Interactions from lipid membrane deformations

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Chapter 1

Introduction

Biological cells are the basic building blocks of all living organisms, from single-celled microorganisms to complex multicellular organisms like humans. Every cell is enclosed by a membrane that not only acts as a barrier but also plays crucial roles through the proteins covering more than half of it. These proteins are peripherally attached or embedded in the fluid membrane, and responsible for a wide range of cellular processes. For these proteins to function effectively on the membrane, their proper organization is essential, as irregularities can lead to diseases. In addition to well-known forces such as electrostatic and hydrophobic interactions, the deformation induced by proteins themselves generates a force. This force acts on the protein due to its deformation on the cell membrane, regardless of the chemical compound involved. Therefore, this is an interesting problem for physicists.

This thesis explores how mimicry of proteins by colloidal particles interacts with and through lipid membranes. Using a combination of experimental, simulation and theoretical methods, we investigate the mechanisms underlying the adhesion and uptake, as well as the deformation-mediated interaction and aggregation of colloids on lipid membranes. Our results demonstrate that the interaction between colloids and lipid membranes strongly depends on the curvature they cause on the membrane. They therefore suggest the significant role of shape in these interactions. We also measure the non-additive interaction of many-body interactions for the first time and compare it to the pairwise interaction, where colloids self-assemble into ordered and disordered structures. In addition, lipid membranes can be useful for assembling colloids into a variety of structures suitable for a range of applications including biomedicine, drug delivery, and nanotechnology. Overall, this thesis provides a comprehensive understanding of the interactions of colloids mediated by lipid membranes, which is essential for a better understanding of membrane-mediated interactions and the development of a range of applications in various fields. In this section, we provide an in-depth background on the significance of lipid membranes and their interactions with colloidal particles. We will also offer an overview of previous research in this field and outline the specific research questions addressed in this thesis, along with the methods employed to investigate them.

1.1 Lipid Membranes

The cell is the fundamental structural and functional unit of all living organisms. It consists of a cytoplasm surrounded by a membrane and houses organelles, numerous biomolecules, proteins, DNA, RNA, and various small molecules such as

metabolites and nutrients [1], see Figure 1.1a. Cells come in various sizes, ranging from 8 to 100 μm depending on their function [2].

Cell membranes, also called plasma membranes (PM), separate cells and their organelles from the internal and external environment. The PM is shared by all living cells, from bacteria to sophisticated human cells, to protect cellular functions. Phospholipid molecules are the main component of the cell membrane, illustrated in Figure 1.1b and c. They are amphiphilic and have a hydrophilic head group and a fatty hydrophobic tail. The head group can be charged or uncharged, depending on the specific phospholipid. The tails are composed of long hydrocarbon chains that are typically saturated or unsaturated, depicted in Figure1.1d, and can vary in length and degree of saturation [3, 4].

The organization of proteins on cell membranes is a crucial aspect of cellular function. Membrane proteins perform a variety of functions, including signal transduction [5], transport [6], and structural support [7]. Proper localization and arrangement of membrane proteins is essential for proper cellular communication, and disruption of this process can lead to a variety of diseases such as Alzheimer's disease [8] and diabetes [9].

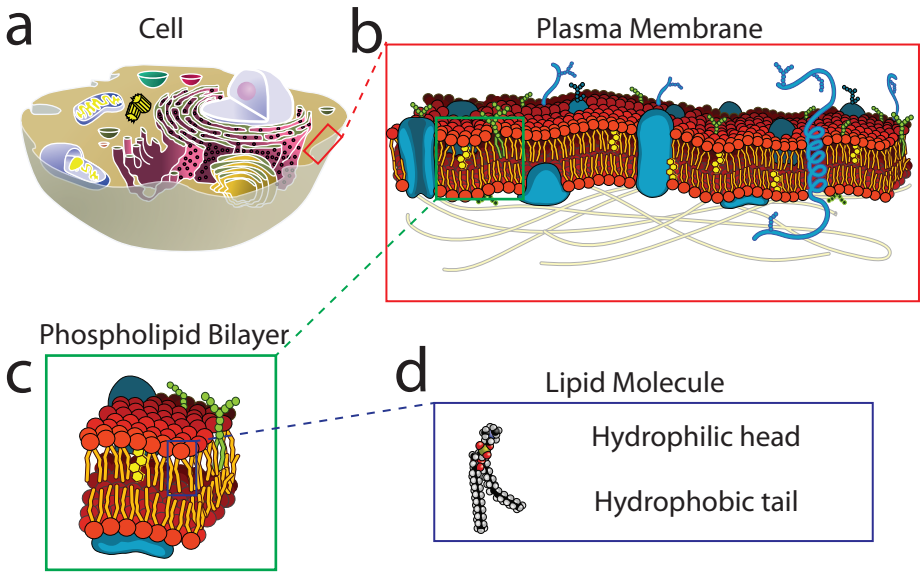

Figure 1.1: Biological cell membrane: (a) a schematic cross-section of a biological cell showing all organelles; (b) a small schematic cross-section of the plasma membrane included transmembrane proteins, peripheral proteins, and glycoproteins; (c) a magnified section through the phospholipid bilayer with phospholipids and cholesterol; (d) a single typical phospholipid molecule with a hydrophilic head and a hydrophobic tail. Illustrations taken from the references [10, 11].

The organization of proteins on cell membranes is highly dynamic and influenced by several factors. These factors include lipid composition [12], protein-protein interactions [13], and cellular signaling pathways. The lipid composition of cell membranes can play a crucial role in protein localization, with specific lipids binding to and stabilizing certain proteins in specific regions of the membrane [14, 15]. Additionally, protein-protein interactions can promote the clustering of proteins in specific domains of the membrane, further influencing their shape and deformation of the membrane [16]. One important form of protein organization on membranes is caused by the membrane-mediated interaction.

There are several types of membrane-mediated interactions between proteins on the cell membrane. One mechanism is interactions mediated by different composition of lipids or other membrane components [12,17]. For example, certain lipids can bind to stabilize specific proteins in specific regions of the membrane, promoting their localization and function [18]. Another important membrane-mediated interaction called curvature-mediated interaction stems from any deformation of cell membrane [19]. Understanding the mechanisms of these interactions is critical for understanding cellular processes and for developing new therapies to treat diseases caused by dysfunction in membrane-mediated protein interactions.

Proteins are stiffer than lipid membranes by a factor of 2 to 400 [20], so they can cause out-of-plane deformation when they are contact with the lipid bilayer [21, 22]. Interestingly, proteins not only shape the membrane, but the membrane can also sort them and promote their aggregation in regions of strong curvatures [23]. Theoretical predictions showed that this membrane-mediated interaction is a long-range interaction that decays with d^{-4}, where d is the distance between objects. This is interesting because most forces such as Van der Waals and even electrostatic interactions have a very short range due to the high salinity of the inner and outer solution.

1.1.1 Lipid membranes in theory

From a theoretical prospective, membranes are often considered as a two-dimensional flat sheets. Since area compression modulus of the membrane is orders of magnitude higher than bending modulus [24] it can be considered incompressible. Because of its fluidity and the fact that the distance between lipid molecules are constant, it is tensionless and has been described by the Helfrich Hamiltonian [25]):

$$\Delta H = \iint dA[\frac{\kappa}{2}\ (c_1 + c_2 - c_0)^2 + \bar{\kappa}\ c_1 c_2] \tag{1.1}$$

where A is membrane area, κ is bending rigidity, c_1 and c_2 are local principle curvatures, c_0 spontaneous curvature, $\bar{\kappa}$ denotes as Gaussian bending rigidity, c_1 and c_2 are depicted in Figure 1.2a. The second term refers to the Gaussian curvature, in which the integral is constant over a closed membrane according to the Gauss-Bonnet theorem.

Even though the surface area is preserved and the energy required to increase the surface area is very high, an effective membrane tension can be defined that relates to the area to volume ratio of the membrane [26]. This effective membrane tension, expressed as the force per unit length acting on a membrane cross-section [27]. It can be measured using various techniques, including thermal fluctuations [28], micropipette aspiration [26], and optical tweezers membrane tethering [29, 30].

In the presence of adhesion between the membrane and an object, bending and adhesion energy will compete. This competition can be described mathematically by taking to account of the adhesion energy term to the Hamiltonian of the system [31]. Hence, assuming the spontaneous curvature is negligible, the simplified Hamiltonian can be written as follows:

$$\Delta H = \iint dA[\frac{\kappa}{2}\ (c_1 + c_2)^2 + \sigma - u_{ad}] \tag{1.2}$$

where σ is effective membrane tension that we will refer to as membrane tension throughout this thesis, and u_{ad} is adhesion energy per unit of area.

1.1.2 Lipid membranes in experiment

To study the cell membrane experimentally, actual cells prove too complex for many purposes. Within cells, organelles, cytoskeletal elements, and various proteins can introduce interference when quantifying membrane deformation-induced interactions. Consequently, researchers often employ model systems, such as giant plasma membrane vesicles (GPMVs) or giant unilamellar vesicles (GUVs). GPMVs represent segments of the cell's plasma membrane, comprising several types of phospholipids, cholesterol, and proteins, while lacking organelles or a cytoskeleton [32, 33]. In contrast, GUVs offer even greater simplicity, typically composed of only one or two types of phospholipids and often containing a sugar solution, making them an excellent choice as a model for the simple cell membrane [34]. A typical GUV is shown in Figure 1.2b in magenta.

Colloidal particles serve as biological materials Colloidal particles, which typically are between 1 and 1000 nm in size, offer unique properties that make them

attractive substitutes for a wide range of biological materials, such as proteins, viruses, and bacteria. Through surface modification, colloidal particles can be engineered to interact with lipid membranes in precise ways. For example, they can mimic the behavior of viruses or bacteria during cell entry (endocytosis) [35,36] and exit (exocytosis) [37]. They perform as protein-deforming membranes and demonstrated membrane-mediated interactions [38,39] or more recently, they have been shown to replicate the activity of cells [40]. These properties make colloidal particles a valuable tool for studying cellular processes in the laboratory setting. An example of a model-system is shown in Figure 1.2b where green colloids are adhered to GUVs.

1.1.3 Lipid membranes in compute simulations

Computer simulation methods have become a valuable tool for studying cell membranes, and better understanding of physical properties, structural organization, and dynamic behavior of cell membranes. Some common simulation methods used in cell membrane research include all atom simulations, molecular dynamics simulations, Monte Carlo simulations, or numerically solving equation 1.1. All atom simulations are computationally expensive [41] Consequently, other coarse-grained techniques are often used to reduce calculation time as well as complexity. The numerical models were built using two main approaches: first, the vesicle membrane was simulated using a one-particle thick bead connected to a tether forming a triangular network [42–45], an example is shown in Figure 1.2c. This could model spherical vesicles with bending rigidity or other properties of GUVs. Second, the lipid membranes were modelled as a flat surface using software such as Surface Evolver, which could simulate different forces and constraints for various shapes [46]. By using this model, a solution for large deformations could be easily obtained [47, 48] which may not be achievable through analytical methods for solving equation 1.1.

1.2 Engulfment of objects

The investigation of colloidal engulfment is of great significance, not only as a model for comprehending endocytosis or nutrient uptake, but also due to the growing use of microparticles in various industrial applications such as in foods, paints, and powders [51] and their uptake and interaction by animals and humans. Engulfment can be broadly classified into active and passive mechanisms. Active components are used to study endocytosis and phagocytosis in cells [52, 53].

However, in order to gain a better understanding of the passive endocytosis mechanism, giant unilamellar vesicles (GUVs) have emerged as a unique model system. GUVs have allowed researchers to identify different pathways for the wrapping of spherical particles [35, 36, 54–58], as shown in Figure 1.3a$_1$, a$_2$, b$_1$, and b$_2$. The binding between GUVs and colloids has been facilitated by a strong ligand-receptor bond [55,56] and a depletion force [57,58]. The ligand-receptor wrapping allowed a specific attachments [59] while it made permanent stickiness of lipid molecules and colloids (Figure 1.3 a$_1$, a$_2$), and controlling adhesion energy was only was possible through varying number of receptors on colloids. The depletion force on the other hand allowed more control on adhesion energy while it applied unspecifically on any objects(Figure 1.3 b$_1$, b$_2$) [57, 58].

This passive wrapping of a sphere has two distinct regimes: in the first regime, where the membrane bending and tension predominate, the particle cannot be fully wrapped by the membrane. In the second regime, where the adhesion energy overcomes the bending and tension in equation 1.2, the particle becomes completely wrapped by the membrane. The critical size of the particles and the adhesion energy for wrapping to occur, and their pathways were simulated in ref. [60] and later calculated in [61–63]. While spherical particles are commonly utilized in studies and applications, other shapes that resemble biological objects have yet to be experimentally examined. Simulations have predicted that an ellipsoidal particle can be wrapped by a vesicle when it is rotated to increase the area from the tip side [64], as depicted in Figure 1.3c. Rotation upon wrapping has also been

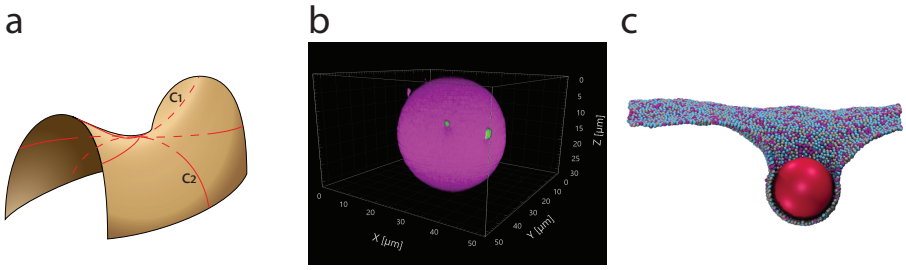

Figure 1.2: Simplified model of lipid membranes in theory, experiment and simulation (a) an illustration of a deformed sheet showing the two principal curvatures as c$_1$ and c$_2$, this illustration is reproduced from ref. [49]; (b) a 3D reconstructed confocal image of a giant unilamellar vesicle composed mainly of a single type of phospholipids (1,2-dioleoyl-sn-glycero-3-phosphocholine), shown in magenta, and colloidal particles functionalized to adhere to the vesicle, in green; (c) A cross section of a simulated vesicle with a tick bead model in blue and deformed by a red colloid, image printed from ref. [50].

observed in nanorods [65, 66], as illustrated in Figure 1.3d. Similarly, it has been discovered that the shape and orientation of nonspherical particles affect their cellular uptake, with cylindrical and ellipsoidal particles oriented perpendicular to the cell surface showing the highest efficiency [48, 67].

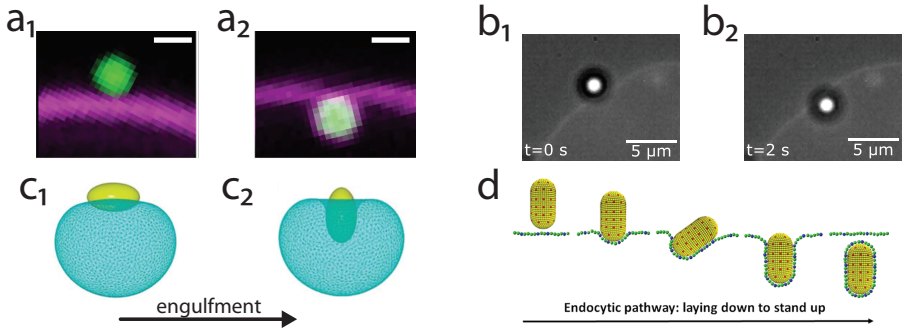

Figure 1.3: Engulfment of colloidal objects by membranes: (a_1) a single sticky sphere adhered to a vesicle; (a_2) a sphere engulfed by a membrane due to low membrane tension and high adhesion energy, scale bar denotes 1 μm. These images are reprinted from ref. [55]; (b_1 and b_2) the dynamic wrapping of a single colloidal sphere by a floppy vesicle, where adhesion energy is created by a depletion force, These images are reprinted from ref. [58]; (c_1 and c_2) The process of engulfment of a prolate ellipsoidal particle obtained through energy minimization of a triangulated vesicle. (c_1)The particle is only partially bound to the membrane, and it adheres preferentially to the weakly curved surface segment. (c_2) if the particle is strongly bound to the membrane, the particle is reoriented such that its strongly curved tip points toward the interior of the vesicle and the wrapped surface fraction is increased. These results were reported in [64]; (d) the dynamic endocytosis of a spherocylindrical particle occurs such that the particle is tilted with respect to the membrane to become fully engulfed, printed from [66] and with permission from The Royal Society of Chemistry.

1.3 Membrane-mediated interaction

In addition to direct forces caused by electric charges or van der Waals interactions, the cell membrane also mediates interactions between objects on the membrane based on their tilt angle or deformation, irrespective of their chemical composition. These interactions have been the subject of intense study by physicists, who have approached the problem using geometric methods wherein the membrane shape and height has been approximated [68–71], or using a field theory approach due to its similarity to gravity in general relativity and the way curvature propagates on the membrane [72]. This type of interaction is depicted in Figure 1.4b. Another kind of interaction occurs when objects perturb the thermal undulation of the fluid membrane and cause an interaction analogous to the Casimir force [73]. This

interaction is also shown in Figure 1.4c. These two types of interactions have led to many interesting results, and have been the focus of research for several decades. In this chapter, these two types of interactions mediated by the cell membrane will be further examined and their implications will be explored.

1.3.1 Fluctuation-mediated forces

Thermal motion of liquids constantly perturbs the membrane, leading to both short and long fluctuation waves on the membrane [74]. Proteins peripherally attached to this membrane affect the waves. It has been found that these proteins interact through this membrane perturbation [73, 75, 76]. Noteworthy, this interaction was bending rigidity invariant [19, 77], as shown in below:

$$U(d) = -6k_BT\frac{a^4}{d^4} \tag{1.3}$$

where d is distance between the objects and a it's radius (see Figure 1.4a).This force also was studied with other methods [78–82] and all found a similar Casimir-like force between sheets [83] and similarly attraction forces between objects adhere to membrane.

1.3.2 Curvature-mediated forces

Interaction of two symmetrical objects: Membrane proteins can locally induce deformation in the membrane. As evident from equation 1.2, the membrane does not naturally favor additional curvature beyond its undeformed state. Proteins are stiffer than the membrane [20], they induce deformation in the membrane, leading to a long-range interaction mediated by the membrane to reduce overall curvature. By solving equation 1.1, Goulian et al. [19, 84] analytically predicted a repulsive force for two cones with radii of a to be equal to:

$$U(d) = 4\pi\kappa(\alpha_1^2 + \alpha_2^2)\frac{a^4}{d^4} \tag{1.4}$$

where κ is bending rigidity, α_1 and α_2 are the contact angles, and d distance between the objects. Equation 1.4 suggests that interactions are consistently repulsive, regardless of whether the objects are on the same side of the membrane or have opposite curvatures when one is upside down, meaning that they have opposite curvature. Membrane tension was a key parameter that was excluded in the first calculation. Including membrane tension, the interaction remains similarly repulsive for two identical cones adhering to one side of the membrane, while cones on opposite sides have been shown to be attractive [85]. Other theories took

other geometric approaches [70, 71] or considered a defect propagating like a field around it and applying effective field theory [72, 86] where all showed repulsive interaction of two symmetric objects. So far all these calculations, the membrane was always considered to be a flat sheet. In the presence of symmetrical defects on a spherical membrane, the interaction was even stronger, although it remained repulsive [87]. Despite all progress, the analytical solutions were valid only for small perturbations of the vesicle because of the taken approximations of the underlying fourth-order nonlinear partial differential equations.

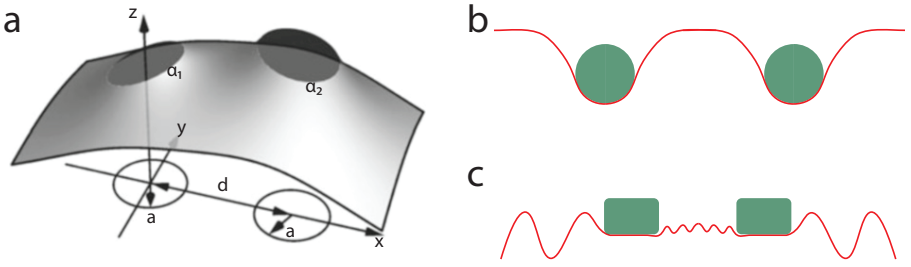

Figure 1.4: Schematic of lipid membrane (a) A calculated shape of a membrane containing two disc-shaped inclusions with contact angles α_1 and α_1. The shape of the membrane analytically defined by its height h with a Monge gauge for the small deformations on a tensionless membrane where height is calculated relative to the plane $z = 0$, while the radii of the inclusions are denoted by a and their centre-to-centre distance by d. The image is taken from [82]. (b) A schematic one-dimensional representation of two membrane deforming objects (c) A schematic one-dimensional representation of the thermal undulations of the membrane disturbed by two objects. Illustration is replicated from [88].

In order to describe curvature-mediated force over a wider range of deformations, a step was taken to develop experimental methods to study larger deformations. To isolate the contributions from deformation-mediated interactions, a model system which consists of giant unilamellar vesicles (GUVs) as a simplified membrane and sticky colloids deforming the membrane were used in experiments [38, 55, 89–91]. The interaction between two spheres fully wrapped by the membrane was observed to be attractive with an maximum strength of $3.3k_BT$ (Figure 1.5a$_1$), while particles that did not deform the membrane did not interact, see Figure 1.5a$_2$ [55]. Another experimental system confirmed the interaction to be attractive however quantified the attractive force to be on the order of 100 k_BT [89]. In the experimental setting, it was challenging to apply controlled and reproducible deformations to lipid membranes so that some particles would adhere readily to the GUVs and others would be completely wrapped by the vesicles [57]. This variation in the area of adhesion of the particles to the GUVs may have been

the reason for the variation in interaction energy.

It is more straightforward to control adhesion in numerical and molecular dynamics simulation. Using these approaches, it was found that attraction is possible in the case of strong nonlinear deformation of particles [92, 93], in contrast to the earlier results obtained for the linearized equation. For two colloids, deformation was found to play a crucial role and could shift interactions from repulsion to attraction [47, 94].

Many-body Interactions: The curvature-mediated interaction is not simply pairwise additive. It is related to the integral over the square of the total curvatures across the entire surface, as expressed in the equation 1.2. Consequently, the prediction of the interaction between three or more particles cannot be deduced directly from the two-body interaction. Interestingly, it has been shown that for many objects on the membrane, this non-pairwise additive interaction enables the formation of a stable cluster despite the assumption of pairwise repulsion [95], see Figure 1.5b. Hexagonal clustering of colloids was found between oppositely charged micro-spheres and for tense membranes, while decreasing the tension disordered the aggregation [39], see Figure 1.5c_1 and c_2.

Anisotropy was found to lead to pairwise attraction at small deformations, and it was shown that nonspherical objects assemble into linear chains, circular and compact structures, egg cartons [96], and aggregates that are in a state similar to a gas phase [97]. Surprisingly, simulations without the assumption of pairwise interactions again resulted in a linear aggregation, where the free energy of a three spheres assembled in a line was lower than that of three spheres arranged in a triangular orientation [98], see Figure 1.5d_1 and d_2. Although there have been some efforts to study the multiple lipid membrane deforming objects, the non-additive and non-pairwise effects of this force have not been extensively studied.

Interaction of anisotropic objects: Similar to isotropic particles, the study of the interaction of anisotropic objects was initially explored with analytical theories in which repulsion was found between two cylinders on a planar membrane with or without lateral tension [68, 99, 100]. A saddle curvature was found to promote an attractive potential between the colloids [72, 86]. For instance, ellipsoidal particles were found to aggregate tip-to tip [101], see Figure 1.6a_1 and a_2 . Other shapes clustered based on their aspect ratio in various forms where particles with a lower aspect ratio arranged in a gas-like phase (Figure 1.6b_1); in an intermediate range they arranged like a ring (Figure 1.6b_2), and finally, at high aspect ratios, a linear aggregation was found, see Figure 1.6b_3 [97].

Simulations and experiments often focused on the crescent-shaped inclusions because they resemble proteins of the BAR family, which are responsible for deforming membranes or sensing curvature in cells [21,102,103]. Studies have shown interesting properties of BAR family proteins which can induce membrane tubes due to their anisotropic shape [104], see Figure 1.6c_1, c_2 and c_3. These proteins can accumulate in regions with higher curvature, for instance, they sense an extruded tube and collect there [105]. In Figure 1.6d_1 and d_3 a collection of I-BAR proteins was attached to the outside of the membrane, and in Figure 1.6d_2 and d_4, they were encapsulated inside the membrane. Because of their accurate shape, they preferentially adhere to the membrane section that matches the shape. Obviously, proteins that adhere to the outside of the vesicle here accumulate on the vesicle and proteins that are added from the inside preferentially accumulate on the tube

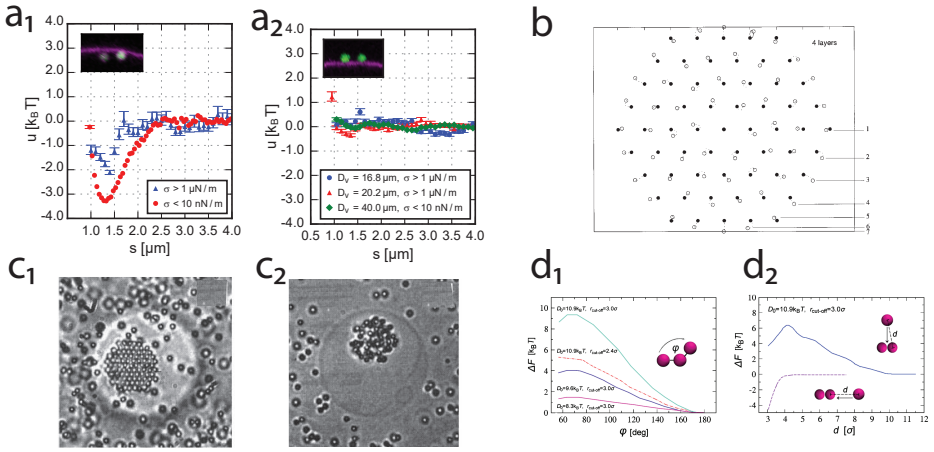

Figure 1.5: Membrane-mediated interaction of symmetric objects: (a_1,a_2) The interaction energy $u(s)$ between two spherical colloids with separation distance s; (a_1) a long-ranging attraction potential was measured between two fully membrane-wrapped particles; (a_2) no significant interaction was found between two particles only adhered to a vesicle in the same experimental setup. (a_1,a_2) printed from [55]. (b) A cluster of 61 proteins assuming pairwise repulsion between proteins deforming a flat tensionless membrane, with the initial position shown as black circles and the final position shown as open circles that shows a stable cluster despite pairwise repulsion. The image taken form [95]. (c_1, c_2) The self-assembly of polystyrene particles on a oppositely charged vesicle. The adhesion between particles and the vesicles was granted by the Coulomb interaction and the pattern is mediated by the vesicle. (c_1) Hexagonal cluster of polystyrene spheres on a tense membrane; (c_2) Disordered cluster of spheres on floppy membrane, this image printed from [39]. (d_1) The angular free-energy profile of three particles, each bound to and wrapped by a membrane (d_2) The free energy varies as a third particle approaches a two particles located at a fixed distance along their long axis (indicated by a dashed line) or perpendicular to it (indicated by a full line), as a function of their separation distance. The image is taken from [98].

region [23]. Under different circumstances, two BAR proteins may form a dimer structure in a parallel orientation, leading to an increase in their concentration. Additionally, multiple BAR proteins may induce significant out-of-plane deformations, as studied in several studies [106–111]. Many studies have attempted to understand the interaction between anisotropic proteins. However, because the individual proteins cannot be seen with optical microscopes, it has not been possible to study the evolution of single anisotropic objects, so a suitable model system is needed.

1.4 Aim and Outline of this thesis

The aim of this thesis is to investigate shape dependence ligand-receptor wrapping of objects by the membrane and quantify interactions of colloids that deforming the lipid membranes and their non-additive effects. The study begins with the quantitative analysis of the engulfment of a dumbbell-shaped particle as an anisotropic object. In subsequent chapters, the thesis delves deeper into understanding the membrane-mediated forces of membrane-deforming colloids and the effects of induced curvature. Furthermore, the thesis uses a new approach to investigate the membrane-mediated interaction between many particles and non-spherical particles. Overall, this work aims to provide valuable insights into the fundamental physics of membrane-mediated interactions and their applications in various fields, including soft matter physics, biophysics, and drug delivery.

In **Chapter 2** we quantitatively studied engulfment of a dumbbell shape particle as a simple anisotropic object. This is the first experimental observation of passive ligand-receptor mediated wrapping of an anisotropic object by a membrane. Subsequently, we have specifically distinguished the wrapping pathways and their dynamics that may contribute to a better understanding of the role of shape in the uptake of nutrients or drugs into cells.

Moving forward, we focus on understanding the membrane-mediated forces between membrane-deforming colloids. In **Chapter 3**, we investigate the arrangement of three membrane-deforming objects. This interaction cannot be predicted solely by knowing the two-body force, therefore we employ a model system consisting of GUVs and sticky spherical colloids wrapped by the membrane. Through several experiments, we quantify the arrangement of three particles and the energy associated with each arrangement. In **Chapter 4**, we probe the effect of induced curvature on membrane-mediated interactions by experimentally investigating the interaction of two particles that induce opposite membrane curvature. Using a similar system with GUVs and sticky spheres, we induce a positive cur-

vature by pulling a tube from the GUV with an optical trap and quantify its interaction with colloids that induce a negative curvature. By controlling the wrapping fraction, we prove that the amount of deformation can significantly alter the membrane-mediated interaction.

To further investigate the curvature-mediated interaction, in **Chapter 5** we

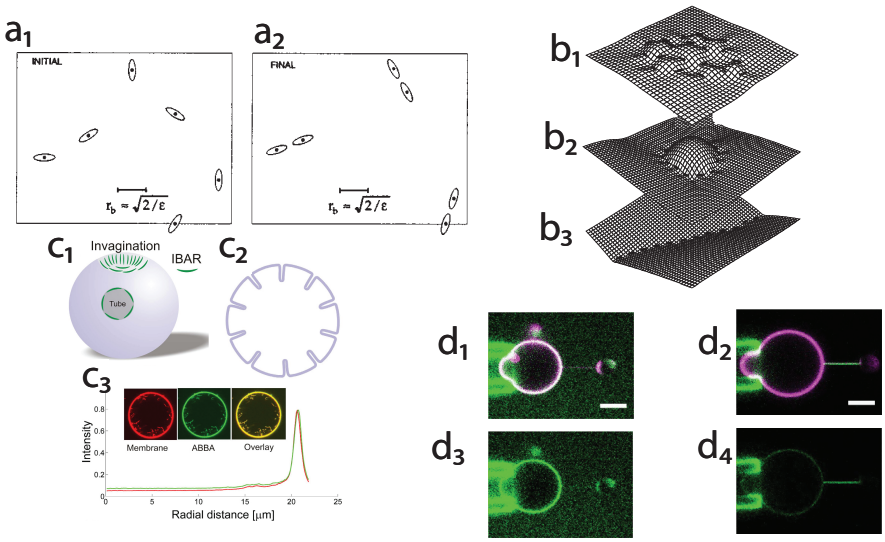

Figure 1.6: Membrane-mediated interaction of anisotropic objects (a_1) Initial configuration of six ellipsoidal inclusions on a membrane (a_2) Final configuration of six ellipsoidal inclusions on a membrane, showing that they aggregate tip by tip. These images were printed from ref. [101] (b_1,b_2,b_3) The final clustering of N=20 identical anisotropic inclusions deforming a flat membrane that equilibrium aggregates were obtained from a Monte Carlo simulation: (b_1) Particles induce zero mean curvature and they aggregate is in a gas-phase; (b_2) Particles induce medium range mean curvature and anisotropic curvature so they aggregate in a ring; (b_3) Particles induce high mean curvature and medium anisotropic curvature and they aggregate in a line; (b_1,b_2,b_3,b_4) are reproduced from ref. [97]. (c_1)Schematic shown that I-BAR proteins which adhere to the outside of a membrane create tubes on the membrane; (c_2) Schematic of the membrane cross-section and extruded tubes; (c_3) A confocal cross section images of a GUV incubated with I-BAR proteins where red shows the GUV, green indicates the proteins and yellow shows the overlay of both. (c_1,c_2,c_3) are taken from ref. [104]. (d_1) A confocal cross-section of a GUV held by micropipette aspiration from the left and a membrane tube pulled by a colloid pulled by an optical trap; the GUV was incubated with I-BAR proteins from the outside of the vesicle, where magenta represents the GUV and proteins are denoted by the green color; (d_2) The same setup as in d_1 but now incubated with I-BAR proteins from the inside of the vesicle, where magenta represents the GUV and proteins are denoted by green; (d_3) Green channel of (d_1) that shows I-BAR most of proteins are collected to the vesicle border when they are added to the outside of the membrane; (d_4) Green channel of (d_2) that shows I-BAR most of proteins are accumulated on the membrane tube when they are added to the inside of the membrane; (d_1,d_2,d_3,d_4) are printed from ref. [23].

develop a new and versatile experimental setup and confirmed it by numerical simulations. Using an attachment-free method, we sandwiched colloids between of a heavy deflated vesicle and a flat substrate. Since particles are stiffer than the vesicle, they induce a deformation, and two colloids can interact through this deformation. We are able to perform a series of experiments between two, three, and many spheres. Subsequently, we precisely roll out the effect of many-body interaction from pair interaction. This thesis ends with **Chapter 6**, which shows the ability of our model system to study membrane-mediated forces between different particle shapes. Through a simple experimental system, we find that the final arrangement and orientation of a cluster includes ellipsoids, tetrahedra, dumbbells, crescents, cubes, and scalene triangles based on the initial configuration.