Lipid Membranes-Mediated Interactions and self-assembly of spherocylindrical Nanoparticles

Abash Sharma

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LIPID MEMBRANES-MEDIATED INTERACTIONS AND SELF-ASSEMBLY OF SPHEROCYLINDRICAL NANOPARTICLES

by

Abash Sharma

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ACKNOWLEDGMENTS

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Self-assembly is a fascinating natural process that spontaneously organizes specific structures from multiple components. In the field of nano-cluster fabrication, where there is a high demand for large volumes, particularly in biomedical applications like bio-sensing, drug delivery, and gene therapy, the difficulty of top-down fabrication in nano-scale structures has led to growing interest in exploring bottom-up techniques. These techniques involve self-assembling basic units into desired structures through physical or chemical forces. Recently, significant progress has been made in self-assembling precisely organized nano-clusters, including dimers, helices, polyhedra, and superlattices.

Lipid membranes play a vital role in life as they form the basis of cells and many cellular organelles, acting as a barrier between the internal and external environments. Consequently, the study of lipid membranes has been ongoing for several decades. Due to their fluidity, flexibility, and relatively strong adhesion between nanoparticles (NPs) and lipid head groups during endocytosis, researchers have considered using lipid membranes as a medium for fabricating specific nanoclusters. Since most work has been done on spherical NPs, we intend to bridge this gap by systematically studying lipid membrane-mediated adhesion and interaction of SCNPs. In this work, we propose to study the adhesion and clustering of spherocylindrical NPs (SCNPs) and provide a solution to address the challenges associated with endocytosis by utilizing functionalized nanoparticles.

Throughout this dissertation, we have thoroughly investigated the interaction and adhesion of SCNPs on lipid membranes and explored the potential of lipid membranes as a medium for self-assembling SCNPs into specific nanoclusters.

Chapters 3 and 4 of this dissertation are respectively centered on articles [1] and [2] which I published in peer-reviewed journals as the lead author, i.e.,

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Chapters 5 and 6 are centered on two other articles which will be published in peer-reviewed journals in the near future. These are


In addition to the work I presented in my dissertation, I also contributed to four other publications during my Ph.D. study. These correspond to one publication as the lead author [3], and three publications [4–6] as the second author.


ABSTRACT

This dissertation highlights the pivotal role of nanoparticles (NPs) anisotropy on their adhesion and potential self-assembly on lipid membranes. The research, which is performed in the context of spherocylindrical NPs (SCNPs), offers valuable insights into the complex interaction between NPs and lipid membranes and the utilization of lipid membranes as a medium for SCNPs self-assembly, potentially opening the door to various advanced applications. Modeling SCNPs as triangulated hollow shells, the adhesion modes and endocytosis of SCNPs on tensionless planar membranes are investigated using molecular dynamics simulations of a coarse-grained implicit-solvent model. The SCNPs are shown to adhere to the membrane through two main modes, namely a parallel mode and a normal mode. We found that increasing the aspect ratio of the SCNPs facilitates the transition from the parallel to the normal mode. This exploration was extended to investigate the adhesion modes, dimerization, and endocytosis of two SCNPs on planar lipid membranes. The SCNPs exhibit five different modes of adhesion, depending on their diameter, aspect ratio, and adhesion strength values. Furthermore, when two SCNPs adhere in close proximity to a membrane, they tend to dimerize, forming either wedged or tubular dimers. In contrast, they adhere in the monomeric normal mode when their initial separation is substantial. Moreover, highly ordered nanoclusters with diverse geometries are obtained when surface-modified Janus SCNPs, such that one moiety interacts more attractively with the lipid head groups, and the other moiety that is hydrophilic and interacts more favorably with the solvent are placed on lipid vesicles. Similarly, the arrangement of uniform SCNPs on the inner side of lipid vesicles is explored, revealing their self-assembly into highly ordered polygonal and quasi-two-dimensional star-like structures, a phenomenon not observed when NPs adhere to the vesicle’s outer side. The geometries of the SCNPs’ nanoclusters are influenced by their aspect ratio, number, and adhesion strength. These structures are characterized by analyzing various quantities, including their symmetry point groups, coplanarity, and nematic order parameter.
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Chapter 1

INTRODUCTION

1.1 Background

The plasma membrane, a major component of all living cells, plays many major biological roles. In particular, the plasma membrane acts as a topological separation between the cell’s interior from the outside environment and acts as a support of a complex molecular machinery that is crucial for the normal functions of the cell \[7\]. The plasma membrane regulates the substances from entering into and out of cells. The plasma membrane also encapsulates many organelles within the cell. The permeability of the cell membrane is vital to the cells since it enables the passage of critical nutrients and specific molecules into the cell and waste materials out of the cell. Similarly, oxygen, carbon dioxide, water, and other smaller molecules can freely diffuse across the membrane, but the passage of amino acids, sugars, and larger molecules is carefully regulated. These membranes are very flexible, which enables them to grow, change shape, and often undergo topological transformations. Membranes in biological systems can perform all these functions by exploiting the physical properties of constituents that make up the membranes. All cell membranes are composed of a wide variety of fatty acids, proteins, and cholesterol. The presence of hydrophobic and hydrophilic sites on each molecule determines the alignment and structure of these constituents within the membrane \[8\].

Fatty acids are strongly hydrophobic and do not readily dissolve in water. They are typically not found in a free state in cells; instead, they chemically bond to a hydrophobic group, such as glycerol, which leads to the formation of lipid molecules. Therefore, fatty acids are the fundamental building blocks for all lipids in living organisms. Replacing one fatty acid with a polar group enhances the affinity of fatty acid for water. In
plants and animals, the length of the chains formed by these fatty acids can range from two to thirty-six, thereby influencing the thickness of the cell membrane and function it carries [9]. Hence, a wide array of lipids can be generated simply by varying fatty-acid chain length, degree of saturation, polar head group, and types of glycosylation.

Lipids are the primary constituents of all biological membranes and comprise most of the mass and volume in cell membranes. They are amphiphilic due to a hydrophilic polar end and a hydrophobic non-polar end. Amphipathic molecules tend to aggregate when placed in a solvent spontaneously. This aggregation minimizes energetically unfavorable interactions between the lipid hydrophobic tails and the solvent while maximizing favorable interactions between the lipid head groups and the surrounding solvent. As a result, lipids typically arrange themselves into bilayers of various topologies when immersed in water. The molecular arrangement of the aggregate depends upon the solvent and the details of the amphipathic structure of the lipid, and depending on the display, it leads to the formation of many molecular aggregates through spontaneous self-assembly. They can also form tubes and micelles depending on their concentrations and mixtures. The three major classes of membrane lipid molecules are phospholipids, sphingolipids, and sterols [10].

Phospholipids are specialized lipids that make significant components of the plasma membrane. While fats typically comprise three fatty acid chains attached to a glyco-
Phosphate
Glycerol
Hydrophilic head
Hydrophobic tail
(A) Phospholipid
Saturated
Fatty acid
Unsaturated
Fatty acid
Sphingosine backbone
Fatty acid residue
(B) Ceramide
(C) Cholesterol
steroid nucleus
hydrocarbon side chain
hydroxyl group

Figure 1.2: Different Classes of Lipids. (A) A phospholipid. (B) A ceramide, which is a type of Sphingolipid. (C) Cholesterol, the most common sterol found in animal cell membranes.

erol backbone, phospholipids generally have just two fatty acid chains ester-linked to the glycerol, and a third carbon is ester-linked to a modified phosphate group. Different phospholipids have different modifiers on the phosphate group, with choline (a nitrogen-containing compound), serine (an amino acid), and inositol being common examples [10]. Different modifiers give phospholipids different properties and roles in a cell. On the other hand, Sphingolipids are a less abundant class of membrane lipids with a backbone formed from sphingosine, an amino alcohol with a long hydrocarbon chain. Ceramide is a simple sphingolipid with a hydrophobic fatty acid tail linked to the amino group of the sphingosine. Esterification of additional groups to the terminal hydroxyl group of the sphingosine backbone gives rise to other types of sphingolipids, such as sphingomyelin (a polar phosphoryl-choline head group) and glycolipids (a carbohydrate group) [10].

Sterols, a major lipid component of cell membranes, possess a single polar hydroxyl head group attached to a rigid steroid ring structure and a short non-polar hydrocarbon tail. Cholesterol is the principal sterol component of animal cell membranes. Different sterols are found in other eukaryotic cell membranes, such as ergosterol (in yeast
and fungi) as well as sitosterol and stigmasterol (in plants) \[10\]. Interestingly, prokaryotic cell membranes do not contain sterols. Sterols insert into the lipid bilayer with their hydroxyl head groups oriented with the phospholipid polar groups. This aligns the rigid ring structure of the sterol with the phospholipid hydrocarbon tail, which decreases phospholipid mobility. While this stiffening effect reduces the water-soluble permeability of the bilayer, it does not affect the membrane’s fluidity.

Lipid molecules form aggregates with various geometries in the presence of water, the most common of which is the bilayer. Lipid bilayers exhibit two main phases corresponding to the gel phase at low temperatures and the liquid phase at high temperatures \[9\]. In the gel phase, the conformations of the lipid tails are ordered and arranged in a two-dimensional, typically triangular, lattice. In the liquid phase, however, the conformations of the lipid tails are disordered, and their in-plane positions in the bilayer lack long-range order. In the liquid phase, the lipid molecules are fairly diffusive, in contrast to the gel phase. The melting temperature of lipid bilayers depends strongly on the degree of saturation of the lipids and decreases with increasing degree of saturation. The plasma membrane, whose physiological functions require high fluidity and compartmentalization, comprises many types of saturated and unsaturated lipids. Sphingomyelin is the main saturated lipid of animal cells. Because of its high melting temperature, sphingolipid-rich regions are highly mixed with cholesterol in order to provide fluidity and, therefore, biological function to these regions \[9\]. For example, the human body maintains a stable internal temperature of around 37°C. The fluidity of the plasma membrane is ensured by the fact it is comprised of many types of unsaturated lipids, whose melting temperatures are well below the body temperature, and by the enrichment of membrane regions, composed mainly of saturated lipids such as sphingomyelin and dipalmitoyl phosphatidylcholine, with cholesterol \[11\].
Figure 1.3: Characteristic colors emitted by different sizes of gold NPs \cite{15}. The color of the solution changes due to variations in aspect ratio, nanoshell thickness, and gold concentration. The alteration of any of the above-discussed factors influences the absorption properties of the NPs, and hence, different absorption colors are observed.

1.2 Nanoparticles and their Applications

Nanoparticles (NPs) are particles with at least one spatial dimension ranging from 1 to 100 nanometres and are made of carbon, organic matter, metal, or metal oxides \cite{13}. NPs demonstrate distinct physical, chemical, and biological properties at the nanoscale compared to their larger-scale counterparts. These phenomena result from the large surface area of NPs compared to bulk materials, i.e., the large ratio between the number of atoms on an NP’s surface and the number of atoms in its bulk, which increases with the decrease of the NP’s dimensions. This leads to increased reactivity in chemi-
cal processes and enhanced mechanical strength, among many other factors [14]. Bes-
sides, NP properties are sensitive to altering their size, shape, or morphology. For
instance, Dreaden et al. illustrated that Gold (Au) NPs synthesized with different sizes
exhibit characteristic colors and properties that depend on the size and shape of the
NPs [15]. Recent advances in nanoscience and nanotechnology have accelerated the
fabrication of nanomaterials with different properties, compositions, and geometries.
These materials can be tailored at extremely small scales with optimal sizes, shapes,
and surface properties [16]. Depending upon the overall shape and size, these particles
can be zero-dimensional (spheres), one-dimensional (nanorods, nanowires, nanotubes),
two-dimensional (triangles, plates and sheets, ribbon), or three-dimensional (pyramids,
stars, nanoboxes, nanocubes) [17].

NPs can be broadly divided into categories depending on their morphology, size, and
chemical properties, namely carbon-based NPs, metallic NPs, semiconducting NPs, ce-
ramics NPs, polymeric NPs, and lipid-based NPs [18]. Fullerene, a spherical hollow
structures, graphene, and carbon nanotubes (CNTs) are major examples of carbon NPs.
These NPs are extensively utilized because of their unique physical, chemical, and me-
chanical characteristics such as electrical conductivity, electron affinity, high strength,
etc [19,20]. Metallic NPs are composed of metals such as copper (Cu), silver (Ag), iron
(Fe), aluminum (Al), and gold (Au) and possess distinct optical and electrical prop-
erties. For example, these NPs have enhanced absorption and scattering of light near
localized surface plasmon resonance (LSPR) [14,18], and hence, exhibit a wide absorp-
tion range within the visible regime of the electromagnetic spectrum. Hence, controlled
synthesis of metal NPs, with precise control over their facets, size, and shape, has vari-
ous applications across different research fields [15]. For instance, coating on gold NPs
(AuNPs) enhances the electronic stream and is commonly used in scanning electron
microscopy (SEM) to produce high-quality images [18]. Similarly, semiconductor NPs
possess properties between those of metals and nonmetals. Since these NPs have wide
bandgaps, bandgap tuning leads to a significant alteration in their properties and has at-
tracted interest from researchers for applications in water splitting [21], photocatalysis, photo optics, and electronic devices [18, 22, 23].

Ceramic NPs are solid inorganic and nonmetallic and are synthesized through heating and subsequent cooling processes. They can be found in various forms, including amorphous, polycrystalline, dense, porous, or hollow structures [18, 24]. These NPs have numerous applications in catalysis, photocatalysis, dye photodegradation, and imaging applications [25]. Polymeric NPs are organic NPs that can either have polymeric molecules adsorbed to the outer boundary of their solid matrix or have solid mass encapsulated within the particle completely [26]. These NPs can be functionalized for numerous applications [18]. Lastly, lipid NPs include solid lipid nanoparticles, nanostructured lipid carriers, and cationic lipid–nucleic acid complexes [27]. These particles are typically spherical in shape, with their size ranging from 10 to 1000 nm in diameter [18]. The timing and location of drug release can be highly regulated by decorating the NPs with ligands that bind to specific receptors expressed by the targeted cells [29, 30]. A recent application of these lipid NPs includes their use in COVID-19 mRNA vaccines to convey mRNA to cells efficiently [27].

NPs are synthesized using various approaches that can be broadly classified as top-down approaches or bottom-up approaches. The top-down approach involves starting with larger molecules or bulk materials and decomposing them into smaller nanoscale units. These units are ultimately converted into suitable NPs. Some of the widely used top-down methods for NP synthesis are grinding/milling, chemical vapor deposition (CVD), physical vapor deposition (PVD), laser ablation, sputtering, and thermal decomposition [14, 31]. On the other hand, the bottom-up approach, also known as the building-up approach, involves the build-up of NPs from relatively simpler substances, such as atoms or molecules. These smaller units of atoms or molecules are assembled to form desired NPs. Sol-gel, atomic layer decomposition, self-assembly, spinning biochemical synthesis, etc., are common bottom-up approaches to synthesizing NPs. Recent advances have led to the bottom-up synthesis of NPs biologically [32, 33],
an approach that is environmentally more friendly than the other approaches. For in-
stance, studies have shown that specific bacteria, fungi, and plants can synthesize mettal-
ic nanoparticles intracellularly or extracellularly [34]. This method requires low energy
input and is more cost-effective than physical or chemical synthesis methods.

The application of nanomaterials in various fields has increased tremendously. The
increased production and use of NPs, including consumer products, has led to increased
entry into living organisms, including the human body. This leads to increased inter-
action of various NPs with living cells. It is important to note that some NPs and
nanomaterials may introduce certain toxicities to humans and cells. For instance, while
magnetic NPs have many advantages arising from their small size, high reactivity, and
great capacity, these factors might be lethal, inducing adverse and toxic cellular effects.
In addition to direct effects on humans, the growing use of NPs in a wide range of prod-
ucts results in their introduction into water environments. Subsequently, these NPs can
dissolve and release ions or initiate other reactions, which might prove detrimental to
aquatic life. For example, studies have linked the use of Ag NPs to toxic effects on
aquatic organisms, including bacteria, algae, fish, and plankton [35].

Development and nanomaterials synthesis and functionalization imply that NPs can
be employed in various biological applications due to the rapid progress and early ac-
ceptance of nano-biotechnology. However, the potential adverse health effects from
prolonged exposure to different concentration levels of NPs in living cells and the envi-
ronment are poorly understood. With increasing applications of nanomaterials, there is
a growing need to study and understand nanotoxicity and how to minimize it [37–39].
To mitigate the potential toxicity of nanomaterials, it is vital to understand the in-
teractions of NPs with biomembranes since these act as an entry point for all living
cells [36,39].
1.3 Interaction of Nanoparticles with Membranes

The plasma membrane is an effective barrier for all living cells from the outside world since the membrane is highly selective and strongly limits the passage of large macromolecules in and out of the cells [40]. NPs overcome this barrier to enter living cells through various endocytic pathways or via passive penetration of the plasma membrane [41]. When NPs undergo endocytosis, they remain encapsulated within early endocytic vesicles, which prevent them from immediately being transported into the cytosol. In contrast, NPs are transferred directly into the cytoplasm when internalized via membrane penetration [42].

The cellular uptake of NPs by the plasma membrane usually happens through the membrane engulfment of the particle [46]. However, NPs also have the ability to enter living cells through passive penetration of the plasma membrane [42]. When NPs are internalized through membrane engulfment, they are enclosed within early endocytic vesicles and not directly transported into the cytosol [42]. In contrast, membrane penetration allows direct transfer of internalized NPs into the cytoplasm, which can be harnessed for drug delivery [47].

Cellular uptakes of NPs through membrane engulfment occur through two main processes, known as phagocytosis and endocytosis. Phagocytosis involves the engulfment of particles larger than 1 $\mu$m by the membrane by specialized cells such as monocytes/macrophages, neutrophils, and dendritic cells. In the process of engulfing particles, these cells can form intracellular phagosomes [48]. On the other hand, endocytosis refers to the cellular uptake of smaller particles and is often mediated by specialized proteins, Clathrin and Caveolin [49]. Clathrin-mediated endocytosis is initiated through specific ligand-receptor interactions and results in the uptake of NPs through entrapment inside endosomal/lysosomal vesicles [50]. Caveolae-dependent, also known as raft-dependent endocytosis, involves clustering lipid raft domains on the plasma membrane, forming invaginated flask-shaped structures known as caveolae [51]. These
structures are formed due to membrane interactions with various proteins, especially Caveolin. However, protein-independent endocytosis of NPs has also been observed. Studies have demonstrated that Au and Titanium dioxide (TiO$_2$) NPs with diameters ranging between 20 and 200 nm enter red blood cells, which lack the capacity to induce phagocytosis or clathrin and caveolin-mediated endocytosis [52]. Tahara et al. showed a spontaneous internalization of soft poly(D, L-lactide-co-glycolide) (PLGA) NPs by multicomponent giant unilamellar vesicles [53]. Similarly, Le Bihan et al. observed the spontaneous internalization of surface-modified gold and silica NPs by liposomes, following an endocytic-like process, without the help of any energy or proteins [54]. The same group found that the liposomes did not internalize smaller particles with diameters less than 30 nm. These findings highlight the need to understand better the adhesion of NPs adhesion on lipid membranes and their passive internalization.

Many studies have been performed to understand the interaction between NPs and
Figure 1.5: A schematic representation of a single spherical NP (orange) that is partially wrapped by the membrane (black).

biomembranes [43–45]. However, it is hard to study the effects of NPs’ adhesion on the conformation of membranes due to limitations in the spatial resolution of current microscopy tools. Moreover, lipid membranes are highly dynamic. As a result, morphological changes of the membrane induced by NPs’ adhesion happen over very short time scales. Hence, most of our understanding of NPs’ adhesion and interaction with lipid membranes is based on theoretical models and numerical simulations of lipid membranes. Recent advances have paved the way for a large number of computational studies during the last 15 years, making them very essential to our understanding of the interaction between NPs and lipid membranes [46, 47, 55–68].

The fluidity of lipid membranes and their out-of-plane curvature deformations make their interactions with NPs particularly interesting. NPs deform the membrane once they come in contact with it. The onset and modes of adhesion of NPs to lipid membranes are determined by a competition between the adhesive energy and the membrane elasticity [46, 69, 70]. In the simplest case of a spherical NP with diameter $D$ in contact with a planar tensionless membrane (see Fig. 1.5), the free energy of the membrane-NP composite is given by

$$F = E_{adh} + E_{curv}. \quad (1.1)$$

where $E_{adh}$ is the adhesion energy of the NP’s surface to the membrane, and $E_{curv}$ is the curvature energy of the membrane due to its deformation from its preferred (usually planar) geometry. Assuming that the interaction between the NP and the membrane is
short-ranged, $E_{adh}$ can be written as

$$E_{adh} = -\pi \nu D^2 \xi, \quad (1.2)$$

where $\xi (> 0)$ is the adhesion energy density and $\nu$ is the fraction of the NP’s surface in contact with the membrane. $E_{curv}$ is described by the Helfrich Hamiltonian [71], i.e.,

$$E_{curv} = \sigma A + \int da \frac{\kappa}{2} \left( \frac{c_1 + c_2}{2} - c_0 \right)^2 + \int da \kappa' c_1 c_2, \quad (1.3)$$

where $\sigma$ is the tension of the membrane, $\kappa$ is its bending modulus and $\kappa'$ is its saddle-splay bending modulus. The first term in Eq. (1.3) is typically very small. Similarly, the last term is a topological constant, given $\kappa'$ is independent of space, as per the Gauss-Bonnet theorem [72]. Hence, only the second term in Eq. (1.3) contributes to the curvature energy in a membrane system that does not undergo topological change.

It is crucial to highlight that the free energy stated in Eq. (1.1) assumes two conditions: firstly, the diameter of the NP is significantly larger than the membrane’s thickness, and secondly, the interaction between the NP and the membrane is a short-range contact interaction.

Minimization of Eq. (1.1) in the case of a tensionless lipid membrane ($\sigma = 0$) shows two distinct states separated by a first-order transition that occurs at the adhesion strength $\xi^* = 8\kappa A / D_{NP}^2$ [73]. When $\xi < \xi^*$, the NP is unbound to the membrane, while for $\xi > \xi^*$, the NP becomes fully wrapped by the membrane. This model suggests that the NP is either unbounded when $\xi < \xi^*$ or endocytosed when $\xi > \xi^*$. However, as tension is introduced into the membrane, this transition becomes continuous, and the NP is either unbound, partially wrapped, or completely wrapped by the membrane.

In contrast to the previously mentioned model, a molecular dynamics study conducted by Spangler et al. on the adhesion of spherical NPs to tensionless lipid membranes yielded different findings [46]. They presented a more microscopic approach in which the lipid membrane is treated as a self-assembled bilayer of coarse-grained
Figure 1.6: Interaction of a spherical NP placed on a tensionless lipid bilayer for different values of adhesion strength. (A) Snapshots (i)–(vii) correspond to increasing adhesion strength values. (B) The degree of wrapping, defined as $Z = (1 - \cos \theta)/2$, vs. adhesion strength for the cases corresponding to (A). From Spangler et al. (2016) [46].

lipids. Importantly, this study incorporates random thermal fluctuations, and the diameter of the NP is comparable to the membrane’s thickness. Similarly, the interaction of the NP with a portion of the bilayer in the neck region is accounted for in this study, as opposed to the model proposed earlier by Deserno et al., which approximates that their interaction with the membrane is only a contact potential. Spangler et al. showed that the degree of wrapping of an NP increases continuously as the adhesion strength increases, as demonstrated by Fig. 1.6(B). At very low adhesion strengths, the NP remains unbound to the membrane. However, at high adhesion strengths, the NP undergoes endocytosis, as depicted by snapshot (vii) in Fig. 1.6(A). Hence, Deserno et al.’s theory is only valid for NPs or colloidal particles that are much larger in size compared to the thickness of the membrane.

Various studies have demonstrated that at low or intermediate adhesion strength,
the adhesion of many particles to lipid membranes results in membrane deformations. These deformations occur over distances much larger than the size of the particles, inducing long-range interactions between them. Membrane deformations overlap long before any direct interaction occurs when two particles approach one another, subsequently promoting clustering to minimize the curvature energy. These long range-interactions arising from the adhesion of particles adhering to lipid membranes and the consequent membrane deformations can lead to the self-assembly of these particles, as confirmed by some experimental studies [74–79].

![Figure 1.7: Self-assembly of particles presented in different experimental studies, arising from membrane-mediated interactions. (1) A time sequence of several 0.9-µm-diameter beads placed on a palmytroyloleoylphosphatidylcholine (POPC) vesicle, taken via light microscopy. From Safinya et al. (1999) [75]. (2) Cryogenic transmission electron microscopy (Cryo-TEM) images of citrate-coated AuNPs (cAuNPs) on dipalmitoyl phosphatidylcholine (DPPC) vesicles, showing the self-assembly of the adsorbed cAuNPs, upon heating the composite above \(T_m\). From Sugikawa et al. (2016) [77]. (3) Confocal images of non-wrapped particles that self-assemble irreversibly via lipid structures. Image in (c) separately shows the membrane fluorescence from (b). From Van der Wel et al. (2016) [78].](image)
Safinya et al. [75] experimented with sub-micrometer latex colloidal particles by either chemically bounding or physisorbed the particles to palmytoyleoleylphosphatidylcholine (POPC) or dimristoylphosphocholine (DMPC) vesicles. Using light microscopy, they demonstrated the self-assembly of these particles into linear clusters. This result is one of the pioneering experimental works in the field of NP self-assembly on lipid vesicles (see Fig. 1.7 (1)). Later, Sugikawa et al. [77] showed that citrate-coated Au NPs, which were much smaller in size compared to the latex colloidal particles used by Safinya et al., adsorbed on dipalmytoylphosphatidylcholine (DPPC) liposomes, and formed aggregates of in-plane linear chains at temperature, $T > T_m$ (see Fig. 1.7 (2)). Similarly, Van der Wel et al. [78] showed that giant unilamellar vesicles composed of dioleyolphosphatidylcholine (DOPC) induced an effective attraction between micrometer-sized polystyrene particles adhering to the vesicle, even though the interaction between the colloidal particles, in solution is repulsive (see Fig. 1.7 (3)).

Wang et al. [79] showed that temperature played a crucial role in tuning the interactions between thermoresponsive microgel particles and giant unilamellar vesicles. They manipulated the tunable properties of the microgels, such as softness, deformability, hydrophobicity, and thermosensitivity, to guide the adsorption and assembly behavior. The study presented that phospholipid bilayers can be used as versatile molecular templates for defined and highly ordered interfacial assemblies, 2D colloidal crystals, and selective patterning as imposed by the membrane’s fluidity. Specifically, they found that the microgel NPs exhibited self-assembly behavior at low temperatures, forming two-dimensional hexagonal structures. However, when the vesicle transitioned into a more solid gel phase, the microgel NPs detached from the vesicle surface (see Fig. 1.8).

Several computational models have been used to investigate and understand lipid membrane-induced interactions between spherical NPs [44, 46, 47, 55, 64, 66, 68]. Reynwar et al. [55] used a coarse-grained implicit solvent lipid model and performed molecular dynamics simulations to investigate the aggregation of caps and Janus capsids, representing surface proteins, on lipid membranes. They discovered that the NPs expe-
Figure 1.8: Effect of temperature on the interaction of soft thermoresponsive NPs arising from the membrane. (A) Schematic representation of dimyristoylphosphocholine (DMPC) membrane and thermoresponsive poly(N-isopropyl acrylamide) NPs as a function of temperature. (B–G) Fluorescence confocal micrographs showing a giant DMPC vesicle with the NPs at 30°C (B and E), at 24°C (C and F), and at 17°C (D and G). From Wang et al. (2019) [79].

rienced an attractive force due to the lipid membrane curvature. Similarly, Li et al. [44] used dissipative particle dynamics to present the aggregation of more complex patterned spherical NPs on lipid membranes. However, the aggregation was primarily driven by explicit anisotropic interactions between the NPs rather than the lipid membrane curvature. Moreover, Angelikopoulos et al. [80] performed molecular dynamics simulations of 3-nm spherical NPs coated with hydrophobic and anionic ligands on DPPC lipid membrane, using MARTINI force model [81]. In this study, the NPs partially penetrated the hydrophobic region of the lipid molecule and self-assembled into chains as a result of hydrophobic mismatch. Xiong et al. [66] performed molecular dynamics simulations using an implicit-solvent model to investigate the cooperative behavior of spherical and ellipsoidal NPs. They observed that the aggregation behavior of spherical
NPs depends on their size and number. The larger particles form out-of-plane chains, and smaller particles form out-of-plane triangular trimers and out-of-plane ring structures with a hemifused region in the middle.

The bending modulus of the lipid membranes is shown to play an important role in the aggregation of NPs, as shown by studies. For example, Saric and Cacciuto [61, 62] investigated the effects of adhesion strength and bending modulus of fluid lipid membranes on the aggregation of spherical NPs, by employing a dynamic triangulation Monte Carlo (DTMC) model [82]. In this model, the lipid membrane is treated as a fluid elastic sheet [71]. For moderate values of the bending modulus of the membrane, they found that the adsorbed NPs exhibited a two-dimensional gas state for low adhesion strength, in-plane linear chains for moderate adhesion strength, and tubular chains for high adhesion strength. However, for very low or very high values of the lipid membrane bending modulus, they observed that the NPs formed two-dimensional in-plane triangular lattices. Using the same model, Bahrami et al. [60] performed Monte Carlo energy minimization to find that both two and three NPs aggregated into out-of-plane tubular dimers or trimers on liposomes with high excess area, but not at low excess area. Both Saric and Cacciuto’s and Bahrami et al.’s simulations assumed that the lipid membrane’s local elastic properties are uniform and unaffected by the NPs’ adhesion. However, experiments [83] and recent simulations [46, 67] have shown that NPs’ adhesion on lipid membranes can alter their structural and elastic properties.

Laradji’s group [46, 67] treated the lipid membrane as a self-assembled bilayer of coarse-grained lipids to investigate spherical NPs with sizes comparable to the thickness of the membrane. They also considered the renormalization of the lipid membrane’s local structural and elastic properties due to NP adhesion. By performing systematic simulations as a function of adhesion strength, NPs diameter, and their number density on planar lipid membrane, they observed that the NPs exist in a two-dimensional gas phase for low values of adhesion strength and out-of-plane single-row tubular chains at high values of the adhesion strength, which is in agreement with the results obtained by
earlier works using DTMC model \cite{61,62}. However, for intermediate adhesion strength values, the NPs form in-plane chains when they are part of small aggregates. For larger aggregates, they assemble into out-of-plane two-row tubular structures (bitubes) or annular (ring) chains. They showed that the adhesion strength required to form NPs’ clusters decreases with the increase in the number density of the NPs or with an increase in their diameter. Similarly, the phase diagram of NPs’ aggregation is strongly influenced by the size of the NP aggregates, as shown in Fig. 1.9.

It is particularly interesting when two spherical NPs adhere to a lipid vesicle. Unlike when NPs adhere to a flat membrane, the interaction with a relatively small vesicle causes the entire vesicle to deform globally. As a result, the adhesion modes between the NPs and the vesicle depend not only on the NP diameter and the strength of the adhesive interaction but also on the diameter of the vesicle itself. To better understand the extent of this interaction, Spangler and Laradji calculated the free energy of a vesicle containing two spherical NPs as a function of the distance between their cen-
Figure 1.10: Free energy vs. distance between the center of uniform spherical NPs with corresponding snapshots at the local minima of the free energy for different values of adhesion strength. From Spangler and Laradji (2021) [68].

ters (referred to as the reaction coordinate) [67]. As Fig. (see Fig. 1.10) illustrates, the membrane-mediated interaction between NPs can be attractive or repulsive. At low adhesion strengths ($\xi \lesssim 0.6 \frac{k_B T}{\text{nm}^2}$), this interaction is repulsive. However, in this range, free energy dependence on distance is relatively weak, suggesting that the NPs exhibit a significant degree of diffusion on the vesicle. On the other hand, at higher values of $\xi$, the free energy displays two local minima. The first minimum corresponds to a dimeric state where the NPs are in contact with each other, forming either an in-plane or out-of-plane dimer. The second local minimum represents the monomeric state. In this regime, the dimeric state is more stable than the monomeric state, and the energy barrier between the two states is relatively small ( a few $k_B T$’s), indicating that thermal fluctuations can facilitate dimerization. Consequently, achieving stable NP structures on lipid membranes, where the NPs are separated from each other, becomes challenging.

Many NP properties are significantly influenced by their morphology. For example, the optical properties of AuNPs, as discussed earlier, can be finely tuned through morphological changes, making them promising for various biomedical applications.
Various studies have demonstrated that the size and geometry of NPs play a crucial role in how they adhere to lipid membranes and get internalized within cells. Unfortunately, most of the understanding is based on spherical NPs, and there is a significant lack of systematic experimental investigations on the adhesion and internalization of non-spherical NPs using simple model lipid membranes.

1.4 Interaction of Elongated NPs with Lipid Membranes

The arguments supporting spherical NPs can be extended to cylindrical NPs with a diameter $D$ and an extremely high aspect ratio, such that its two ends can be disregarded. The NP adheres to the membrane in these conditions when $\xi > \xi^* = 2\kappa / D^2$. The mean curvature in this case is smaller compared to the case of a spherical NP with the same diameter. Hence, the adhesion threshold of a cylindrical NP is reduced. Dasgupta et al. [84] investigated the modes of adhesion for cylindrical NPs with finite aspect ratios and blunt circular ends, using a Monte Carlo energy minimization method on dynamically triangulated planar membranes. They found that for low values of $\xi$, the NP adheres shallowly to the membrane through one of its blunt surfaces when the curvature, $c_{\text{edge}}$, of the circular edges between the NP’s cylindrical side and blunt surfaces, is high. The NP adheres similarly at low values of its aspect ratio, $\rho$. However, the NP adheres shallowly to the membrane through its cylindrical side for low values of $c_{\text{edge}}$ or high values of $\rho$. As $\xi$ increases, they predicted a discontinuous transition to a deep wrapping state, in which the principal axis of the NP is perpendicular to the membrane, irrespective of the value of $\rho$ or $c_{\text{edge}}$. Subsequently, a discontinuous transition occurs as $\xi$ is further increased, and the membrane fully wraps the NP.

Recent advances in nanomaterials synthesis methods have led to the engineering of anisotropic NPs with an ever-increasing number of geometries, dimensions, and surface properties. Of particular interest to the researching community are gold (Au) nanorods, whose optical and photothermal properties are highly dependent on their aspect ratio [85]. Moreover, Au nanorods have stronger cross-stream drift during flow.
Figure 1.11: (i) Representative TEM images of Cetyltrimethylammonium bromide (CTAB)-coated Au nanorods (AuNRs) with different values of the aspect ratio, $\rho$. (A) AuNRs with $\rho = 1$ (CTAB-1). (B) AuNRs with $\rho = 2$ (CTAB-2). (C) AuNRs with $\rho = 3$ (CTAB-3). (D) AuNRs with $\rho = 4$ (CTAB-4). (ii) Rate of cellular uptake as a function of $\rho$ and surface coating of the AuNRs. (A) Effect of the aspect ratio of CTAB-coated AuNRs on their uptake. (B) Effect of the coating chemistry AuNRs on the uptake of AuNRs with $\rho = 1$ and 4. (iii) TEM images showing the cellular uptake process, which involves the aggregation of the Au nanorods, followed by the internalization of the aggregates into vesicles and further into the lysosomes. From Qiu et al. (2010) [92].
Figure 1.12: (i) Representative TEM images of functionalized AuNRs with different values of $\rho$ and different coatings. (A) AuNRs with $\rho = 32$ coated with tannic acid (TA). (B) AuNRs with $\rho = 32$ coated with thiol carboxylated polyethylene glycol (PEG-COOH). (C) AuNRs with $\rho = 3$ coated with TA. (D) AuNRs with $\rho = 3$ coated with PEG-COOH. (ii) Rate of cellular uptake of the different types of AuNRs. From De-Brosse et al. (2013) [93].

This significantly increases their circulation times in capillaries compared to spherical NPs [86]. These properties make Au nanorods promising for various biomedical applications. Similarly, it was shown that magnetic nanorods adhering to a lipid membrane and in an oscillating magnetic field can lead to increased local fluidization of the membrane and its lysis [87]. A recent study showed that tracking of nanorods, such that they adhere side-wise to lipid membranes, can be used to probe the mechanical properties of membranes, including their bending modulus and surface tension [217].

Experimental studies present conflicting results regarding the impact of nanorods’ aspect ratio on their uptake by cells [89–94]. Qiu et al. [92] demonstrated that the uptake rate of Au nanorods, by HeLa cells, decreases with increasing aspect ratio (see Fig. 1.11(ii)). They used TEM images to show that the longer rods readily tend to form larger aggregates with loose and irregular structures and theorized that these aggregates
potentially make the internalization of these NPs more difficult (see Fig. 1.11 (iii)). Conversely, DeBrosse et al. observed that the internalization of Au nanorods by keratinocyte cells increases with \( \rho \) [93], as shown in Fig. 1.12 (ii). Moreover, they showed that thiol-carboxylated polyethylene glycol (PEG-COOH) functionalized nanorods exhibited lower uptake when compared to tannic acid functionalized nanorods. This also reiterates that surface chemistry plays a vital role in the internalization of NPs by cells.

The discrepancies in these experimental results highlight the challenges in translating our understanding of NP-cell interactions to those between NPs and simple lipid membranes. This difficulty arises because the internalization of NPs by cells is usually an active process that is mediated via various types of proteins, and these proteins may vary depending on the cell type [95]. Furthermore, even in the case of passive internalization, the plasma membrane is adjacent to the actin cortex on the cytoplasmic side. This renormalizes the elastic properties of the plasma membrane, and consequently, the actin cortex influences the NP adhesion to the plasma membrane [96]. Complicating matters further, the typical size of NPs is comparable to the mesh size of the actin cortex, which is approximately 100 nm [97]. Additionally, cooperative effects are anticipated to play a role in the adhesion and internalization modes of NPs, including nanorods.

A more detailed understanding of how nanorods interact with lipid membranes has been mainly derived from computer simulations. However, these simulation studies have been only done in the case of a single nanorod, adhering to a planar membrane [84, 98–101]. These studies all demonstrate that the details of the geometry of the nanorods significantly influence their adhesion modes and the kinetics of their internalization. Of particular interest are two studies, done by V’acha et al. [98] and Huang et al. [99], on the adhesion and endocytosis of spherocylindrical NPs on lipid membranes using coarse-grained molecular dynamics simulations. V’acha et al. investigated the uptake of ligand-coated NPs by tensionless phospholipid membrane with receptors. They found that nanorods adhered through their cylindrical side to the membrane but were endocytosed while remaining nearly parallel to the membrane’s plane [98], as
Figure 1.13: Simulation snapshots of the pathway for spherocylinder endocytosis when a SCNP is initially placed perpendicular to the membrane, performed by (i)Vácha et al. [98] (2011), and (ii)Huang et al. [99] (2013). (i) Representative snapshots of SCNP endocytosis pathway, where NP is endocytosed while nearly parallel to the membrane plane. (ii) Representative snapshots of SCNP endocytosis pathways for NPs with different aspect ratios of (A) $\rho = 1$ (spherical), (B) $\rho = 1.5$ and (C) $\rho = 2$. The NP gets endocytosed while nearly perpendicular to the membrane plane, for all $\rho > 1$. 

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shown in Fig. 1.13 (i). They also found that larger spherical NPs underwent endocytosis more easily compared to smaller ones and attributed this to a favorable compromise between bending rigidity and surface adhesion energy. Furthermore, they showed that the shape of the particles also plays a crucial role in their uptake efficiency. Specifically, spherocylinders with a prolate shape demonstrated more efficient delivery than spheres of the same diameter. They also observed that cylindrical NPs did not undergo endocytosis due to the sharp edges, regardless of ligand-receptor binding strength.

In contrast, Huang et al., who also looked at the receptor-mediated endocytosis of spherocylindrical NPs, presented an alternate endocytosis pathway of SCNP on a lipid membrane. When the spherocylindrical NPs were initially placed on an upright docking position on the membrane plane, Huang et al. found that an NP with an aspect ratio higher than about two adheres initially to the membrane through its cylindrical side. As the NP gets about half-wrapped, it rotates to a perpendicular orientation. Finally, the NP is endocytosed while nearly perpendicular to the membrane plane, as shown in Fig. 1.13 (ii). They also showed that both NP size and shape play crucial roles in modulating the kinetics of endocytosis. They observed that spherical NPs had the shortest endocytosis time. The shape of the NP influences the curvature energy landscape’s symmetry, which, in turn, dictates the specific endocytic pathway. Through a free energy analysis, they concluded that the size of the NP primarily determines whether endocytosis could successfully complete [99]. These studies collectively provide valuable insights into the complex processes of nanorod-membrane interactions and offer essential information for understanding nanomedicine and other related fields.

It is important to note that both these studies used NPs with ligands that bound to a fraction of lipids (receptors). Moreover, they only investigated the cases where the adhesion energy density was relatively high. In addition, studies confirm that the details of the geometry of the nanorods play a significant role in their adhesion mode and their internalization process. However, an intensive understanding of the effect of aspect ratio on the kinetic pathway of the endocytosis in the case of a nanorod /
spherocylindrical NP remains mostly lacking. Similarly, different modes of adhesion of a spherocylindrical NP with the lipid membrane as a function of their adhesion strength and aspect ratio have never been investigated.

Membrane-mediated interaction between nanorods was investigated analytically in the asymptotic limit of NPs with infinitely long aspect ratio [102, 103]. These calculations, therefore, consider the case where the long axes of the NPs are parallel and can only account for the side-wise adhesion mode of the NPs. For instance, in the case of a flat membrane under lateral tension, calculations from Weikl showed that two cylinders adhering to the same side of the membrane repel each other, while cylinders adhering to opposite sides attract each other [102]. On the other hand, in the case of a membrane in an external harmonic potential, the interaction between the cylinders is always attractive, regardless of their positions on the membrane. However, our understanding of the interaction between nanorods with finite aspect ratio is lacking. This shows that there exists a big gap in the understanding of cooperative behavior in the case of two or more nanorods, and thus, highlights the need for more research on nanorods and their interactions with lipid membranes.

Lastly, there has been a growing interest more recently in arranging NPs into specific structures. A variety of potential new NP superstructures formed from these arrangements further increases the range of applications of nanomaterials [104]. These structures are created via top-down methods and bottom-up methods. In particular, the self-assembly of the NPs is a well-established bottom-up approach, which involves the spontaneous organization of NPs into highly ordered structures. This spontaneous assembly can result from physical interactions or chemical bonds [105]. Self-assemblies can be mediated by electrostatic, steric, magnetic, or van der Waals forces [106]. This thermodynamics-driven spontaneous assembly of NPs often leads to unpredictable structures. Hence, precise control over assemblies is vital to obtain a specific structure for a particular application. This requires precise control over the size, shape, and inter-NP spacing of the nanoassemblies. Various types and geometries of nanoassemblies
have been synthesized, including but not limited to dimers [107, 108], trimers [109],
tetramers [110], icosamers [111] and pyramids [112]. Ordered NP assemblies have a
much wider range of tunable properties than isolated NPs. This makes them ideal for ap-
lications in biomedicine including biosensing [113–115], drug delivery [16,116–119],
gene therapy [120,121], diagnostics [122], magnetic hyperthermia [123], and photother-
mal therapy [124,125].

Studies have shown that NP interactions with lipid membranes can be fine-tuned by
controlling factors such as system free energy, pH, temperature, size of the well-defined
template, or by applying external fields [44, 46, 47, 55–64, 66–68, 75, 77–81, 83]. While
the self-assembly of NPs and nanorods opens the door for a wide range of applica-
tions, lipid membrane-induced self-assembly of nanorods with well-controlled distance
between the NPs is something that has never been achieved before. This requires pre-
cise control over the NP properties and, more importantly, the NPs to be apart at some
specific distance, suppressing dimerization. As discussed earlier, NPs with uniform
surfaces can either be apart from each other or aggregate as clusters on lipid vesicles.
However, even when they are apart from each other, they tend to be fairly diffusive
at low adhesion strengths. Furthermore, NPs with uniform surfaces can be endocy-
tosed spontaneously at higher adhesion strengths. At intermediate values of adhesion
strengths, where the NP gets fairly wrapped without being endocytosed, the degree of
wrapping of NPs with uniform surfaces is determined by the interplay between the ad-
hesion strength and curvature energy, which increases rather rapidly with increasing
adhesion strength.

The goal is to design NPs whose degree of wrapping is more controllable and can-
not easily be endocytosed. This problem is mitigated by surface modification of the
NPs into Janus NPs [4]. Specifically, these Janus NPs are surface-modified such that
one moiety interacts more attractively with the lipid head groups, and the other is hy-
drophilic and interacts more favorably with the solvent. Surface modification gives the
ability to regulate the degree of wrapping of these NPs based on their level of Janusity.
Figure 1.14: Snapshots of a single Janus spherical NP of diameter equal to 20 nm adhering to a planar membrane for different values of the area fraction $J$ of the NP that interact attractively with the lipid head group (colored yellow). The blue beads interact repulsively with the lipid head group. (a) to (f) correspond to $J = 0.1, 0.3, 0.5, 0.7, 0.9, \text{ and } 1$. Snapshots are generated from simulations performed at adhesion strength of $1.56 \text{ nm}^2/k_B T$, between yellow beads and red lipid head group. The snapshots show that surface modification of NPs gives good control over the amount of wrapping and suppresses endocytosis. From Zhu (2023) [128].
Additionally, this alteration significantly enhances the energy barrier between the monomeric and dimeric states, leading to the stabilization of monomeric states, hence ensuring that the NPs are apart from each other. Also important, this modification effectively suppresses the endocytosis of these NPs. Interestingly, as we will see later, when placed inside the vesicle, uniform SCNPs suppress clustering and dimerization and do not exocytose easily for a wide range of adhesion strengths. This ensures that uniform SCNPs self-assemble into interesting structures, even without surface modifications, when placed inside a vesicle.

Finally, this brings us to the purpose of this thesis, where I intend to present my work on understanding the interactions of nanorods with lipid membranes. Firstly, a spherocylindrical NP(SCNP) is designed to mimic the geometry of a nanorod. The synthesis of a SCNP is discussed in detail in the methods section. The membrane is modeled using a mesoscale implicit-solvent model for self-assembled lipid bilayers, in which a lipid molecule is coarse-grained into a short semi-flexible chain that is composed of one hydrophilic bead and two hydrophobic tail beads. The interaction of a single SCNP with a lipid bilayer is investigated using molecular dynamics (MD) simulations, and this investigation is then extended in the case of two SCNPs. Once the interaction of SCNPs with lipid bilayer is understood, the self-assembly of many SCNPs is explored by placing it outside and inside a vesicle. Analysis codes for calculating angles, generating radial distribution functions, performing Voronoi analysis, calculating nematic order parameter, calculating coplanarity, etc., was written using C++, Python, MATLAB, awk, and bash script, and graphs were generated using Xmgrace. Besides, I also worked with my collaborators, resulting in publications as a second author.

The work in this dissertation was supported by a grant from the National Science Foundation (DMR-1931837). Most simulations were performed on computers of the High-Performance Computing Facility at the University of Memphis. Some of the simulations were conducted as part of a user project at the Center for Nanophase Materials Sciences (CNMS), which is a US Department of Energy, Office of Science User Facility.
at Oak Ridge National Laboratory, and using computational resources of the Oak Ridge Leadership Computing Facility, which is a DOE Office of Science User Facility supported under Contract DE-AC05-00OR22725. Snapshots in this article were generated using VMD version 1.9.3 [127].

1.5 Goals of the Study

The specific goals of this dissertation are listed as follows:

1. To model spherocylindrical NPs such that the number of degrees of freedom associated with NPs is reduced, such that the model is computationally efficient,

2. To investigate the modes of adhesion of SCNPs and their endocytosis through an approach that accounts for thermal fluctuations. The focus is to determine the phase diagram of adhesion and endocytosis of spherocylindrical NPs as a function of their aspect ratio, dimensions, and strength of the adhesive interaction,

3. To investigate the effect of aspect ratio and strength of adhesive interaction on the dimerization of two spherocylindrical NPs and study the effect of dimerization on the kinetic pathways of endocytosis, and

4. To investigate the self-assembly of many SCNPs on the outside and inside of vesicles and characterize different structures resulting from the self-assembly of these SCNPs.

1.6 Structure Overview

The dissertation is organized as follows:

In this Chapter[1] I introduced the context and background of the study. I presented the research goals, outlined the research questions, and argued the significance of the work.

In Chapter[2] various models and computational approaches are presented. I will present the details of our implicit-solvent coarse-grained model and the molecular dy-
namics approach used in this study. I will also present some details of the weighted histo-
togram analysis method (WHAM) \([187]\) and a free energy calculation approach based
on a local formulation of the Helfrich Hamiltonian \([46]\). These free energy calculations
were heavily used to determine the stability of the spatial arrangements of the NPs.
Other numerical methods used in this dissertation to calculate the equation of a plane,
using the method of least squares and the nematic order parameter, are also presented
in Chapter 2.

Chapter 3 focuses on the numerical investigation of the adhesion modes and endo-
cytosis pathway of spherocylindrical nanoparticles (NPs) on tensionless planar mem-
branes, using molecular dynamics simulations of a coarse-grained implicit-solvent model.
An approximate adhesion phase diagram of an SCNP is determined using simplified
theoretical arguments based on a balance between the Helfrich’s curvature energy \([71]\)
of the membrane and the adhesion energy. Similarly, results from simulations per-
formed over a range of values of the SCNP’s diameter, aspect ratio, and adhesion energy
density are presented.

Chapter 4 focuses on the numerical investigation of the modes of adhesion and
endocytosis of two SCNPs on planar and tensionless lipid membranes. The study is
performed using systematic molecular dynamics simulations with varying SCNPs’ ad-
hesion strength, aspect ratio, and diameter values. Moreover, the effect of the initial
placement of the SCNPs and their orientation on the membrane is investigated in detail.

Chapter 5 focuses on highly ordered nanoclusters with a plethora of geometries de-
pendent on the aspect ratio of the Janus SCNPs, their number on the vesicle, and the
size of the vesicle. Similarly, the rigidity of the geometry is investigated as a function
of aspect ratio and the size of the vesicle. The details of the geometries of these JSCNP
nanoclusters are analyzed in terms of the radial distribution function, bond angle distri-
butions, and a nematic order parameter.

Chapter 6 focuses on the arrangement of many SCNP uniform NPs on the inner
side of lipid vesicles. Membrane-mediated interactions between two SCNPs adhering
on the inside of a lipid vesicle, as a function of adhesion strength, are discussed. Self-assembly of SCNPs into interesting ordered nanocluster, with a detailed investigation of lipid vesicle arrangement into two-dimensional geometry, as a function of SCNP number is presented.

Chapter 7 summarizes my dissertation. The main idea of this work is that the aspect ratio of the NP plays an essential role in the adhesion of an elongated NP on lipid membranes, and the membrane induces different kinds of interactions between the particles. This knowledge can be harnessed to induce the self-assembly of SCNPs on lipid vesicles. The majority of results in this dissertation are either already published or will be published in the following articles.


Chapter 2

MODEL AND COMPUTATIONAL METHODS

Cellular processes occur over a wide range of length and time scales. It is often vital to understand processes that occur at the atomistic level to understand microscale phenomena fully. Moreover, some processes in cells occur so abruptly that it is nearly impossible to capture them with the tools that exist in today's world. Existing microscopy tools lack the spatial and temporal resolution to characterize the details of cellular processes that occur at these small length scales and time scales. Computer simulations can be used as an alternative to these shortcomings.

Recent advances in computers have proven vital for our understanding of cells. Besides enabling the extraction of short time-scale and length-scale information, they offer the ability to pause and modify simulations, revisit acquired data, and compare experimental results. Corrections to simulation parameters can be made based on these comparisons. Moreover, simulations allow for the study of physical properties without altering the sample and, hence, do not leave any physical artifacts. These advantages make computer simulations a versatile tool for the study of cell membranes.

2.1 Lipid Models

Most of our understanding of cell membrane through computer simulations are based on lipid models. A large number of computational studies have been conducted to add to our understanding of lipid membranes, their elasticity, interaction with NPs and macromolecules, and much more. Various theoretical approaches have been proposed and used to model and study lipid membranes. These existing modeling approaches range from fine to coarse-grained representations. Depending on the length scales of interest, computational studies on lipid membranes have been carried out via three main approaches corresponding to (1) continuum membrane elasticity models, (2) atomistic
molecular dynamics models, and (3) mesoscale molecular dynamics models. Each of these methods can vary in their level of coarseness and their ability to mimic a specific range of substructures.

### 2.1.1 Continuum Membrane Elasticity Models

Continuum membrane elasticity models treat the lipid membrane as a thin, elastic medium while leaving out the detailed atomistic interactions between individual lipid molecules. Elastic models are usually based on the Helfrich Hamiltonian (Eq. (1.3)) [71]. A lot of studies have been performed to study lipid membranes using these models [129–131,133–141]. These continuum membrane elasticity models enable the study of deformations in the membrane structure that occur over relatively large length scales compared to the molecular size of individual lipids. These models offer a simplified description of the lipid bilayer’s behavior, making them computationally more efficient and easier to handle when compared to atomistic molecular dynamics simulations. Similarly, continuum models can be easily simplified and approximated through mean-field theories. Hence, continuum membrane bending models are suitable for analytical calculations, Langevin simulations, or Monte Carlo simulations.

Owing to these advantages, continuum models are extensively used for investigating long-wavelength phenomena and have been widely applied to investigating a wide range of problems on lipid membranes over the years. For instance, Maio et al. explored the various shape transitions of fluid lipid-bilayer vesicles and their relation to the differences in vesicle leaflet areas [130]. Mukhopadhyay et al. investigated the role of membrane elasticity and bending energy to study the shapes of human blood cells [131]. Deserno and Bickel studied the elastic deformation of a fluid membrane arising from the adhesion of a spherical colloidal particle with the membrane [73]. Similarly, interactions between particles and lipid membranes were numerically investigated by several other studies [138–140]. It is important to note that all these studies were performed using continuum models where the elastic thin membrane is assumed to
Figure 2.1: Dynamically triangulated membranes generated using Monte Carlo simulations, from (i) Okuda and Eiraku (2017) [133] and (ii) Iyer et al. (2023) [135].

be continuous. Continuum elasticity models have also been employed as dynamically triangulated vertices over the years. The membrane is discretized using dynamically triangulated vertices linked to each other through some potential (see Fig. 2.1). The membrane bending elasticity in discretized continuum models is usually approximated using Helfrich’s Hamiltonian. Kumar et al. used this model to examine the phase behavior of lipid membranes with multiple components [141]. This discretization of the membrane also allows for fluctuations in the membrane. Dynamically triangulated membranes have been extensively used to investigate the interactions between NPs and lipid membranes [60, 84, 135].

Continuum models are mostly useful for studying the macroscopic equilibrium behavior of the lipid membrane system. However, they may lack precision at the molecular level, which is often required to gain a more comprehensive understanding of complex interactions of lipid membranes with NPs. Similarly, the size of the membrane is
infinitesimally small in this approximation; however, the size of the membrane and the size of the NPs are comparable in our study. Moreover, these models are ineffective in studying the kinetics of NP-membrane interaction and often fail to capture the intermediate states during the time evolution. Hence, although computationally very efficient and used in multiple studies, we opted not to use the continuum membrane elasticity models to model our membrane as part of our study.

2.1.2 Atomistic Molecular Dynamics

It is often necessary to employ modeling techniques that simulate atomic-level processes to understand phenomena at the microscopic level. Thanks to significant advances in computers, effective models such as classical density functional theory, atomistic molecular dynamics (MD), and phase-field crystal models exist that can capture processes at the atomic level [3]. Atomistic MD models, in particular, have been extensively employed to examine the microscopic details of molecular processes in various fields, including physics and material sciences. An MD model is basically a many-body system where each particle, usually an atom, is represented as a dot, and the interactions between each of these dots are specified. The inter-particle interactions modeled using some potential energy function, which can be either attractive or repulsive, determine the accuracy of the resulting system. The method of MD was first formulated using Lennard-Jones potentials in the mid-1960s. To this day, Lennard-Jones potential is commonly used as a hard inter-atomic potential in atomic MD simulations and is given as,

\[ V(r) = 4\epsilon \left[ \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^{6} \right], \quad (2.1) \]

where \( r \) is the distance between the particles and \( \epsilon \) is the strength of the potential energy. The minimum of the potential occurs at a distance \( 2^{1/6}\sigma \approx 0.89\sigma \). The \( r^{-12} \) is the repulsive term and represents the Pauli exclusion principle at short ranges. This makes
the interactions at short distances strongly repulsive. Similarly, $r^{-6}$ term gives the long-range attractive force and represents the van der Waal’s forces. The forces are then summed up, and the net force is calculated by solving Newton’s equations of motion for a system of interacting particles at each time step. A large number of iterations of the equations of motion result in the evolution of the system’s structure, from which many observables are extracted.

Atomistic MD models focusing on lipid membranes have been extensively used over the last two decades [142–145]. Since atomistic MD models simulate a few thousand lipid molecules over a few microseconds, these models are particularly useful in determining atomistic-scale or molecular-scale structures and kinetics of lipid membranes. This approach allows for studying lipid membranes with different kinds of lipids or sim-
ulating the interaction of specific proteins with specific lipid membranes (see Fig. 2.2). This also enables the predictions of some thermal and transport properties of lipid membranes.

Niemelä et al. used atomistic MD simulations to study lipid rafts by investigating the properties of ternary raft mixtures with cholesterol, palmitoylsphingomyelin, and palmitoyloleoylphosphatidylcholine [142]. Yesylevskyy et al. investigated the influence of curvature on the properties of the atomistic model of the mammalian plasma membrane with asymmetric lipid content of monolayers and a realistic concentration of cholesterol [144]. More recently, Ardham et al. used atomistic MD simulations to predict the membrane capacitance from the dielectric profile of lipid bilayers [145]. Several studies have used atomistic MD simulations to investigate the interactions of NPs with lipid membranes [146–150]. For instance, using atomistic MD simulations, Bedrov et al. studied the passive transport of C$_{60}$ fullerenes through a lipid membrane [146]. Heikkilä et al. simulated the interactions of an anionic AuNP (AuNP$^-$) with asymmetric model lipid membranes [147]. Van et al. investigated the pathway for insertion of amphiphilic NPs into defect-free lipid bilayers [148], Ou et al. studied the interactions between amphiphilic Janus NPs and lipid bilayers, and looked at the effects of lipid ordering and leaflet asymmetry [149], and Liu et al. studied the interactions between thiolate-protected gold nanoclusters and phospholipid membranes [150].

Atomistic MD simulations provide a much more detailed model and capture microscopic details of lipid membranes. However, it is important to note that atomistic MD simulations are typically limited to relatively small systems, usually containing a few tens of thousands of particles. Moreover, the simulation time is limited to a few microseconds. Hence, with current computational resources, this approach is not ideal for investigating the equilibrium behavior and kinetics of large NPs and/or multiple NPs, which occur over more extended diffusive time scales. Hence, MD simulations are often complemented with other numerical approaches to bridge this gap and gain a mesoscale understanding of lipid membranes.
2.1.3 Mesoscale Molecular Dynamics

Mesoscale MD models have the ability to explore mesoscopic length and time scales while still retaining some molecular-level details. This makes this approach valuable for studying lipid membranes with a large number of molecules and longer time scales. In such models, the lipid molecules are coarse-grained into short chains of amphiphilic beads, treating each bead as an interacting point particle. Although mesoscale MD models might overlook important details such as shape anisotropy and polarity of certain molecules, this approach extends the simulation scale to several hundred nanometers or even microseconds. Hence, a large number of studies focusing on interactions between NPs and lipid membranes have been carried out using mesoscale particle-based methods. The solvent can be modeled explicitly, through coarse-graining of a number of molecules as beads or implicitly, replacing the hydrophobic interactions between the tail and solvent that leads to the self-assembly of the lipid membrane with effective, attractive interactions between the lipid tails. These coarse-grained models can be system-specific or generic.

Marrink et al. proposed a system-specific coarse-grained model, known as the Martini model [81, 151], to describe specific lipid membranes in explicit solvent (see Fig. 2.3). This approach involves the coarse-graining of four heavy atoms (e.g., carbon, oxygen, nitrogen, and phosphorus) that are bonded with hydrogen atoms into one bead. Complex structures such as cholesterol are coarse-grained two-to-one instead. The bonded beads interact via interactions that are harmonic, three-body, and even dihedral. The model considers four main types of interaction sites: polar, nonpolar, apolar, and charged. Each particle type is linked to a number of sub-types, which correspond to the chemical nature of the underlying atomic structure. Hence, this provides an accurate picture of the atomic or molecular structures. In the Martini approach, the Lennard-Jones interaction is used for pairs of non-bonded beads, and Coulomb interactions are used in addition to other interactions if the beads are charged. The Martini
parameters are obtained through a process of trial and optimization. This allows for reproducing the experimental values of hydration-free energies and the partitioning coefficient of specific molecules in water and selected alkane solvents. This model was used to quantitatively reproduce the well-known condensing effect of cholesterol on the area per lipid [81]. The Martini approach has been very popular due to its coarse-grained nature and its system specificity and has been used in many investigations of the interactions between NPs and lipid membranes [152–155]. Despite the popularity of the Martini approach, it remains limited to relatively small systems and has at times produced erroneous predictions [156–158].

Many mesoscale coarse-grained models have been developed to predict generic
properties of lipid membranes with different levels of coarse-graining. Generic particle-based mesoscale models offer a simplified representation of all lipid membranes and capture their essential behaviors and characteristics. The dissipative particle dynamics (DPD) model of lipid membranes (see Fig. 2.4) is a widely used generic coarse-grained approach that models the solvent explicitly [159–161]. The DPD model incorporates soft pairwise conservative ($F_{ij}^C$), dissipative ($F_{ij}^D$), and random ($F_{ij}^R$) forces between the beads. Hence, the total non-bonded force acting on any DPD bead due to all other DPD beads in the system is a sum of these forces, i.e.,

$$f_i = \sum_{i \neq j} \left( F_{ij}^C + F_{ij}^D + F_{ij}^R \right).$$ (2.2)

The pairwise nature of all forces in DPD allows for local momentum conservation and, therefore, a correct account for the long-range hydrodynamic interactions [162]. The use of soft pairwise interactions instead of Lennard-Jones potential or other steep potentials significantly increases the order of simulation time step, hence allowing for simulations over time scales orders of magnitude larger than can be achieved with atomistic MD. This approach has mainly found its usefulness in studies involving soft materials that are usually characterized by slow kinetics. The coarse-graining within this approach can be easily controlled, and hence, this approach has been used in a large number of studies of lipid membranes.

Explicit solvent models of lipid membranes, such as DPD and Martini, involve a
solvent occupying most of the volume of the simulation box. As a result, a large amount of computational time is dedicated to calculating interactions and forces between solvent beads. The inclusion of explicit solvent is crucial for studying various transport and dynamic properties in order to accurately account for the hydrodynamic interactions. However, it is unnecessary to spend time explicitly on the solvent when investigating the equilibrium properties of the system. In such cases, the hydrophobic interactions between lipid tail beads and solvent beads, responsible for the lipid bilayer self-assembly, can be effectively modeled by introducing an attractive force between the lipid tail beads. Following this simplification, several implicit-solvent particle-based models have been developed to model lipid membranes [126,163–169].

Drouffe et al. presented the pioneering work on an implicit solvent model, where they treated the bilayer as a monolayer of self-assembled anisotropic hard spheres [163]. Each hard sphere was subdivided into a hydrophobic core around the equator and hydrophilic caps on the top and bottom of the sphere. These spheres are self-assembled
as a result of pairwise interactions between them, as well as multi-body interactions, as shown in Fig. 2.5. This model successfully predicted both the gel and fluid phase of lipids through MD simulations. However, it predicted much weaker values of the bending modulus as compared to experimental values. A few years later, improved coarse-grained implicit models were proposed by Noguchi and Takasu [166], as well as Brannigan et al. [168], in which lipid molecules are treated as rigid rods, and spherocylindrical shape respectively. Simulations using these implicit-solvent coarse-grained models successfully reproduced stable gel and fluid membranes, whose elastic and structural properties qualitatively agreed with those obtained from experiments. However, the beads in these approaches are very coarse-grained and, hence, do not allow for internal degrees of freedom and their conformational changes. To further improve on this, Cooke and Deserno [169] proposed an implicit-solvent particle-based model by coarse-graining the lipids into semi-flexible chains of point beads. The bonded beads interact through the finitely extensible nonlinear elastic (FENE) potential [170]. The bending rigidity of the lipid chains is achieved through a harmonic interaction between the end beads. The non-bonded beads in this model interact through the Lennard-Jones potential. This model has been used in many studies of lipid membranes, including interactions between lipid membranes and NPs [55, 171–173].

Laradji’s group further improved on the model of Cooke et al. [169, 174] and proposed an even more efficient implicit solvent coarse-grained model of semi-flexible chains of point beads [126]. The main difference between this approach and that of Cooke et al. is that in Laradji et al.’s model, point beads interact via soft-body interactions instead of the Lennard-Jones, and the bonded beads interact through harmonic potential instead of the FENE potential. Furthermore, the bending rigidity of the lipid chains in Laradji et al.’s model is maintained through a three-body interaction instead of a harmonic interaction between the ends of the lipid chain. A comparison of the tail-tail interaction potential from Laradji et al.’s model and Cooke et al. is shown in Fig. 2.6 Using soft potential instead of Lennard-Jones allows for larger
time steps, making Laradji et al.’s model computationally more efficient. This model successfully reproduces the self-assembled lipid membranes, and the bending modulus of phospholipid membranes estimated from simulations using this model agree with experimental values [126]. This efficient approach has since been used to investigate many problems associated with lipid membranes, such as the phase behavior of lipid membranes [126], cytoskeleton-induced blebbing of lipid membranes [175], phase behavior of liposomes [176], the combined effect of transmembrane proteins and cytoskeleton on lipid membrane compositional heterogeneities such as lipid rafts [177], and the phase behavior of substrate-supported lipid bilayers [178]. Similarly, this approach has been extensively used to investigate the interactions of NPs with lipid membranes [46, 65, 68, 126, 179, 180].

Laradji et al.’s approach has various advantages over the previous lipid models. Firstly, this model involves the coarse-graining of the lipid molecules into semi-flexible chains of point beads and implicitly takes care of the solvent through the addition of effective, attractive interactions between lipid tails. This leads to a significant decrease
in the number of degrees of freedom while allowing for conformational changes in the lipid membrane. Secondly, Cooke et al.’s model model [169] uses interaction, which diverges as the distance between the particles approaches zero. Laradji et al.’s approach uses soft interactions between the non-bonded beads to tackle this issue, hence allowing for much longer time steps, as compared to Cooke et al.’s model [169] or other mesoscale models. This allows for simulations of large systems over longer time scales. Hence, in this dissertation, I use the coarse-grained model introduced by Laradji et al. [126].

2.1.4 Coarse-Grained Implicit-Solvent Model of Lipids Membranes

In this approach [46, 126], a lipid molecule is coarse-grained into a short semiflexible chain that is composed of one hydrophilic head (h) bead and two hydrophobic tail beads (t) (see Fig. 2.7). The potential energy of the lipid bilayer has three contributions and is given by

\[
U ( \{ r_i \} ) = \sum_{i,j} U_0^{\alpha_i \alpha_j} (r_{ij}) + \sum_{(i,j)} U_{\text{bond}}^{\alpha_i \alpha_j} (r_{ij}) + \sum_{(i,j,k)} U_{\text{bend}}^{\alpha_i \alpha_j \alpha_k} (r_i, r_j, r_k),
\]

(2.3)
where \( \mathbf{r}_i \) is the coordinate of bead \( i \), \( r_{ij} = |\mathbf{r}_i - \mathbf{r}_j| \), and \( \alpha_i \) \((= h \text{ or } t)\) represents the type of bead \( i \). The first term in Eq. (2.3), \( U_i^{\alpha_i \alpha_j} \) is a soft two-body potential between neighboring beads of types \( \alpha \) and \( \beta \) and is given by

\[
U_0^{\alpha \beta}(r) = \begin{cases} 
(U_{\alpha \beta}^{\max} - U_{\alpha \beta}^{\min}) \frac{(r_m-r)^2}{r_m^2} + U_{\alpha \beta}^{\min} & \text{if } r \leq r_m, \\
-2U_{\alpha \beta}^{\min} \frac{(r_c-r)^3}{(r_c-r_m)^3} + 3U_{\alpha \beta}^{\min} \frac{(r_c-r_m)^2}{(r_c-r_m)^2} & \text{if } r_m < r \leq r_c, \\
0 & \text{if } r > r_c,
\end{cases} 
\tag{2.4}
\]

where \( U_{\alpha \beta}^{\alpha \beta} > 0 \) and \( U_{\alpha \beta}^{\min} \leq 0 \) for any pair \((\alpha, \beta)\) \([46]\). \( U_{\alpha \beta}^{\alpha \beta} = 0 \) implies a fully repulsive interaction between \( \alpha \) and \( \beta \), and \( U_{\alpha \beta}^{\min} < 0 \) implies a short-range attraction between the two beads. The self-assembly of the lipids into thermodynamically stable bilayers is ensured by choosing \( U_{hh}^{\min} = U_{ht}^{\min} = 0 \) and strong enough negative value of \( U_{tt}^{\min} \) \([46]\). Due to the absence of explicit solvent in this model, the self-assembly of the lipid chains into bilayers is achieved through a short-range attractive interaction between the \( t \)-beads. Otherwise, \( h-h \) and \( h-t \) interactions are repulsive \([46]\).

In Eq. (2.3), the second summation is over bonded pairs within the lipid chains. The angular bracket in the summation indicates that \( i \) and \( j \) are part of the same lipid chain. The harmonic potential, \( U_{\text{bond}}^{\alpha \beta} \), ensures these beads \( \alpha \) and \( \beta \) within a chain are connected, and is given by

\[
U_{\text{bond}}^{\alpha \beta}(r) = \frac{k_{\text{bond}}^{\alpha \beta}}{2} (r - a_{\alpha \beta})^2, \tag{2.5}
\]

where \( k_{\text{bond}}^{\alpha \beta} \) is the bond stiffness coefficient and controls the strength of the bond. \( a_{\alpha \beta} \) is the preferred distance between the beads \( \alpha \) and \( \beta \). We note that bonded beads in a lipid chain also interact via the two-body potential in Eq. (2.4).

Finally, the third summation in Eq. (2.3) is over the triplets of beads constituting each lipid chain. The angular bracket in the summation here indicates \( i, j, \) and \( k \) are part of the same lipid chain. \( U_{\text{bend}}^{\alpha \beta \gamma} \) is a three-body potential that provides bending stiffness.
Table 2.1: Lipid model interaction parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$U_{hh}^{max}$, $U_{ht}^{max}$</td>
<td>100$\epsilon$</td>
</tr>
<tr>
<td>$U_{tt}^{max}$</td>
<td>200$\epsilon$</td>
</tr>
<tr>
<td>$U_{hh}^{min}$, $U_{ht}^{min}$</td>
<td>0</td>
</tr>
<tr>
<td>$U_{tt}^{min}$</td>
<td>$-6\epsilon$</td>
</tr>
<tr>
<td>$k_{bond}$, $k_{bond}$</td>
<td>100$\epsilon/r_m^2$</td>
</tr>
<tr>
<td>$k_{bend}$</td>
<td>100$\epsilon$</td>
</tr>
<tr>
<td>$r_c$</td>
<td>2$r_m$</td>
</tr>
<tr>
<td>$a_{ht}$, $a_{tt}$</td>
<td>0.7$r_m$</td>
</tr>
</tbody>
</table>

to the lipid chains and is given by

$$U_{bend}^{\alpha\beta\gamma}(r_\alpha, r_\beta, r_\gamma) = \frac{k_{bend}^{\alpha\beta\gamma}}{2} \left( \cos \varphi_0^{\alpha\beta\gamma} - \frac{r_\alpha \cdot r_\gamma}{r_\alpha r_\gamma} \right)^2,$$  \hspace{1cm} (2.6)

where $k_{bend}^{\alpha\beta\gamma}$ is the bending stiffness coefficient. The strength of $k_{bend}^{\alpha\beta\gamma}$ controls the bending rigidity of the membrane, in addition to the hydrophobic interaction, $U_{tt}^{\alpha\beta}$. $\varphi_0^{\alpha\beta\gamma}$ is the preferred splay angle of the lipid chain taken to be 180°.

The specific values of the model interaction parameters used to simulate the lipid bilayer are provided in Table 2.1. As mentioned before, the solvent is incorporated implicitly in this model. Depending on the initial conditions or geometry of the confirmation and the system size, lipids self-assemble into different confirmations. This approach has successfully modeled stable planar membranes, vesicles, and cylindrical membranes, as shown in Fig. 2.8. The bending modulus of the bare bilayer, as extracted from the spectrum of the height fluctuations of the bilayer, is $\kappa \approx 30k_BT$. This value is comparable to that of a DPPC bilayer in the fluid phase. By comparing the thickness of the model bilayer in the fluid phase, which is about $4r_m$, with that of a typical fluid phospholipid bilayer, $\approx 4$ nm, the length scale can be estimated to be $r_m \approx 1$ nm. Hence, in the remainder of this dissertation, all lengths are expressed in nanometers, and the energies are expressed in $k_BT$. 

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Figure 2.8: Self-assembly of lipids obtained from molecular dynamics simulations using the coarse-grained implicit solvent model of Laradji et al. The lipids self-assemble into various geometries, including a) a tensionless planar membrane [46], b) a tensionless vesicle [68], and c) a tensionless cylindrical membrane [180].

2.2 Modelling of Spherocylindrical Nanoparticle

A spherocylindrical NP (SCNP) is modeled as a hollow object; hence, all degrees of freedom of the NP are localized at the surface. This ensures that the number of degrees of freedom within an NP in this model is significantly smaller than that of an NP modeled as a three-dimensional crystalline lattice of beads [182]. SCNP is constructed as a fairly rigid triangulated mesh, following the approach shown in Fig 2.9. At first, a geodesic polyhedron is created as a good approximation to a sphere starting from an icosahedron. An icosahedral grid, which circumscribes a sphere of radius 10 units, was triangulated three times, resulting in 642 vertices and 1280 elementary triangles [183]. The coordinates of these beads were ultimately projected onto the circumscribed sphere, ensuring that all the vertices lie on the sphere and are at a distance of 10 units from its center, as shown by Fig 2.9(a). The final vertices all have six nearest neighbors, except [182].
Figure 2.9: Steps showing the construction of a spherocylindrical NP. (a) Original tessellated sphere with 642 vertices. (b) Two hemispherical caps of the NP are constructed by splitting the original sphere. (c) Rearrangement of the vertices on the great circle such that the vertices are equidistant. (d) Generation of the vertices of the cylindrical portion of the NP. (e) Final tessellated NP.

for 12 initial icosahedron vertices, which have five nearest neighbors. The average distance between the nodes is 1.5 units.

The sphere is then split symmetrically through the plane \((x = 0)\) into left and right hemispheres, which will form the two ends of the SCNP (Fig 2.9(b)), such that the NP principal axis is along the \(x\)-axis. The points that lie on the \(yz\)-plane (great circle) are shared by both hemispheres. The right hemisphere is then translated by a distance \(\Delta x\) to the right, which is determined from the NP’s desired aspect ratio, \(\alpha\). For instance, to generate an NP of \(\alpha = 1.6\), \(\Delta x = 1.6 \times 20 - 20 = 12\). Hence, all vertices on the right hemisphere are translated by 12 units towards the right along the \(x\)-axis. It is important to note that the points on the circumference of the great circle are not equidistant, as demonstrated by the left configuration in Fig 2.9(c). To mitigate this problem, the points on the great circle are rearranged such that they are at a uniform distance from each other (see Fig 2.9(c)).

The next step generates the vertices on the surface of the cylindrical portion of the NP. First, replicas of the great circle are constructed between the left and right great circles and are separated by a distance of 1.5 units. The number of these replicas de-
pends on $\alpha$. Vertices are then added at the center of each quadrilateral formed by four nearest neighbors, (e.g., dashed quadrilateral in Fig. 2.9(d)). We note that this approach leads to some defects in the regions where the cylindrical surface meets the two hemispheres. These defects are formed because of the rearrangement of the points on the circumference of the great circles of the hemispheres. In particular, there are a few vertices in these regions with only four nearest neighbors. These points are connected to their neighbors with some bonds that are longer than 2 units. This problem is solved by identifying the defect regions and inserting vertices in the middle of the defect region.

Finally, we project all the points on the surface of hemispheres at a distance of 10 units from their respective centers and project the points on the surface of the cylindrical portion on a cylinder with a radius of 10 units. The final constructed SCNP is shown in Fig. 2.9(e). The NP is then scaled conformally to the desired diameter, $D_{np}$.

Every $n$-bead of the NP is connected to its nearest neighbors by the harmonic potential, Eq. (2.5), with a bond stiffness $k_{nn}^{bond}$ and a preferred bond length $a_{b}^{nn}$. The value of $a_{b}^{nn}$ is determined from the initial configuration of the NP. Since the NP is hollow, the two-body interaction is not sufficient to provide a very rigid structure to the NP. This problem is solved by introducing an additional bead, $c$, at the center of mass of the NP, that is connected to all $n$-beads by a harmonic bond given by Eq. (2.5), with a bond stiffness $k_{cn}^{bond}$ and a bond length determined by the initial configuration. Short-range attractive interaction between the $n$ beads of the NP and a lipid head bead is insured using $U_{min}^{nh} < 0$. To prevent partial insertion of the NP in the hydrophobic core of the lipid bilayer, $U_{min}^{nt} = 0$. The parameters, $k_{cn}^{bond}$ and $k_{nn}^{bond}$, can be tuned to control the rigidity and the roughness of the NP’s surface. The specific values of the SCNP interaction parameters used in the simulations are shown in Table 2.2.
Table 2.2: Single SCNP interaction parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$U_{\text{nh}}^\text{max}, U_{\text{nt}}^\text{max}$</td>
<td>$200\epsilon$</td>
</tr>
<tr>
<td>$U_{\text{nh}}^\text{min}$</td>
<td>$-\epsilon$</td>
</tr>
<tr>
<td>$U_{\text{nt}}^\text{min}$</td>
<td>$0$</td>
</tr>
<tr>
<td>$k_{\text{bond}}^{nn}$</td>
<td>$500\epsilon/r_m^2$</td>
</tr>
<tr>
<td>$k_{\text{bond}}^{nc}$</td>
<td>$10\epsilon/r_m^2$</td>
</tr>
<tr>
<td>$a_{cn}$</td>
<td>variable</td>
</tr>
</tbody>
</table>

### 2.3 Numerical Approaches

The classical microstate of a system consisting of $N$ point beads can be denoted as

$$\{r_1, r_2, \ldots, r_N; p_1, p_2, \ldots, p_N\}, \quad (2.7)$$

where $r_i$ and $p_i$, with $i = 1, 2, \ldots, N$, are the positions and momenta of particle $i$. At a particular time, $t$, the state of the system can be described by a Hamiltonian,

$$\mathcal{H} = E_k + E_p, \quad (2.8)$$

where $E_k$ is the total kinetic energy and $E_p$ is the total potential energy of the system. $E_p$ has contributions from two-body interactions, three-body interactions, etc. The positions and momenta of the beads satisfy Hamilton’s equations,

$$\dot{r}_{i,\nu} = \frac{\partial \mathcal{H}}{\partial p_{i,\nu}} = \frac{p_{i,\nu}}{m_i},$$

$$\dot{p}_{i,\nu} = -\frac{\partial \mathcal{H}}{\partial r_{i,\nu}} = f_{i,\nu}, \quad (2.9)$$

where $\nu = x, y$ or $z$, and $p_{i,\nu} = m_i v_{i,\nu}$. The trajectory of this system of $N$ particles is tracked using the molecular dynamics approach. The interactions between the beads are described as a force field, calculated using Eq. (2.8). For a thermodynamic system in contact with a thermal reservoir at some fixed temperature $T$, non-conservative thermal and dissipative forces must be added to Eq. (2.9) to account for the interaction between...
2.3.1 Langevin Dynamics

The beads in the system are moved using an MD scheme in conjunction with a Langevin thermostat, using the following equations of motion,

\[ \dot{r}_i(t) = v_i(t), \]
\[ m\dot{v}_i(t) = -\nabla_i U - \Gamma v_i(t) + \sigma \Xi_i(t), \]

where \( r_i \) and \( v_i \) are the position and velocity of particle \( i \) respectively at time \( t \). Similarly, \( m \) is the mass of the particles, and \( \Gamma \) is a bead’s friction coefficient. \( \sigma \Xi_i(t) \) is a random force originating from a heat bath and is uncorrelated for different particles, different times, and different components. \( \Xi_i(t) \) is generated as a random vector from a uniform distribution with zero mean and obeys \( \langle \Xi_i(t) \rangle = 0 \) and \( \langle \Xi_{i,\mu}(t) \Xi_{j,\nu}(t') \rangle = \delta_{\mu\nu} \delta_{ij} \delta(t-t') \), where \( \delta_{ij} \) is the Kronecker delta and \( \delta(t) \) is the Dirac delta function. \( \Gamma \) and \( \sigma \) are inter-related through the fluctuation-dissipation theorem leading to \( \Gamma = \sigma^2/2k_B T \), where \( k_B \) is the Boltzmann’s constant. This ensures that the system reaches thermal equilibrium.

2.3.2 Velocity-Verlet Integration

Numerical integration methods involve the Taylor expansions to integrate the equations of motion, like the one in Eq. (2.11). These methods range from simple first-order methods like Euler integration to more advanced techniques for higher-order expansions such as the third-order algorithm with an error of \( O(\Delta t^3) \), leap-frog integration method with an error of \( O(\Delta t^3) \), Runge-Kutta integration with an error of \( O(\Delta t^3) \), etc. Higher-order methods offer improved accuracy; however, they can be computationally very inefficient. First-order methods are computationally very efficient. However, the error accumulation over time using these methods is much higher compared to
second or higher-order integration methods. Velocity-verlet method is a second-order integration approach, which has lesser error accumulation while ensuring the relative computational efficiency [185]. Hence, this approach is adopted in this work.

In the velocity-Verlet approach, discretized equations are obtained by expanding the equations of motion to the second order. Hence, by expanding Eq. (2.9) to the second-order, the position and the velocity of the $i$th bead at time $t + \Delta t$ is given by

\[
\begin{align*}
\mathbf{r}_i(t + \Delta t) &= \mathbf{r}_i(t) + \mathbf{v}_i(t) \Delta t + \frac{f_i(t)}{2m_i} \Delta t^2, \\
\mathbf{v}_i(t + \Delta t) &= \mathbf{v}_i(t) + \frac{f_i(t) + f_i(t + \Delta t)}{2m_i} \Delta t,
\end{align*}
\]

(2.12)

where $f_i(t) = -\nabla_i U (\{r_i\}) - \Gamma \mathbf{v}_i(t) + \sigma \Xi_i(t)$. In Eq. (2.12), $t$ is the current time, and $\Delta t$ is the time step.

### 2.3.3 Simulations Details

All beads have the same mass, $m$, in the simulations. Additionally, simulation boxes have periodic boundary conditions along the $x$-, $y$- and $z$-axes. The simulations of planar membranes are conducted in the $\gamma NVT$ ensemble, where $\gamma$ represents the surface tension experienced by the bilayer, and $V$ is the volume of the system ($V = L_x L_y L_z$). Due to thermal fluctuations and the surface tension, the projected area of the planar membrane is not constant.

Simulations of lipid vesicles are performed in the NVT ensemble, with $V = L^3$ and no $\gamma$. Hence, the simulations are performed following molecular dynamics, as compared to the cases where $N$ particles are simulated in the box with non-constant volume. A hybrid approach is used to simulate the box with non-constant volume [46]. In this case, the coordinates and velocities of the particles are still updated using the MD approach. However, the system size is updated using a Metropolis Monte Carlo scheme [186]. In the case of a planar membrane that is overall parallel to the $xy$-plane.
with a lateral surface tension, $\gamma$, applied to it, the effective Hamiltonian is given by,

$$\tilde{H} = \mathcal{U}(\{r_i\}) + \gamma L^2. \quad (2.13)$$

At every few time steps, a new linear system size along the $xy$-plane, $L' = L + \Lambda$, is attempted, with $\Lambda$ being a small random perturbation in the interval $(-0.1r_m, 0.1r_m)$, resulting in an attempted new state $\tilde{H}$. Consequently, attempted new coordinates of all beads are conformally rescaled to $x'_i = x_i L'/L$, $y'_i = y_i L'/L$, and $z'_i = z_i L'/L$, to keep the volume constant. The attempted change is then accepted or rejected using the standard Metropolis criterion with the Hamiltonian in Eq. (2.13). The ratio between the probability of the new state and the old state is given by,

$$\alpha = \frac{P'}{P} = \exp \left[ -\beta (\tilde{H}' - \tilde{H}) \right], \quad (2.14)$$

where $\beta = 1/k_B T$. If $\alpha \geq 1$, the attempted state is accepted. Otherwise, a random number, $\rho$, is generated in the range $[0, 1)$, and the attempted state is then accepted if $\rho \leq \alpha$.

The simulations are typically executed at $k_B T = 3.0 \epsilon$, and with a time step $\Delta t = 0.02\tau$, where $\tau = r_m (m/\epsilon)^{1/2}$. Simulations involving SCNP with an aspect ratio greater than 2.5 are performed for $\Delta t = 0.01\tau$. The diameter of the SCNP varies between 10 nm and 20 nm, and the aspect ratio is varied between 1.08 to 5.5. The number of lipids in planar membranes varies between 50,000 and 200,000, corresponding to projected areas ranging from 16,000 to 65,000 nm$^2$. Lipid vesicle simulations are performed with vesicle diameters between 44.8 and 157.8 nm, corresponding to the number of lipids ranging from 22,000 to 270,000. The duration of a simulation typically spans from $5 \times 10^6$ to $4 \times 10^7$ time steps, which translates to a time span between 100,000 and 800,000$\tau$. Random numbers are generated in the range $[0, 1)$ via the Mersenne Twister (MT19937) pseudo-random number generator algorithm.
2.4 Free Energy Calculations

Simulations in this study often produce results that can differ due to different initial conditions or variable parameters. While some results can be inferred qualitatively by simply looking at the snapshots from simulations or through some analysis of the positions of the beads, most systems yield results that are hard to understand through these methods. More importantly, NPs adhere to the lipid membrane through different modes of adhesion, and it is often impossible to determine the stability of a system with multiple NPs simply through direct MD simulations. This is simply because the kinetics of the adhesion often cause the NP to be stuck in some metastable state. Hence, to address this issue and better understand the stability of the systems in our study, the free energy of the system is calculated via two methods: Weighted histogram analysis method and Local Monge representation of the Helfrich curvature Hamiltonian.

2.4.1 Weighted Histogram Analysis Method

The weighted histogram analysis method (WHAM) \cite{187} is a statistical method frequently used to analyze simulation data and infer the relative stability of different states in complex systems. In this method, the free energy is calculated as a function of some reaction coordinate. This reaction coordinate is a collective variable that characterizes the configurations of the system and helps in understanding the overall behavior of the system. In this study, the distance between the centers of two NPs is often used as the reaction coordinate. Using distance as the reaction coordinate is particularly useful in studying the interactions between two SCNPs and understanding their tendency to aggregate or dissociate.

WHAM involves biased MD simulations of the system with two SCNPs, with the addition of a bias harmonic potential energy given by,

\[
u_i(x) = \frac{k_i}{2} (x - a_i)^2, \tag{2.15}\]
where \( x \) is the distance between the center beads of two NPs (reaction coordinate) and \( a_i \) is the preferred distance between the center beads of the two NPs. \( k_i \) is an arbitrary bias strength, the value of which can vary between simulations and systems. Because of the bias potential between the SCNPs, they oscillate around a distance at their equilibrium state. Hence, from each simulation based on the biased potential given by Eq. (2.15) \( n_i(x) \) number of points of the histogram is obtained for each \( x \) in the interval \([x, x + \delta x]\). Hence, the total number of points in the histogram obtained from simulation \( i \) is given by

\[
N_i = \sum_x n_i(x).
\]  

(2.16)

The probability, \( P(x) \), that \( x \in [x, x + \delta x] \) in a biased simulation \( i \) can be calculated by [187],

\[
P_i(x) = \frac{n_i(x)}{N_i} = \frac{e^{-\beta u_i(x)}}{\langle e^{-\beta u_i(\hat{x}(R))} \rangle} P(x),
\]  

(2.17)

where \( \langle e^{-\beta u_i(\hat{x}(R))} \rangle \) is the thermal average of the Boltzmann factor of the bias potential energy, with \( R \) representing the coordinates of all particles in the system. However, the thermal average is calculated on a system without bias and is given by

\[
\langle e^{-\beta u_i(\hat{x}(R))} \rangle = \int dR e^{-\beta[H(R) - u_i(x(R))]} dR e^{-\beta[H(R)]}
\]  

(2.18)

where \( dR = \prod_{k=1}^N dx_k dy_k dz_k \), and \( N \) is the total number of particles in the system. Using \( f_i = -k_B T \ln \langle e^{-\beta u_i(\hat{x}(R))} \rangle \), Eq. (2.17) becomes

\[
n_i(x) = N_i P(x) e^{\beta(f_i - u_i(x))}.
\]  

(2.19)

Eq. (2.19) once summed over the total number of simulations becomes

\[
P(x) = \frac{\sum_{i=1}^M n_i(x)}{\sum_{i=1}^M N_i e^{-\beta(f_i - u_i(x))}},
\]  

(2.20)

where \( M \) is the total number of biased simulations. Combining Eqs. (2.19) and (2.16),
we then obtain

\[ N_i = \sum_x n_i(x) = \sum_x N_i P(x)e^{\beta(f_i - u_i(x))} = e^{-\beta F_i} \sum_x N_i P(x)e^{\beta u_i(x)}, \quad (2.21) \]

which leads to

\[ f_i = -k_B T \ln \sum_x P(x)e^{-\beta u_i(x)}. \quad (2.22) \]

Finally, by self-consistently calculating \( P(x) \) using Eqs. (2.20) and (2.22), the free energy, \( F(x) \), can be calculated as a function of the reaction coordinate, given by

\[ F(x) = -k_B T \ln P(x). \quad (2.23) \]

The minima obtained from the free energy curve, as a function of distance, correspond to the stable states of the system and help us understand the energy barriers between different modes of adhesion, given that they exist. The free energy obtained from WHAM gives an accurate picture of the nature of the free energy. However, obtaining free energy using this method is computationally very expensive, as it requires a lot of biased simulations. Moreover, this method fails if there is a discontinuous transition in the modes of adhesion. Hence, in this work, free energy is also calculated using a different approach.

### 2.4.2 Local Monge Representation of the Helfrich Curvature Free Energy

The free energy of the membrane with the adhering SCNPs is given by the sum of the potential energy between the NPs and the lipids, which is calculated using Eq. (2.4), and the curvature energy of the lipids membrane. In order to calculate the curvature energy, we use a local Monge representation of the Helfrich Hamiltonian \([71]\). This approach is valid since the Helfrich Hamiltonian is invariant under arbitrary rotation. This approach does not account for the entropic contributions to the free energy of the lipid membrane \([67]\). Therefore, \( E_c \) is an approximation of the curvature free energy.
The calculation proceeds through the following steps (See Fig. 2.10):

1) For each lipid \( i \), all neighboring lipids within the same leaflet and within a range \( \lambda = 0.865 r_m \) are identified, and their average normalized end-to-end vector, \( \hat{n}_i \), is calculated.

2) A unit vector, tangent to the membrane at lipid \( i \), \( \hat{t}_i = \hat{n}_i \times \hat{z} \), is determined.

3) The portion of the membrane, composed of all lipids within the distance \( 3\sqrt{2} \lambda \) from lipid \( i \) and belonging to the same leaflet, is then rotated around the tangent vector \( \hat{t}_i \) by the angle \( \theta = \cos^{-1}(\hat{z} \cdot \hat{n}_i) \), using the following matrix \( [188] \)

\[
R = \begin{bmatrix}
    t_{i,x}^2(1 - c) + c & t_{i,x}t_{i,y}(1 - c) - t_{i,z}s & t_{i,x}t_{i,z}(1 - c) + t_{i,y}s \\
    t_{i,x}t_{i,y}(1 - c) + t_{i,z}s & t_{i,y}^2(1 - c) + c & t_{i,y}t_{i,z}(1 - c) - t_{i,x}s \\
    t_{i,x}t_{i,z}(1 - c) - t_{i,y}s & t_{i,y}t_{i,z}(1 - c) + t_{i,x}s & t_{i,z}^2(1 - c) + c 
\end{bmatrix} \tag{2.24}
\]

where \( t_{i,x} \), \( t_{i,y} \) and \( t_{i,z} \) are the components of the unit tangent vector \( \hat{t}_i \), \( c = \cos \theta \) and \( s = \sin \theta \). The \( z \)-axis is now locally normal to the region of the membrane around lipid \( i \).

4) The tangent plane to the rotated portion of the membrane leaflet around lipid \( i \) is then discretized into small squares of area \( a_p = (2\lambda)^2 \), such that the projection of the head bead of lipid \( i \) is at the center of its projection square, as shown by Fig. 6 in Ref. [67].

5) The local heights of the leaflet in the neighborhood of \( i \) are then determined, which allow for the calculation of the first order partial derivatives \( h_{i,\hat{x}} \), \( h_{i,\hat{y}} \), and second order partial derivatives \( h_{i,\hat{x}\hat{x}} \), \( h_{i,\hat{x}\hat{y}} \) and \( h_{i,\hat{y}\hat{y}} \) using the finite difference method based on both nearest and next-nearest neighbors.

6) The local extrinsic curvature of the leaflet at lipid \( i \) is then calculated using the following approximation,
Figure 2.10: A schematic representation of a portion of the lipid bilayer, centered at lipid $i$ before rotation (A) and after rotation (B). In (A), $\hat{n}_i$ is a unit vector in the direction of the mean of the end-to-end vectors of lipids in a small region around $i$, as explained in the text. $\hat{t}_i = \hat{n}_i \times \hat{z}$ is a local tangent vector that is normal to both the $z$-axis and $\hat{n}_i$. The bilayer is rotated around the vector $\hat{t}_i$ by the angle $\theta$ between $\hat{n}_i$ and the $z$-axis. Note that the bilayer is not discretized before rotation. The discretized $xy$-plane in (B) is parallel to the tangent plane to the membrane at $i$. The vertical purple arrows indicate the heights of the discretized parts of the leaflet containing lipid $i$. From Spangler et al. [67].
\[ K_i = \frac{(1 + h_{i,\tilde{x}}^2) h_{i,\tilde{x}x} + (1 + h_{i,\tilde{y}}^2) h_{i,\tilde{y}y} - 2h_{i,\tilde{x}} h_{i,\tilde{x}y} h_{i,\tilde{x}\tilde{y}}}{(1 + h_{i,\tilde{x}}^2 + h_{i,\tilde{y}}^2)^{3/2}}. \] (2.25)

7) The curvature energy of the bilayer is then calculated as

\[ E_c = \sum_{i=1}^{N_{lip}} \Delta A_i \frac{\kappa}{4} K_i^2, \] (2.26)

where 4 in the denominator accounts for the fact that the curvature energy is calculated for each leaflet separately, and \( \kappa \) is the bending modulus of the bilayer. \( \Delta A_i \) in the equation above is the local area of the leaflet at lipid \( i \) and is given by

\[ \Delta A_i = \left( \frac{a_p}{n_i} \right) \left( 1 + h_{i,\tilde{x}}^2 + h_{i,\tilde{y}}^2 \right)^{1/2}, \] (2.27)

where \( n_i \) is the number of lipids whose projections fall onto the local square centered at \( i \) in the locally rotated coordinate system.

### 2.5 Analysis Techniques

Besides visualizing the system, the data is usually analyzed to harness detailed information and enhance understanding of the simulations. In this work, the simulation data were analyzed using various analysis techniques. Some basic information extracted from the simulations included the angle made by an SCNP with the z-axis, angles between the NPs, distance between the SCNPs, and degree of wrapping of SCNP by the membrane. Apart from generic analysis of the data, more complex approaches were used in the study. These approaches are discussed in the following sections.

#### 2.5.1 Voronoi Diagrams and Delaunay Triangulations

Voronoi diagram and the Delaunay triangulation are powerful approaches used to analyze the spatial relationship of a set of discrete points in space. These methods are dual to each other. The Delaunay triangulation connects groups of three points to form
sub-triangles in two dimensions. The triangle is connected while ensuring that no other points lie inside the circumcircle of the triangle. This approach maximizes the minimum angle among all the triangles, leading to more regular and well-behaved triangles [189]. The vertices of these triangles are then linked, making them nearest neighbors to each other.

Delaunay triangulation is used in this study to determine the nearest neighbors of the SCNPs adhering to vesicles. This is illustrated in Fig. 2.11. This approach was implemented using two steps. Firstly, the SCNP centers are projected onto a sphere’s surface. This was followed by simply employing an algorithm from the Python Scipy library [191], which allowed us to extract each bead’s first and second neighbors. This extracted information is used for analytical graph generation of the system and calculation of bond angles.
2.5.2 Nematic Order Parameter

SCNPs position themselves on the lipid vesicles with some order in terms of the positions of their centers of mass and their orientations. Correlations between their positions are characterized by their radial distribution function. The relative orientations of the SCNPs are quantified using the nematic order parameter, which is often used to characterize the degree of order in liquid crystals. The nematic order parameter basically informs us on the degree of alignment of the SCNPs regardless of their locations. This approach involves two steps. First, we calculate the elements of the $3 \times 3$-nematic order parameter tensor $Q$ of the SCNPs as

$$Q_{ij} = \frac{1}{n} \sum_{k=1}^{n} \frac{1}{2} (3u_{k,\mu}u_{k,\nu} - \delta_{\mu\nu}),$$  \hspace{1cm} (2.28)$$

where $u_{k,i}$ and $u_{k,j}$ are the $\mu$th and $\nu$th components of the director unit vector $\hat{u}_k$ of SCNP $k$, $\mu, \nu = x, y, z$, and $n$ is the total number of SCNPs [192,193]. The director vector of a SCNP is simply the unit vector along the axis passing by its two poles. The brackets indicate an ensemble average. Once the tensor elements $Q_{ij}$ are calculated, the eigenvalues of the tensor are then determined. The largest eigenvalue, $S$, is then defined as the nematic order parameter of the SCNPs. The SCNPs exhibit nematic order if $S > 0.3$, planar nematic order if $S < -0.3$, and isotropic order for intermediate values between $-0.3 < S < 0.3$ [192].

2.5.3 Least-Square Plane

The least-squares method is a statistical technique that is used to find the best-fitting line or curve to a set of data points. It is useful in data fitting to determine the relationship between variables used in a regression analysis. This method minimizes the sum of the squares of the differences between the observed data points and the corresponding values predicted by the model [194]. In this study, the least-square method is used to calculate the equation of a plane that best fits the centers of mass of an ensemble of
SCNPs once they adhere inside a lipid vesicle. The distance between the centers and this mid-plane is calculated to infer how coplanar are the centers of the SCNPs.

The equation of a plane in three dimensions is given by,

\[ z = ax + by + c, \tag{2.29} \]

where \( x, y, \) and \( z \) are the Cartesian coordinates of an arbitrary point in the plane, and \( a, b, \) and \( c \) are real constants. For a given set of \( n \) points, where \( n \geq 3 \), the mid-plane equation is calculated by minimizing the following equation \( S \),

\[ S = \sum_{k=0}^{n-1} (ax_k + by_k + c - z_k)^2, \tag{2.30} \]

such that the condition,

\[ \frac{\partial S}{\partial a} = \frac{\partial S}{\partial b} = \frac{\partial S}{\partial c} = 0 \tag{2.31} \]

is satisfied. Therefore, \( a, b, \) and \( c \) are determined from solving the following set of linear equations

\[
\begin{bmatrix}
\sum x_k^2 & \sum x_k y_k & \sum x_k \\
\sum x_k y_k & \sum y_k^2 & \sum y_k \\
\sum x_k & \sum y_k & n
\end{bmatrix}
\begin{bmatrix}
a \\
b \\
c
\end{bmatrix}
= 
\begin{bmatrix}
\sum x_k z_k \\
\sum y_k z_k \\
\sum z_k
\end{bmatrix}.
\tag{2.32}
\]

The degree of coplanarity of the SCNPs’ centers is then calculated as the mean deviation of the SCNPs’ centers from this plane, \textit{i.e.}, as \( d = \sqrt{S} \), using Eq. (2.30) with the values of \( a, b, \) and \( c \) obtained from Eq. (2.32).
Chapter 3

MODES OF ADHESION OF SPHEROCYLINDRICAL NANOPARTICLES TO TENSIONLESS LIPID BILAYERS

3.1 Introduction

Recent advances in nanoscience and nanotechnology have accelerated the fabrication of nanomaterials with different geometries, dimensions, and surface properties [195]. Engineered nanomaterials can be designed for use in different applications, including chemical sensing [115, 196], electronics [197], data storage [198], food industry [199], and cosmetics [200]. A particularly astonishing progress has been made in the development of nanomaterials for various biological and medical applications. These include gene therapy [120], cancer drug delivery [16], cancer diagnostics [201], biosensing [113], and the probe of intracellular structure [202]. With the ever-increasing range of applications of nanomaterials, there is also a growing need to investigate their potential toxicity and ways to minimize it [37–39]. The development of effective and safe nanomaterials requires an understanding of how nanoparticles (NPs) interact with biomembranes, which act as the entry point of living cells.

Many NPs’ properties depend highly on their morphology. For example, gold NPs with tailored optical properties can be synthesized by tuning their morphology, making them uniquely promising for a range of biomedical applications [203]. Several studies have shown that NPs’ dimensions and geometry play a major role in their adhesion to lipid membranes and their internalization [84, 89, 91, 98, 99]. Gratton et al.’s study, in particular, showed that internalization of cylindrical NPs with a given aspect ratio is enhanced with increasing the NPs’ diameter [91]. The uptake of NPs by living cells is complicated by the presence of several active processes facilitating their internalization. Furthermore, the actomyosin cytoskeleton may hinder the adhesion mode in
which the NPs protrude to the inner side of the cell. The understanding of the effect of NPs’ morphology on their adhesion to cells and their subsequent internalization would greatly benefit from studies of the adhesion of NPs on simple lipid membranes. Unfortunately, systematic experimental investigations of the adhesion and internalization of non-spherical NPs by simple model lipid membranes have thus far been largely lacking. This makes computer simulations a useful alternative tool to understand the adhesion and internalization of non-spherical NPs by lipid membranes.

The onset and modes of adhesion of NPs to lipid membranes can be estimated from the balance between the adhesive energy and the membrane elasticity energy. In the simplest case of a spherical NP with diameter $D$, its adhesion energy is $E_{\text{adh}} = -\pi \nu D^2 \xi$, where $\xi (> 0)$ is the adhesion energy density and $\nu$ is the fraction of the NP’s surface in contact with the membrane. In the case of a tensionless planar membrane, its excess elastic energy, resulting from its curvature deformation due to the adhesion of the NP, is $E_{\text{curv}} = 8\pi \nu \kappa$, where $\kappa$ is the membrane bending modulus. The minimization of the total energy leads to an adhesion threshold $\xi^* = 8\kappa/D^2$, below which the NP is unbound and above which the NP is fully wrapped by the membrane [73, 204]. This argument assumes that the NP and the membrane interact with each other only when they are in contact and that the curvature energy of the non-contact portion of the membrane is zero. This argument was shown to not be correct if the NPs’ diameter is comparable to, or a few times, the thickness of the lipid membrane or if the finite range of the NP-membrane interaction is accounted for [46, 179, 205].

The arguments above can be extended to the case of a cylindrical NP, with diameter $D$ and a very high aspect ratio, such that its two ends can be ignored. In this case, one finds that the NP adheres to the membrane for $\xi > \xi^* = 2\kappa/D^2$. The decrease in the adhesion threshold of a cylindrical NP is due to its reduced mean curvature compared to that of a spherical NP with the same diameter. The adhesion of spherocylindrical NPs on lipid membranes and their endocytosis was investigated through coarse-grained molecular dynamics (MD) simulations by Vácha et al. [98] and Huang et al. [99]. Both
studies focused on the kinetics of spontaneous endocytosis of NPs with ligands that bind to a fraction of lipids (receptors) and on cases where the adhesion energy density is relatively high. Huang et al. found that an NP, with an aspect ratio higher than about 2, first adheres through its cylindrical side to the membrane [99]. When the NP is about half-wrapped, it rotates to a perpendicular orientation [99]. Finally, the NP is endocytosed while nearly perpendicular to the membrane plane [99]. On the other hand, Vácha et al. found, using a more microscopic membrane model, that while the NP adheres through its cylindrical side to the membrane, they are endocytosed while they remain nearly parallel to the membrane’s plane [98].

The modes of adhesion of cylindrical NPs, with finite aspect ratio and blunt circular ends, were investigated by Dasgupta et al. [84] using a Monte Carlo energy minimization method of dynamically triangulated planar membranes. For low values of $\xi$, the NP adheres shallowly to the membrane through one of its blunt surfaces for high values of the curvature, $c_{\text{edge}}$, of the circular edges between the NP’s cylindrical side and blunt surfaces or for low values of the NP’s aspect ratio, $\alpha$. However, for low values of $c_{\text{edge}}$ or high values of $\alpha$, the NP adheres to the membrane shallowly through its cylindrical side [84]. As $\xi$ is increased, they predicted a discontinuous transition to a deep wrapping state, in which the principal axis of the NP is perpendicular to the membrane regardless of the value of $\alpha$ or $c_{\text{edge}}$. This state is then followed, through another discontinuous transition, by the full wrapping of the NP as $\xi$ is further increased [84].

The goal of the present study is to investigate the modes of adhesion of spherocylindrical NPs and their endocytosis through an approach that accounts for thermal fluctuations. To this end, we used MD simulations of a coarse-grained implicit solvent model of self-assembled lipid membranes in which the lipid molecules are coarse-grained as short, semi-flexible amphiphilic chains. To reduce the total number of degrees of freedom associated with spherocylindrical NPs, they are modeled as triangulated hollow shells. The focus of the study is to determine the phase diagram of adhesion and endocytosis of spherocylindrical NPs as a function of their aspect ratio, dimensions, and
3.2 Model and Numerical Approach

The lipid membrane is modeled using the implicit solvent model discussed in section (2.1.4), and the SCNP is modeled using the approach discussed in section (2.2). All simulations are performed on tensionless membranes ($\Sigma = 0$). Typically the initial size ($L_x, L_y, L_z$) = (100$r_m$, 100$r_m$, 100$r_m$). The total number of lipids in the membrane is $N_{lip} = 10,000$. The simulations are executed at $k_B T = 3.0\epsilon$, with a time step $\Delta t = 0.02\tau$, where $\tau = r_m(m/\epsilon)^{1/2}$. Eqs. (2.10 and 2.11) are integrated using the velocity-Verlet algorithm with $\Gamma = \sqrt{6m/\tau}$.

The adhesion energy density is defined as $\xi = |U_{adh}|/A_{adh}$, where $U_{adh}$ is the net potential energy between the NP and the membrane and $A_{adh}$ is the area of the NP adhering to the membrane. To determine the adhesion energy density, simulations of a spherical NP of diameter $D$ adhering to a tensionless planar bilayer are performed at
different values of $\mathcal{E}$. Here, a bead of type $n$ adheres to the membrane if it interacts with at least one $h$ bead of the membrane, i.e., if its distance from the $h$ bead is less than $r_c$. Fig. 3.1 depicts the adhesion energy density versus distance $\mathcal{E}$ for NPs with diameter $D = 10, 15$, and $20$ nm. This figure shows that $\xi$ dependence on $\mathcal{E}$ is not linear for low values of $\mathcal{E}$ but becomes linear as $\mathcal{E}$ further increases. This figure also shows that for a given value of $\mathcal{E}$, $\xi$ decreases with $D$. This is simply due to the fact that the number of beads on a spherical NP is fixed (642 beads), regardless of its size.

### 3.3 Elasticity Theory Phase Diagram

Before presenting our results from the MD simulations, it is instructional first to determine an approximate adhesion phase diagram of a spherocylindrical NP using simplified theoretical arguments based on a balance between the Helfrich’s curvature...
energy \[71\] of the membrane and the adhesion energy. Assuming that the NP only deforms the portion of the membrane that is in contact with it, the excess free energy of the membrane is then given by \[71\]

\[ F = \int_A da \frac{\kappa}{2} (c_1 + c_2 - c_0)^2 + \int_A da \tilde{\kappa} c_1 c_2 - \xi A, \tag{3.1} \]

where \( A \) is the NP-membrane contact area. \( \kappa \) and \( \tilde{\kappa} \) are the bending modulus and saddle splay modulus, respectively. \( c_1 \) and \( c_2 \) are the local principal curvatures of the membrane in the contact region. Due to the absence of transbilayer asymmetry of the membrane, the spontaneous curvature \( c_0 = 0 \). Assuming that the adhesion of the NP does not locally modify the saddle-splay bending modulus, the second term in Eq. (3.1) reduces to an integral of the Gaussian curvature \( c_1 c_2 \) over space, which is a topological invariant. Therefore, this term is not included in the subsequent calculations.

Motivated by the MD results, four adhesion modes of the NP on the membrane, shown schematically in Fig. 3.2, are considered in the calculations. These correspond to the unbound state, the parallel adhesion mode, the partially wrapped perpendicular mode, and the strongly wrapped perpendicular mode, shown by (A), (B), (C), and (D), respectively. The free energy of the system in the different modes is given by

\[ \frac{F}{2\pi\kappa} = \begin{cases} 
0 & \text{for (A),} \\
(2 - \tilde{\xi})(1 - \cos \psi) + \frac{\alpha - 1}{\pi}(1 - 2\tilde{\xi})\psi & \text{for (B),} \\
(2 - \tilde{\xi}) + (\alpha' - 1)(1 - 2\tilde{\xi}) & \text{for (C),} \\
(2 - \tilde{\xi})(1 + \sin \varphi) + (\alpha - 1)(1 - 2\tilde{\xi}) & \text{for (D),} 
\end{cases} \tag{3.2} \]

where \( \tilde{\xi} = \xi D^2 / 4\kappa \) is the reduced adhesion energy density, \( D \) is the NP’s diameter, \( \alpha = (L + D)/D \) is the NP’s aspect ratio, and \( \alpha' = (L' + D)/D \), where \( L' \) is the partial height of the cylindrical portion of the NP that is wrapped by the membrane. The wrapping angles \( \psi \) and \( \varphi \) are defined in Fig. 3.2. We emphasize that this theoretical approach is fairly crude as (1) it assumes that the interaction between the NP and the membrane
Figure 3.3: Theoretical adhesion phase diagram in terms of the reduced adhesion energy density, $\tilde{\xi}$, and NP's aspect ratio, $\alpha$, obtained from the minimization of the free energy given by Eq. (3.2). The transition line from the unbound mode to the parallel mode is blue, the transition line from the parallel mode to the perpendicular mode is pink, and the transition line from the perpendicular mode to the fully wrapped mode is orange. Note that the transition line from the unbound to the parallel mode (blue line) asymptotically approaches the adhesion threshold of an infinitely long cylindrical NP ($\tilde{\xi} = 0.5$) as $\alpha$ is increased.

is a contact interaction, (2) it does not account for the fact that the NP deforms the membrane beyond the contact region, (3) it is only valid in the limit where the NP’s dimensions are much larger than the membrane thickness, and (4) it does not account for fluctuations.

The phase diagram of the adhesion modes, in terms of $\tilde{\xi}$ and $\alpha$, obtained from the minimization of the free energy in Eq. (3.2) is shown in Fig. 3.3. The degree of wrapping of the NP $f$, defined as the fraction of the NP’s area that is wrapped by the membrane, is shown as a function of $\tilde{\xi}$ for four different values of the aspect ratio in Fig. 3.4.
Figure 3.4: The degree of wrapping of the NP’s area, obtained from the minimization of the free energy in Eq. (3.2), as a function of the reduced adhesion energy density, for different values of the aspect ratio.

Fig. 3.3 shows that regardless of the aspect ratio, the NP adheres to the membrane in the parallel mode at low values of the adhesion energy density. In this state, the degree of wrapping $f$ gradually increases with increasing $\tilde{\xi}$ toward a maximum value that increases with the aspect ratio, as shown by Fig. 3.4. This is contrasted to the case of a spherical NP, which can either be unbound ($f = 0$) or completely wrapped ($f = 1$). The cylindrical side of a spherocylindrical NP, therefore, allows for its partial wrapping. As the adhesion strength is further increased, the NP mode of adhesion changes to the perpendicular mode, in which the whole cylindrical side of the NP is wrapped by the membrane (i.e., $L' = L$). The phase diagram also shows that the adhesion energy density, at which the NP flips from the parallel to the perpendicular mode, decreases with increasing the aspect ratio. Interestingly, the range of $\tilde{\xi}$ over which the parallel mode is stable decreases with increasing $\alpha$ and eventually vanishes in the marginal case of infinitely long NPs. The transition from the perpendicular mode to the fully wrapped
mode occurs at $\tilde{\xi} = 2$ regardless of the NP’s aspect ratio. In state (D), the NP is always fully wrapped, i.e., $\varphi = \pi/2$ regardless of $\tilde{\xi}$. This value also corresponds to the transition adhesion energy density of a spherical NP from the unbound to the fully wrapped state.

### 3.4 Numerical Results

We now turn to the modes of adhesion obtained from the MD simulations. Fig. 3.5 depicts a sequence of typical equilibrium configurations for different values of the adhesion energy density in the case of $\alpha = 1.60$. These modes of adhesion are essentially the same as those in Fig. 3.2, with some differences that will be discussed later. The endocytosis mode in Fig. 3.5 at $\xi = 1.56 k_B T/\text{nm}^2$ is identified with the completely wrapped state in Fig. 3.2.

The adhesion phase diagram, from the simulations, as a function of the adhesion energy density and aspect ratio in the case of NPs with $D = 20 \text{ nm}$ is shown in Fig. 3.6. The degree of wrapping of the NP as a function of adhesion energy density for different values of aspect ratio for $D = 20 \text{ nm}$ is shown in Fig. 3.7. Fig. 3.6 shows a $\xi$-$\alpha$ phase diagram that is qualitatively in agreement with the theoretical one in Fig. 3.3.
the NP is unbound at very low values of $\xi$ and adheres in the parallel mode as $\xi$ is increased. As $\xi$ is further increased, the NP adheres in the perpendicular mode and is endocytosed at high values of $\xi$. The qualitative dependence of the transition lines on $\xi$ from the MD simulations agrees with those in Fig. 3.3. In particular, the transition adhesion energy density from the unbound state to the parallel adhesion mode and that from the parallel to the perpendicular mode decreases with increasing the aspect ratio. These results imply that the higher the aspect ratio of the NP, the easier it is for a spherocylindrical NP to adhere to a planar membrane. Likewise, the NP becomes more prone to flip from the parallel to the perpendicular mode as its aspect ratio is increased. Fig. 3.7 shows that the degree of wrapping exhibits a discontinuity at the transition from the parallel mode to the perpendicular mode, also in qualitative agreement with
A notable qualitative difference between the MD and theoretical phase diagrams is that a spherical NP \((\alpha = 1)\), in the MD simulations, is partially wrapped over a range of values of \(\xi\), with the contact area fraction \(f\) that increases continuously with \(\xi\), as shown by Fig. 3.7 and by earlier studies [46, 179]. In contrast, the theoretical argument predicts that the NP is either unbound or completely wrapped as demonstrated by the phase diagram in Figs. 3.3 and 3.4 at \(\alpha = 1\). Another important difference between the phase diagrams in Figs. 3.3 and 3.6 is that the theoretical arguments predict that the degree of wrapping of the NP in the perpendicular mode is independent of \(\xi\), as shown by Fig. 3.4. This is due to the fact that the theory predicts that the membrane does not wrap the top hemispherical cap of the NP in this mode. In contrast, the MD simulations show that the degree of wrapping increases continuously in the perpendicular mode, as shown by Fig. 3.6.
Figure 3.8: $\xi - 1/D^2$ adhesion phase diagram, from the MD simulations, as a function of adhesion energy density and inverse of the square of NP’s diameter, for the case of $\alpha = 1.75$. The transition line from the unbound state to the parallel adhesion mode is blue, the transition line from the parallel mode to the perpendicular mode is pink, and the transition line from the perpendicular mode to endocytosis is orange. Note that the blue line is very close to the $y$-axis.

The effect of the NP’s diameter on its adhesion is shown by the $\xi - 1/D^2$ phase diagram in Fig. [3.8] for the case of $\alpha = 1.75$. This figure shows that the adhesion energy densities corresponding to the transition from the parallel to the perpendicular mode and the transition from the perpendicular mode to endocytosis decrease with the diameter of the NP. Fig. [3.8] shows that the value of the adhesion energy density at these transitions, $\xi^* \sim 1/D^2$, is in agreement with the theoretical predictions. The particularly strong dependence of $\xi^*$ on the NP’s diameter at the transition from the perpendicular mode to endocytosis and the lack of dependence of $\xi^*$ on the length of the NP, as shown by the $\xi - \alpha$ phase diagram in Fig. [3.6], implies that the onset of endocytosis of spherocylindrical NPs is controlled by their diameter and not by their length.

In the parallel mode, the NP’s orientation is mainly parallel to the $xy$-plane, as shown by the distribution of the latitude angle, $P(\theta)$ depicted in Fig. [3.9] for the case of $D = 20$ nm and $\alpha = 1.6$. This figure shows that the distribution peaked at $\theta = \ldots$
90°, consistent with an NP adhesion in the parallel mode. However, the amount of fluctuations exhibited by the NP’s orientation increases with increasing the adhesion strength. This implies that as the transition from the parallel mode to the perpendicular mode is approached, the NP’s wobbling around its horizontal orientation is increased.

The simulations show that in the perpendicular mode, the NP typically adopts an orientation in which its principal axis is parallel to the z-axis for relatively low values of $\xi$ (see snapshot at $\xi = 0.86k_B T/\text{nm}^2$ in Fig. 3.5). However, for relatively high values of $\xi$, the NP’s principal axis is mostly tilted with respect to the z-axis, as shown by the snapshot at $\xi = 1.33k_B T/\text{nm}^2$ in Fig. 3.5. To infer the origin of the tilt of the NP in the perpendicular mode, the net adhesion potential energy of the NP vs. its latitude angle is shown in the scatter plot in Fig. 3.10, determined from a large set of configurations at equilibrium. This figure shows that for low values of $\xi$, within the perpendicular state, the range of the NP’s latitude angle is small, for example, up to about 15° and an average of about 5° for $\xi = 0.86k_B T/\text{nm}^2$. This indicates that the NP’s principal axis is mostly perpendicular to the $xy$-plane at low values of $\xi$ within the perpendicular mode. However, as $\xi$ is increased, the range of the latitude angle sampled by the NP is increased, with an average value of about 18° at $\xi = 1.10k_B T/\text{nm}^2$ and 23° at $\xi = 1.33k_B T/\text{nm}^2$, which indicates a preferred tilt of the NP in the perpendicular mode at high $\xi$.

The adhesion energy, shown in Fig. 3.10 is essentially independent of the angle $\theta$ for a given $\xi$. This is explained by the fact that as the latitude angle $\theta$ of the NP changes, the size of the neck connecting the bud containing the NP to the membrane remains roughly constant, and therefore, the amount of NP’s wrapping is independent of $\theta$. Since the curvature of the membrane in the contact region also remains unchanged by the tilt of the NP, the preferred tilt must be due to a reduced curvature energy of the non-contact region of the membrane. An interesting feature of the tilted orientation of the NP (see snapshots in Fig. 3.10) is that at the azimuthal angle corresponding to the tilt angle $\theta$, the membrane does not adhere to the top hemispherical cap, whereas
Figure 3.9: Distribution of the latitude angle $\theta$, defined in the inset, for an NP with $D = 20\,\text{nm}$ and $\alpha = 1.6$ in the (A) parallel mode and (B) normal mode, at different values of the adhesion energy density. The solid lines are guides to the eye.
at the supplementary azimuthal angle, the membrane degree of wrapping of the top hemispherical cap is maximized. Such a configuration must correspond to a reduced curvature energy of the non-contact region of the membrane than in the case where the NP is perpendicular to the $xy$-plane.

### 3.5 Kinetics of Endocytosis of Spherocylindrical NPs

In this section, we focus on the effect of the adhesion energy density, aspect ratio, and diameter of the NP on the kinetics of its endocytosis. Fig. 3.11 depicts the time evolution of the NP’s latitude angle along with the fraction of its area wrapped by the membrane for the case of $D = 20\,\text{nm}$ and $\alpha = 1.6$, at different values of $\xi$ within the endocytosis regime. Here, the NP is initially placed such that its axis is perpendicular to the membrane (i.e., $\theta(0) = 0$). Time series of snapshots corresponding to $\xi = 1.56$ and $2.77k_B T/\text{nm}^2$ are also shown in Fig. 3.11. This figure shows that regardless of

![Figure 3.10: Scatter plot of the NP’s net adhesion energy, $U_{\text{adh}}$, versus its tilt angle $\theta$ for the case of $D = 20\,\text{nm}$, $\alpha = 1.6$ at $\xi = 1.33k_B T/\text{nm}^2$. The inset shows the average latitude angle of the NP versus adhesion energy density.](image-url)

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Figure 3.10: Scatter plot of the NP’s net adhesion energy, $U_{\text{adh}}$, versus its tilt angle $\theta$ for the case of $D = 20\,\text{nm}$, $\alpha = 1.6$ at $\xi = 1.33k_B T/\text{nm}^2$. The inset shows the average latitude angle of the NP versus adhesion energy density.
the adhesion strength, upon its adhesion on the membrane via one of its hemispherical caps, the NP rotates from the perpendicular to the parallel orientation within a short time. While the NP remains temporarily in the parallel orientation for a short duration, the degree of wrapping $f$, at which the orientation of the NP is parallel to the $xy$-plane, increases with time, with a value around 0.5. The NP’s latitude angle then decreases with time as the degree of wrapping further increases. For relatively low values of $\xi$, such as $\xi = 1.56k_B T/\text{nm}^2$ (i.e. values of $\xi$ close to the endocytosis transition line in the phase diagram), the NP almost completely rotates again to an orientation perpendicular to the $xy$-plane (snapshot (e) in Fig. 3.11). However, as the amount of wrapping...
increases, such as the neck connecting the main bilayer to the invagination containing the NP is well formed, the latitude angle increases again such as the NP adopts a tilted orientation (snapshot (f) in Fig. 3.11) that is reminiscent of that in the perpendicular adhesion mode at high values of \( \xi \) (snapshot at \( \xi = 1.33 k_B T/\text{nm}^2 \) in Fig. 3.5). These kinetic pathways are in agreement with that observed by Huang et al. [99] using a more coarse-grained model.

At higher values of \( \xi \) (e.g. \( \xi = 2.02 \) and \( 2.92 k_B T/\text{nm}^2 \)), Fig. 3.11 shows that the NP does not rotate almost completely to the perpendicular orientation (snapshots (iv to vi) in Fig. 3.11), with the minimum value of \( \theta \) achieved by the NP that increases with increasing \( \xi \). This is due to the fact that the higher curvature energy of the intermediate orientations (iv-to vi) is compensated by a higher adhesion energy (in absolute value) at high values of \( \xi \). As a result, the NP is endocytosed while nearly parallel to the membrane at high values of \( \xi \). The details of the endocytosis pathway, therefore, depend on the adhesion strength.
The effect of the diameter of the NP on the endocytosis kinetics is shown in Fig. 3.12 for the case of $\alpha = 1.75$ at $\xi = 6k_B T/\text{nm}^2$. Here, the NPs are initially placed parallel to the membrane. This value of $\xi$ is very close to the endocytosis transition for $D = 10\text{ nm}$, as shown by the phase diagram in Fig. 3.8. Fig. 3.12 shows that the tendency of the NP to rotate from the horizontal orientation decreases with the difference, $\Delta \xi = \xi - \xi_{\text{end}}(D)$, where $\xi_{\text{end}}(D)$ is the value of the adhesion energy density at the endocytosis transition for an NP of diameter $D$. This is due to the fact that as $D$ is increased, $\Delta \xi$ is increased, and therefore, the resistance to the wrapping of the NP by the membrane, due to the curvature energy, is decreased. As a result, the angle by which the NP rotates toward the perpendicular orientation is diminished as $D$ is increased.

### 3.6 Summary

The adhesion modes and endocytosis of spherocylindrical NPs on tensionless planar membranes are investigated in this chapter using molecular dynamics simulations of a coarse-grained implicit-solvent model. The NPs are efficiently modeled as triangulated hollow shells. The simulations were performed over a range of values of the NP’s diameter $D$, aspect ratio $\alpha$, and strength of the adhesion energy density $\xi$. The NPs exhibit two modes of adhesion, which depend on $\xi$, $\alpha$, and $D$. For low values of $\xi$, the NP adheres through its side, i.e., its principal axis is parallel to the membrane with an increasing amount of wobbling with increasing $\xi$. As $\xi$ is increased, the NP’s adhesion mode flips to the perpendicular mode, in which the principal axis is mainly perpendicular to the membrane. The value of $\xi$ corresponding to the transition from the parallel mode to the perpendicular mode decreases with increasing the NP’s aspect ratio or its diameter, in agreement with theoretical arguments based on the Helfrich Hamiltonian. An interesting feature of the perpendicular adhesion mode is that once the neck of the bud containing the NP is well formed, the NP’s principal axis is tilted from the $z$-axis by an angle that increases with $\xi$. The lack of correlation between this tilt angle and the net adhesion energy of the NP implies that the preferred tilt angle in the perpendicular
adhesion mode is due to a combination of the finite range of the interaction between the lipid head beads and the NP and the curvature energy of the non-contact region of the membrane.

The sequence of adhesion modes observed in this study is in qualitative agreement with those based on the energy minimization approach [84]. However, in their approach, the NP caps are blunt with varying degrees. Due to the bluntness of the caps, the transition between the partially wrapped perpendicular mode and the fully wrapped perpendicular mode is discontinuous. In contrast, the present study shows that the degree of wrapping increases continuously with increasing $\xi$.

As $\xi$ is further increased, the NP undergoes spontaneous endocytosis. The value of $\xi$ corresponding to the transition from the perpendicular adhesion mode to endocytosis ($\xi_{end}$) is found to be independent of the NP’s aspect ratio. However, $\xi_{end}$ decreases with the diameter, as $1/D^2$, in agreement with the theoretical arguments. The independence of this transition from the NP’s aspect ratio is due to the fact that the neck linking the NP’s invagination to the main membrane in the perpendicular mode is near the hemispherical cap and is therefore independent of the length of the cylindrical portion.

The kinetics of endocytosis depends strongly on $\xi$ and $D$. Quickly after its adhesion, the NP rotates to a parallel orientation until its degree of wrapping is about 0.5. The NP then rotates toward the perpendicular orientation while its degree of wrapping increases with time. A minimum orientation angle of almost 0 is achieved in the case of relatively weak values of $\xi$. The almost vertical orientation of the NP is achieved once the cylindrical portion of the NP is fully wrapped. As the degree of wrapping further increases, the tilt of the NP’s orientation increases. This implies that as the NP’s invagination detaches from the membrane, the NP is not perpendicular to the $xy$-plane at low values of $\xi$. As $\xi$ increases, within the endocytosis region of the phase diagram, the minimum angle it achieves, as it rotates away from its parallel orientation, decreases with increasing $\xi$. As such, the NP’s orientation as it is endocytosed becomes increasingly parallel to the $xy$-plane as $\xi$ is increased. The tendency of the NP to rotate from the
parallel mode to the perpendicular mode during its endocytosis pathway is also found to decrease with increasing its diameter. However, it is fairly independent of its aspect ratio. The observed endocytosis pathways agree with earlier ones performed in the limit of high adhesion strength [98] and in the limit of relatively weak adhesion strength [99].
Chapter 4

MEMBRANE-MEDIATED DIMERIZATION OF SPHEROCYLINDRICAL NANOPARTICLES

4.1 Introduction

Owing to their unique properties, stemming from their high area-to-volume ratio, nanomaterials have a wide range of promising applications including data storage [206], light harvesting [207], non-linear optics [208], catalysis [209], drug delivery [210], biosensing [115] and food formulation [211]. In many biomedical applications, nanoparticles (NPs) come in direct contact with living cells. Since the plasma membrane is the point of entry of all living cells, the understanding of the interaction between NPs and lipid membranes is crucial for the development of safe and effective nanomaterials for biomedical applications. This understanding can also be leveraged for the use of lipid membranes as an alternative tool for bottom-up fabrication of ordered nanostructures [79].

Competition between the adhesive energy of the NPs on a lipid membrane and the curvature energy of the membrane, due to its local deformation to conform to the NPs surfaces, leads the membrane to partially or fully wrap the NPs [46, 55, 68, 171, 179, 212-215]. These deformations, which extend over length scales that are longer than the NP’s dimensions, lead to an interesting membrane curvature-mediated interaction between the NPs and can result in their aggregation. Studies of membrane-mediated interactions between two spherical NPs show four modes of adhesion, depending on their adhesion energy per unit of area, $\xi$ [60, 61, 68]. For low values of $\xi$, spherical NPs are weakly wrapped by the membrane, and as a result, they are highly diffusive, and their positions are uncorrelated [216]. For intermediate values of $\xi$, two NPs dimerize into an in-plane dimer. At higher values of $\xi$, the NPs dimerize into an out-plane dimer (tube). Finally,
the two NPs are endocytosed at even higher values of $\xi$. In the case of many spherical NPs, membrane curvature-mediated many-body effects are important and lead to their aggregation into in-plane or out-of-plane linear chains \[61\][62][67] as well as long-lived transient states, including out-of-plane bitube and ring aggregates \[67\].

Recent advances in nanomaterial synthesis methods have led to engineering anisotropic NPs with an ever-increasing number of geometries, dimensions, and surface properties. Particularly interesting anisotropic NPs are gold (Au) nanorods, which are endowed with optical and photothermal properties that are highly dependent on their aspect ratio \[85\]. Another advantage of nanorods is that, due to their stronger cross-stream drift during flow, their circulation times in capillaries are longer than those of spherical NPs \[86\]. A recent study showed that tracking of nanorods, with sidewise adhesion to lipid membranes, can be used to probe the mechanical properties of membranes, including their bending modulus and surface tension \[217\]. In another study, it was shown that magnetic nanorods adhering to a lipid membrane and in an oscillating magnetic field can lead to increased local fluidization of the membrane and to its lysis \[87\].

Conflicting experimental results have been reported on the effect of nanorods aspect ratio $\rho = l/D$, where $l$ and $D$ are the length and diameter of the nanorod, respectively, on their cellular uptake \[89\][94\]. For example, Qui et al. showed that the rate of uptake of Au nanorods by HeLa and human breast adenocarcinoma cells decreases with increasing $\rho$ \[92\]. In contrast, DeBrosse et al. showed that the internalization of Au nanorods by keratinocyte cells increases with $\rho$ \[93\]. The discrepancies between these experimental results underscore the difficulty in transferring our understanding of the interaction between NPs and living cells to that between NPs and simple lipid membranes. This is due to the fact that the internalization of NPs by cells is typically an active process that is mediated by various types of proteins, which may be different for different cell types \[95\]. Furthermore, even in the case of passive internalization, since the plasma membrane is apposed on the cytoplasmic side to the actin cortex, which renormalizes its elastic properties \[96\], the adhesion of NPs to the plasma membrane should be af-
fected by the actin cortex. The internalization process of NPs is further complicated by the fact that their typical size is of the same order as the mesh size of the actin cortex, which is about 100 nm \[97\]. Moreover, cooperative effects are expected to play a role in the modes of adhesion and internalization of NPs, including nanorods.

A more detailed understanding of the interaction of nanorods with lipid membranes has been mainly extracted from computer simulations, which thus far have only been carried in the context of a single particle adhering to a planar membrane \[1, 84, 98-101\]. These studies demonstrate that the details of geometry of the nanorods play a major role in their adhesion mode and the internalization process. Namely, for low values of \(\xi\), an adhering nanorod lies mainly parallel to the membrane. However, as \(\xi\) is increased, the nanorod adhesion mode undergoes a first-order transition to the normal mode, in which it is in a tubular pit that is mainly perpendicular to the membrane \[1, 84\]. The value of \(\xi\) at the transition from the parallel to the normal mode decreases with increasing \(D\) or \(\rho\) \[1\]. The nanorod undergoes spontaneous endocytosis at adhesion strengths beyond a threshold value \(\xi^*\), which decreases with \(D\) but is independent of \(\rho\) \[1\].

Membrane-mediated interaction between nanorods was investigated analytically in the asymptotic limit of NPs with infinitely long aspect ratio \[102, 103\]. These calculations, therefore, consider the case where the long axes of the NPs are parallel and can only account for the side-wise adhesion mode of the NPs. However, our understanding of the interaction between nanorods with finite aspect ratio is lacking. The aim of the present study is to investigate the adhesion modes of two nanorods on lipid membranes, their modes of dimerization, and their spontaneous endocytosis. Specifically, we investigate membrane-mediated interactions between spherocylindrical nanoparticles (SC-NPs) through molecular dynamics (MD) simulations of a coarse-grained model with implicit solvent \[46, 126\]. The study is performed systematically with varying values of the adhesion strength and geometric details of the SCNP. To determine the relative stability of the different modes of adhesion, free energies are calculated using the weighted histogram analysis method \[187\].
Table 4.1: Two SCNP interaction parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$U^{n_{1\text{h}}, n_{1\text{t}}}<em>{\text{max}}$, $U^{n</em>{2\text{t}}}_{\text{max}}$</td>
<td>200$\epsilon$</td>
</tr>
<tr>
<td>$U^{n_{2\text{h}}, n_{2\text{t}}}<em>{\text{min}}$, $U^{n</em>{1\text{t}}}_{\text{min}}$</td>
<td>$-\epsilon$</td>
</tr>
<tr>
<td>$U^{n_{1\text{h}}, n_{2\text{h}}}<em>{\text{min}}$, $U^{n</em>{1\text{h}}, n_{2\text{t}}}_{\text{min}}$</td>
<td>0</td>
</tr>
<tr>
<td>$U^{n_{1\text{h}}, n_{2\text{h}}}<em>{\text{max}}$, $U^{n</em>{1\text{h}}, n_{2\text{t}}}_{\text{max}}$</td>
<td>200$\epsilon$</td>
</tr>
<tr>
<td>$a_{cn_{1}, a_{cn_{2}}}$</td>
<td>variable</td>
</tr>
<tr>
<td>$k_{\text{bond}}^{n_{2\text{h}}}$, $k_{\text{bond}}^{n_{2\text{t}}}$</td>
<td>$500\epsilon/r_m^2$</td>
</tr>
<tr>
<td>$k_{\text{bond}}^{n_{1\text{h}}}$, $k_{\text{bond}}^{n_{1\text{t}}}$</td>
<td>$10\epsilon/r_m^2$</td>
</tr>
</tbody>
</table>

4.2 Model and Numerical Approach

The present work is based on a mesoscale implicit-solvent model for self-assembled lipid bilayers, presented in Sec. 2.1.4, and the SCNP are modeled using the approach discussed in Sec. 2.2. In addition to the model parameters (table 2.1) and NP parameters (table 2.2), specific values of the interaction parameters used in the simulations in this study are included in table 4.1. $D$ is varied between 10 and 20 nm, and $\rho$ is varied between 1 and 2.5.

4.3 Results

As shown by earlier studies, a single SCNP exhibits two modes of adhesion to a planar lipid membrane, depending on the values of $\xi$, $\rho$, and $D$. At low values of $\xi$, the effect of membrane curvature dominates over that of adhesion, leading the SCNP to adhere sidewise (parallel mode), with its degree of wrapping that increases with $\xi$. Beyond some value of $\xi$, the effect of adhesion becomes dominant, leading the SCNP to adhere such that its long axis is perpendicular to the membrane (normal mode). The SCNP undergoes spontaneous endocytosis at even higher values of $\xi$.

In Fig. 4.1, transient and equilibrium configurations of two SCNP on a tensionless lipid membrane at different values of $\xi$ are shown for the case of $D = 10\text{ nm}$ and $\rho = 1.75$. Here, the SCNP are initially placed above an equilibrated bare membrane, such
Figure 4.1: Transient and equilibrium snapshots at different values of ξ in the case of SCNPs with \( D = 10\,\text{nm} \) and \( \rho = 1.75 \). The SCNPs are placed initially at random orientations and at an initial distance \( d_0 = 38\,\text{nm} \) between their centers of mass. The equilibrium state in (a) corresponds to the parallel monomeric state, in which the SCNPs lie parallel to the membrane and are fairly diffusive, without a preferred distance between them; (b) the wedged dimeric state, in which the splay angle is obtuse (acute) at relatively low (high) \( \xi \); (c) the tubular dimeric state, in which the SCNPs are dimerized in a tube parallel to the \( z \)-axis; (d) the normal monomeric state, in which the SCNPs are parallel to the \( z \)-axis; and finally (e) the endocytosis state, in which the SCNPs are endocytosed as monomers (as shown by snapshot (e)) if they initially adhere to the membrane at large distances, or as a dimer if they initially adhere at nearby locations.
that their long axes are parallel to the $xy$-plane, their centers of mass are at a distance $d_0 = 38 \text{ nm}$, and their orientations are random. The initial distance between the long axes of the SCNPs and the average height of the head groups of the proximal leaflet of the membrane is 7.5 nm. This figure demonstrates that two SCNPs exhibit five adhesion modes on the membrane with increasing $\xi$. These correspond to (a) the parallel monomeric mode, in which the SCNPs lie parallel to the membrane and are highly diffusive, as demonstrated by Fig. 4.2; (b) the wedged dimeric mode, in which the SCNPs are dimerized, and their long axes are at an angle $\varphi \leq 90^\circ$ from the $z$-axis; (c) a tubular dimeric mode, in which the SCNPs form a tubular dimer that is mainly parallel to the $z$-axis; (d) a monomeric normal mode, in which the SCNPs are apart from each other and are perpendicular to the $xy$-plane; and (e) the endocytosis mode. Interestingly, whether the SCNP dimerizes or remains in the monomeric mode is found not only to depend on $D$, $\rho$, and $\xi$ but also on the initial distance between them. Likewise, whether the SCNPs endocytose as monomers or as dimers also depends on the initial distance between them. This implies that kinetic effects also play an important role in the adhesion and internalization modes of SCNPs.
Figure 4.3: (A) Angle between the SCNPs’ long axes as a function of time during the dimerization process for different values of the initial angle, $\theta_0$, between them. (B) Distance between the same SCNPs in (A). The simulations are performed in the case of two SCNPs with $D = 10$ nm and $\rho = 2.13$ at $\xi = 1.83 \frac{k_B T}{\text{nm}^2}$. The initial distance between the NPs is $d_0 = 30$ nm. Inset of (A): a snapshot of the system at $t = 1.25 \times 10^4 \tau$ in the case of $\theta_0 = 105^\circ$. Inset of (B): A snapshot of the wedged dimer at equilibrium.

4.3.1 Effects of Initial Angle and Distance between SCNPs on their Adhesion Mode and Kinetics of Dimerization

We first inferred the effect of the initial angle, $\theta_0$, between two SCNPs at the onset of their adhesion to the membrane through a series of simulations at a value of $d_0 = 30$ nm in the case of $D = 10$ nm and $\rho = 2.13$ at $\xi = 1.83 \frac{k_B T}{\text{nm}^2}$. Here, $\theta_0$ is defined as the largest angle between the long axes of the SCNPs, as indicated by Fig. 4.4. In these
simulations, the SCNPs are initially placed on the membrane such that their long axes are parallel to the $xy$-plane, and the long axis of the second SCNP intersects the long axis of the first SCNP at the center of mass of the latter. This choice of initial condition is in line with earlier studies on single SCNPs, which demonstrate that regardless of their initial orientation with respect to the membrane plane, upon their adhesion, the SCNPs quickly rotate to the parallel mode \[1, 99\].

The time evolution of the angle $\theta(t)$, starting from $\theta_0$, and the distance between the two SCNPs are shown in Figs. 4.3(A) and (B), respectively. Movies 1 and 2 (in Electronic Supplementary of Ref. \[2\]) illustrate these kinetics in the case of $\theta_0 = 180^\circ$ and $90^\circ$, respectively. Fig. 4.3 and Movies 1 and 2 indicate that independent of $\theta_0$, the SCNPs dimerize into a wedged configuration such that the equilibrium angle between them is around $30^\circ$, and the equilibrium distance between their centers of mass is around 14 nm. Fig. 4.3(A) indicates that the dimerization proceeds first through in-plane rotation of the SCNPs’ long axes such that they become temporarily almost aligned, with $\theta \gtrsim 150^\circ$. This is then followed by a rapid decrease in $\theta$, accompanied by an invagination of the membrane by the dimer. As a result, SCNPs that are initially already aligned (i.e., cases with $\theta_0 \gtrsim 150^\circ$) dimerize faster than SCNPs with smaller values of $\theta_0$. These
Figure 4.5: Distance between the centers of mass of two SCNPs, with $D = 10\text{ nm}$ and $\rho = 2.13$, vs. time for different values of $d_0$. $\xi = 0.88\ \kappa_B T/\text{nm}^2$ for (A), $1.45\ \kappa_B T/\text{nm}^2$ for (B), $1.83\ \kappa_B T/\text{nm}^2$ for (C), and $2.24\ \kappa_B T/\text{nm}^2$ for (D). The dashed pink line in (A) indicates a distance $d = l + 1\text{ nm}$, where $l$ is the length of the SCNPs. In (B-D), the dashed pink line indicates $d = D + 1$. The insets show the equilibrated snapshots of the systems and cross-sections around the centers of the SCNPs.
results imply that the initial angle $\theta_0$ has no effect on the final state of the SCNPs on the membrane, for the case where $d_0 \approx 30 \text{ nm}$.

We now turn to the effect of the initial distance, $d_0$, between two SCNPs on their final adhesion mode. To this end, we performed a series of simulations in the case of $D = 10 \text{ nm}$ and $\rho = 2.13$ at $\xi = 0.88, 1.45, 1.83$ and $2.24 \frac{k_B T}{\text{nm}^2}$, with different values of $d_0$ ranging between 25 and 70 nm. The SCNPs are initially placed on the membrane such that their long axes are parallel to the $xy$-plane and such that they are colinear. Fig. 4.5(A) shows that in the case of $\xi = 0.88 \frac{k_B T}{\text{nm}^2}$, the SCNPs generally dimerize into a wedged dimer with a large obtuse angle if $d_0 \lesssim 40 \text{ nm}$, as shown by the snapshot in the same figure. This is also demonstrated by the fact that the final distance between the SCNPs, when dimerized, is very close to their length. However, for higher values of $d_0$, they remain in the parallel monomeric state and are highly diffusive. This figure shows, as well, that in the instance of $d_0 = 32 \text{ nm}$, after spending a considerable amount of time in the monomeric mode, the SCNPs eventually dimerize at $t \approx 1.9 \times 10^5 \tau$. This indicates the existence of a relatively weak energy barrier from the monomeric state to the dimeric state at this value of $\xi$. However, once dimerized, the SCNPs never undimerize. This implies that the energy barrier from the dimeric to the monomeric state at $\xi = 0.88 \frac{k_B T}{\text{nm}^2}$ is fairly high. Fig. 4.5(A) hints to a location of this energy barrier at $d^* \approx 35 \text{ nm}$.

In contrast to Fig. 4.5(A), Fig. 4.5(B) shows that in the case of $\xi = 1.45 \frac{k_B T}{\text{nm}^2}$, the SCNPs dimerize into a wedged dimer for all considered values of $d_0$. The kinetics of dimerization at this value of $\xi$ in the case of $d_0 = 55 \text{ nm}$ is also demonstrated by Movie 3 (in electronic supplementary of Ref. 2). The dimer at equilibrium is a highly acute wedge in which the SCNPs are nearly parallel (see snapshots in Fig. 4.5(B)). This concurs with the fact that the distance between their centers of mass is slightly higher than $D$. The fact the SCNPs dimerize for all values of $d_0 \lesssim 70 \text{ nm}$ implies that the energy barrier between the monomeric and dimeric states at this value of $\xi$ must be at a relatively high value of $d$ that exceeds $70 \text{ nm}$. This can be explained by the fact that
Figure 4.6: Distance between the centers of mass of two SCNPs, with $D = 10 \text{nm}$ and $\rho = 2.13$, vs. time for different values of $d_0$ at $\xi = 1.83 \frac{k_BT}{\text{nm}^2}$. The initial seeds of the random number generator in these simulations are different from those in Fig. 3(C). This figure demonstrates that the results are independent of initial randomness.

At $\xi = 1.45 \frac{k_BT}{\text{nm}^2}$, monomeric SCNPs are in the parallel mode \cite{1}, and induce a relatively large deformation of the membrane, which must lead to relatively long-range membrane curvature-mediated attraction between them.

Figs. 4.5(C) and (D) show that, in the cases of $\xi = 1.83$ and $2.24 \frac{k_BT}{\text{nm}^2}$, the SCNPs dimerize if $d_0 \lesssim 59 \text{nm}$ and $48 \text{nm}$, respectively, into acute wedged dimers. The distance between the SCNPs in this state slightly increases with $\xi$. This is due to the increased amount of membrane wrapping of individual SCNPs with increasing $\xi$, which tends to increase the angle of the wedge and pull the SCNPs apart, as demonstrated by the cross-section snapshots shown in Figs. 4.5(B-D). The kinetics of dimerization is illustrated by Movie 1 (in Electronic Supplementary of Ref. \cite{2}) at $\xi = 1.83 \frac{k_BT}{\text{nm}^2}$ and $d_0 = 30 \text{nm}$ and Movie 4 (in Electronic Supplementary of Ref. \cite{2}) at $\xi = 2.24 \frac{k_BT}{\text{nm}^2}$ and $d_0 = 45 \text{nm}$. For large values of $d_0$, the SCNPs individually flip to the normal monomeric state and do not dimerize, as also illustrated by Movie 5 (in Electronic Supplementary of Ref. \cite{2}) at $\xi = 2.24 \frac{k_BT}{\text{nm}^2}$ and $d_0 = 62 \text{nm}$.  

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Therefore, for intermediate values of $\xi$, at which the monomeric state is the normal mode, there must exist an energy barrier between the monomeric and dimeric state, whose location decreases with increasing $\xi$. We note that our simulations indicate that once the SCNPs are in the normal monomeric state, they never dimerize. This hints at the presence of a fairly high energy barrier between the normal monomeric state and the dimeric states. We note that although each graph in Fig. 4.5 is based on a single simulation, the results shown in this figure are, in fact, repeatable, as shown in Fig. 4.6.

The effect of initial distance on the final adhesion mode of the SCNPs was also investigated in the case where they form a tubular dimer (see snapshot (c) in Fig. 1). Fig. 4.7 shows that in the case of $\xi = 2.68 \, k_B T/\text{nm}^2$, the SCNPs form a tubular dimer if $d_0 = 25 \, \text{nm}$. However, if placed at a larger distance, they individually rotate to the normal mode and do not dimerize (see the right snapshot in Fig. 4.7 at $\xi = 2.68 \, k_B T/\text{nm}^2$). Fig. 4.7 shows that at $\xi = 3.94 \, k_B T/\text{nm}^2$, the SCNPs are endocytosed as a dimer, following their dimerization into a tubular dimer. In contrast, however, if initially placed at
a large distance, they individually flip to the normal mode but do not endocytose (see right snapshot in Fig. 4.7 at $\xi = 3.94 k_B T/\text{nm}^2$). This implies that the dimerization of the SCNPs promotes their endocytosis. At $\xi = 5.21 k_B T/\text{nm}^2$, Fig. 4.7 shows that the SCNPs are endocytosed as a dimer for low values of $d_0$ or as monomers for high values of $d_0$.

### 4.3.2 Kinetics of Dimerization into Wedged Dimers

We now look at the kinetics of dimerization of two SCNPs in the case of $\xi = 1.83 k_B T/\text{nm}^2$, at an initial distance $d_0 = 45 \text{ nm}$ and angle $\theta_0 = 135^\circ$. Fig. 4.5(C) shows that at this adhesion strength, the final state is an acute wedged dimer. The kinetics of the SCNPs dimerization is characterized by the time dependence of the distance $d$ and angle $\theta$ between them, shown in Fig. 4.8(A), the angle $\phi$ between the SCNPs long axes and the $z$-axis, shown in Fig. 4.8(C), the number $n$ of lipid head beads that are in contact with the SCNPs, shown in Fig. 4.8(D), and the distance $h$ along the $z$-axis between the SCNPs centers of mass and the membrane’s average height, shown in Fig. 4.8(E). Side and top views of snapshots during the dimerization process are shown in Fig. 4.8(A) and (B), respectively. This kinetics is also illustrated by Movie 6 (in Electronic Supplementary of Ref. [2]).

Fig. 4.8(A) shows that during the first stage (Stage I), which occurs during $0 < t \lesssim 2 \times 10^4 \tau$, the SCNPs quickly rotate such that the angle $\theta$ between them increases to almost $180^\circ$, i.e., the SCNPs long axes become aligned while they remain mostly perpendicular to the $z$-axis ($\phi \approx 90^\circ$) as shown in Fig. 4.8(C). This alignment implies that the SCNPs experience an effective interaction that is mediated by the membrane curvature. Note that during this alignment stage, the distance between the SCNPs increases from 45 nm to about 50 nm, as shown by Fig. 4.8(A). Fig. 4.8(D) shows that this early stage is associated with a rapid increase in the amount of contact between the SCNPs and the membrane, which results in a rapid increase in the depth $h$ of the NPs in the membrane (see Fig. 4.8(E)).
Figure 4.8: Kinetics of dimerization of two SCNPs with $D = 10 \text{ nm}$ and $\rho = 2.13$ at $\xi = 1.83 k_B T / \text{nm}^2$. Here, the initial distance and angle between the SCNPs are $d_0 = 45 \text{ nm}$ and $\theta_0 = 135^\circ$, respectively. (A) Distance and angle between the SCNPs vs. time. Also shown in (A) are side-view snapshots at different times indicated by the arrows. Respective top-views of the snapshots in (A) are shown in (B). (C) Angles, $\varphi$, between the SCNPs and the $z$-axis (normal to the membrane). (D) Numbers of lipid head beads in contact with the SCNPs. (E) Depths of the SCNPs, along the $z$-axis, with respect to the average height of the membrane.
Stage II ($2 \times 10^4 \tau \lesssim t \lesssim 6 \times 10^4 \tau$) is characterized by an emerging attraction between the SCNPs, which leads to the decrease in the distance between their centers (see as shown by Fig. 4.8(A)) while they remain colinear. This is demonstrated by the high angle between them ($\theta \approx 180^\circ$), as shown by Fig. 4.8(A), and by the fact that they are perpendicular to the $z$-axis ($\varphi \approx 90^\circ$), as shown by Fig. 4.8(C). However, Fig. 4.8(A) shows that the SCNPs approach each other during Stage II while their degrees of wrapping keep increasing, as demonstrated in Fig. 4.8(D). Eventually, the SCNPs come in contact ($d \approx 25$ nm) at the end of Stage II (see snapshots (iv) in Figs. 4.8(A) and (B)). The wedged dimer forms during Stage III, which occurs during the interval $6 \times 10^4 \tau \lesssim t \lesssim 7 \times 10^4 \tau$. This stage is characterized by the rapid decrease in the distance $d$ and angle $\theta$ between the SCNPs, shown in Fig. 4.8(A), and rapid decrease of the angles $\varphi$ between the SCNPs long axes and $z$-axis. Finally, local equilibrium is reached at $t \approx 7 \times 10^4 \tau$.

In summary, Fig. 4.8 shows that SCNPs’ dimerization into a wedged dimer proceeds through four stages. These correspond to the alignment stage, followed by a stage during which the SCNPs are colinear and move toward each other, followed by the third stage during which the wedged dimer forms. The wedged dimer is fully formed in the fourth stage. We note that if this numerical experiment is repeated such that $d_0 \gtrsim 60$ nm, the SCNPs individually flip to the normal monomeric mode and do not dimerize (see Fig. 4.9).

4.3.3 $d_0$-$\xi$ Phase Diagram

The phase diagram of the final states of two SCNPs, as a function of $\xi$ and $d_0$, is shown in Fig. 4.10 for the case of $D = 10$ nm and $\rho = 2.13$. This phase diagram is determined from simulations based on particles adhering simultaneously to the membrane. This figure demonstrates that the final state of the SCNPs is dependent on the initial separation between them, particularly at high values of $\xi$. Fig. 4.10 shows that the SCNPs remain monomeric and in the parallel mode at low values of $\xi$ regardless of
Figure 4.9: Kinetic pathway to the normal monomeric mode in the case where two SCNPs, with $D = 10 \text{ nm}$ and $\rho = 2.13$ at $\xi = 1.83 k_B T / \text{nm}^2$, initially at a distance $d_0 = 50 \text{ nm}$ and angle $\theta_0 = 45^\circ$. (A) Distance $d$ between the SCNPs vs. time (left $y$-axis) and angle $\theta$ vs. time (right $y$-axis). Also shown in (A) is the side-view snapshot of the equilibrated system. (B) Angles between the SCNPs and the $z$-axis. (C) Numbers of lipid head beads in contact with the SCNPs. (D) Depths of the SCNPs, along the $z$-axis, with respect to the average height of the membrane.
Figure 4.10: Adhesion modes phase diagram in terms of the adhesion strength, $\xi$, and initial distance between the centers of mass of the SCNPs, in the case of $D = 10$ nm and $\rho = 2.13$. This phase diagram is extracted from the case where the SCNPs adhere simultaneously to the membrane, and their long axes are initially parallel to the $xy$-plane. Solid lines indicate boundaries between monomeric and dimeric states. Dotted lines indicate boundaries between two monomeric states or two dimeric states.

As $\xi$ is slightly increased, the SCNPs dimerize into the obtuse-wedged dimeric mode at low values of $d_0$ (orange squares). However, they remain in the monomeric parallel mode at high values of $d_0$, an indication of the presence of an energy barrier at a distance between them of about 35 nm, as discussed earlier, and as implicitly demonstrated by Fig. 4.5(A). However, since this energy barrier is weak, the SCNPs are expected to dimerize eventually. To indicate this, the region between the vertical solid red line and dashed green line, in the phase diagram of Fig. 4.10, is partially shaded orange.

With further increase of $\xi$, the SCNPs dimerize in the acute wedged dimeric mode (green symbols in Fig. 4.10). For relatively low values of $\xi$ within this region of the phase diagram, the SCNPs dimerize for all considered values of $d_0$, as discussed in Subsection 3.1. This is correlated with the fact that SCNPs in the monomeric state
are in the parallel mode at these values of $\xi$. The data shown in the phase diagram is based on simulations of membranes with a system size of $150 \times 150 \text{ nm}^2$. Finite size effects should become important for large values of $d_0$. This implies that the region of the phase diagram with $1 k_B T/\text{nm}^2 \lesssim \xi \lesssim 1.8 k_B T/\text{nm}^2$ may be different for larger membranes at large values of $d_0$, although we found that the SCNPs dimerize even on a $300 \times 300 \text{ nm}^2$ membrane at $\xi = 1.45 k_B T/\text{nm}^2$, when initially placed at $d_0 = 55 \text{ nm}$, as demonstrated by Movie [7] (in Electronic Supplementary of Ref. [2]). This implies that finite size effects in the simulations based on $150 \times 150 \text{ nm}^2$-membranes must be weak.

For values of $1.8 k_B T/\text{nm}^2 \lesssim \xi \lesssim 2.5 k_B T/\text{nm}^2$, the SCNPs dimerize only if $d_0 < d_0^*$, where $d_0^*$ decreases with increasing $\xi$, as also demonstrated by Figs. 4.5(C) and (D). It is notable that, in this range of values of $\xi$, a single SCNP is in the normal monomeric state [1]. This reduction of $d_0^*$ with $\xi$ can be understood from a comparison of the time scale of dimerization $\tau_{dimer}$ and the time scale $\tau_{flip}$, associated with the flip of a single SCNP from the parallel to the normal mode. The transition from the wedged dimeric mode to the normal monomeric mode should occur when $\tau_{dimer} \approx \tau_{flip}$. Since $\tau_{dimer}$ increases with increasing $d_0$ and $\tau_{flip}$ decreases with increasing $\xi$ [1], $d_0^*$ should decrease with $\xi$.

For values of $\xi \gtrsim 2.4 k_B T/\text{nm}^2$, two SCNPs dimerize into tubular dimers if $d_0 \lesssim 37 \text{ nm}$ (brown triangles in Fig. 4.10). However, for larger values of $d_0$, the SCNPs are in the normal monomeric mode. Tubular dimers endocytose at $\xi \gtrsim 3 k_B T/\text{nm}^2$ if $d_0$ is small (blue diamonds). However, SCNPs in the normal monomeric mode endocytose at $\xi \gtrsim 5 k_B T/\text{nm}^2$ (blue squares). Important conclusions drawn from this phase diagram are that the initial distance plays an important role in the final state of the SCNPs at intermediate or high values of $\xi$. Furthermore, their dimerization promotes their endocytosis.
Figure 4.11: Kinetics of dimerization of two SCNPs with $D = 10 \text{ nm}$ and $\rho = 2.13$ at $\xi = 1.63 k_BT/\text{nm}^2$. Here, the initial distance between the centers of mass of the SCNPs is $d_0 = 70 \text{ nm}$. (A) Distance between the SCNPs vs. time. Also shown in (A) are side-view snapshots at different times. (B) Angles between the SCNPs and the $z$-axis. Snapshots in (A) correspond to configurations of the system at times indicated by the arrows.

4.3.4 Non-Simultaneous Adhesion of the SCNPs

The results presented so far are based on simulations of two SCNPs that adhere simultaneously to the membrane. In a typical experimental situation, however, NPs do not adhere simultaneously to a lipid membrane. In this subsection, we focus on the effect of non-simultaneous adhesion of SCNPs on their final adhesion mode at values of $\xi$, at which the first SCNP is already in the normal monomeric state. Fig. 4.11 shows the distance $d$ between the two SCNPs, as well as the angles $\varphi$ between their principal axes and the $z$-axis in the case of $D = 10 \text{ nm}$ and $\rho = 2.13$ at $\xi = 1.63 k_BT/\text{nm}^2$. Here, the initial
distance between the SCNPs is $d_0 = 70 \text{ nm}$. This kinetics is also illustrated by Movie\textsuperscript{8} (in Electronic Supplementary of Ref. [2]). This figure shows that following the adhesion of the second SCNP, the distance between their centers of mass steadily decreases while the first one remains mostly parallel to the $z$-axis (green curve in Fig. 4.11(B)) and the second one is mostly perpendicular to the $z$-axis (pink curve in Fig. 4.11(B)). During this regime, the second SCNP increasingly invaginates the membrane. Once the two SCNPs come in contact, they align to form a tubular dimer, as demonstrated by their angles $\phi$ becoming equal at $t \approx 9 \times 10^4 \tau$. The tubular dimer then rapidly rotates such that the angles of the SCNPs with the $z$-axis are almost 0. This final state is then very different from the acute wedged dimeric state, which occurs in the case where the two SCNPs adhere simultaneously to the membrane at $\xi = 1.63 k_B T/\text{nm}^2$ (see Section III.B and Fig. 4.8). These results are interesting in that the value of $\xi$ of the simulation in Fig. 4.11 which is $1.63 k_B T/\text{nm}^2$, is lower than that in Fig. 4.8 ($\xi = 1.83 k_B T/\text{nm}^2$), at which the SCNPs form an acute wedged dimer. A question that arises then is which of the wedged dimeric state or tubular dimeric state is more stable.

4.3.5 Relative Stability of Different States

To determine the relative stability of the dimeric and monomeric states for a given value of $\xi$, we carried a large number of umbrella sampling simulations with a reaction coordinate corresponding to the distance between the SCNPs’ centers of mass \textsuperscript{219}. The weighted histogram analysis method (WHAM) \textsuperscript{187} was then used to obtain the unbiased free energy of the lipid bilayer with two SCNPs as a function of the distance $d$ (see Sec. 2.4.1 for details). Since WHAM calculations are very costly, in general, we limited our calculations to $d_{\text{bias}} < 50 \text{ nm}$.

The resulting free energies for the case of two SCNPs with $D = 10 \text{ nm}$ and $\rho = 2.13$ at $\xi = 0.45, 0.88, 1.15, 1.30,$ and $1.83 k_B T/\text{nm}^2$ are shown in Fig. 4.12. In the case of the weakest adhesion strength ($\xi = 0.45 k_B T/\text{nm}^2$), $F(d)$ decreases monotonically with $d$. Therefore, the monomeric state is the only stable state in this case, in accordance
Figure 4.12: Free Energy, obtained from WHAM, versus distance between the centers of mass of two SCNPs with $D = 10\, \text{nm}$ and $\rho = 2.13$ at different values of $\xi$. Snapshots (a) and (b) correspond to configurations at low $\xi$ when the SCNPs are relatively close to and far from each other, respectively. Snapshot (c) corresponds to the minimum free energy obtuse wedged dimeric state at $\xi = 0.88\, k_B T/\text{nm}^2$. Snapshot (d) corresponds to a configuration of the undimerized SCNPs in the parallel monomeric mode at $\xi = 0.88\, k_B T/\text{nm}^2$. Snapshot (e) corresponds to a configuration of the SCNPs in the acute wedged dimeric state at $\xi = 1.15\, k_B T/\text{nm}^2$. Snapshot (f) indicates a configuration of the SCNPs in the obtuse wedged dimeric state at $\xi = 1.15\, k_B T/\text{nm}^2$. Snapshots (g) and (h) correspond to configurations of the SCNPs in the acute wedged dimeric state at $\xi = 1.30$ and $1.83\, k_B T/\text{nm}^2$, respectively.

with the phase diagram shown in Fig. 4.10. However, Fig. 4.12 shows that, at $\xi = 0.88\, k_B T/\text{nm}^2$, the free energy has a local minimum at $d \approx 23\, \text{nm}$, which corresponds to the obtuse wedged dimeric state, shown by snapshot (c) in the same figure. The free energy, at the same value of $\xi$, displays a local maximum at $d \approx 40\, \text{nm}$, beyond which $F(d)$ decreases steadily with $d$, albeit at a very weak rate. The free energy should asymptotically approach a constant as $d$ is increased. The slow decrease of the free energy with $d$ for large values of $d$ implies that non-dimerized SCNPs at this value of $\xi$ can relatively easily get close to each other and overcome the small energy barrier.
This explains Fig. 3(A). It is possible that the weak decrease in the free energy at large distances for $\xi = 0.88 \frac{k_B T}{\text{nm}^2}$ is due to finite size effects. If so, then for very large systems, there should not be an energy barrier from the monomeric to the dimeric state. However, we believe this may not be the case since the largest distance of the free energy at this value of $\xi$ is about a third of the linear system size. The energy barrier from the obtuse wedged dimeric state to the monomeric state, at $\xi = 0.88 \frac{k_B T}{\text{nm}^2}$, is, in contrast, relatively high (about $25k_B T$). This implies that once dimerized, the SCNPs do not undimerize, again in agreement with Fig. 3(A).

Fig. 4.12 shows that the free energies, at $\xi = 1.15, 1.30,$ and $1.83 \frac{k_B T}{\text{nm}^2}$, exhibit clear minima corresponding to the wedged state, with configurations shown by snapshots (e), (g) and (h), respectively. We note that we were unable to detect local maxima of the free energies at higher values of $d$ for $\xi \geq 1.15 \frac{k_B T}{\text{nm}^2}$ within the range of considered values of $d$. However, the fact that Figs. 4.5 and 4.10 show that the SCNPs do not dimerize at high values of $d_0$ implies that there must be an energy barrier at some value of $50 \text{ nm} < d < 70 \text{ nm}$. Noting that we were never able to observe a dimerization of the SCNPs once they are in the normal monomeric state, the energy barrier between the wedged dimeric state and the normal monomeric state should be very high.

We were also unable to determine the relative stability of the wedged dimeric state and the tubular state, using WHAM with the distance $d$ as a reaction coordinate, due to the lack of stable biased intermediate states between the two dimeric states. To overcome this difficulty, we used an alternative approach, which we discussed earlier, for calculating the free energy based on the Helfrich Hamiltonian [71] in conjunction with a local Monge representation [67]. The free energy of the membrane with the adhering SCNPs is given by

$$F(\xi) \approx F_{\text{curv}}(\xi) + E_{\text{adh}}(\xi),$$

(4.1)

where $F_{\text{curv}}$ is the curvature energy, which is estimated using the Helfrich Hamilton-
Figure 4.13: Free energies of the different observed phases versus adhesion energy density, $\xi$ for the case of $D = 10\text{ nm}$ and $\rho = 2.13$.

The approximated free energies of the different observed adhesion modes, calculated using Eq. (4.1) are shown in Fig. 4.13 as a function of $\xi$ in the case of $D = 10\text{ nm}$ and $\rho = 2.13$. This figure shows that, excluding the endocytosis states, there are only three most stable states of two SCNPs on a lipid membrane. These correspond to the parallel monomeric state at low values of $\xi$, the obtuse wedged dimeric state at intermediate values of $\xi$, and the tubular dimeric state at high values of $\xi$. Of course, at even higher values of $\xi$, the endocytosis states become most stable. Interestingly, this phase diagram shows that the free energy of the acute wedged dimeric state is higher than that of the tubular dimeric state, regardless of $\xi$. From our simulations, however, the acute wedged dimeric state never spontaneously transforms to the tubular dimeric...
Figure 4.14: Average Angle between two SCNPs as a function of $\xi$ for the case of $D = 10 \text{ nm}$ at different values of aspect ratio.

state unless $\xi \geq 2.4 k_B T / \text{nm}^2$. This implies that the energy barrier between these two states is high for $\xi \lesssim 2.4 k_B T / \text{nm}^2$. Fig. 4.13 also shows that the free energy of the normal monomeric state is always higher than that of any other state, except at high values of $\xi$, at which the normal monomeric state becomes more stable than the tubular dimeric state. Since we never observed spontaneous transitions of the SCNPs from the normal monomeric state to other states, except the monomeric endocytosis state at $\xi \gtrsim 5 k_B T / \text{nm}^2$, the energy barrier from the normal monomeric state to the other states (excluding the endocytosis state at high $\xi$) must be substantially higher than the thermal energy.

4.3.6 Effect of Aspect Ratio on the Dimerization of SCNPs into the Wedged State

As stated earlier, there seem to be two wedged states in which the splay angle is either high at relatively low $\xi$ (obtuse wedged dimeric state) or low at relatively high $\xi$ (acute wedged dimeric state). The distinction between these two states is also hinted
at by the free energy, shown in Fig. 4.12 for the case of $D = 10 \text{ nm}$ and $\rho = 2.13$ at $\xi = 1.15 k_B T/\text{nm}^2$ (blue curve), which displays an interesting shoulder to the right of the absolute minimum, whose configuration is shown by snapshot (f) in the same figure. Likewise, the free energy in the case of $\xi = 1.30 k_B T/\text{nm}^2$ also displays a shoulder, albeit weaker, to the right of the absolute minimum. These two states are of course indistinguishable for spherical or near-spherical NPs [67]. To infer whether these two dimerization modes are distinct states and determine the effect of the aspect ratio on the transition between them, we performed a series of simulations with varying values of $\xi$ for $1.23 \leq \rho \leq 2.50$. Fig. 4.14 shows that independent of the value of $\rho$, the average of the splay angle between the SCNPs, $\langle \theta \rangle$, decreases monotonically with $\xi$ (except in the case of $\rho = 2.13$ and 2.50 at large values of $\xi$, which will be discussed below), with the sharpest decrease at some value of $\xi$, which decreases with increasing $\rho$. In the case of the lowest aspect ratio ($\rho = 1.23$), the decrease is gradual. In contrast, however, the decrease is fairly abrupt for $\rho \gtrsim 1.75$. This indicates that there are actually two distinct equilibrium wedged states, namely the obtuse and acute wedged dimeric states, at relatively low and high values of $\xi$, and that a first-order transition likely separates them.

An interesting feature, shown by Fig. 4.14, is the weak increase in $\langle \theta \rangle$ with $\xi$ at high values of $\xi$ within the acute wedged dimeric state, which is particularly clear for large values of $\rho$ (e.g., blue curve). This increase is due to the increased amount of wrapping of each SCNP by the membrane as $\xi$ is increased, which tends to separate the SCNPs from each other. This increased amount of wrapping is demonstrated by the cross-sections of the dimers shown in Figs. 4.5(B) to (D).

To further investigate the obtuse and acute wedged dimeric states, we conducted many umbrella sampling simulations at $\xi = 1.3 k_B T/\text{nm}^2$, i.e., close to the transition between these two states at $\rho = 1.75$, as shown by Fig. 4.14. The free energies, shown in Fig. 4.15, obtained through WHAM from these simulations, demonstrate two clear local minima for the case of $\rho = 1.75$, with the absolute minimum corresponding to the
obtuse dimeric state. However, the free energy for $\rho = 1.38$ exhibits a single minimum, corresponding to the obtuse state. This agrees with Fig. 4.14, which shows that for this value of $\rho$, the SCNPs are in the obtuse wedged dimeric state at $\xi = 1.3 k_B T / \text{nm}^2$.

In contrast, the free energies for $\rho = 2.13$ and $2.50$ exhibit a single minimum, corresponding to the acute state, which also agrees with Fig. 4.14. The presence of two local minima with an energy barrier at $\rho = 1.75$ indicates that the transition between the two states is likely discontinuous.

4.3.7 $\xi$-$\rho$ and $\xi$-$D$ Phase Diagrams

The results, thus far presented in this article, were mostly for the case of $D = 10 \text{ nm}$ and $\rho = 2.13$. The effects of $D$ and $\rho$ on the adhesion phase diagram of two SCNPs and their endocytosis are obtained from a series of simulations at values of $D$ ranging between 10 and 20 nm, and value of $\rho$ ranging between 1 (spherical NPs) and 2.50. The phase diagram is obtained by initially placing the SCNPs at nearby locations on the
Figure 4.16: $\xi - \rho$ adhesion phase diagram for 2 SCNPs in the case of $D = 10$ nm. The dotted lines are the transition lines between various phases. This phase diagram is based on MD simulations of two SCNPs with nearby initial positions, corresponding to $d_0 = l + 5$ nm.

membrane, corresponding to $d_0 = l + 5$ nm.

Fig. 4.16 which depicts the $\xi - \rho$ phase diagram in the case of $D = 10$ nm, demonstrates the general sequence of phases with increasing $\rho$, i.e., the parallel monomeric state, the obtuse wedged dimeric state, the acute wedged dimeric state, the tubular dimeric state, and then the endocytosis state. Naturally, for $\rho = 1$ (i.e., spherical NPs), there is no difference between the two wedged dimeric states. The acute wedged dimeric state emerges at some value of $\rho$ lower than 1.38. Overall, this phase diagram shows that the values of $\xi$ corresponding to the transitions between different states decrease with $\rho$, except for the transition between the acute wedged dimeric state and the tubular dimeric state. This implies that the increase in the aspect ratio of the SCNPs for a given diameter promotes their dimerization, tubulation, and endocytosis. Our re-
Figure 4.17: $\xi - D$ adhesion phase diagram for 2 SCNPs in the case of $\rho = 1.75$. The dotted lines are the transition lines between various phases. This phase diagram is based on MD simulations of two SCNPs with nearby initial positions, corresponding to $d_0 = l + 5 \text{ nm}$.

Results qualitatively agree with DeBrosse et al., who showed that the internalization of Au nanorods by keratinocyte cells increases with increasing $\rho$ [93].

Fig. 4.13 shows that the tubular dimeric state is more stable than the acute wedged dimeric state. Therefore, if we account only for the most stable states in the phase diagram, the green region, corresponding to the acute wedged dimeric state, disappears at the expense of the tubular dimeric state (maroon region). The effect of the SCNPs’ diameter on their adhesion phase diagram is shown by the $\xi-D$ phase diagram in Fig. 4.17 for the case of $\rho = 1.75$. This figure shows that all transition lines between different phases, decrease monotonically with $\xi$. This implies that increasing the SCNPs’s diameter for a given aspect ratio promotes the dimerization of the NPs into wedged dimers, their dimerization into tubular dimers, and their endocytosis. As in Fig. 4.16 if we were to only account for the most stable states, the green region (acute wedged...
dimeric phase) of the phase diagram in Fig. 4.17 should be replaced by the maroon region (tubular dimeric phase).

### 4.3.8 Kinetic Pathway of Endocytosis of Two Spherocylindrical NPs

Finally, we focus on the kinetic pathway of the endocytosis of 2 SCNPs in the case where they simultaneously adhere to the membrane at nearby locations. A time sequence of snapshots depicting this process is depicted in Fig. 4.18 for the case of 2 SCNPs with $D = 10$ nm and $\rho = 2.13$ at $\xi = 3.10 k_B T/\text{nm}^2$. This kinetics is also illustrated by Movie 9 (in Electronic Supplementary of Ref. [2]). The angle and distance between the SCNPs, and the number of lipid head beads in contact with the SCNPs are shown vs. time in Fig. 4.19 for $\xi = 3.1, 4.15$ and $5.24 k_B T/\text{nm}^2$. Fig. 4.18 shows that, upon their adhesion, the SCNPs quickly form a wedged dimer whose splay angle decreases with time, as the SCNPs are increasingly wrapped by the membrane up to $t \approx 5500 \tau$ in the case of $\xi = 3.10 k_B T/\text{nm}^2$. This is followed by a regime up to about $15500 \tau$, in which the SCNPs become increasingly wrapped while remaining in the wedged state, leading to slight increases in both $d$ and $\theta$. The upper tip of one of the two SCNPs becomes increasingly wrapped by the membrane, leading to a fast increase in $d$ and $\theta$. This leads the SCNPs to become increasingly colinear (see snapshots at $t = 17000$ and $18000 \tau$) and eventual formation of a tubular dimer. The dimer is then

![Figure 4.18: Snapshot series corresponding to the case of $\xi = 3.1k_B T/\text{nm}^2$, $\rho = 2.13$, $D = 10$ nm and $d_0 = 25$ nm.](image-url)
endocytosed at $t \approx 19000\tau$. Since the effect of adhesion becomes more dominant than that of curvature with increasing $\xi$, the speed of this endocytosis process is increased with $\xi$, as shown by Fig. 4.19.

If the SCNPs adhere simultaneously but far from each other, they first adhere through the membrane in the parallel mode, then rotate to the normal mode [1]. The SCNPs remain attached to the membrane in the normal monomeric mode if $\xi \lesssim 5k_B T/\text{nm}^2$, and endocytose individually if $\xi \gtrsim 5k_B T/\text{nm}^2$. The same final states are also observed if the
SCNPs do not adhere to the membrane simultaneously, even if \( d_0 \) is very small. We note that if the two SCNPs in the dimeric tubular mode become fully normal to the membrane, their endocytosis threshold should be the same as that of a single SCNP, with the same \( D \). This is because, in this case, endocytosis is controlled by the free energy of the deformation of the neck. However, as demonstrated by Fig. 4.18, the SCNP dimer does not fully become normal to the membrane. Endocytosis, in fact, occurs before the two SCNPs become colinear, as shown by Fig. 4.19. This implies that kinetic effects play a role in the endocytosis of dimerized SCNPs, i.e., endocytosis of dimerized SCNPs is not only controlled by deformation of the membrane in the neck region. This result implies that the dimerization of the SCNPs promotes their endocytosis. However, it is emphasized that endocytosis of the SCNPs as a dimer occurs only when the SCNPs adhere to the membrane simultaneously and at close locations.

4.4 Summary

Details of the adhesion modes, dimerization, and endocytosis of two spherocylindrical NPs on tensionless planar membranes are investigated in this chapter using molecular dynamics simulations of a coarse-grained implicit-solvent model. The SCNPs are efficiently modeled as triangulated hollow shells [1, 4]. This allows for a relatively low number of degrees of freedom associated with the SCNPs and, therefore, simulations of SCNPs up to about 20 nm in diameter and 35 nm in length. The simulations were systematically performed over a range of values of the SCNPs diameter \( D \), aspect ratio \( \rho \), strength of the adhesion energy density \( \xi \), and initial distance between their centers of mass \( d_0 \).

The SCNPs exhibit five different modes of adhesion, depending on the values of \( \xi \), \( \rho \), and \( D \). At small \( \xi \) values, they are in the monomeric-gas state and are highly diffusive. Increasing \( \xi \) leads to their aggregation into wedged dimers with an obtuse splay angle. As \( \xi \) is further increased, the obtuse wedged dimers transform into acute wedged dimers, with this transition becoming increasingly abrupt with increasing \( \rho \).
The SCNPs dimerize into tubular dimers at higher values of $\xi$ and are endocytosed at even higher values of $\xi$. Increasing the value of $D$ or $\rho$ promotes dimerization, tabulation, and endocytosis of the SCNPs.

We also found that the final adhesion mode of the SCNPs depends strongly on the initial distance $d_0$ at intermediate and high values of $\xi$. Namely, the SCNPs dimerize into acute wedged dimers or tubular dimers at intermediate values of $\xi$ if they adhere to the membrane at nearby locations. In contrast, they remain in the monomeric normal mode if the initial distance between them is high.

Free energy calculations based on the Helfrich Hamiltonian, in conjunction with a local Monge representation [67], show that the sequence of most stable adhesion modes of two SCNPs, with increasing $\xi$, corresponds to the parallel monomeric mode, the obtuse wedged dimeric mode, the tubular dimeric mode, then the endocytosis mode. On the other hand, the free energy of the acute wedged dimeric mode is always higher than that of the tubular mode. Furthermore, the free energy of the normal monomeric mode is higher than that of the wedged or tubular dimeric modes. However, dimerization of the SCNPs initially in the normal monomeric mode was never observed. This implies a large energy barrier between the normal monomeric state and the wedged dimeric or tubular dimeric state. Likewise, the free energy of the monomeric normal mode is also higher than that of the other states. We note that the SCNPs never dimerize such that their long axes are parallel to each other. This implicitly implies that SCNPs experience a repulsive effective interaction when they are parallel to each other. This agrees with earlier analytical studies [102, 218].

In experimental situations, NPs are not expected to adhere simultaneously to the membrane. Our simulations of non-simultaneous adhesion of two SCNPs at nearby locations, in which one of the two SCNPs is already in the normal monomeric mode, show that the final state at intermediate values of $\xi$ is the tubular dimeric mode, instead of the wedged dimeric mode. At higher values of $\xi$, the SCNPs do not dimerize a tubular dimer and then endocytose. Instead, both SCNPs adopt the normal monomeric mode.
This implies that the two SCNPs endocytose as a dimer only when they adhere almost simultaneously and at nearby locations. Otherwise, they endocytose as monomers.

Thus far, experimental studies of SCNP interaction with lipid membranes have been mainly conducted in the context of living cells. Our simulations agree qualitatively with DeBrosse et al. [93] in that longer Au nanorods are more easily internalized by keratoocytes than shorter nanorods. However, a detailed comparison between our results and existing experimental results is not possible due to the fact that the plasma membrane of living cells is apposed to the cortical cytoskeleton, which affects the elasticity of the lipid membrane, and to the presence of active effects in living cells. Experimental studies of many SCNPs interacting with simple planar lipid membranes or lipid vesicles would be very useful to validate our results.
Chapter 5

SELF-ASSEMBLY OF JANUS SPHEROCYLINDRICAL NANOPARTICLES
Mediated by Lipid Vesicles

5.1 Introduction

In this chapter, we explore the possibility of inducing self-assembly of elongated nanoparticles (NPs) on lipid vesicles. In the previous chapters, we gained a good understanding of how an SCNP interacts with planar lipid membranes and the different modes of adhesion. Various applications require self-assemblies of NPs in which the NPs remain separated from each other. However, the ability of lipid membranes to achieve such arrangements is constrained by the fact that NPs can be dispersed on lipid membranes, maintaining a distance from each other only when adhesive energies are weak. At weak adhesion strengths, the NPs are weakly wrapped by the membrane and are fairly diffusive in nature. This is shown in detail by the study done in previous chapters. On the other hand, at high adhesive energies, the NPs become tightly wrapped by the membrane, making them prone to endocytosis. This narrows down the range over which NPs form stable self-assemblies on lipid membranes. As shown by previous works, these assemblies are often linear and thus lack structural variety. Moreover, neighboring NPs within these assemblies are in close proximity, practically in contact [2, 61, 62, 67, 77].

Many experimental [74–79] and theoretical studies [1, 2, 4, 5, 46, 55, 68, 179, 212–215] demonstrated that lipid membranes can self-assemble NPs. These self-assemblies arise from the deformation of the membranes when NPs adhere to the membrane. The flexibility of the membrane leads to the membrane deformation to conform to the NP’s surface. Competition between the adhesive energy of the NPs on the lipid membrane and the curvature energy of the membrane decides the extent of these deformations. As
discussed in the earlier sections and shown in the previous chapter, these deformations extend over length scales longer than the NPs’ dimensions. These deformations often lead to an interesting membrane-curvature-mediated interaction between the NPs. This interaction can result in the NPs’ aggregation into in-plane or out-of-plane linear chains as well as long-lived transient states, including out-of-plane bitubes and rings \[61, 62, 66, 67, 75, 77\].

The limitation of lipid membranes in self-assembling NPs into aggregates with different geometries, in which they are apart from each other, can be mitigated by their surface modification into Janus NPs (JNPs) \[5, 6\]. Specifically, these JNPs are composed of two apposed moieties. One moiety interacts more attractively with the lipid head groups, and the other interacts more attractively with the solvent, while the overall hydrophilic character of the JNPs is maintained \[4, 5, 220\]. Such surface modification promotes strong adhesion of only one moiety of the JNPs while the other moiety remains exposed to the solvent. This leads to a suppression of spontaneous endocytosis of the JNPs and induces a repulsive interaction between them \[5\]. As a result, the adhesion of spherical JNPs on lipid vesicles leads to their self-assembly into a wide range of ordered nanoclusters, including a few Platonic solids, in which the JNPs are apart from each other \[5\]. More recently, we also showed that the adhesion of spherical JNPs onto planar membranes can lead to their self-assembly into non-closed packed hexagonal lattices, with a lattice constant that is determined by their number density on the membrane \[6\].

Thanks to recent advances in nanomaterial synthesis, anisotropic nanoparticles with varying geometries, dimensions, and surface properties can be engineered \[17\]. A question that arises is how would an additional shape anisotropy to Janus NPs affect their arrangement on lipid vesicles. To answer this question, we performed a systematic set of molecular dynamics simulations of a coarse-grained implicit solvent model of several simultaneously adhering Janus spherocylindrical NPs (JSCNPs) on lipid vesicles. More specifically, we investigated the effects of the aspect ratio of the JSCNPs and their
Table 5.1: Many Janus SCNPs interaction parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$U_{n_a}^{h}$, $U_{n_a}^{t}$</td>
<td>200$\epsilon$</td>
</tr>
<tr>
<td>$U_{n_b}^{h}$, $U_{n_b}^{t}$</td>
<td>200$\epsilon$</td>
</tr>
<tr>
<td>$U_{n_a}^{h}$, $U_{n_b}^{h}$, $U_{n_a}^{t}$, $U_{n_b}^{t}$</td>
<td>$-\xi$</td>
</tr>
<tr>
<td>$U_{n_a}^{h}$, $U_{n_b}^{h}$, $U_{n_a}^{t}$, $U_{n_b}^{t}$</td>
<td>0</td>
</tr>
<tr>
<td>$U_{n_a}^{h}$, $U_{n_b}^{h}$, $U_{n_a}^{t}$, $U_{n_b}^{t}$</td>
<td>200$\epsilon$</td>
</tr>
<tr>
<td>$k_{bond}^{n_a}$, $k_{bond}^{n_b}$</td>
<td>500$\epsilon/r_m^2$</td>
</tr>
<tr>
<td>$k_{bond}^{n_a}$, $k_{bond}^{n_b}$</td>
<td>10$\epsilon/r_m^2$</td>
</tr>
<tr>
<td>$J$</td>
<td>0.4</td>
</tr>
</tbody>
</table>

number on the vesicle on their arrangement on the vesicle. Our results show that the additional geometric anisotropy of the Janus NPs leads to a range of novel highly ordered nanoassemblies.

5.2 Methods

The present work is based on a mesoscale implicit-solvent model for self-assembled lipid bilayers, presented in Sec. 2.1.4, and the SCNPs are modeled using the approach discussed in Sec. 2.2. In addition to the model parameters (table 2.1) and NP parameters (table 2.2), specific values of the interaction parameters used in the simulations in this study are included in table 5.1. Simulations are performed for $D = 20$ nm.

The simulations are performed on vesicles with diameter, $D_V$, ranging between 42 and 160 nm. Here, $D_V$ is defined as twice the average distance between the positions of the $h$-beads of the outer leaflet and the vesicle’s center of mass. This corresponds to a total number of lipid chains in a vesicle ranging between 25,000 and 300,000. The diameter of the JSCNPs is fixed at $D_N = 20$ nm. All simulations are performed over an adhesion strength per unit of area $\xi = 4.11 k_B T / \text{nm}^2$. A JSCNP’s Janusity, defined as the fraction of $n_a$ beads on the JSCNP, is fixed at $J = 0.4$. The aspect ratio, $\alpha$, of the JSCNPs is varied between 1.23 and 5.5. A snapshot of the Janus SCNP is shown in Fig. 5.1. In a typical simulation, $n$ JSCNPs, initially placed close to an equilibrated vesicle at random positions, quickly adhere to the vesicle. We consider values of $n$
corresponding to 3, 4, 6, 7 and 8. Unless indicated, the ratio $A_V/nA_N \approx 1.2$ in all simulations, where $A_V$ is the area of the outer leaflet of a vesicle and $A_N$ is the area of a JSCNP. Most simulations were run over $4 \times 10^7$ time steps.

### 5.3 Results

We first performed a series of simulations of two JSCNPs adhering to a lipid vesicle to determine their preferred placement on the vesicle at equilibrium. Fig. 5.2(A) shows the time dependence of the distance between the centers of two JSCNPs, with aspect ratio $\alpha = 1.6$ and 4, starting from a configuration in which they are placed very close and parallel to each other and very close to an equilibrated vesicle. This figure demonstrates that right after their adhesion, the two JSCNPs drift away from each other toward an equilibrium state in which they are apart, regardless of their aspect ratio. Fig. 5.2(A) shows that the relative equilibrium positions of the JSCNPs do not exhibit a lot of fluctuations. However, Fig. 5.2(B) shows that the amplitude of orientational fluctuations increases with $\alpha$ but remains overall small, indicating that the JSCNPs prefer conformations in which they are close to parallel.

To confirm that the non-dimerized, i.e., monomeric, state of two JSCNPs on a vesicle is preferred, we performed free energy calculations using the weighted histogram
Figure 5.2: (A) Distance between the centers of two JSCNPs versus time for two aspect ratio values. The two JSCNPs are initially placed adjacent to each other on an equilibrated vesicle. (B) The angle between the two JSCNPs in (A) versus time. (C) Free energy of a vesicle with two JSCNPs as a function of distance between their centers in the case of $\alpha = 1.6$. The snapshots show the time evolution of the configuration of the system. In these snapshots, the membrane is icy blue, and the moiety of the JSCNPs that interacts attractively (repulsively) with the lipid membrane is yellow (blue).
analysis method (Sec. 2.4.1). The reaction coordinate, here, corresponds to the distance between the JSCNPs’ c-beads. The obtained free energy versus the distance between the JSCNPs’ centers, shown in Fig. 5.2(C) for the case of $\alpha = 1.6$, demonstrates that the absolute minimum of the free energy corresponds to the state where the JSCNPs are apart (monomeric state). The value of the distance corresponding to the absolute minimum ($\approx 37$) is very close to the equilibrium value of the distance in Fig. 5.2(A). Fig. 5.2(C) shows another local minimum at short distances corresponding to the dimeric state. Despite the presence of this dimeric state, the JSCNPs drift away from each other following their adhesion at nearby locations instead of dimerizing, as demonstrated by Fig. 5.2(A).

The reason why the JSCNPs do not form a metastable dimeric state upon their adhesion, as demonstrated by Fig. 5.2(A), is because right after their adhesion, the JSCNPs are only weakly wrapped by the membrane and are therefore fairly mobile. The conformation of the vesicle following the initial adhesion of the JSCNPs is almost spherical and, therefore, substantially different from the well-deformed conformation of the vesicle when the JSCNPs are in the dimeric state (far left snapshot in Fig. 5.2(C) versus far left snapshot in Fig. 5.2(B)). Furthermore, Fig. 5.2(C) indicates that the energy barrier from the monomeric to the dimeric state is much larger than $k_B T$. This implies that the JSCNPs do not revert to the dimeric state once in the monomeric state.

The upshot of the calculations above is that JSCNPs’ adhesion to a vesicle results in a substantial deformation of the vesicle, and leads to an effective repulsive interaction between the JSCNPs. The remainder of this article focuses on determining and characterizing the preferred arrangements of more than two JSCNPs on lipid vesicles. These arrangements are characterized by the radial distribution function (RDF) of the JSCNPs’ center beads, the angle $\theta$ between the axes of JSCNPs in any pair (regardless of whether the JSCNPs are nearest neighbors), and the bond angle distribution (BAD), based on the JSCNPs’ center beads.

Fig. 5.3(A) shows that in the case of $n = 3$, the JSCNPs form a highly ordered
nanocluster, in which their centers form an equilateral triangle, similar to the case of spherical Janus NPs [5]. This is confirmed by the corresponding RDFs in Fig. 5.3(B), which exhibit a single peak regardless of $\alpha$. Fig. 5.3(A) shows that the 3 JSCNPs are arranged such that they are perpendicular to the plane containing their center beads. The general features of nanoclusters composed of three JSCNPs are, therefore, independent of their aspect ratio.

Configuration snapshots, at equilibrium, of the JSCNPs and the vesicle, in the cases of $n = 4, 6, 7, \text{ and } 8$ at $\alpha = 1.23, 1.6, 1.9 \text{ and } 2.5$, are shown in Fig. 5.4. Here, for each value of $n$, the first and third rows depict different views of the system. The second and fourth rows show corresponding polyhedra whose vertices (red points) are time-averaged positions of the JSCNPs’ center beads at equilibrium. The edges (blue segments), which are links between nearest neighbor JSCNPs, are obtained using spher-
Figure 5.4: Snapshots of the vesicle with the JSCNPs at values of $n$ ranging between 4 and 8 and for different aspect ratio values. The first and third rows for each value of $n$ show two views of the vesicle with the JSCNPs. The second and fourth rows for each value of $n$ show different views of the geometries of the JSCNPs’ nanoclusters obtained from time averages of their positions at equilibrium. Red spheres correspond to the center beads of the JSCNPs. Yellow segments represent the axes of the JSCNPs. Blue segments correspond to links between nearest neighbor JSCNPs obtained from the Delaunay triangulation. Names of the geometries of the vesicles and corresponding nanoclusters for different values of $n$ are shown on the top and bottom tables, respectively.

The yellow segments correspond to the time-averaged directions of the JSCNPs. Fig. 5.4 demonstrates that JSCNPs’ adhesion to lipid vesicles leads them to form strikingly ordered nanoassemblies with details that depend strongly on $n$. In particular, the JSCNPs’ centers form mostly highly symmetric, strictly convex polyhedra and the JSCNPs’ orientations tend to be highly correlated and depend on the locations of the respective JSCNPs on the
Figure 5.5: (A) Radial distribution function, $g(r)$, of the JSCNPs’ centers of mass for different values of $n$. (B) Average angle, $\bar{\theta}$, between the directions of two JSCNPs versus the index of the pair for different values of $n$. Here, the indices are ordered, such as $\bar{\theta}$ is an ascending function of the index. Note that for each value of $n$, there are $n(n - 1)/2$ values of the pair index. (C) Bond angle distribution, $P(\phi)$, for different values of $n$. (D) Different views of the geometries of the JSCNP nanoclusters, obtained from time averages of their positions at equilibrium. Red beads correspond to the center of the JSCNPs in the case of $\alpha = 1.23$, and green beads correspond to the center of the JSCNPs in the case of $\alpha = 1.9$. 

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Snapshots in the second and fourth rows of Fig. 5.4, corresponding to $n = 4$ at $\alpha = 1.23$, demonstrate that the centers of the JSCNPs form a tetrahedron, similar to that of 4 Janus spherical NPs on a lipid vesicle [5]. The almost regular nature of this structure is demonstrated by the fact that its corresponding RDF, shown by the red curve in Fig. 5.5(A) for $n = 4$, exhibits a single peak [5]. However, this peak seems to be the superposition of two very close peaks, implying that the center beads’ polyhedron is slightly distorted from the regular polyhedron. This distortion is confirmed by the corresponding BAD, shown in Fig. 5.5(C), which exhibits two peaks that are close to $60^\circ$, again in contrast to a single well-defined peak at $60^\circ$ in the case of spherical Janus NPs [5]. Although the JSCNPs’ centers are localized spatially, their orientations exhibit a high degree of fluctuations at low values of $\alpha$, as demonstrated by the time dependence of the angles between their axes shown in Fig. 5.6(A) for the case of $\alpha = 1.23$.

Fig. 5.5(A) shows that the single peak of the RDF, in the case of $n = 4$ at $\alpha = 1.23$, bifurcates into two clear peaks as the aspect ratio is increased. The corresponding BADs, shown in Fig. 5.5(C), also exhibit two clear peaks, one larger than $60^\circ$ and the other smaller than $60^\circ$. Therefore, the geometry of the nanocluster of the JSCNPs’ centers for $n = 4$, which is a regular tetrahedron at low values of $\alpha$, evolves into a disphenoid, as demonstrated by the corresponding snapshots in Fig. 5.4. In this case, the disphenoid comprises four congruent acute isosceles triangles whose vertex angle decreases with increasing $\alpha$.

Figs. 5.6(A) and (B) show that the amount of fluctuations in the angles between the JSCNPs’ axes drastically decreases with increasing $\alpha$ in the case of $n = 4$. This is also demonstrated by the averages of the angles between the JSCNPs’ axes, shown in Fig. 5.5(B). Therefore, while the JSCNPs’ centers form a well-ordered disphenoid nanocluster for the considered values of the aspect ratio, the relative orientations of the JSCNPs are fluid at low $\alpha$, but become increasingly rigid with increasing $\alpha$. Furthermore, the two JSCNPs at neighboring apexes become increasingly aligned with
Figure 5.6: The angle between the JSCNPs’ axes versus time for different values of \( n \) and \( \alpha \). The angles shown are between the JSCNP, indicated by the pink segment in the snapshots, and the other \((n-1)\) JSCNPs.

Based on the above, the following question arises: Why is the arrangement of 4 JSCNPs not equivalent to that of 3 JSCNPs shown in Fig. [3.3]? In other words, why are the 4 JSCNPs not parallel to each other, with their center beads arranged in a square or a rectangle? To answer this question, we performed a biased simulation that favors an arrangement of 4 JSCNPs such that they are parallel to each other and their centers form a rectangle. These biased simulations are based on an additional set of three-body potential energies. These correspond to a set of three-body potential energies between
the center beads to bias the JSCNPs to adopt a rectangular geometry and a set of three-body potential energies between the JSCNPs’ poles to bias them to be parallel. The first set of biased interactions acts between the center beads of any three JSCNPs (red beads in Fig. 5.7). It is given by

$$U_{\text{bend}}^{\alpha\beta\gamma}(\mathbf{r}_i, \mathbf{r}_j, \mathbf{r}_k) = \frac{k_{\text{bias}}}{2} \left( \frac{\mathbf{r}_{ij} \cdot \mathbf{r}_{kj}}{r_{ij} r_{kj}} \right)^2.$$  (5.1)

This interaction favors a bond angle that is equal to $90^\circ$. The second set of biased interactions is between the poles of any pair of JSCNPs (blue beads in Fig. 5.7) and is also given by Eq. (5.1). $k_{\text{bias}}$ is taken to be $= 6000 \epsilon$ in these simulations.

The biased simulations resulted in a long-lived intermediate state in which the JSCNPs’ center beads form a square configuration and are parallel, as shown by snapshots (A) in Fig. 5.8. Interestingly, the square state then evolves into a rectangular state, shown by snapshots (B) in Fig. 5.8. This implies that the biased rectangular state is more stable than the square state. Once the biased interaction is turned off, the rectangular configuration rapidly transforms into the same disphenoid structure obtained from the unbiased simulation (see snapshots (C) and (D) in Fig. 5.8). Therefore, the disphenoid nanocluster must be more stable than the square or rectangular nanocluster.

To further confirm the stability of the disphenoid structure against the square and
Figure 5.8: Time dependence of the curvature energy of a vesicle in the case of $n = 4$ and $\alpha = 2.5$. Red and blue curves correspond to the curvature energy of the vesicle in the case where the arrangement of the JSCNPs is biased such that they are parallel to each other and their centers of mass form a rectangle. The red curve corresponds to the case where the centers of the JSCNPs form a square (snapshot (A)). The blue curve corresponds to the case where the centers of the JSCNPs form a rectangle (snapshot (B)). The green curve corresponds to the curvature energy of the vesicle after removal of the bias. In this case, the JSCNPs quickly rearrange to form a disphenoid (snapshot (C)). The black curve corresponds to the curvature energy obtained from an unbiased simulation, yielding a disphenoid arrangement of the JSCNPs (snapshot (D)). In the legends, (b), (r), and (u) stand for biased, relaxed and unbiased simulations.

Rectangular configurations, we calculated the free energy of the three states. Note that the net adhesion energy of the JSCNPs on the membrane is independent of the JSCNPs’ arrangement (see Fig. 5.9). Therefore, the relative stability of the different structures is dictated by the curvature energy of the vesicle. The curvature energy of the vesicle, with the JSCNPs forming the square, rectangular, or disphenoid nanocluster, is calculated using an approach based on the Helfrich Hamiltonian [71] in conjunction with a local Monge representation [67] (Sec. 2.4.2). Fig. 5.8 demonstrates that the curvature energy of the disphenoid nanocluster is lower than those of the rectangular and square nanoclusters and, therefore, confirms the higher stability of the disphenoid nanocluster against the square or rectangular nanoclusters.
Figure 5.9: Potential energy in the case of $n = 4$ at $\alpha = 2.5$ for different geometries. The red and blue curves are the adhesion energies, corresponding to the case where the JSCNPs form a square and rectangular nanocluster, respectively. These are based on biased simulations. The green curve is that of the vesicle after the bias potential is turned off. This corresponds to a disphenoid nanocluster of the JSCNPs. The black curve corresponds to the adhesion potential energy obtained from a non-biased simulation.

We now turn our attention to the case of $n = 6$, whose configurations are shown in Fig. 5.4 for different values of $\alpha$. At $\alpha = 1.23$, the nanocluster formed by the centers of the 6 JSCNPs is almost a regular octahedron, and the geometry of the corresponding vesicle is close to a cube, very similar to that of their counterparts in the case of 6 Janus spherical NPs [5]. The regular octahedral structure of the 6-JSCNPs’ nanocluster is demonstrated by its corresponding RDF, shown in Fig. 5.5(A), which exhibits two peaks with a ratio between their positions being very close to $\sqrt{2}$. This octahedral structure is also confirmed by its corresponding BAD, shown in Fig. 5.5(C), which exhibits a single peak at $60^\circ$. Despite the ordered placement of the centers of 6 JSCNPs at $\alpha = 1.23$, their relative orientations exhibit a high degree of fluctuations, as demonstrated by Fig. 5.6(C), similar to the case of 4 JSCNPs. Interestingly, however, Fig. 5.6(C) shows that these fluctuations are localized around either low or large values, with frequent transitions between them.

Fig. 5.6(D) shows that the amplitude of the fluctuations of the relative orientations of the JSCNPs decreases with increasing $\alpha$, again similar to the case of 4 JSCNPs. This
Figure 5.10: (A) Projected views of the structures shown Centers of JSCNPs in the case of $n = 6$, for all $\alpha$s shown in 5.4. This view projects the structure as a 2D structure. (B) Angles, $\theta$, between the projected centers of JSCNP with their first nearest neighbor ($\gamma$) and their second nearest neighbor in a counter-clockwise direction ($\beta$) for the systems in (A). Inset of B schematically defines the angles $\beta$ and $\gamma$. (C) Average values of $\beta$ and $\gamma$ as a function of $\alpha$. (D) Average of the angle $\phi$ between the orientation of a JSCNP and the plane containing the centers. Note that the value of $\phi$ converges to $55^\circ$, which is the theoretical value in the case where the centers of the JSCNPs form a regular octahedron, the vesicle has the geometry of a cube, and the JSCNPs axes are parallel to the edges of the cube.
figure demonstrates that a JSCNP is almost parallel to one of the other five JSCNPs (blue graph in Fig. 5.6D), while the angles with the other four JSCNPs are high. This is also demonstrated by Fig. 5.5B. Namely, the angle of three pairs of JSCNPs is relatively low, i.e., the JSCNPs in each of these three pairs are almost parallel. The angles of the remaining 12 pairs are, however, reasonably high. An interesting feature of the time dependence of the angles between the JSCNPs’ axes, shown in Fig. 5.6D, is that the fluctuations of the angles between a given JSCNP and the 4 JSCNPs that are not parallel to it are not uncorrelated, as demonstrated by the correlation between the black and green curves, and the correlation between the red and orange curves. These correlations result from the fact that these 4 JSCNPs are composed of two parallel pairs, as stated above.

Inspection of the 6 JSCNPs’ snapshots, in Fig. 5.4, leads us to conclude that the general geometry of their center beads polyhedra is a triangular gyroprism. This is more clearly demonstrated by the snapshots in Fig. 5.10(A), which show that the JSCNPs’ center beads are clustered into two parallel equilateral triangles. The equilateral nature of these triangles is demonstrated by the angle $\beta \approx 120^\circ$, shown by Fig. 5.10(B) and (C), regardless of $\alpha$. Here, the vertex of $\beta$ is the center of mass of the triangle, as defined schematically by the snapshot in Fig. 5.10(B). The base equilateral triangles of the 6-JSCNPs’ gyroprism are offset by an angle $\gamma$ that is about $60^\circ$ for low values of $\alpha$, as shown by Fig. 5.10(B) and (C) (i.e., the center-beads nanocluster for $\alpha = 1.23$ corresponds to a regular octahedron, as discussed above), and decreases with increasing $\alpha$. The 6 JSCNPs are interestingly oriented in a chiral fashion, such that they are not parallel to each other in each of the triangular bases of the nanocluster. However, each JSCNP in a triangular base is parallel to its closest JSCNP on the other triangular base. The angle, $\phi$, between a JSCNP and the plane of the triangular base, to which it belongs, decreases with increasing $\alpha$, as demonstrated by Fig. 5.10D), i.e., the JSCNPs’ axes in each triangular base become increasingly coplanar with increasing $\alpha$. The ordered chiral nanocluster of 6 JSCNPs is unique, and to our knowledge, such a structured self-
assembled nanocluster has not been observed in earlier studies.

Fig. 5.4 shows that in the case of \( n = 7 \), the JSCNPs’ center beads form a pentagonal bipyramid in which the 5 JSCNPs of the pentagonal base are perpendicular to the pentagon’s plane and the JSCNPs at the apex and bottom vertices of the polyhedron are parallel to the pentagonal base. The angles of JSCNPs pairs, shown in Fig. 5.5(B), for the case of \( n = 7 \), indicate that the JSCNPs are either almost parallel or perpendicular to each other, regardless of \( \alpha \), except in the case of \( \alpha = 1.23 \), for which there is a pair whose average angle is about 50°. This angle, which corresponds to the pair composed of the apex and bottom JSCNPs, increases toward 90° with increasing \( \alpha \). Fig. S6 shows that this is due to fluctuations in this angle, whose amplitude decreases with increasing \( \alpha \).

Fig. 5.4 shows that 8 JSCNPs form a uniquely interesting and highly ordered nanocluster with general details of its geometry that are independent of \( \alpha \) in this range of aspect ratios. In this case, the polyhedron formed by JSCNPs’ centers corresponds to the digonal gyrobianticupola, and that of the vesicle is its dual, i.e., the elongated gyrobiastigium. We note that the digonal gyrobianticupola is a distorted snub disphenoid. It was recently shown that 8 Janus spherical NPs self-assemble into a polyhedron that is intermediate between the snub disphenoid and square antiprism. It is therefore not surprising that 8 JSCNPs into a nanocluster with a digonal gyrobianticupola geometry.

Fig. 5.4’s second and fourth rows, for \( n = 8 \), demonstrate that the JSCNPs exhibit two orientations, with two sets of parallel JSCNPs. This is demonstrated by the fact that the angles of the JSCNPs’ pairs, shown in Fig. 5.5(B), are either close to 0 or 90°, with a degree of fluctuations that decreases with increasing aspect ratio, as shown by their time dependence in Figs. 5.6(G) and (H). The centers of the JSCNPs in each set are coplanar, and the directions of the JSCNPs in each set are parallel. It is interesting to note that the RDF and BAD of 8 JSCNPs at low values of the aspect ratio (\( \alpha = 1.23 \)) are qualitatively different from their spherical Janus NP counterparts [5]. The behavior of 8 JSCNPs, even when they are fairly short, is therefore qualitatively different from
that of nanoclusters composed of a smaller number of JSCNPs, with small aspect ratio, whose RDF and BAD are quite similar to their spherical Janus NPs counterparts [5].

The results above were presented for the case where the ratio, \( \rho \), between the surface area of the vesicle and the net surface area of the JSCNPs is \( \rho = 1.2 \). We examined the effect of this ratio on the geometry of the nanocluster by also performing simulations for the cases of \( n = 4 \) and 6 with \( \alpha = 1.9 \) at \( \rho = 3.6 \). The equilibrium configurations, in Figs. 5.11(A) and (B), seem to not be very dependent on \( \rho \). Namely, 4 JSCNPs form a tetrahedron, and 6 JSCNPs form a triangular gyroprism. However, the RDF of the case
of \( n = 4 \) exhibits a single peak, albeit broad, instead of two peaks. This indicates that the 4-JSCNPs’ nanocluster with \( \rho = 3.6 \) has a geometry closer to the regular tetrahedron than in the case of \( D_{ves} = 1.2 \). Likewise, the RDF of 6-JSCNPs with \( D\rho = 3.6 \) exhibits two peaks only, which implies that the geometry of the 6-JSCNPs’ nanocluster is close to that of a regular octahedron. Furthermore, the angles between the JSCNPs versus time, shown in Figs. 5.11(C) to (F), demonstrate increased fluctuations with increasing \( \rho \). This implies that an increase in the membrane area available to the JSCNPs allows for increased rotational fluidity while the positions of their centers of mass remain fairly localized. One may also conclude that increasing \( \rho \) for given values of \( n \) and \( \alpha \) is equivalent to decreasing \( \alpha \) for given values of \( n \) and \( \rho \).

We now focus on the effect of increasing the aspect ratio beyond 2.5 on the geometries of the nanocluster. We, therefore, performed another set of systematic simulations for the same number of JSCNPs, with \( \alpha \) ranging between 3 and 5.5. We note simulations on even longer JSCNPs become quickly computationally very expensive. Hence, the largest explored value of \( \alpha \) was 5.5. Fig. 5.12 depicts equilibrium configurations of systems with \( n = 4, 6, 7 \) and 8 at \( \alpha = 3.25, 4 \) and 5.5. Surprisingly, this figure shows that, except for \( n = 4 \), increasing \( \alpha \) beyond values in Fig. 5.4 leads to a dramatic effect on the nanoclusters’ geometry.

In the case of \( n = 4 \), Fig. 5.12 shows that for \( \alpha \leq 4 \), the general geometry of the JSCNPs’ nanocluster is the same as that shown in Fig. 5.4. Namely, the nanocluster’s geometry is a disphenoid. Interestingly, however, the nanocluster’s geometry for \( \alpha = 5.5 \) is rectangular, which was found to be unstable for lower values of \( \alpha \) (see Fig. 5.8). Interestingly, the disphenoid structure at \( \alpha = 5.5 \) was observed as a long-lived transient state. This indicates that the disphenoid nanocluster, in the case of \( n = 4 \), for relatively large values of the aspect ratio becomes metastable.

Although the nanocluster for \( n > 4 \) and relatively large values of \( \alpha \), shown in Fig. 5.12 are largely different from those in Fig. 5.4, the structures themselves at high \( \alpha \) are symmetric. The point groups associated with the symmetries of these structures are
Figure 5.12: Different JSCNP nanoclusters with different snapshots of vesicles obtained from simulations at high aspect ratios in the case of $n = 4, 6, 7$ and 8. The membrane is shown as icy blue. JSCNPs moiety that interacts attractively (repulsively) with the vesicle is yellow (blue). Simulations are performed on JSCNPs with $J = 0.4$ at $\xi = 4.11 \text{nm}^2/k_B T$. The table at the bottom indicates the point group of each of the above structures.
Figure 5.13: Values for the nematic order parameter \( \langle S \rangle \) for different values of \( n \) as a function of \( \alpha \)

listed in the table at the bottom of Fig. 5.12. We emphasize that although these structures are reproducible from independent simulations, in a few cases, we found that the JSCNPs form dimers in which the JSCNPs are side by side and almost in contact with each other. We note that configurations in which the JSCNPs dimerize are not observed for \( \alpha \lesssim 3.25 \). We believe that they are the result of crowding due to the increased aspect ratio.

Figs. 5.4 and 5.12 show qualitatively that \( \alpha \) and \( n \) affect the orientational ordering of the JSCNPs on the vesicles. The orientational order of the JSCNPs is characterized by the nematic order parameter, \( \langle S \rangle \), which is often used to characterize the degree of order in liquid crystals. The details of the calculation of \( S \) are presented in Sec. 2.5.2. The brackets in \( S \) indicate an ensemble average.

The JSCNPs order parameter is shown as a function of \( \alpha \) for different values of \( n \) in Fig. 5.13. This figure shows that, except in a few cases, \( \langle S \rangle \) increases with \( \alpha \). In the case of 3 JSCNPs, \( \langle S \rangle \) is close to 1 even for the smallest value of \( \alpha \) values, suggesting that the JSCNPs align parallel to each other for all given values of \( \alpha \). In case of \( n = 4 \),
\( \langle S \rangle \approx 0.4 \) at very small \( \alpha \). This is a result of the closest JSCNP pairs aligning at an angle with each other. However, as \( \alpha \) is increased in the case of \( n = 4 \), this angle between the closest JSCNP pairs starts decreasing and the JSCNPs start aligning in the same direction, hence increasing \( \langle S \rangle \) values, as shown in Fig. 4. \( \langle S \rangle \) reaches the maximum value of 1 at \( \alpha = 5.5 \), when the JSNCPs align parallel to each other.

Fig. 5.13 shows that in the case of 6 JSCNPs, \( \langle S \rangle \) decreases initially as \( \alpha \) is increased from 1.15 to around 1.6, suggesting the change of geometry of the nanocluster such that the JSCNPs are less aligned with each other. This change is visually illustrated in Fig 5.4 in the case of \( n = 6 \) when the geometry of the nanocluster goes from an octahedron at \( \alpha = 1.23 \) to an elongated triangular gyroprism at \( \alpha = 1.6 \). The geometry of the nanocluster and its corresponding \( \langle S \rangle \) remain unchanged over \( 1.6 \lesssim \alpha \lesssim 2.2 \), with 3 JSCNPs’ pairs aligned in different directions. Interestingly, at \( \alpha = 2.5 \), the JSCNPs align in such a way that both sets of 3 JSCNPs are pointing towards a common vertex, as shown in the case of \( n = 6 \) at \( \alpha = 2.5 \) in Fig. 5.4, leading to \( \langle S \rangle \approx 0.25 \). As \( \alpha \) is further increased to \( 2.8 \lesssim \alpha \lesssim 3.5 \), the JSCNPs align such that two sets of 3 JSCNPs seem to point to a specific point, making \( \langle S \rangle \approx 0.5 \). As \( \alpha \geq 4 \) in the case of 6 JSCNPs, the JSCNPs align such that 5 JSCNPs all point towards a common vertex (as shown in Fig. 5.12), and \( \langle S \rangle \) value reaches around 0.65. While it is very likely that all the JSCNPs will align parallel to each other at some \( \alpha \geq \alpha^* \), current computational efficiency prevents us from reaching that limit using our present model.

Fig. 5.13 shows that 7 JSCNPs align with \( \langle S \rangle \approx 0.55 \) over a wide range of \( \alpha \) (1.15 \( \lesssim \alpha \lesssim 2.8 \)), reconfirming our statement earlier that nanocluster geometry in case of \( n = 7 \) remains unchanged, with 5 JSCNPs orienting in the same direction, in the given range of \( \alpha \). However, in the given range of \( \alpha \), the geometry gets distorted with increasing \( \alpha \). Hence, \( \langle S \rangle \) decreases, although minutely, with increasing \( \alpha \). At \( \alpha = 3.25 \), the geometry clearly changes, and the JSCNPs in this new geometry are temporarily less ordered than in the cases of \( \alpha < 3.25 \). However, the JSCNP alignment becomes more ordered as \( \alpha \) is increased to 3.4 or above, even though the structure of nanocluster in the case of
\[ \alpha = 3.4 \] looks qualitatively similar to that of \( \alpha = 3.25 \), hinting that the JSCNPs just align themselves, and become more ordered as \( \alpha \) is increased from 3.24 to 3.4 and above.

Lastly, Fig. 5.13 shows that 8 JSCNPs self-assemble into a digonal gyrobiaticupola geometry, with \( \langle S \rangle \approx 0.27 \) at \( \alpha \lesssim 3.6 \). Interestingly, as \( \alpha \) is increased to 4, simulations show that the final geometry of the nanocluster is dependent on the initial placement of the particles, and we obtain three different structures through repeated simulations with different initial conditions, in the case of 8 JSCNPs at \( \alpha = 4 \). One such structure obtained for \( \alpha = 4 \) is a digonal gyrobiaticupola, which, as expected, has \( \langle S \rangle \approx 0.26 \) (shown by dotted pink line in Fig. 5.13). However, analytical calculations shown by Fig. 5.14 and discussed in detail in the next paragraph shows that the most stable structure in case of 8 JSCNPs with \( \alpha = 4 \) is completely different from a digonal gyrobiaticupola, with 6 of the JSCNPs pointing towards a same line(see Fig. 5.12), with \( \langle S \rangle \approx 0.55 \). The geometry of the nanocluster changes again and becomes less symmetric as \( \alpha \) is further increased to 5, and the alignment order parameter of the JSCNPs is close to 0.5. Similar to the case of \( \alpha = 4 \), the JSCNPs, when initially placed into a digonal gyrobiaticupola orientation, equilibrate to a structure similar to digonal gyrobiaticupola, with an order parameter value of \( \langle S \rangle \approx 0.28 \).

We now focus on the case of 8 JSCNPs with \( \alpha = 4 \) to understand the stability of the three numerically observed nanostructures. To achieve this, we performed 10 independent simulations (with initial placements of the JSCNPs) over a long time until the systems reached equilibrium. Since the net adhesion energy of the JSCNPs is independent of the geometry of the final nanocluster, the relative stability of the different geometries is inferred from the curvature energy, \( F_{\text{curv}} \), of the vesicle in the three structures. The curvature energy is calculated using our approach based on the Helfrich Hamiltonian in conjunction with a local Monge representation (Sec. 2.4.2). \( F_{\text{curv}} \), shown in Fig. 5.14 demonstrates that the structure with configuration (2) in Fig. 5.14 is most stable and that the structures with configurations (1) and (3) (i.e., digonal gyrobiaticupola) have almost same free energy. This shows that the initial placement of the NPs does influ-
Figure 5.14: The curvature energy of the vesicle in configurations (1), (2), and (3), shown above, as a function of time for the case of \( n = 8 \) at \( \alpha = 4 \).

ence the final structure of the self-assembly at high values of \( \alpha \). For low values of \( \alpha \), 8 JSCNPs always self-assemble into the digonal gyrobiaticupola, which implies that other configurations are not stable at \( \alpha \lesssim 3.25 \).

### 5.4 Summary

This chapter’s central contribution lies in demonstrating the unique ability of lipid vesicles to orchestrate the self-assembly of JSCNPs into intricately ordered nanoclusters, a phenomenon unattainable with uniformly surfaced SCNPs. We extensively explored the membrane-mediated self-assembly of Janus spherocylindrical nanoparticles (JSCNPs), adhering to the outside of the vesicles, using molecular dynamics simulations of a coarse-grained implicit-solvent model. The SCNPs are efficiently modeled as triangulated hollow shells. Simulations are systematically performed over a range of aspect ratios, \( \alpha \), at \( \xi = 4.11 \text{ } k_B T/\text{nm}^2 \) and \( D = 20 \text{ nm} \). This self-assembly of the JSCNPs on the vesicle is ensured by implementing two conditions: using the SCNPs with
a Janusity of 0.4, such that this fraction of the SCNP interacts more attractively with the lipid head groups, and the adhesion strength is kept sufficiently high to induce complete wrapping of the Janus moieties by the vesicle, while simultaneously preventing their endocytosis [5]. The investigation is performed for different numbers of JSCNPs, including \( n = 2, 3, 4, 6, 7, \) and 8.

It was consistently observed in the case of \( n = 2 \) that JSCNPs exhibited mutual repulsion regardless of \( \alpha \). We showed that the number of the JSCNPs determines the geometry of the vesicles-induced ordered nanoclusters. These structures can be tuned by changing the aspect ratio (\( \alpha \)) of the JSCNPs. We found that increasing the length of the JSCNPs elongates the vesicle in one, two, or all three directions, resulting in elongated vesicle geometries. For \( \alpha < 2.5 \), the JSCNPs self-assemble into nanocluster geometries corresponding to the equilateral triangle (\( n = 3 \)), disphenoid (\( n = 4 \)), triangular gyroprism (\( n = 6 \)), pentagonal bipyramid (\( n = 7 \)) and digonal gyrobianticupola (\( n = 8 \)). Correspondingly, the vesicle equilibrates as a triangular prism, disphenoid, triangular gyrotegum, pentagonal prism, and elongated gyrobufastigium in the case of \( n = 3, 4, 6, 7, \) and 8 respectively. Systematic simulations show that the geometry of these nanoclusters change abruptly at \( \alpha \gtrsim 3 \), and the JSCNPs align in a specific direction with respect to each other at higher \( \alpha \). Interestingly, these SCNPs are always arranged such that they have some symmetry. Calculation of the nematic order parameter shows that the order parameter reaches or tends to reach a value of 1 as \( \alpha > \alpha^* \), and is verified in the case of \( n = 3 \) and \( n = 4 \).

Interestingly, for a constant membrane area per JSCNP, the degree of fluctuations in their orientations decreases with increasing \( \alpha \). Within these vesicle-induced ordered nanoclusters, JSCNPs maintain distinct separations from one another, their nearest neighbor distances contingent on both \( n \) and the size of the vesicle. The overall geometry of these nanoclusters is intricately determined by \( n \). Moreover, the results indicate that the effect of \( \alpha \) within these nanoclusters decreases as the vesicle’s diameter increases. Remarkably, even when the distance between nearest neighbor JSCNPs
reaches approximately 100 nm, these nanoclusters maintain a considerable degree of organization. This work provides valuable insights into the intricacies of JSCNP-vesicle interactions and paves the way for further exploration in the field.

The results presented in this article are very exciting in that they demonstrate that lipid vesicles have the potential to be used as an alternative medium for self-assembling JSCNPs into highly ordered nanoclusters. Moreover, this shows that the geometry of the self-assembly can be tuned by not only changing the number of the JSCNPs but by changing their lengths. Although challenging, experimental studies are warranted to validate our results.
Chapter 6

SELF-ASSEMBLY OF SPHEROCYLINDRICAL NPS WITH UNIFORM SURFACES INSIDE LIPID VESICLES

6.1 Introduction

We have established so far that a single SCNP exhibits two modes of adhesion to a planar lipid membrane, depending on the values of $\xi$, $\alpha$, and $D$ \cite{1,84}. At low values of $\xi$, the effect of membrane curvature dominates over that of adhesion, leading the SCNP to adhere sidewise (parallel mode), with its degree of wrapping that increases with $\xi$. Beyond some value of $\xi$, the effect of adhesion becomes dominant, leading the SCNP to adhere such that its long axis is perpendicular to the membrane (normal mode). The SCNP undergoes spontaneous endocytosis at even higher values of $\xi$. When two SCNPs are introduced into the system, membrane-mediated interactions result in the dimerization of the SCNPs at intermediate or higher adhesion strengths. Moreover, we saw that when two Janus SCNPs are placed on a vesicle, they tend to avoid dimerization and move further apart. Multiple Janus SCNPs self-assemble into interesting regular nanoclusters on vesicles, and these geometries can be tuned by altering the JSCNPs’ aspect ratio, number of NPs, and vesicle size.

In this Chapter, we shift our focus to the adhesion and interactions of spherocylindrical nanoparticles (SCNPs) placed inside lipid vesicles, which presents a departure from the previous observations of numerous Janus SCNPs adhering to the outer surface of vesicles. Building upon the findings presented in Chapter 4, which revealed that Janus NPs can self-assemble into well-ordered nanoclusters when attached to the vesicle’s exterior through effective membrane-mediated repulsion, we propose the possibility of a similar self-assembly phenomenon occurring when these NPs adhere to the inner side of lipid vesicles.
It is worth noting that prior studies examining NP-vesicle interactions have primarily explored scenarios where NPs attach to the vesicle’s outer surface. Notably, recent research by Gompper’s group looks into the influence of active Janus NPs adhering to the vesicle’s inner side on vesicle morphology. Their investigations presented intriguing, non-equilibrium steady-state morphologies, showing local tethers, dendritic structures, and prolate and bola-like configurations \([135,221]\). However, the behavior of passive NPs adhering to the inner side of vesicles remains relatively uncharted territory.

We recently examined the adhesion and self-assembly behavior of uniform and Janus spherical NPs adhering inside the vesicle. We saw that even the spherical NPs assembled in interesting 2D and 3D structures. Given the area available per NP is large enough, we noticed that the NPs preferred to stay away from each other at all given instances. This gives rise to the following question: Do SCNPs assemble inside lipid vesicles to form similar interesting 2D structures, and if so, how does the anisotropy of the SCNPs affect these structures?

This chapter presents comprehensive simulation results detailing the behavior of numerous uniform SCNPs adhering to the inner side of vesicles. Our findings reveal that these uniform SCNPs organize into intricate two-dimensional and three-dimensional nano assemblies for a wide range of adhesion strengths, shedding light on this less-explored facet of NP-vesicle interactions.

### 6.2 Model and Computational Method

The present work is based on a mesoscale implicit-solvent model for self-assembled lipid bilayers, presented in Sec. 2.1.4 and the SCNPs are modeled using the approach discussed in Sec. 2.2. In addition to the model parameters (table 2.1) and NP parameters (table 2.2), specific values of the interaction parameters used in the simulations in this study are included in table 6.1. Simulations are performed for \(D = 20\, \text{nm}\).

The simulations are performed on vesicles with diameter, \(D_V\), ranging between 42
Table 6.1: Many SCNPs inside vesicle interaction parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$U_{nht,max}, U_{nvt,max}$</td>
<td>$200\epsilon$</td>
</tr>
<tr>
<td>$U_{nh,min}$</td>
<td>$-\epsilon$</td>
</tr>
<tr>
<td>$U_{nt,min}$</td>
<td>$0$</td>
</tr>
<tr>
<td>$U_{m1,m2,max}$</td>
<td>$200\epsilon$</td>
</tr>
<tr>
<td>$U_{m1,m2,min}$</td>
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<td>$500\epsilon/r_m^2$</td>
</tr>
<tr>
<td>$k_{cn,bond}$</td>
<td>$10\epsilon/r_m^2$</td>
</tr>
</tbody>
</table>

and 90 nm. Here, $D_V$ is defined as twice the average distance between the positions of the \( h \)-beads of the outer leaflet and the vesicle’s center of mass. This corresponds to a total number of lipid chains in a vesicle ranging between 20,000 and 220,000. The diameter of the SCNPs is fixed at $D_N = 20$ nm. In a typical simulation, $n$ SCNPs, which are initially placed inside an equilibrated vesicle at random positions, adhere to the vesicle at different times. We consider values of $n$ corresponding to 2, 3, 4, 5, and 6. Throughout the study, the ratio $A_V/nA_N \approx 1.8$ in all simulations, where $A_V$ is the area of the outer leaflet of a vesicle and $A_N$ is the area of a SCNP. The system is then equilibrated over a large number of steps (typically over at least $4 \times 10^5 \tau$ for $n = 2$ and $8 \times 10^5 \tau$ for all other values of $n$), and the results are collected once equilibrium is reached. The time step in these simulations is $\Delta t = 0.02\tau$.

6.3 Results

6.3.1 Adhesion of 2 SCNPs inside a Vesicle

We performed a series of coarse-grained molecular dynamics simulations based on the model and numerical approach presented in Section 2.3. The simulations are performed for $n = 2, 3, 4, 5$ and 6 at various values of adhesion strength, $\xi$. The aspect ratio, $\alpha$, is increased to investigate its effect on the geometry of the nanocluster.

First, we explored the adhesion behavior of two uniform SCNPs when placed inside a vesicle. We performed a series of simulations of two SCNPs adhering to a lipid vesicle to explore their adhesion modes on the vesicle at equilibrium. The simulations
Figure 6.1: Equilibrium snapshots at different values of $\xi$ in the case of two SCNPs adhering inside the vesicle. The simulations are performed at $D = 20\text{nm}$ and $\alpha = 1.9$.

are started from a configuration in which they are placed randomly, at random orientations, inside a vesicle. The SCNPs interact with the lipid head groups with given adhesion strengths as they diffuse inside the vesicle. Fig. 6.1 shows different equilibrium configurations of two SCNPs on a vesicle to varying values of $\xi$, obtained from MD simulations for the case of $D = 20\text{nm}$ and $\rho = 1.9$. This figure demonstrates that two SCNPs exhibit several adhesion modes inside the vesicle for different values of $\xi$.

In particular, Fig. 6.1 shows that the SCNPs adhere in the parallel mode up to a specific value of $\xi$. Similar to what we saw in Chapter 4, when two SCNPs are placed on a planar membrane, the SCNPs inside vesicles also transition to the normal mode at some intermediate value of $\xi$. Finally, the SCNPs are exocytosed at high values of $\xi$.

Fig. 6.1 shows that at $\xi = 0.08k_B T/\text{nm}^2$ and $\xi = 0.16k_B T/\text{nm}^2$, the SCNPs lie parallel to the membrane and diffuse around the membrane. Hence, the SCNPs are highly diffusive at very low $\xi$ values. The vesicle maintains its spherical shape in the equilibrated configuration at these lower values of $\xi$. The SCNPs remain diffusive and experience a lot of fluctuations even after $\xi$ is further increased. To better understand the effect of the adhesive interaction on the diffusive nature of SCNPs, we plotted in Fig. 6.2 the closest distance between the SCNPs at low values of $\xi$, as a function of time. Fig. 6.2 shows that the SCNPs remain confined and next to each other at very
Figure 6.2: Closest Distance between the SCNPs, in the case of $D = 20$ nm and $\alpha = 1.9$, vs. time for lower values of $\xi$. The dashed pink line indicates the minimum interaction distance $d = 1$ nm between any two beads. The insets show the configurations during the evolution of the system in the case of $\xi = 0.16 k_B T/nm^2$, corresponding to a (i)dimeric and (ii)monomeric state.

Low values of $\xi$. However, as $\xi$ is increased from $0.08 k_B T/nm^2$ to $0.11 k_B T/nm^2$ or $0.16 k_B T/nm^2$, we see that the SCNPs tend to undergo dimerization, which is followed by undimerization. This suggests that the free energy should exhibit two local minima corresponding to the dimeric and monomeric state, with some energy barrier between the two states. Interestingly, the vesicle gets flatter along the direction perpendicular to the axes containing the SCNPs’ principal axes, with the increase in the value of $\xi$. Hence, the vesicle becomes quasi-two-dimensional from a three-dimensional sphere, and the SCNPs start aligning in the same plane as $\xi$ increases.

As $\xi$ is further increased to $0.43 k_B T/nm^2$, the SCNPs remain apart from each other such that their axes are parallel. The SCNPs remain in this parallel state for a wide range of adhesion strengths. The SCNPs move further from each other due to their
increased wrapping by the membrane, and the thickness of the vesicle in the direction normal to the SCNPs’ axes decreases as $\xi$ is increased. Hence, the vesicle turns from a quasi-two-dimensional to a two-dimensional geometry at high adhesion strength values in this mode. In this regime, the principal axes of the SCNPs are coplanar. This is discussed in detail later (Section 6.3.4). Fig. 6.3 depicts the angles made by both the SCNPs’ axes with the center of the vesicle and the angles made between the axes of SCNPs. The SCNPs make approximately $55^\circ$ with the center of the vesicle and roughly $10^\circ$ with each other. This shows that the SCNPs are relatively parallel in this range of $\xi$ values.

As $\xi$ is further increased, the SCNPs’ transition from the parallel to the normal mode, in which they are encapsulated by a tubular vesicle, as shown by the configuration at $\xi = 0.63k_B T/\text{nm}^2$ in Fig. 6.1. Interestingly, the SCNPs adhere to the vesicle in this mode over a wide interval of $\xi$ values. The effect of increasing $\xi$ on this type of adhesion mode is explored by calculating the angle, $\theta$, between an SCNP’s axis and the segment.
connecting its proximal pole to the vesicle’s center of mass, as defined schematically by the snapshot in Fig. 6.4. This figure shows that $\theta \approx 180^\circ$ for relatively low values of $\xi$ within this regime. This implies that the SCNP are colinear in this range; however, as $\xi$ is increased, $\theta$ decreases, an indication that the SCNP in this regime form a boomerang nanocluster (see snapshot at $\xi = 3.57 k_B T/\text{nm}^2$ in Fig. 6.1). This configuration results from a reduction of the membrane’s neck at the poles of the SCNP, with increasing $\xi$. The tilt of the SCNP in this conformation is similar to that of the adhesion of SCNP to planar membranes, as discussed earlier in Chapter 3. Finally, the SCNP are engulfed by the membrane into two individual vesicles as $\xi$ is further increased, as shown by the snapshot at $\xi = 3.7 k_B T/\text{nm}^2$ in Fig. 6.1.

### 6.3.2 Adhesion of Many SCNP inside a Vesicle

Now that we have a good idea of the adhesion behavior of two SCNP inside a vesicle, we extend this study to explore the adhesion behavior of many SCNP inside a
Figure 6.5: Equilibrium snapshots for different values of $n$ and $\xi$ of SCNPs with $\alpha = 1.9$ inside vesicles.

lipid vesicle. This is achieved through a large set of systematic simulations for different values of the number of SCNPs, $n$, in the case of $\alpha = 1.9$, at different values of $\xi$. At equilibrium, configuration snapshots of the SCNPs and the vesicle for $n = 2, 3, 4, 5$ and 6 at different values of $\xi$ are shown in Fig. 6.5. The last two rows depict equilibrium configurations at $\xi$ right before the SCNPs undergo exocytosis and a value of $\xi$ where all SCNPs are exocytosed. The value of $\xi$ is indicated next to its corresponding snapshot in these two regimes.

Fig. 6.5 demonstrates that the general adhesion modes of the SCNPs depend mainly on the value of $\xi$. In the most generic sense, Fig. 6.5 shows that the SCNPs adhere in the parallel mode at low values of $\xi$ and the normal mode at intermediate values of $\xi$. The SCNPs are exocytosed at high values of $\xi$. 

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Figure 6.6: (A) Radial distribution function, $g(r)$, of the SCNPs’ centers of mass for different values of $n$ at $\xi = 0.43 k_B T / \text{nm}^2$. Inset of (A) shows schematically the first, second, and third nearest neighbors of a SCNP. (B) Distribution of the angle, $\phi$, between the SCNPs’ axes and the vector connecting the vesicles’s center of mass with the SCNP’s pole closest to the center, as explained schematically by the inset in (B). The simulations are performed in the case of $\alpha = 1.9$.

Fig. 6.5 shows that at $\xi = 0.08 k_B T / \text{nm}^2$, the SCNPs are diffusive, their positions are weakly correlated, and the vesicles are fairly spherical, regardless of the value of $n$. At $\xi = 0.16 k_B T / \text{nm}^2$, the SCNPs form chain-like structures. These structures are fairly dynamic in that the SCNPs at their end continuously adsorb and desorb from them. As seen earlier in the case of $n = 2$ (see Fig. 6.2), the configurations remain bimodal in this range of values of $\xi$. As $n$ increases, in this range of values of $\xi$, the SCNPs tend to stay clustered in chains. We believe that this is due to the fact that these chains are two-dimensional and the overall geometry of the vesicle is fairly flat. The perimeter of the chain decreases with $n$, although the area of the vesicle is proportional to $n$.

The SCNPs align themselves to form a regular polygonal geometry for all values of $n$ at $\xi = 0.43 k_B T / \text{nm}^2$, as shown by Fig. 6.5. The SCNPs align themselves such that their axes are coplanar, and the vesicle is quasi-two-dimensional. The projection of the vesicle is effectively a regular $n$-polygon with the edges conforming to the side of the SCNPs. This geometry is fairly rigid for all considered values of $n$, as shown by their
Figure 6.7: (A) Radial distribution function, $g(r)$, of the SCNPs’ centers of mass for different values of $n$ at $\xi = 0.98k_BT/nm^2$. Inset of (A) shows schematically the first, second, and third nearest neighbors of a SCNP. (B) Distribution of the angle, $\phi$, between the SCNPs’ axes and the vector connecting the vesicles’s center of mass with the SCNP’s pole closest to the center, as explained schematically by the inset in (B). The simulations are performed in the case of $\alpha = 1.9$.

RDF in Fig. 6.6(A) and by the distribution of the angles between the SCNPs’ axes and the legs connecting their poles with the vesicle’s center of mass, shown in Fig. 6.6(B).

Fig. 6.5 shows that the SCNPs flip to the normal mode and form star-like geometries at $\xi = 0.98k_BT/nm^2$ for all considered values of $n$. These star self-assemblies are fairly rigid, as shown by their RDFs in Fig. 6.7(A). The SCNPs form these assemblies over a wide interval of $\xi$. The SCNPs remain almost coplanar to each other, as confirmed by the distribution of angles made by the SCNPs with the center of the vesicle in Fig. 6.7(B), which peaks at a value close to $180^\circ$. Similar to the case of $n = 2$, discussed earlier, Fig. 6.5 shows a decrease in the size of the neck and, hence, an increase in the tilt of the SCNPs as $\xi$ is increased. The structure is fairly two-dimensional at the lower end of the interval of $\xi$, where the star nanoclusters are observed. However, as $\xi$ increases, the SCNPs tilt increases, resulting in the SCNPs pointing toward different directions from the plane containing the center of the vesicle and one end of the SCNPs. This is clearly depicted in Fig. 6.8.
Figure 6.8: Average of the tilt angle $\Theta$ versus $\xi$ for different values of $n$. $\Theta$ is defined as the angle between the axes of an SCNP’s axis and the plane that fits best all endpoints of the SCNPs.

### 6.3.3 $n - \xi$ Phase Diagram

The phase diagram of the equilibrium states of many SCNPs adhering to the inside of a vesicle, in terms of $\xi$ and $n$, is shown in Fig. 6.9 for the case of $D = 20\text{nm}$ and $\alpha = 1.9$. This phase diagram is obtained from simulations based on particles initially placed at random positions and orientations inside the vesicle. This figure demonstrates that the final state of the SCNPs depends on their number, particularly at high values of $\xi$. Fig. 6.9 shows that the SCNPs occupy random positions and have random orientations in the parallel mode at low values of $\xi$ regardless of $n$ (red circles in Fig. 6.9). As $\xi$ is slightly increased, the SCNPs form chain-like structures (orange squares). The geometry of these chains becomes increasingly more rigid for high values of $n$. This is due to a decrease in the perimeter available per SCNP for its diffusion.

With a further increase in $\xi$, the vesicles become more two-dimensional, and the SCNPs self-assemble into polygonal geometries (green squares in Fig. 6.9).
Figure 6.9: Adhesion modes phase diagram in terms of the adhesion strength, $\xi$, and the number of SCNPs, $n$, in the case of $D = 10$ nm and $\alpha = 1.9$. This phase diagram is extracted from the case where the SCNPs adhere randomly inside the membrane. Solid lines indicate boundaries in different states. The blue dotted line indicates the start of the coexistence region between the polygonal mode and star-like mode.

shows a coexistence region between the polygonal nanocluster mode and the star-like nanocluster mode, especially at high values of $n$. This region is an indication that there exists a high energy barrier between the two modes. This energy barrier arises from the energy barrier between the parallel and normal modes for a single SCNP, discussed earlier in Chapter 4. However, we also saw that in the non-simultaneous adhesion of two SCNPs, the SCNPs reach the tubular (normal) mode for values of $\xi$ where parallel mode is expected. Hence, many factors such as initial placement, orientation at which the SCNPs approach the vesicle, size of the SCNPs, and size of the membrane play a significant role in their final modes of adhesion, resulting in a range of $\xi$ values that correspond to the coexistence region. Thus, in this range, we might see no flips, some flips, or all flips of the SCNPs from the parallel mode to the normal mode. In the case of Fig. 6.9, the final adhesion mode in the phase diagram is selected through statistical
analysis, where multiple simulations are submitted at different initial configurations, and the equilibrated configuration that is most observed is selected. To indicate this coexistence phase, a blue dashed line is drawn to show the start of the region, and the region between the vertically dashed blue line and the curved pink line in the phase diagram of Fig. 6.9 is shaded blue.

As $\xi$ is increased beyond $\xi = 0.98 k_B T / \mu m^2$, the SCNPs self-assemble into star-nanoclusters, shown by the pink symbols in Fig. 6.9, regardless of $n$. This suggests the decrease in the energy barrier with the increase in $\xi$. These interesting nanoclusters occur over a wide interval of $\xi$. However, the extent of this range is highly dependent on the value of $n$. For relatively higher values of $\xi$ within this region of the phase diagram, one or more SCNPs get exocytosed from the membrane. The value of $\xi$ at which at least one SCNP is exocytosed decreases with $n$. This indicates strong many-body effects in these systems that are mediated by the curvature of the vesicle. The blue symbols in Fig. 6.9 show the exocytosis phase. Important conclusions drawn from this phase diagram are that the number of SCNPs plays an important role in their equilibrium placement inside vesicles at intermediate and high values of $\xi$. Furthermore, different states may coexist in the phase diagram for a given $\alpha, \xi, D$, and the size of the vesicle.

### 6.3.4 Coplanarity of the SCNPs’ Nanoclusters

We have established that, for any given value of $n$, the SCNPs self-assemble into interesting polygonal and star nanoclusters, depending on the adhesion strength. In both these structures, regardless of $n$, the vesicle remains two-dimensional, and interestingly, the SCNPs tend to be coplanar. To quantify this behavior as a function of $\xi$ and $n$, we calculated a quantity called "coplanarity", which measures how co-planar are the ends of the SCNPs. The coplanarity, $d$, of a system is defined as the mean square of the distance of the SCNPs’ ends to the best-fit plane of these points. The details of the calculation of $d$ are given in Section 2.5.3. The lower the value of $d$, the more coplanar the SCNPs are.
Figure 6.10: Coplanarity $d$ as a function of $\xi$ for different values of $n$ values. The data correspond to the points shown in Fig. 6.9. Data here correspond to $\alpha = 1.9$.

Fig. 6.10 depicts the coplanarity of the equilibrated configurations as a function of $\xi$ for different values of $n$. High values of $d$ at very low $\xi$ confirm that the SCNPs’ positions are uncorrelated and are distributed in three dimensions on the vesicle. However, as $\xi$ increases, the deviation drops sharply regardless of $n$, confirming that the system becomes coplanar. Notice that $d$ remains much smaller than the diameter of the SCNPs, which is 20 nm in this case. The lowest values of $d$ correspond to the case where the SCNPs form polygonal nanoclusters. As $\xi$ is increased, $d$ steadily increases, suggesting that the system becomes less coplanar. This is because of the increase in tilt angle of the SCNPs in the star-like geometry, which is associated with the increased constriction of the neck with increased degree of the SCNPs’ wrapping. The SCNPs point in different directions with the increase in $\xi$, making them less coplanar. However, the part of the vesicle not in contact with the SCNPs is still very flat and remains quasi-two-dimensional.
6.4 Summary

Details of the adhesion modes of two spherocylindrical NPs adhering to the inside of the vesicles are investigated in this chapter using molecular dynamics simulations of a coarse-grained implicit-solvent model. The SCNPs are efficiently modeled as triangulated hollow shells \[1, 2, 4\]. These SCNPs have a relatively low number of degrees of freedom, allowing for the simulations of SCNPs with high diameter and aspect ratio. The simulations were systematically performed over a range of adhesion strength values \(\xi\), at a specific aspect ratio of \(\alpha = 1.9\) and a specific diameter of 20 nm. This study was extended for the case of \(n = 3, 4, 5,\) and 6, and the effect of increasing the number density is investigated. Notably, all the simulations are performed keeping the ratio of the area of the outer leaflet of a vesicle and the area of an SCNP constant at \(\approx 1.65\).

In the case of two SCNPs, the SCNPs exhibit high diffusivity and remain parallel to the membrane at very low \(\xi\) values, causing the vesicle to maintain its spherical shape. As \(\xi\) is slightly increased, SCNPs tend to dimerize and undimerize, indicating the presence of low energy barriers between these states. The vesicle becomes quasi-two-dimensional with increasing \(\xi\), and SCNPs align in the same plane. At higher \(\xi\), SCNPs move further apart due to increased membrane wrapping, transitioning from quasi-two-dimensional to two-dimensional geometry. In this mode, SCNPs’ principal axes become coplanar. The angles between SCNPs’ axes and their orientation relative to the vesicle change with varying \(\xi\). At very high \(\xi\), SCNPs are engulfed by the membrane, resulting in exocytosis. This research provides valuable insights into understanding the complex dynamics of two SCNPs adhesion inside the membrane at different adhesion strengths.

Cooperative effects become dominant as \(n\) is increased. At higher \(n\) values, SCNPs exhibit chain-like formations at low values of \(\xi\). SCNPs self-assemble into highly ordered polygonal and star-like two-dimensional structures for all \(n\) over a wide range of intermediate adhesion strength values. The rigidity of these structures is confirmed.
through the analysis of their radial distribution functions and angular distributions. Notably, a coexistence region exists between the polygonal and star-like geometries at higher $n$ values, influenced by factors like initial placement and size of the vesicle. Significantly higher $\xi$ values result in the exocytosis of the SCNPs. This critical $\xi$ value highly depends on $n$, and it decreases with increase in $n$. This underscores the pivotal role of the density of SCNPs in determining their final states, particularly at intermediate or high $\xi$ values.

The results presented in this article are very exciting and demonstrate that lipid vesicles have the potential to be used as an alternative medium for self-assembling SCNPs into ordered nanoclusters. The geometry of the self-assembly can be tuned by changing the density of SCNPs inside the vesicles. Moreover, the aspect ratio, the diameter of the SCNPs, and the size of the vesicle are expected to play vital roles in the self-assembly process. It is worth noting that experimental studies are warranted to validate our results.
Chapter 7

CONCLUSIONS AND FUTURE WORK

This dissertation comprehensively investigates the adhesion mechanisms of spherocylindrical nanoparticles (SCNPs) on lipid membranes and their endocytosis. It also explores the self-assembly of SCNPs on lipid membranes, which has far-reaching implications for numerous advanced applications in nanotechnology and materials science.

The SCNPs are efficiently modeled as a hollow triangular mesh of beads with constant topology. This approach substantially reduces the degrees of freedom associated with the SCNPs, enhancing the efficiency of the study and enabling a more detailed exploration of their behavior. An implicit-solvent model using a coarse-grained approach is implemented to simulate lipid membranes through molecular dynamics simulations. Using the velocity-Verlet method to integrate the equations of motion facilitated the execution of the simulations over more extended time scales than can be achieved from other computational methods. Advanced free energy calculation using the weighted histogram analysis method (WHAM) and a local formulation of the Helfrich Hamiltonian enhanced our understanding of the interactions within the system and the adhesion mechanisms.

The findings of this study added to our fundamental understanding of the adhesion modes and endocytosis of a single SCNP on tensionless planar membranes, revealing two distinct modes of adhesion, namely the parallel mode and the normal mode. The study reveals that a SCNP always adheres to the membrane in the parallel mode, irrespective of its initial orientation with respect to the membrane. Increasing the SCNP’s aspect ratio or the length promotes a transition from the parallel to the normal mode. Further investigations extended to explore the adhesion modes, dimerization, and endocytosis of two SCNPs on lipid membranes revealed five different adhesion behaviors influenced by parameters such as diameter, length, and initial distance of the SCNPs.
Dimerization of the SCNPs is shown to facilitate the endocytosis of SCNPs. Moreover, SCNPs dimerize for lower adhesion values as their aspect ratio increases. Furthermore, an energy barrier between the monomeric and dimeric states at intermediate values of the adhesion strength is much higher than the thermal energy. This implies that the SCNPs dimerize only when they adhere to the membrane at nearby locations. Otherwise, the SCNPs prefer to be distant from each other and adhere in the normal monomeric mode.

One of this dissertation’s most notable contributions is the thorough exploration of the self-assembly of the SCNPs induced by lipid vesicles. The formation of highly ordered nanoclusters with diverse geometries, controlled by the aspect ratio of Janus SCNPs, adhesion energy density, and NP number density, highlights the intricate interplay between these anisotropic nanoparticles and lipid membranes. Moreover, the formation of unique polygonal and quasi-two-dimensional star-like structures formed when SCNPs adhere to the inner side of lipid vesicles adds a novel dimension to our understanding of self-assembly phenomena and paves the way in harnessing the lipids membrane as a tool to induce self-assembly of NPs.

The study of membrane-mediated interactions of Janus SCNPs placed on the surface of a vesicle demonstrated highly ordered nanoclusters. These vesicle-induced ordered nanoclusters highly depend on the number of JSCNPs, and the geometry can be tuned by varying their aspect ratio, $\alpha$. Mutual repulsion among Janus spherocylindrical nanoparticles (JSCNPs) is constantly observed in cases involving two JSCNPs ($n = 2$), regardless of $\alpha$. Increasing the length of the JSCNPs induces elongation of the vesicle in one, two, or all three dimensions, leading to elongated vesicle geometries. For $\alpha \leq 2.5$, the JSCNPs self-assemble into nanocluster geometries resembling various polyhedra, such as equilateral triangles ($n = 3$), disphenoids ($n = 4$), triangular gyroprisms ($n = 6$), pentagonal bipyramids ($n = 7$), and digonal gyrobi anticupolas ($n = 8$). Correspondingly, the vesicle adopts shapes like triangular prisms, disphenoids, triangular gyrotegums, pentagonal prisms, and elongated gyrobufastigium, respectively. Sys-
tematic simulations revealed that the nanocluster geometry undergoes abrupt changes at \( \alpha \gtrsim 3 \), with JSCNPs aligning in specific directions at higher \( \alpha \) values. Remarkably, these SCNPs consistently exhibit symmetrical arrangements within the nanoclusters, a trend further analyzed through calculations of the nematic order parameter, which approaches a value of 1 for \( \alpha > \alpha^* \). Additionally, it was found that for a constant membrane area per JSCNP, the degree of orientation fluctuations decreases as \( \alpha \) increases. The organization within these vesicle-induced nanoclusters is intricately determined by \( n \) and the vesicle’s size, with the effect of \( \alpha \) diminishing as the vesicle’s diameter increases. This arrangement of JSCNPs into ordered nanoclusters is seen even for big vesicles. This research provides valuable insights into the complexities of JSCNP-vesicle interactions and lays the foundation for further exploration in this field.

Lastly, lipid vesicle-induced self-assembly of uniform SCNPs on the inside of a vesicle is investigated. The research systematically explores various adhesion strength values (\( \xi \)) at a fixed aspect ratio of \( \alpha = 1.9 \) and diameter of \( D = 20 \text{ nm} \). For two SCNPs, it is observed that at very low \( \xi \), they exhibit high diffusivity and remain parallel to the membrane, preserving the vesicle’s spherical shape. Intermediate \( \xi \) values prompt a transition to two-dimensional geometry, with SCNPs’ axes becoming coplanar. The degree of coplanarity, however, decreases with a further increase of \( \xi \), ultimately leading to the exocytosis of SCNPs at very high \( \xi \). Additionally, the study reveals cooperative effects as the number of SCNPs, \( n \), increases, with higher \( n \) values leading to chain-like formations at low \( n \) and highly ordered polygonal and star-like two-dimensional structures across a wide range of intermediate values of \( \xi \). Notably, a coexistence region emerges between these geometries at higher \( n \) values, influenced by initial placement and vesicle size. Furthermore, exocytosis of SCNPs occurs at significantly higher \( \xi \) values, with the \( \xi \) value at which the transition occurs decreasing as \( n \) is increased, highlighting the density of SCNPs’ role in determining their final states, especially at intermediate or high \( \xi \) values. This research offers valuable insights into the intricate dynamics of SCNPs’ adhesion within vesicles across varying adhesion strengths and
number density.

The scope of this work opens the door to plenty of future research opportunities. The study primarily focuses on the adhesion of uniform SCNP pairs on planar membranes, limiting our understanding of cooperative behavior in systems involving multiple SCNP pairs. Given the intriguing findings in this dissertation, it is evident that studying the interactions of multiple SCNP pairs, similar to studies on spherical nanoparticles, holds significant potential. Investigating the cooperativity of multiple SCNP pairs on lipid membranes could provide valuable insights into complex biological processes involving proteins and other elongated particles interacting with membranes. Similarly, the dissertation employed a coarse-grained approach, which, while efficient, lacks the details captured by atomistic simulations with explicit solvent. Future research endeavors may consider transitioning to atomistic simulations with explicit solvents, despite the associated dramatic increase in computational time and memory, for a more comprehensive understanding of the kinetics of these complex biological processes. It is worth noting that while the dissertation has presented promising results and valuable theoretical insights, the lack of experimental evidence to validate the findings remains a limitation. This underscores the importance of bridging the gap between theoretical predictions and experimental validation in future research endeavors.

In summary, this dissertation has revealed the critical role of the anisotropy nanoparticles on their adhesion to lipid membranes and highlights the potential for inducing intricate self-assembly of SCNP pairs on lipid vesicles. The scalability and control of nanocluster geometry through adjustments in the SCNP pairs' number and dimensions pave the way for innovative applications in nanotechnology and materials engineering. These findings open new avenues for research and development, with the potential for tailoring SCNP pairs' self-assembly to meet specific needs in a wide range of applications. As the field continues to evolve and mature, the knowledge generated by this dissertation will undoubtedly inspire further studies and innovations in nanomaterial applications and biophysical research in the future.
References


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