This set of simulations was performed with the GAFF force field and the initial unit cell was adjusted by increasing its y-dimension by 0.1 nm. System with a star (*) indicate that the initial unit cell for the outer wall was further adjusted (increased by 2.4% in the y- and z-dimension) to imitate a change in the monomer density.

	QM C1C1	Final C1C1	QM C8S3	Final C8S3
H1	0.0476	0.03	0.0007	0.02
C2	-0.0442	-0.03	-0.0265	-0.02
C3	-0.2366	-0.25	-0.292	-0.27
C4	-0.2694	-0.25	-0.2548	-0.27
C5	0.6774	0.66	0.6788	0.67
C6	0.6675	0.66	0.6545	0.67
H7	0.0244	0.03	0.0586	0.06
H8	0.0459	0.03	0.0611	0.06
N9	-0.3486	-0.39	-0.3785	-0.39
N10	-0.3406	-0.39	-0.381	-0.39
N11	-0.3786	-0.39	-0.4205	-0.39
N12	-0.3614	-0.39	-0.4236	-0.39
C13	0.282	0.28	0.3152	0.3
C14	0.2937	0.28	0.3378	0.3
C15	0.2799	0.28	0.3125	0.3
C16	0.2828	0.28	0.2895	0.3
C17	-0.1822	-0.16	-0.2054	-0.2
C18	-0.1893	-0.16	-0.2321	-0.2
C19	-0.1893	-0.16	-0.2321	-0.2
C20	-0.1991	-0.16	-0.2118	-0.2
C21	0.002	0.02	0.0198	0.02
C22	0.0338	0.02	0.0135	0.02
C23	0.0765	0.02	0.0263	0.02
C24	0.0346	0.02	0.027	0.02
H25	0.0971	0.11	0.133	0.13
H26	0.1006	0.11	0.1592	0.13
H27	0.0797	0.11	0.1135	0.13
H28	0.1067	0.11	0.0977	0.13
CL29	-0.0323	-0.03	-0.0805	-0.09
CL30	-0.0347	-0.03	-0.1019	-0.09
CL31	-0.0343	-0.03	-0.0792	-0.09
CL32	-0.0346	-0.03	-0.0913	-0.09
H/C33	0.2016	0.2	0.1314	0.16
H/C34	0.192	0.2	0.1141	0.16
H/C47	0.176	0.2	0.1262	0.16
H/C48	0.183	0.2	0.1957	0.16

Table 6.4: QM and final MM partial charges for C1C1 and C8S3 (left). Atom order (right).

	QM C8S3	Final C8S3
C33	0.1314	0.16
C34	0.1141	0.16
C35	0.0844	0.0
C36	0.0946	0.0
C37	-0.1451	-0.12
C38	-0.1505	-0.12
S39	1.1588	1.16
S40	1.1583	1.16
O41	-0.6065	-0.62
O42	-0.6602	-0.62
O43	-0.6054	-0.62
O44	-0.6061	-0.62
O45	-0.6611	-0.62
C47	0.1262	0.16
C48	0.1957	0.16
C49	0.034	0.0
C50	0.009	0.0
C51	-0.0065	0.0
C52	0.0079	0.0
C53	0.0166	0.0

	QM C8S3	Final C8S3
C54	0.0006	0.0
C55	-0.0009	0.0
C56	0.006	0.0
C57	0.0069	0.0
C58	0.0117	0.0
C59	0.0414	0.0
C60	0.044	0.0
C61	-0.0442	0.0
C62	-0.0422	0.0

Table 6.5: QM and final MM partial charges for C8S3 side chains(left). Atom order (right).

System	COM distance (nm)	Electron density distance (nm)	Average distance of small patches (nm)
1	2.407±0.009	2.354±0.007	2.291±0.012
2	2.590±0.010	2.883±0.013	2.498±0.002
3	2.325±0.011	2.504±0.005	2.247±0.020
4	2.398±0.012	2.423±0.001	2.286±0.005
5	2.551±0.009	2.811±0.001	2.571±0.037
6	2.355±0.009	2.330±0.005	1.862±0.010
7	2.452±0.014	2.366±0.001	2.358±0.015
8	2.553±0.011	2.797±0.012	2.218±0.059
9	2.344±0.010	2.462±0.001	2.271±0.025
10	2.585±0.008	2.576±0.001	2.452±0.012
11	2.759±0.007	2.720±0.001	2.757±0.352
12	2.521±0.011	2.551±0.001	2.384±0.037

Table 6.6: Comparison of the C8S3 bilayer thickness measured with different approaches.

6.1. Insights on the structure of C8S3 nanotubes

System	Tail order parameter	Area per molecule (nm ²)	Diffusion (μm ² /s)
1	0.202±0.005	0.8904±0.0001	3.239±1.045
2	0.149±0.005	0.8029±0.0003	6.203±6.605
3	0.112±0.004	0.8864±0.0001	6.427±2.425
4	0.208±0.005	0.9000±0.0001	5.869±0.241
5	0.143±0.006	0.8137±0.0002	8.393±3.347
6	0.103±0.004	0.8810±0.0010	7.825±1.954
7	0.194±0.005	0.8982±0.0005	8.179±5.825
8	0.194±0.005	0.8982±0.0005	8.179±5.825
9	0.142±0.005	0.8290±0.0008	8.285±3.609
10	0.114±0.005	0.8887±0.0001	10.440±3.653
11	0.309±0.011	0.8682±0.0001	3.628±2.724
12	0.301±0.010	0.8180±0.0001	6.157±1.332

Table 6.7: Bilayer properties in different simulations.

System	Inner radius (nm)	Outer radius (nm)	Thickness (nm)
Brickwork 1	4.55 ± 0.39	6.93 ± 0.36	2.37 ± 0.04
Brickwork 2	3.86 ± 0.49	6.18 ± 0.37	2.31 ± 0.04
Herringbone 1	4.09 ± 0.51	6.40 ± 0.40	2.31 ± 0.03
Herringbone 2	4.73 ± 0.47	7.12 ± 0.34	2.39 ± 0.03
Herringbone Long 1	3.71 ± 0.07	6.19 ± 0.06	2.48 ± 0.04
Herringbone Long 1*	3.68 ± 0.05	6.31 ± 0.07	2.63 ± 0.05

Table 6.8: Final dimensions of C8S3 nanotubes simulations. The system with a star (*) was performed with the GAFF force field.

System	Initial inner radius (nm)	Initial outer radius (nm)	Final inner radius (nm)	Final outer radius (nm)
1	3.72	5.49	3.76 ± 0.02	5.48 ± 0.02
2	3.54	5.27	3.50 ± 0.01	5.24 ± 0.01
3	3.73	5.44	3.72 ± 0.02	5.43 ± 0.01
4*	3.72	5.44	3.71 ± 0.01	5.43 ± 0.02
5	3.73	5.45	3.74 ± 0.04	5.50 ± 0.03
6	3.73	5.44	3.79 ± 0.04	5.53 ± 0.03

Table 6.9: Initial parameters for the preparation of C8S3 nanotubes simulations for the optical spectra calculations. This set of simulations was performed with the GAFF force field and the initial unit cell was adjusted by increasing its y-dimension by 0.1 nm. System with a star (*) indicate that the initial unit cell for the outer wall was further adjusted (increased by 2.4% in the y- and z-dimension) to imitate a change in the monomer density.

6.2 Coarse-graining cyanine dyes

Atom	Model 1	Model 2	Model 3
H1	0.030	0.133	0.224
C2	-0.030	-0.017	0.148
C3	-0.250	-0.448	-0.606
C4	-0.250	-0.448	-0.606
C5	0.660	0.852	0.406
C6	0.660	0.852	0.406
H7	0.030	0.116	0.248
H8	0.030	0.116	0.248
N9	-0.390	-0.539	-0.169
N10	-0.390	-0.539	-0.169
N11	-0.390	-0.539	-0.169
N12	-0.390	-0.539	-0.169
C13	0.280	0.334	0.094
C14	0.280	0.334	0.094
C15	0.280	0.334	0.094
C16	0.280	0.334	0.094
C17	-0.160	-0.227	-0.220
C18	-0.160	-0.227	-0.220
C19	-0.160	-0.227	-0.220
C20	-0.160	-0.227	-0.220
C21	0.020	0.014	0.072
C22	0.020	0.014	0.072
C23	0.020	0.014	0.072
C24	0.020	0.014	0.072
H25	0.110	0.170	0.191
H26	0.110	0.170	0.191
H27	0.110	0.170	0.191
H28	0.110	0.170	0.191
CL29	-0.030	-0.055	-0.065
CL30	-0.030	-0.055	-0.065
CL31	-0.030	-0.055	-0.065
CL32	-0.030	-0.055	-0.065
C33	0.200	0.264	0.206
C34	0.200	0.264	0.206
C35	0.200	0.264	0.206
C36	0.200	0.264	0.206
C37	0.0	0.0	0.024
C38	0.0	0.0	0.024
C39	0.0	0.0	0.024
C40	0.0	0.0	0.024

Table 6.10: Partial charges for C2C2.

Atom	Model 1	Model 2	Model 3
H1	0.030	0.166	0.21
C2	-0.030	-0.006	0.133
C3	-0.250	-0.499	-0.562
C4	-0.250	-0.499	-0.562
C5	0.660	0.902	0.420
C6	0.660	0.902	0.420
H7	0.030	0.164	0.282
H8	0.030	0.164	0.282
N9	-0.390	-0.565	-0.185
N10	-0.390	-0.565	-0.185
N11	-0.390	-0.565	-0.185
N12	-0.390	-0.565	-0.185
C13	0.280	0.338	0.112
C14	0.280	0.338	0.112
C15	0.280	0.338	0.112
C16	0.280	0.338	0.112
C17	-0.160	-0.224	-0.234
C18	-0.160	-0.224	-0.234
C19	-0.160	-0.224	-0.234
C20	-0.160	-0.224	-0.234
C21	0.020	0.011	0.067
C22	0.020	0.011	0.067
C23	0.020	0.011	0.067
C24	0.020	0.011	0.067
H25	0.110	0.159	0.188
H26	0.110	0.159	0.188
H27	0.110	0.159	0.188
H28	0.110	0.159	0.188
CL29	-0.030	-0.085	-0.094
CL30	-0.030	-0.085	-0.094
CL31	-0.030	-0.085	-0.094
CL32	-0.030	-0.085	-0.094
C33	0.200	0.215	0.120
C34	0.200	0.215	0.120
C35	0.0	0.069	0.0
C36	0.0	0.022	0.044
C37	-0.080	-0.062	0.019
C38	0.120	-0.124	-0.093
C39	0.640	0.918	0.865
C40	0.600	0.877	0.878

Atom	Model 1	Model 2	Model 3
O41	-0.780	-0.722	-0.782
O42	-0.610	-0.784	-0.682
O43	-0.780	-0.722	-0.782
O44	-0.570	-0.685	-0.651
C45	0.200	0.192	0.120
C46	0.200	0.192	0.120
C47	0.0	0.031	0.089
C48	0.0	0.031	0.089
C49	0.0	-0.007	-0.001
C50	0.0	-0.007	-0.001
C51	0.0	0.017	0.035
C52	0.0	0.017	0.035
C53	0.0	0.0	-0.009
C54	0.0	0.01	-0.009
C55	0.0	0.016	-0.002
C56	0.0	0.016	-0.002
C57	0.0	0.05	0.062
C58	0.0	0.05	0.062
C59	0.0	-0.05	-0.068
C60	0.0	-0.05	-0.068
H61	0.460	0.455	0.453

Table 6.11: Partial charges for C8O3. Partials charges for the carboxylic tails were adapted from butanoic acid in ATB (Molecule ID: 936 and 6085).

Figure 6.1: Comparison of atomistic and CG bond length distributions for C2C2.

Figure 6.2: Comparison of atomistic and CG dihedral distributions for C2C2.

Figure 6.3: Comparison of atomistic and CG bonded terms distributions for C2S4.

Figure 6.4: Comparison of atomistic and CG bonded terms distributions for C8S3.

Figure 6.5: Analysis of the C2C2 small scale self-assembly simulation at different time periods. RDF of the C3 bead (left). P_2 parameter for angle θ (right).

Figure 6.6: Analysis of the C2S4 small scale self-assembly simulation at different time periods. RDF of the C3 bead (left). P_2 parameter for angle θ (right).

Figure 6.7: Analysis of the C8S3 small scale self-assembly simulation at different time periods. RDF of the C3 bead (left). P_2 parameter for angle θ (right).

Figure 6.8: Analysis of the C2C2 large scale self-assembly simulation at different time periods. RDF of the C3 bead (left). P_2 parameter for angle θ (right).

Figure 6.9: Analysis of the C2S4 large scale self-assembly simulation at different time periods. RDF of the C3 bead (left). P_2 parameter for angle θ (right).

Figure 6.10: Analysis of the C8S3 large scale self-assembly simulation at different time periods. RDF of the C3 bead (left). P_2 parameter for angle θ (right).

6.3 Directed self-assembly of a 2NapFF nanotube

Figure 6.11: Snapshots from a random self-assembly simulation of the 2NapFF molecules at a) 0 ns, b) 30 ns and c) 180 ns. The structure of a random aggregate is highlighted.

6.4 Dimerization of small aromatic rings

Name	Molecule ID	Opt. Solvation Free Energy	Simulation time (ns)
Benzene	804	✓	100
Toluene	21	✓	100
Chlorobenzene	3176	✓	150
Phenol	1061	✓	100
Styrene	1266	✓	100
para-Cresol	17	✓	150
Cyclohexane	17666	✓	100
Naphthalene	820	✓	100
Pyrene	7233	✓	150
Tetracene	33259	✗	150

Table 6.12: ATB entries for the topologies used. Most of the parameters have been optimised to reproduce experimental solvation free energies.

Figure 6.12: a) FES for the dimerization of toluene, b) Statistical error of the FES calculations as a function of the block size.

Figure 6.13: a) FES for the dimerization of chlorobenzene, b) Statistical error of the FES calculations as a function of the block size.

Figure 6.14: a) FES for the dimerization of phenol, b) Statistical error of the FES calculations as a function of the block size.

Figure 6.15: a) FES for the dimerization of styrene, b) Statistical error of the FES calculations as a function of the block size.

Figure 6.16: a) FES for the dimerization of para-cresol, b) Statistical error of the FES calculations as a function of the block size.

Figure 6.17: a) FES for the dimerization of cyclohexane, b) Statistical error of the FES calculations as a function of the block size.

Figure 6.18: a) FES for the dimerization of naphthalene, b) Statistical error of the FES calculations as a function of the block size.

Figure 6.19: a) FES for the dimerization of pyrene, b) Statistical error of the FES calculations as a function of the block size.

Summary

"All models are wrong, but some are useful."

- George E. P. Box, 1919-2013

No matter how complex a model or a theory is, in the end, it is a simpler representation of reality based on our interpretations of how reality works. Consequently, the models we use might not always be either correct or accurate. However, time has shown that employing models and computations together with experimental data can provide useful information on fundamental mechanisms of the systems under study and validate or disprove proposed theories, when experiments or logic alone fail. The aim of this thesis was to study different nanomaterials and chemical compounds and obtain high resolution computational results on their structural and thermodynamic properties. Philosophy, modelling and methods are discussed in **Chapter 1**.

We mainly focused on the structural features of C8S3 aggregates. C8S3 molecules are amphiphilic cyanine dyes that spontaneously self-assemble into nanotubes when solvated in water. The structural and optical properties of C8S3 nanotubes have been experimentally and theoretically studied over the past decades, but there was no study for the arrangement of the C8S3 monomers that incorporated a full spatial description for the C8S3 molecules or produced stable aggregates for the time scales we report. In **Chapter 2**, our results from MD simulations of preformed C8S3 aggregates show that the thickness of the C8S3 bilayers and nanotubes is ~ 2.5 nm, close to the reported values for bilayers from AFM (~ 3.0 nm) and the estimated values for nanotubes from SAXS (~ 2.5 nm), but different from cryo-TEM measurements on nanotubes (~ 3.5 - 4.0 nm). We attribute this difference to the small size of the object and the difficulty to decompose the signal of the inner and outer wall in the cryo-TEM contrast profiles, in order to define the nanotube boundaries. Furthermore, we show that stable C8S3 nanotubes can be simulated, if a reasonable

initial arrangement for the monomers and an optimal thickness is used. The simulated C8S3 aggregates were used for theoretical calculations of the C8S3 absorption spectra that were in agreement with experimental absorption spectra. These findings pave the way for future work on modelling the structural and optical properties of C8S3 nanotubes, studying different initial arrangements of C8S3 monomers and simulating later events of the C8S3 self-assembly, which is the formation of bundles.

In **Chapter 3**, a CG model was prepared for self-assembly simulations of C8S3 molecules and other cyanine dyes, C2C2 and C2S4. The main goal was creating a CG model able to reproduce with great detail structural/spatial properties of the common aromatic core shared among these cyanine dyes. The bonded parameters were thoroughly tuned. Then, self-assembly simulations were attempted. Different structures emerged from each cyanine dye simulation, that did not resemble the experimental determined structures. On the other hand, preformed C8S3 nanotube simulations with the CG model showed that the model is compatible with the nanotube structure. Potential issues of the CG model and the simulation setup are discussed and different solutions are suggested, as future perspectives to improve the performance of the self-assembly simulations. In the same chapter, guidelines and best practices for creating CG models for novel or exotic molecules are also provided.

Regardless of the system and the resolution, simulating a self-assembly process is a complex and time consuming problem. A protocol for preparing initial structures for MD simulations by using experimental data for the dimensions of aggregates is described in **Chapter 4**. The basic idea is that the building blocks of the aggregate are driven and, then, constrained in a restricted area defined by the experimental measurements. After equilibration, the constraints are removed and the system is free to relax. Different properties of the final structures can be calculated, such as the final dimensions or the relative arrangement of the monomers, and compared to the experimental results. The protocol was used on a small amphiphile, 2NapFF, that forms tubular structures. The final structures were stable and the results for the internal molecular structure are compatible with the theory for characteristics of low molecular weight gels, such as 2NapFF. The same protocol can be adapted to obtain initial structures of different nanomaterials and aggregate shapes, when random self-assembly simulations are not an option.

In **Chapter 5**, the convergence of different force fields in calculating the free energy of dimerization of small aromatic rings was evaluated. Our results show that for small molecules the profiles for the dimerization free energy of small molecules are almost identical, and the distance and energy of binding are quite similar. However, as the size of the aromatic molecules increases, so do the deviations from the general trends. Consequently, the results for larger molecules should be interpreted with great care, and further force field optimization and comparison with experimental results are required.

Samenvatting

Hoe complex een model of theorie ook is, uiteindelijk is het een eenvoudigere weergave van de realiteit gebaseerd op onze interpretaties van hoe de werkelijkheid werkt. Derhalve zijn de modellen die we gebruiken niet altijd correct of nauwkeurig. De ervaring heeft echter geleerd dat het gebruik van modellen en berekeningen, samen met experimentele data, nuttige informatie kan verschaffen over fundamentele mechanismen van de bestudeerde systemen en voorgestelde theorieën kan valideren of falsificeren, wanneer experimenten of logica te kort schieten. Het doel van dit proefschrift was om verschillende nanomaterialen en chemische verbindingen te bestuderen en hoge resolutie computationele resultaten van de structurele en thermodynamische eigenschappen te produceren. Filosofie, modelleren en methodiek worden in **Hoofdstuk 1** besproken.

We hebben ons vooral gericht op de structurele kenmerken van C8S3-aggregaten. C8S3-moleculen zijn amfifiele cyaninekleurstoffen die spontaan tot nanobuisjes assembleren wanneer deze in water worden opgelost. De structurele en optische eigenschappen van C8S3-nanobuizen zijn de afgelopen decennia experimenteel en theoretisch bestudeerd, maar er was nog geen onderzoek gedaan naar de organisatie van de C8S3-monomeren met een volledige beschrijving van de structuur van de C8S3 moleculen of een beschrijving die stabiele aggregaten opleverde op de tijdschalen zoals wij die hier vermelden. In **Hoofdstuk 2** laten we zien dat in MD simulaties van voorgevormde C8S3 aggregaten, de dikte van C8S3 dubellagen en wanden van nanobuizen ongeveer 2.5 nm is, hetgeen goed vergelijkbaar is met de waarden gemeten voor dubellagen (ongeveer 3.0 nm) en de geschatte waarden voor nanobuizen gemeten met SAXS (ongeveer 2.5 nm), maar afwijkend van cryo-TEM metingen aan nanobuizen (ongeveer 3.5-4.0 nm). We schrijven dit laatste verschil toe aan de zeer kleine afmetingen van het object en de moeilijkheid de signalen die het fasecontrast in cryo-TEM vormen voor de binnen- en buitenwand van elkaar te onderscheiden. De dikte van de nanobuizen wordt in cryo-TEM bepaald aan de hand van deze fasecontrasten. Verder laten we zien dat stabiele C8S3-nanobuizen gesimuleerd kunnen worden, mits er aanvankelijk een redelijke rangschikking voor de monomeren in en een optimale dikte van de wand van de buizen wordt gebruikt. Deze gesimuleerde C8S3-aggregaten werden voor theoretische berekeningen van de C8S3 absorptiespectra gebruikt, en bleken in overeenstemming met experimentele absorptiespectra. Deze bevindingen maken toekomstig onderzoek mogelijk naar het modelleren van de structurele en optische eigenschappen van C8S3 nanobuisjes, de verschillende initiële rangschikking van C8S3-monomeren en het simuleren van verdergaande C8S3 zelf-assemblage, namelijk de vorming van de bundels.

In **Hoofdstuk 3** werd een zogenaamd coarse-grain (CG-)model opgesteld voor simulaties van de zelf-assemblage van C8S3-moleculen en andere cyaninekleurstoffen, namelijk C2C2 en C2S4. Het voornaamste doel was het creëren van het CG-model

dat in staat is om gedetailleerde structurele- en ruimtelijke eigenschappen weer te geven van de gemeenschappelijke aromatische kern die in al deze cyaninekleurstoffen voorkomt. Eerst werden de parameters die de wisselwerkingen tussen de bouwstenen van het molecuul uitvoerig geoptimaliseerd. Vervolgens werden de simulaties van de zelf-assemblage onderzocht. Verschillende structuren kwamen voort uit iedere cyaninekleurstofsimulatie, maar deze kwamen niet overeen met de experimenteel bepaalde structuren. Daarentegen blijkt uit de simulaties van de voorgevormde C8S3 nanobuis met het CG-model dat het model compatibel is met de nanobuis structuur. Potentiële problemen van het CG-model en de simulatie-instellingen worden besproken en er worden verschillende oplossingen voorgesteld als toekomstperspectieven om de prestaties van de zelf-assemblagesimulaties te verbeteren. In hetzelfde hoofdstuk worden ook richtlijnen en best practices voor het maken van CG-modellen voor nieuwe of exotische moleculen geboden.

Ongeacht het systeem en de resolutie, is het simuleren van een zelfassemblageproces een complex en tijdrovend probleem. Een protocol voor het voorbereiden van initiële structuren voor MD-simulaties met behulp van experimentele gegevens voor de dimensies van aggregaten wordt behandeld in **Hoofdstuk 4**. Het voorname idee is dat de bouwstenen van het aggregaat vanuit het oplosmiddel worden verzameld en vervolgens beperkt in een afgebakend gebied gedefinieerd door de experimentele metingen. Na equilibratie worden de beperkingen verwijderd en is het systeem vrij om te relaxeren tot de meest gunstige structuur. Verschillende eigenschappen van de uiteindelijke structuren kunnen worden berekend, zoals de uiteindelijke afmetingen of de plaatsing van de monomeren ten opzichte van elkaar, en worden vergeleken met de experimentele resultaten. Het protocol werd gebruikt op een kleine amfifiel, 2NapFF, dat buisvormige structuren vormt. De uiteindelijke structuren waren stabiel en de resultaten voor de interne moleculaire structuur zijn compatibel met de theorie voor kenmerken van gels met een laag molecuulgewicht, zoals 2NapFF. Hetzelfde protocol kan worden aangepast om initiële structuren van verschillende nanomaterialen en geaggregeerde vormen te verkrijgen, wanneer willekeurige zelfassemblagesimulaties niet mogelijk zijn.

In **Hoofdstuk 5**, werd de convergentie van verschillende krachtvelden bij het berekenen van de Gibbs dimerisatie-energie van kleine aromatische ringen geëvalueerd. Onze resultaten tonen aan dat de profielen voor de vrije energie van dimerisatie van kleine moleculen bijna identiek is voor verschillende kleine moleculen, en dat de afstand en energie van binding vergelijkbaar zijn. Echter, naarmate de grootte van de aromatische moleculen toeneemt, nemen ook de afwijkingen van de algemene trend toe. Bijgevolg moeten de resultaten voor grotere moleculen voorzichtig worden geïnterpreteerd en verdere krachtveldoptimalisatie en -vergelijking met experimentele resultaten zijn hiervoor nodig.

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List of publications

I. Patmanidis, A. H. de Vries, T.A. Wassenaar, W. Wang, G. Portale and S. J. Marrink. Insights on the structure of C8S3 nanotubes with atomistic simulations and SAXS. *in preparation* (**Chapter 2**)

R. Alessandri, J. Barnoud, **I. Patmanidis**, A. H. de Vries, P. C. Telles de Souza and S. J. Marrink. Martini 3.0 Coarse-Grained Force Field: Ring Structures. *in preparation*

A. Bondarenko, **I. Patmanidis**, R. Alessandri, P. C. T. Souza, T. L. C. Jansen, A. H. De Vries, S. J. Marrink and J. Knoester. Multiscale Modeling of Structural and Optical Properties of Complex Supramolecular Aggregates. *in preparation* (**Chapter 2**)

P. C. T. Souza, R. Alessandri, J. Barnoud, S. Thallmair, I. Faustino, F. Grunewald, **I. Patmanidis**, et al. Martini 3.0: A General Purpose Force Field for Coarse-Grain Molecular Dynamics. *in preparation*

D. G. Kontopoulos, **I. Patmanidis**, T. G. Barraclough and S. Pawar. Higher temperatures worsen the effects of mutations on protein stability. *in preparation*

E. R. Draper, B. Dietrich, K. McAulay, C. Brasnett, H. Abdizadeh, **I. Patmanidis**, S. J. Marrink, H. Su, H. Cui, R. Schweins, A. Seddon and D. J. Adams. Using Small-Angle Scattering and Contrast Matching to Understand Molecular Packing in Low Molecular Weight Gels. *Matter*, 2(3):764-778, 2020. (**Chapter 4**)

P. W. J. M. Frederix, **I. Patmanidis** and S. J. Marrink. Molecular simulations of self-assembling bio-inspired supramolecular systems and their connection to experiments. *Chemical Society Reviews*, 47(10):3470-3489, 2018. (**Chapter 1**)

M. Zhang, T. Peyear, **I. Patmanidis**, D. V. Greathouse, S. J. Marrink, O. S. Andersen and H. I. Ingólfsson. Fluorinated Alcohols' Effects on Lipid Bilayer Properties, *Biophysical Journal*, 115(4):679-689, 2018.

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Tassis, you played a catalytic role in changing the routine in the city and the campus with your caustic humour and commitment in planning and preparation of all kinds of activities. The last is something that I should definitely take up. I am sure you will do fine with your PhD golgotha. Even if you don't, you have a strong, independent woman, **Despoina**, to take care of you. Good luck to both of you.

Andreas, I hope you don't hold a grudge against me, because of my spiteful comments. My intentions are pure. Thank you for all the discussions and advice during the last years. I really appreciate your opinion, even if I don't act like I do. **Katerina**, good luck dealing with him and remember, you have to show understanding, when he is really busy.

Kalli, thank you for all the discussions and the nice walks in Amsterdam. Follow your dreams and take good care of yourself. You deserve it.

Misun, **Friso**, **Nikk**, **Mel** and **César**, for making Wednesdays so special and giving me the opportunity to play music with the cool kids.

Jorge, for being the other crazy guy who always showed up for basketball, the nice rehearsals and gigs, and eventful afternoons and nights. I wish we started hanging out earlier or you stayed here longer. You should definitely visit more often and also take **Marina** with you.

Ciccio, **Lucas**, **Cosimo**, **Tom** and all the other fellow adventurers, for the endless hours of fun (and salt). Never forget that existence is pain without death. It is the journey that matters.

Linda and **Koen**, for the nice time we had together and the "Dutch" dinners that were not so Dutch in the end. All the best for you and **Wouter**.

Chris, the latest tenant of the Ebbinge house. I feel you are worthy of carrying on the legacy. Also, thanks for the Dutch translation of the summary and the private Dutch lessons.

All the borrel people, **Nikola, Simone, Caterina, Milos, Reuben, Rudy, Titia, Andrea, Mohammed, Alejandro, Hemant, Elvira, Juanfer, Mamen, Tilde, Clemens, Lur, Marco, Michele, Spyros, Guillaume**, for the lovely Friday evenings.

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Anna, it is really special to be once again with people with whom you had common experiences and worries many years ago. I hope you enjoy working at RUG and living in Groningen as much as I did. **Panos**, good luck with figuring out what you want to do in your life.

Kiki, thank you for all the beautiful moments we had together, the trips and the food. I really appreciate the effort you are putting into making this thing work.

Μαμά και μπαμπά, θείε Γιάννη, σας ευχαριστώ που όλα αυτά τα χρόνια φροντίζετε να μη μου λείπει τίποτα. Χωρίς εσάς δε θα βρισκόμουν σήμερα εδώ.