

Simulating Membranes, Vesicles, and Cells

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Contents

1	Introduction	2
2	Membrane Models and Simulation Techniques	5
2.1	Coarse-Grained Membrane Models	5
2.2	Solvent-Free Membrane Models	6
2.3	Dynamically-Triangulated Surfaces	8
2.4	Meshless Membrane Models	10
2.5	Calculating Shapes—from Vesicles to Shells	12
2.6	Modeling Hydrodynamics	14
3	Applications	16
3.1	Self-Assembly of Micelles and Bilayers	16
3.2	Vesicle Formation	17
3.3	Thermal Membrane Fluctuations	18
3.4	High-Genus Vesicles and Gaussian Saddle-Splay Modulus	20
3.5	Complex Membranes, Inclusions, and Bud Formation	22
3.6	Vesicles in Capillary Flow	24
3.7	Red Blood Cells – Membrane Fluctuations	26
3.8	Red Blood Cells in Flow	29
4	Conclusions and Outlook	31

1 Introduction

Amphiphilic molecules, in particular lipids, are the basic structural element of the membranes in biological cells. A biomembrane is typically composed of many different lipids, which give a cell many opportunities to control membrane properties by adjusting the membrane composition. This can modify the spontaneous curvature and the bending rigidity, and even lead to phase separation and domain formation of lipids. In addition, a biological membrane contains a large number of trans-membrane proteins, which control the exchange of water, ions, and small molecules between the cell plasma and the extracellular space.

Vesicles are cells stripped down to the minimum, a membrane enclosing a fluid volume. Vesicles are therefore ideal model systems to investigate the physical properties of many components of cells in isolation, without the full complexity of the cellular machinery. Because the systems are well-defined, their properties can be analyzed and studied much more easily from a theoretical perspective. Although vesicles are relatively simple, they are still complex many-body systems. Simulations therefore play a very important role in elucidating their equilibrium and dynamic properties. Here, simulation approaches range from the molecular scale, where the properties of lipids and membrane proteins are studied, over the supramolecular scale, where the self-assembly of lipids and their phase-behavior can be investigated, to the vesicle scale, where shapes and deformations due to external forces and fluid flow are studied. Simulations are also important as the focus of the research is shifting from highly simplified to biologically more relevant multi-component systems. An important example is red blood cells, which have a cortical spectrin cytoskeleton attached to lipid-bilayer membrane inside the cell. This gives the membrane a shear modulus. Moreover, red blood cells do not fluctuate only due to thermal motion, but their fluctuations also have an active, metabolic component.

Because the physical effects in membranes cover a large range of relevant length- and time-scales — from the quantum-mechanical behavior of motions in a single molecule and the hydrogen-bonds between different molecules to the hydrodynamic behavior of vesicles and cells — that no single computer model can capture them all, compare Fig. 1.

Therefore, several different models, which are suitable to study phenomena on a smaller range of length scales as illustrated in Fig. 2, have been developed over the last decades:

- **Microscopic Membrane Models** — On the microscopic scale, *all-atom simulations* are required, in which the positions of the atoms of all molecules as well as the interactions between them are modeled explicitly. The interactions are sometimes treated quantum-mechanically, but are modeled in most cases using classical force fields. This requires the development of reliable force fields that allow a quantitative description of specific structural properties of lipid bilayers, such as area per lipid, volume per lipid, bilayer thickness, order parameter for their tail orientation, and headgroup hydration. Several such force fields have been developed and tested in recent years [4,5]. However, Molecular Dynamics simulations of such models are restricted to a few hundred lipid molecules. All-atom simulations are indispensable whenever the chemical structure of the participant molecules is relevant for the phenomena under investigation. For example, the functioning of membrane proteins that act as an ion pumps can only be understood on the basis of such atomistic models.
- **Coarse-Grained Membrane Models** — If the detailed chemical structure is not relevant

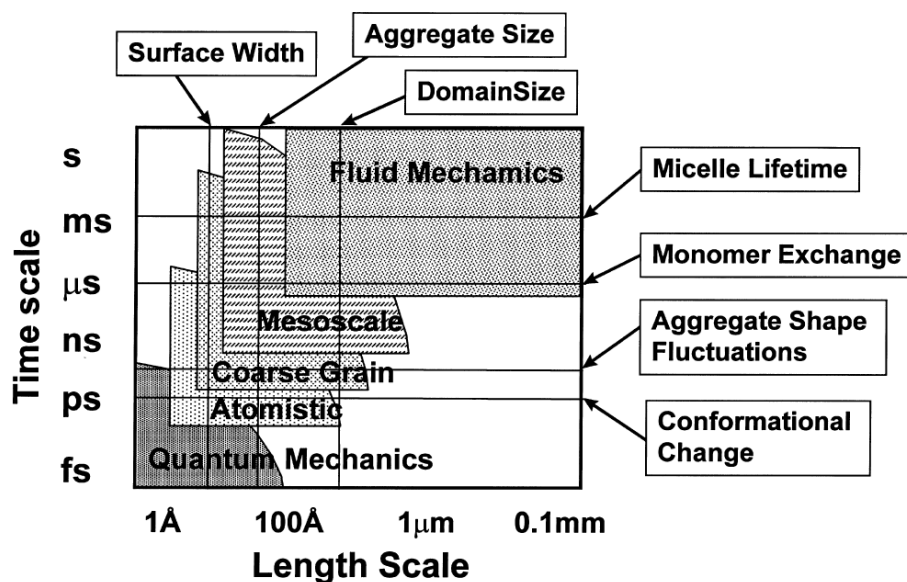


Fig. 1: Characteristic time and length scales in amphiphile solutions. Physical phenomena occurring at the various scales are indicated. Different models and simulation techniques are required to capture the behavior at different scales. Their approximate ranges of validity are shown by the shaded regions. From Ref. [1].

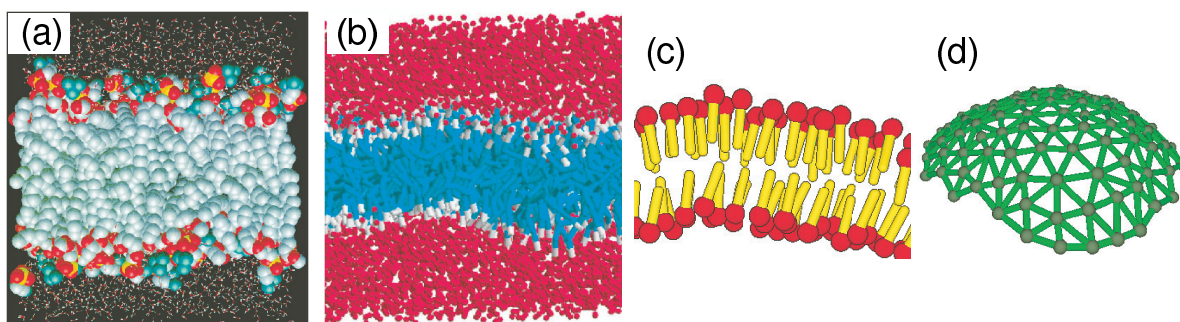


Fig. 2: Membranes models on different length scales. (a) Atomistic model (from Ref. [2]), (b) coarse-grained model (from Ref. [3]), (c) solvent-free bilayer model, and (d) triangulated surface model. Note that the characteristic length in these models is (a) a few Ångstroms, (b),(c) a few nanometers (from Ref. [?]), and (d) tens to hundreds of nanometers (from Ref. [?]).

but more generic properties of amphiphilic molecules are to be studied — like the number of hydrocarbon tails, the chain length of the tails, or mixtures of two different amphiphiles — then a *coarse-grained description* can be used, in which several atoms are lumped into a single unit. These units are typically taken to be Lennard-Jones spheres. In such a model, water becomes a Lennard-Jones fluid with attractive interactions, and amphiphilic molecules become short polymer chains with two kinds of monomers, with attractive or repulsive interactions with the solvent particles and the other monomers [6–8]. The size of such a bead is of the order of a few water molecules or CH_2 groups. Very similar models, with Lennard-Jones interactions replaced by linear “soft” potentials, have also been employed intensively in dissipative-particle dynamics (DPD) simulations [9–13].

The coarse-grained modeling can be taken one step further by taking into account the different chemical nature (and electrical charge) of various head- and tail-groups [14, 15]. This allows to go from a more qualitative to a more quantitative description of membrane properties. Such models allow Molecular Dynamics simulations of few thousand lipids and make it possible to study the formation, structure, and dynamics of small phospholipid vesicles [14, 16].

- **Solvent-Free Membrane Models** — The solvent in a coarse-grained model is required for two reasons. First, it is necessary to stabilize the bilayer structure due to the repulsion between the solvent and the amphiphile tails. Second, it mediates hydrodynamic interactions between different parts of the membrane. However, the simulation of the motion of solvent particles consumes a large fraction of the total simulation time. Therefore, *solvent-free membrane models* have been designed, which work as well as the models with solvent when structural and thermodynamic properties are investigated. Additional interactions between amphiphiles have to be introduced in this case in order to mimic the hydrophobic interactions with the solvent [17–21]. This approach is advantageous in the case of membranes in dilute solution, because it reduces the number of molecules by orders of magnitude. However, the basic length scale is still on the order of magnitude of the size of the amphiphilic molecules.
- **Triangulated Surface Models** — The natural length scale of the previous two classes of membrane models is the size of the head group of a lipid molecule, i.e. roughly 1 nm. This is far too small to describe phenomena on the scale of giant vesicles or cells, which have a diameter on the order of $10\ \mu\text{m}$. In this case, a *continuum description* on the level of *elasticity theory* is required. The building blocks in such models are membrane patches consisting of hundreds or thousands of lipid molecules. In order to make this model amenable to computer simulations, *dynamically-triangulated surfaces* are often employed [22, 23]. The main idea here is to connect membrane “nodes” (or “vertices”) by a triangular network of bonds. The bond potentials are chosen such as to achieve a homogeneous distribution of vertices on the membrane. For polymerized membranes, a fixed connectivity represents the unbreakable bonding between neighboring molecules, and implies a shear elasticity of the membrane. For fluid membranes, the network itself has to be dynamic in order to account for possible “flow” of lipids within the membrane.
- **Meshless Membrane Models** — A different approach to discretize elasticity theory of a two-dimensional surface embedded in three-dimensional space is to employ an ensemble of membrane nodes without connecting them to form a triangulated mesh. Meshless membrane models use instead pairwise and multi-particle interactions to (i) achieve

a roughly homogeneous density of nodes on the membrane, and (ii) to favor smoothly curved membrane conformations [24]. The advantage of meshless membrane models is that open boundaries, which occur for example in membrane rupture, and topology changes, like in vesicle fusion, can be very easily described.

2 Membrane Models and Simulation Techniques

2.1 Coarse-Grained Membrane Models

When the detailed chemical structure of the amphiphilic molecules is not important, a coarse-grained modeling is very useful, where groups of several atoms or molecules are described by only a single position vector. This is important, since it

- reduces the number of degrees of freedom, and therefore allows either to study the system over a longer time range, or to study larger system sizes, or both;
- emphasizes the universal aspects, which are common to many different amphiphilic systems, independent of the detailed chemistry of a particular system.

In coarse-grained models, the solvent molecules are usually treated as spherical particles with attractive Lennard-Jones interactions,

$$U_{LJ}(r) = 4\epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right], \quad (1)$$

where σ is the (effective) hard-core radius. The amphiphilic molecules are modeled as short polymeric chains with head (H) and tail (T) particles, so that neighboring particles in the chain interact via the harmonic-spring potential

$$U_{chain}(r) = k_{chain}(r - \sigma)^2. \quad (2)$$

Different geometries of amphiphilic molecules are shown in Fig. 3. Head and head particles attract each other with the Lennard-Jones potential (1), as well as head and solvent particles. However, the tail particles have a *repulsive* interaction with both the head and the solvent particles. This interaction can be conveniently described by a shifted and truncated Lennard-Jones potential

$$U_{LJ}(r) = \begin{cases} 4\epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right] + \epsilon & \text{for } r < 2^{1/6}\sigma \\ 0 & \text{otherwise} \end{cases}$$

which has the advantage of being continuous and differentiable at the cutoff $r = 2^{1/6}\sigma$. In this model, the Newton's equation of motion for all particle position can be solved by a molecular dynamics simulation employing the velocity-Verlet algorithm [25].

An alternative approach to simulate coarse-grained membrane models is dissipate-particle dynamics (DPD) [9, 11, 26]. An introduction into the DPD simulation technique can be found in Sec. 2.6. In this case, the Lennard-Jones interactions between particle species are replaced by the conservative forces

$$\mathbf{F}_{ij}^C = a_{ij} (1 - r_{ij}/r_0) \hat{\mathbf{r}}_{ij} \quad (3)$$

for distances $r_{ij} < r_0$ and zero otherwise. All conservative forces are taken to be *repulsive*. Water is slightly repelled from the amphiphile head, and is strongly repelled from the amphiphile

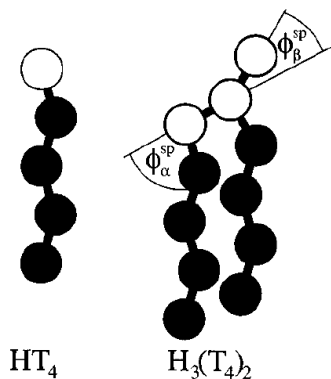


Fig. 3: Typical amphiphilic molecules used in coarse-grained membrane models. The white particles (H) represent the head group, the black particles (T) the tails. From Ref. [6].

tail, which provides the hydrophobic interaction needed to form bilayers. The amphiphile head is hydrophilic and therefore repelled somewhat from its tail.

The coarse-grained membrane approach has been used to address a variety of questions recently, *inter alia* membrane self-assembly and structure [6–9, 12, 27, 28], the spectrum of thermally excited membrane fluctuations [7, 8], phase diagrams of lipid bilayers [29], pore formation in membranes [30–32], domain-formation in multi-component membranes [11], and membrane fusion [14, 26, 33–35].

2.2 Solvent-Free Membrane Models

Simulations of lipid membranes by molecular dynamics require the calculation of the motion of a large number of water molecules in addition to the lipid molecules. To simulate a small patch of a *flat* membrane with an atomistic model, about 30 water molecules per lipid were found to be sufficient [2]. However, much more water molecules are needed for simulations of vesicles, since the formation of a vesicle [see Sec. 3.2] needs a lot of solvent to prevent membrane interactions through the periodic boundary conditions of the simulation box. Similarly, self-assembly of amphiphilic molecules in dilute solutions also requires a lot of water molecules.

In solvent-free models, the solvent is not taken into account explicitly. Instead, the hydrophobic effect is treated by an effective potential between amphiphilic molecules. This reduces the numerical cost of membrane simulations significantly. In particular, the solvent-free model is more efficient for simulations which require a large solvent space. A similar approach is also frequently used in simulations of protein folding.

The first solvent-free model was proposed by Drouffe *et al.* [36]. In this model, a lipid-bilayer membrane consists of a single layer of particles. The characteristic length scale is thus the same as for triangulated-membrane models and the meshless-membrane models discussed in Secs. 2.3 and 2.4 below, respectively – both of which are indeed solvent-free models also. The particles in Ref. [36] possess an orientational degree of freedom and interact with each other via three potentials: a soft-core repulsion, an anisotropic attraction, and a hydrophobic multibody interaction. Particles have been shown to self-assemble into membrane patches and vesicles [36]. Recently, solvent-free models have also been developed to describe bilayer membranes, where the two monolayers are taken into account explicitly [18, 19, 21, 37, 38]. There are several variations of such bilayer models. An amphiphilic molecule is typically modeled as a rigid

or flexible chain, which consists of one hydrophilic segment and two or three hydrophobic segments. The molecules interact with each other with pairwise [19, 21, 38] or multibody [18, 36, 37] potentials. One common feature is the requirement of an attractive potential between hydrophobic segments.

We introduce here one of the bilayer models [37] in more detail. An amphiphilic molecule is modeled as one hydrophilic segment ($j = 1$) and two hydrophobic segments ($j = 2, 3$), which are separated by a fixed distance σ and are fixed on a line. Amphiphilic molecules ($i = 1, \dots, N$) interact via a repulsive soft-core potential U_{rep} and an attractive “hydrophobic” potential U_{hp} , so that the total interaction potential is given by

$$U_{\text{am}} = \sum_{i \neq i'} U_{\text{rep}}(\sigma, |\mathbf{r}_{i,j} - \mathbf{r}_{i',j'}|) + \sum_{j=2,3} U_{\text{hp}}(\rho_{i,j}) \quad (4)$$

with

$$U_{\text{rep}}(r_0, r)/\varepsilon = \exp\{-20(r - r_0)/\sigma\}. \quad (5)$$

The multibody “hydrophobic” interaction is mimicked by a function of the local density of hydrophobic particles,

$$\rho_{i,j} = \sum_{i \neq i', j'=2,3} w_\rho(|\mathbf{r}_{i,j} - \mathbf{r}_{i',j'}|), \quad (6)$$

with the weight function $w_\rho(r) = 1/[\exp\{20(r/\sigma - 1.9)\} + 1]$. Thus, $\rho_{i,j}$ is the number of hydrophobic segments in a sphere with a radius of approximately 1.9σ . The multi-particle potential $U_{\text{hp}}(\rho)$ is then defined by

$$U_{\text{hp}}(\rho)/\varepsilon = \begin{cases} -0.5\rho & (\rho < \rho^* - 1) \\ 0.25(\rho - \rho^*)^2 - c & (\rho^* - 1 \leq \rho < \rho^*) \\ -c & (\rho^* \leq \rho) \end{cases}, \quad (7)$$

where $c = 0.5\rho^* - 0.25$. The values $\rho^* = 10$ and 14 are used for $j = 2$ and 3 (the hydrophobic segments), respectively. At low density ($\rho < \rho^* - 1$), $U_{\text{hp}}(\rho)$ acts as pair-wise potential $-\varepsilon w_\rho(r)$. It is assumed that at $\rho > \rho^*$, the hydrophobic segments are shielded by hydrophilic segments from contact with the solvent molecules and hydrophilic segments of other lipids. Thus, $U_{\text{hp}}(\rho)$ is constant at higher density ($\rho \geq \rho^*$). A similar “hydrophobic” potential is used in other solvent-free membrane and protein models. This multibody potential is employed in order to enhance the molecular diffusion in the membrane and to obtain a wide range of stability of a fluid phase.

A fluid membrane can also be obtained in models with pair-wise attractive potentials only [19, 21, 38]. However, the use of density-dependent potentials seems to be advantageous in obtaining a wide parameter range where the membrane is fluidic.

Meshless membrane models can be studied using Brownian Dynamics and Monte Carlo simulations. In Brownian Dynamics simulations, the motion of the j -th segment of the i -th molecule follows the underdamped Langevin equation with the constraint of a rigid molecule:

$$m \frac{d^2 \mathbf{r}_{i,j}}{dt^2} = -\zeta \frac{d\mathbf{r}_{i,j}}{dt} + \mathbf{g}_{i,j}(t) - \frac{\partial U}{\partial \mathbf{r}_{i,j}}, \quad (8)$$

where m and ζ are the mass and the friction constant of the segments of molecules, respectively. $\mathbf{g}_{i,j}(t)$ is a Gaussian white noise and obeys the fluctuation-dissipation theorem.

A major advantage of meshless membrane models over dynamically triangulated surfaces is that topological changes are easily possible. However, because the model does not explicitly take into account solvent molecules, the volume of a vesicle cannot be kept constant. This is a disadvantage of this type of models. Also, hydrodynamic interactions are not present. However, these interactions can be taken into account by combining a solvent-free model with a mesoscopic solvent technique such as multi-particle collision dynamics (MPC) or dissipative particle dynamics (DPD), as explained in Sec. 2.6.

The solvent-free bilayer models have been applied to a variety of phenomena, such as membrane fusion and fission [17, 18, 39, 40], pore formation in membranes [19, 41], the adhesion of a nanoparticle [39], the fluid-gel phase transition [38, 41], phase separation of lipids [21], protein inclusion in membrane [38], and DNA-membrane complexes [42].

2.3 Dynamically-Triangulated Surfaces

The simulation of membranes and vesicles with characteristic sizes on the order of 100 nm to 10 μm is impossible on the basis of a molecular model, since it would require an enormous number of lipid (and solvent) molecules. Therefore, on this coarse-graining level, a model is necessary in which the individual lipid molecules are no longer “visible”. Instead, the membrane is described by a mathematical surface with an elastic energy which is most appropriate on these mesoscopic length scales [43–45]. The shapes and fluctuations of the membrane are controlled by the curvature elastic energy [46, 47]

$$\mathcal{H}_{\text{curv}} = \int dS [\gamma + 2\kappa(H - C_0)^2 + \bar{\kappa}K + \dots], \quad (9)$$

where the integral extends over the whole membrane surface. The shape of the membrane is expressed by the two principal curvatures c_1 and c_2 — the two eigenvalues of the curvature tensor [48] — at each point of the membrane, which appear in the Hamiltonian (9) as

$$H = [c_1 + c_2]/2, \quad K = c_1 c_2, \quad (10)$$

the mean and Gaussian curvatures, respectively. The parameters of the curvature energy are the surface tension γ , the bending rigidity κ , the saddle-splay modulus $\bar{\kappa}$ and the spontaneous curvature C_0 . These elastic constants of the membrane are the only place where the chemistry, the molecular architecture and the interactions of the constituent lipid and protein molecules enter into this model.

In order to make this model suitable for simulations, the continuous surface has to be approximated by a network of vertices and bonds, see Fig. 4. A triangular network is usually used because it provides the most homogeneous and isotropic discretization of the surface [22]. The simplest potential for the interaction of vertices which are connected by bonds is a *tethering potential*,

$$V(r) = \begin{cases} 0 & \text{if } r < \ell_0 \\ \infty & \text{otherwise} \end{cases} \quad (11)$$

which causes the particles to behave as tethered by a string.

When hard spheres of diameter σ_0 are placed on the vertices, and the bond lengths ℓ_0 are restricted to be $\ell_0 \leq \sqrt{3}\sigma_0$, the surface is *self-avoiding*, since an arbitrary sphere does *not* fit through the holes of the network, so that no interpenetration of different parts of the network is possible.

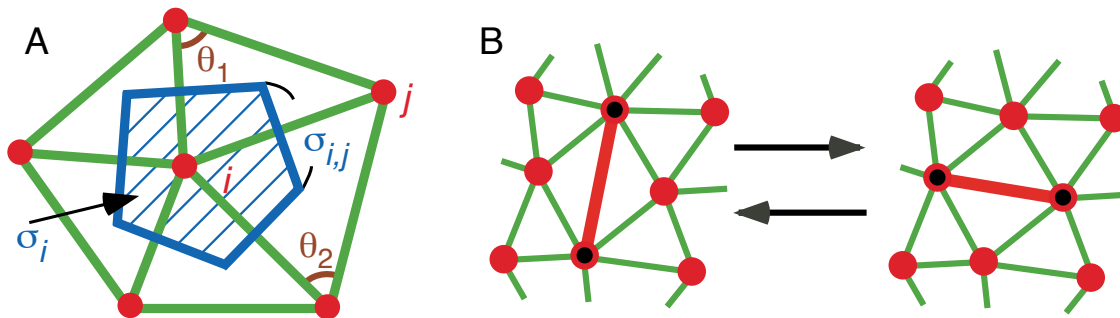


Fig. 4: *Geometry of triangulated surfaces. (A) Bond length and dual lattice, see text. (B) Bond flip in dynamic triangulations.*

The energy, which appears in the Boltzmann weight, is the curvature energy, which can be discretized in different ways [22, 49]. The most commonly used form is [50, 51]

$$E_b = \lambda_b \sum_{\langle ij \rangle} (1 - \mathbf{n}_i \cdot \mathbf{n}_j) \quad (12)$$

where $\mathbf{n}_i$ and $\mathbf{n}_j$ are the normal vectors of neighboring triangles, and the sum runs over all pairs of neighboring triangles. The coupling constant λ_b in Eq. (12) is related to the bending rigidity and saddle-splay modulus by $\kappa = \sqrt{3}\lambda_b/2$ and $\bar{\kappa} = -\kappa$, respectively [49, 52].

The discretization (12) of the curvature energy is not without problems, as discussed in Ref. [49]. In particular, the discretization [49, 53]

$$E_b = \frac{\kappa}{2} \sum_i \frac{1}{\sigma_i} \left\{ \sum_{j(i)} \frac{\sigma_{i,j} \mathbf{r}_{i,j}}{r_{i,j}} \right\}^2 \quad (13)$$

has been found to give reliable results in comparison with the continuum expression (9). Here, the sum over $j(i)$ is over the neighbors of a vertex i which are connected by tethers. The bond vector between the vertices i and j is $\mathbf{r}_{i,j} = \mathbf{r}_i - \mathbf{r}_j$, and $r_{i,j} = |\mathbf{r}_{i,j}|$, as illustrated in Fig. 4. The length of a bond in the dual lattice is $\sigma_{i,j} = r_{i,j}[\cot(\theta_1) + \cot(\theta_2)]/2$, where the angles θ_1 and θ_2 are opposite to bond ij in the two triangles sharing this bond. Finally, $\sigma_i = 0.25 \sum_{j(i)} \sigma_{i,j} r_{i,j}$ is the area of the dual cell of vertex i .

Vesicles with the energy above can be modeled using Monte Carlo method, where one step consists of a random displacement of a randomly selected vertex. This step is accepted with the probability determined by the Boltzmann weight, see Eq. (12), as long as the vertices remain within the maximum bond lengths with their neighbors. In molecular dynamics simulations, smooth bond potentials are usually employed, see Ref. [54].

Polymerized Membranes — Membranes, in which neighboring particles are chemically linked together are called polymerized. Examples of such membranes are the graphite monolayers which are found in fullerenes, or the polymer network attached to the inside of the lipid membranes of red blood cells.

Triangulated surface models for polymerized membranes have first been suggested and studied in the 1980s using Monte Carlo [50, 51, 55, 56] and Molecular Dynamics [54] simulations. Since then, the properties of triangulated surfaces of fixed triangulation have been investigated intensively, see e.g. the reviews of Ref. [22, 23].

Fluid Membranes — For a study of fluid membranes, the connectivity of the membrane vertices cannot remain fixed during the simulation, because otherwise a diffusion of vertices within the membrane is not possible. Therefore, dynamically triangulated surfaces [57–59] have to be used in this case. The essential step of the dynamic triangulation procedure is shown in Fig. 4B. Among the four vertices of two neighboring triangles, the “diagonal” bond is switched from one of the two possible positions to the other. This bond-switching is only allowed if the vertices remain connected to at least three neighbors after the switch. Also, the distance between the newly connected vertices has to be smaller than the maximum bond length. This bond flip has the advantages that [22, 23]

- it is local, i.e. only the vertices of two neighboring triangles are involved, and
- it guarantees that the network retains its two-dimensional connectivity during the whole simulation run. Dynamically triangulated network models of fluid membranes have been applied in recent years to investigate a variety of systems and phenomena, such as phase separation and budding of two-component vesicles [11, 60–62], vesicles with membranes containing curvature-inducing nematogens and membrane tubulation [63, 64], defect scars on flexible vesicles with crystalline order [65], the conformation of charged vesicles [66], particle adhesion to vesicles [67–69], complex formation between a mixed fluid vesicle and a charged colloid [70], vesicle adhesion to surfaces [71], sponge phases [72, 73], and vesicles in shear [74, 75] and capillary flows [76, 77].

2.4 Meshless Membrane Models

The membrane conformation is described by the positions of N particles, which are the membrane “nodes”. These particles either have no internal degrees of freedom [78] or can be characterized by an orientation vector [36]. Models of membrane particles with orientation vector are very similar in spirit to the solvent-free models described in Sec. 2.2 above. Therefore, we focus here on meshless membrane models with particles without internal degrees of freedom, which can be understood as meshless discretization of the curvature energy. The model is well suited to study, for example, vesicle dynamics accompanied by topological changes.

In the model of Ref. [78], the membrane particles interact with each other via a potential

$$U = \varepsilon(U_{\text{rep}} + U_{\text{att}}) + k_{\alpha}U_{\alpha}, \quad (14)$$

which consists of a repulsive soft-core potential U_{rep} with a diameter σ , an attractive potential U_{att} , and a curvature potential U_{α} . All three potentials only depend on the positions $\mathbf{r}_i$ of the particles. The curvature potential is based on the moving least-squares (MLS) method [79, 80]. We briefly outline here the essential aspects of this simulation technique

The MLS method is a least-squares fit of the membrane shape, weighted locally around each particle [78–80]. A Gaussian function is employed as a weight function [78]

$$w_{\text{mls}}(r_{i,j}) = \begin{cases} \exp\left(\frac{(r_{i,j}/\sigma)^2}{(r_{i,j}/r_{\text{cc}})^n - 1}\right) & (r_{i,j} < r_{\text{cc}}) \\ 0 & (r_{i,j} \geq r_{\text{cc}}) \end{cases} \quad (15)$$

where $r_{i,j}$ is the distance between particles i and j . This function is smoothly cut off at $r_{i,j} = r_{\text{cc}}$. Here, the parameters $n = 12$ and $r_{\text{cc}} = 3\sigma$ have been employed.

In the first-order MLS method, a plane is fitted locally to the particle positions by minimizing

$$\Lambda_1(\mathbf{r}_i) = \frac{1}{w_0} \sum_j \{\mathbf{n}(\mathbf{r}_j - \mathbf{r}_0)\}^2 w_{\text{mls}}(r_{i,j}), \quad (16)$$

where the sum is over all points (including i itself) and $w_0 \equiv \sum_j w_{\text{mls}}(r_{i,j})$ is a normalization factor. The normal vector $\mathbf{n}$ of the plane and the point $\mathbf{r}_0$ on the plane are fitting parameters. The minimum of Λ_1 is given by $\Lambda_1^{\text{min}} = \lambda_1$ when $\mathbf{r}_0$ is the weighted center of mass $\mathbf{r}_G = \sum_j \mathbf{r}_j w_{\text{mls}}(r_{i,j})/w_0$ and $\mathbf{n}$ is collinear with the eigenvector $\mathbf{u}_1$ of the lowest eigenvalue λ_1 of the weighted gyration tensor, $a_{\alpha\beta} = \sum_j (\alpha_j - \alpha_G)(\beta_j - \beta_G)w_{\text{mls}}(r_{i,j})$, where $\alpha, \beta = x, y, z$ and $\lambda_1 \leq \lambda_2 \leq \lambda_3$.

We now define the degree of deviation from a plane, the aplanarity, as

$$\alpha_{\text{pl}} = \frac{9D_w}{T_w M_w} = \frac{9\lambda_1\lambda_2\lambda_3}{(\lambda_1 + \lambda_2 + \lambda_3)(\lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1)}, \quad (17)$$

where D_w and T_w are determinant and trace of the weighted gyration tensor, respectively, and M_w is the sum of its three minors, $M_w = a_{xx}a_{yy} + a_{yy}a_{zz} + a_{zz}a_{xx} - a_{xy}^2 - a_{yz}^2 - a_{zx}^2$.

The aplanarity α_{pl} takes values in the interval $[0, 1]$ and represents the degree of deviation from a plane. This quantity acts like λ_1 for $\lambda_1 \ll \lambda_2, \lambda_3$, since $\alpha_{\text{pl}} \simeq 9\lambda_1/(\lambda_2 + \lambda_3)$ in this limit. Therefore, the curvature potential is defined as

$$U_\alpha = \sum_i \alpha_{\text{pl}}(\mathbf{r}_i), \quad (18)$$

where $\alpha_{\text{pl}}(\mathbf{r}_i) = 0$ when the i -th particle has two or less particles within the cutoff distance $r_{i,j} < r_{\text{cc}}$. This potential increases with increasing deviation of the shape of the neighborhood of a particle from a plane, and favors the formation of quasi-two-dimensional membrane aggregates.

The particles interact with each other in the quasi-two-dimensional membrane surface via the potentials U_{rep} and U_{att} . These interaction potentials are necessary to obtain a homogeneous particle density in the membrane plane, and to avoid the membrane from rupturing and falling apart [78]. The particles have an excluded-volume interaction via the repulsive potential

$$U_{\text{rep}} = \sum_{i < j} \exp(-20(r_{i,j}/\sigma - 1) + B)f_{\text{cut}}(r_{i,j}/\sigma), \quad (19)$$

with a cutoff function [78]

$$f_{\text{cut}}(s) = \begin{cases} \exp\{A(1 + \frac{1}{(|s|/s_{\text{cut}})^n - 1})\} & (s < s_{\text{cut}}) \\ 0 & (s \geq s_{\text{cut}}) \end{cases} \quad (20)$$

The factor A in Eq. (20) is determined such that $f_{\text{cut}}(s_{\text{half}}) = 0.5$. In Eq. (19), the parameters $n = 12$, $A = 1$, and $s_{\text{cut}} = 1.2$ were used in Ref. [78].

The attractive interaction mimics the “hydrophobic” interaction. U_{att} is a potential of the local density of particles,

$$\rho_i = \sum_{j \neq i} f_{\text{cut}}(r_{i,j}/\sigma), \quad (21)$$

with the parameters $n = 12$ and $s_{\text{cut}} = s_{\text{half}} + 0.3$ in f_{cut} . Here, ρ_i is the number of particles in a sphere whose radius is approximately $r_{\text{att}} = s_{\text{half}}\sigma$. The density-dependent attractive potential U_{att} is given by

$$U_{\text{att}} = \sum_i 0.25 \ln[1 + \exp\{-4(\rho_i - \rho^*)\}] - C, \quad (22)$$

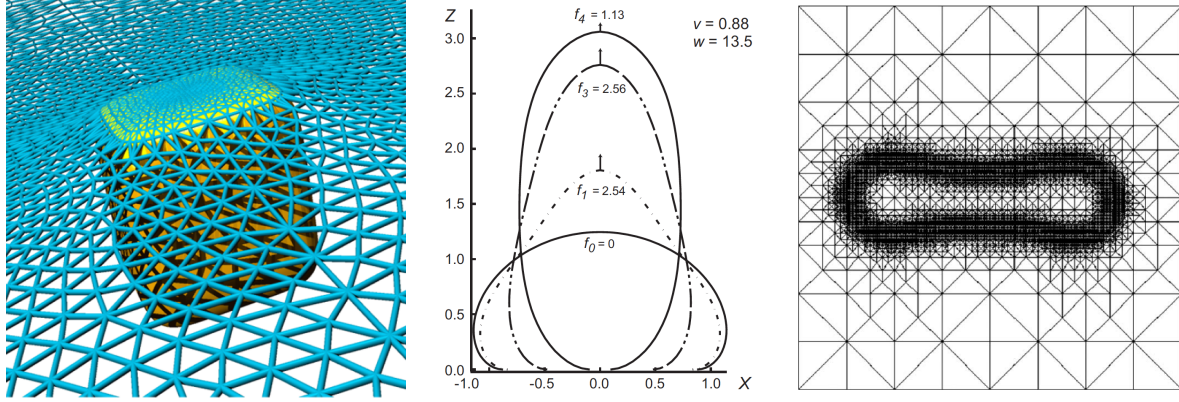


Fig. 5: *Equilibrium membrane shapes obtained using various energy minimization techniques. (a) A cube-like nanoparticle adhered with one face to a triangulated membrane. Reprinted with permission from Ref. [81]. (b) Shapes of a vesicle with reduced volume $v = 0.88$ adhered to a planar substrate with adhesion strength $w = 13.5$ for various pulling forces, calculated using shape equations. Reprinted with permission from Ref. [82]. (c) Adaptively refined mesh for a vesicle shape calculation using a phase field model; the membrane shape can be extracted using isocontours. Reprinted with permission from Ref. [83].*

where $C = 0.25 \ln\{1 + \exp(4\rho^*)\}$. For $\rho_i < \rho^*$, the potential is approximately $U_{\text{att}} \simeq -\rho_i$ and therefore acts like a pair potential with $U_{\text{att}} \simeq -\sum_{i < j} 2f_{\text{cut}}(r_{i,j}/\sigma)$. For $\rho_i > \rho^*$, this function saturates to the constant $-C$. Thus, it is a pairwise potential with cutoff at densities higher than $\rho_i > \rho^*$. A convenient choice of parameters is $r_{\text{att}}/\sigma = 1.8$ and $\rho^* = 6$.

2.5 Calculating Shapes—from Vesicles to Shells

Large vesicles can be simulated using a relatively small number of degrees of freedom using meshless and triangulated membrane models. While for dynamic simulations usually homogeneous discretizations are beneficial, using energy minimisation and triangulated surfaces very accurate results can be achieved by alternate refinement and minimisation steps and by adapting the triangulation to the local membrane curvature [84]. Therefore, membrane shapes with locally very different curvatures can be studied using energy minimization techniques that are challenging for dynamic simulation techniques, such as wrapping of ellipsoidal, cube-like, and rod-like nanoparticles [81, 85]. Compared with other methods to calculate equilibrium shapes, such as numerical solution of shape equations [82, 86, 87] and phase-field models [83], triangulation is a very versatile technique [23]. In particular for giant vesicles, the finite interface width that is used in phase-field models to describe the membrane requires large system sizes in order to obtain a realistic vesicle size compared with the membrane thickness. In experiments, a membrane thickness of 5 nm is negligible at the μm length scale of the vesicle, which is well approximated by the mathematical surface used to model the membrane in triangulation techniques. Energy minimization using shape equations usually exploits cylindrical symmetry of the system, which limits its range of applicability. Examples for systems that have been studied using these three techniques are shown in Fig. 5.

Fig. 6 illustrates the minimization process to obtain an oblate vesicle with reduced volume $v = 0.62$ modeled by a triangulated membrane using the freely available software package

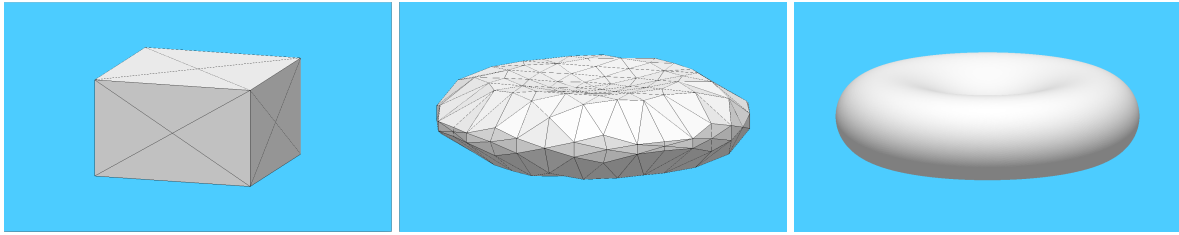


Fig. 6: Vesicle shape determination via energy minimization using triangulated membranes. Starting from a cuboid (a), a first minimization step evolves the shape towards an oblate vesicle (b). The final shape of the oblate vesicle with reduced volume $v=0.62$ is shown in (c). The calculations have been performed using "Surface Evolver" [88].

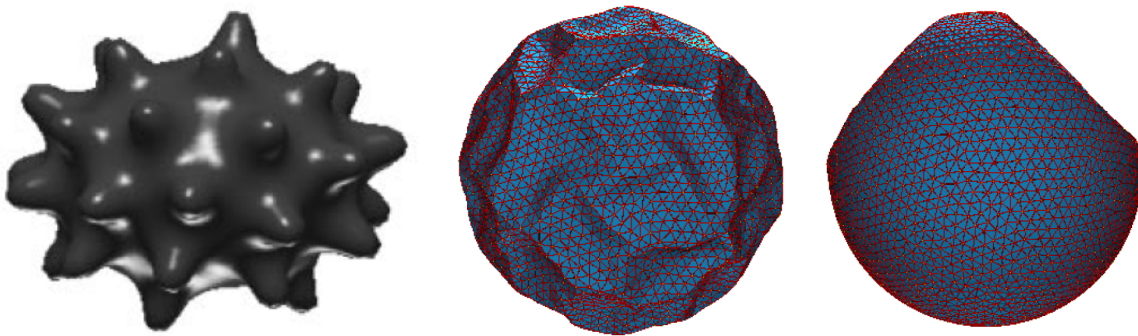


Fig. 7: Shapes for membranes with shear modulus: (a) Echinocytic shape of a red blood cell obtained using energy minimization. Reprinted with permission from Ref. [89]. Shapes of an initially spherical shell that have been deflated by the same volume (b) quickly and (c) slowly obtained using Monte Carlo simulations. Reprinted with permission from Ref. [90].

"Surface Evolver" [88]. The system is set up manually using only eight vertices, twelve edges, and six facets in a cuboidal arrangement. An automatic initial refinement step adds additional vertices, edges, and facets, see Fig. 6 (a). A first energy minimization step using a steepest descent method deforms the shape of the initial structure, see Fig. 6 (b). In order to evolve the system towards the minimal energy state efficiently, it is important not to refine too much as long as the membrane shape is still far from the vesicle's equilibrium shape. After several refinement and minimization steps, energy and shape of the oblate vesicle are obtained, see Fig. 6 (c). Not only membrane mechanics, but also an osmotic pressure that fixes a certain volume of the vesicle and a fixed area of the vesicle can be taken into account using harmonic constraints and can be fixed very accurately if energy minimization techniques are used. Here, the total membrane area is the sum of the areas of all triangles, while the volume can be calculated with the help of the triple product of the position vector of a membrane vertex and of two vectors that connect this vertex with other membrane vertices.

Equilibrium shapes can also be calculated for more complex membranes than homogeneous lipid bilayers that are governed by bending rigidity only, for example for vesicles formed by a lipid bilayer membrane with a finite preferred curvature of the membrane, e.g. induced by an area difference between the two monolayers that form the bilayer. Vesicle shapes have been shown to depend on both, reduced volume of the cell and area difference [87]. In the

area-difference elasticity model, the preferred membrane curvature is taken into account by an additional energy contribution [91],

$$E_{\text{ADE}} = \frac{\pi k_{\text{ADE}}}{2Ah^2} (\Delta A - \Delta A_0)^2, \quad (23)$$

where k_{ADE} is a constant of the order 1, A is the total membrane area of the vesicle, h is the thickness of the lipid bilayer, ΔA is the actual area difference between the two monolayers of the bilayer membrane, and ΔA_0 is the optimal area difference. Ref. [87] reviews in particular calculations of vesicle shapes using shape equations in detail. While the shapes of cylindrically-symmetric vesicles, for which the membrane mechanics is governed by bending rigidity and spontaneous curvature only, are often calculated using shape equations, using triangulated membranes a shear modulus for polymerized membranes and defect structures for crystalline membranes can be taken into account. The complex membrane of red blood cells consists of a lipid bilayer supported by a network of entropic springs, the biochemically well-defined spectrin cytoskeleton [92–95], see also subsections 3.7 and 3.8. These can be modeled using a complex membrane that consists of a fluid membrane with bending rigidity next to a polymerised membrane with shear modulus [96]. Using a membrane model that takes into account bending rigidity of the lipid bilayer membrane, shear elasticity of the cytoskeleton, and an area-difference elasticity, a large variety of experimentally-observed shapes of red blood cells can be reproduced [89].

In particular, spiculated echinocytic shapes of red blood cells can only be obtained if the membrane is modeled with both bending and shear modulus, see Fig. 7 (a) and Ref. [89]. For red blood cells the ratio of shear to bending modulus is characterised by the Föppl-von K’arm’an number, $\Gamma = YD^2/\kappa = 2662$, with Young’s modulus Y , bending rigidity κ , $D = \sqrt{A_{\text{RBC}}}/\pi$, where A_{RBC} is the surface area of the red blood cell [97]. For red blood cells with a thin spectrin cytoskeleton, $Y \approx 4\mu$, where μ is the shear modulus of the membrane. An even stronger dominance of the shear elasticity takes us from cells to shells, where the shape is not only determined by its minimal energy, but also by the pathway and dynamics how the deformation has been achieved [90]. Figures 7 (b) and (c) show shells that have been compressed fast or slow using Monte Carlo simulations with the same volume change. Only the shape obtained with slow compression is close to a minimal-energy shape of the vesicle.

2.6 Modeling Hydrodynamics

Vesicles, as well as cells, are typically studied in an aqueous environment. To describe dynamics and the behaviour under flow, hydrodynamics and hydrodynamic interactions have to be taken into account. Modeling fluid flow of a Newtonian solvent is often performed using the Navier-Stokes (NS) equation or its modifications [98]. The NS equation for an incompressible fluid is given by

$$\begin{aligned} \frac{\partial \mathbf{u}}{\partial t} + (\mathbf{u} \cdot \nabla) \mathbf{u} &= -\frac{1}{\rho} \nabla p + \nu \nabla^2 \mathbf{u}, \\ \nabla \cdot \mathbf{u} &= 0, \end{aligned} \quad (24)$$

where $\mathbf{u}$ is the local fluid velocity, ρ is the density, p is the pressure, and ν is the kinematic viscosity. These equations are derived using conservation laws and continuum assumption. For instance, the upper part in Eq. (24) corresponds to the conservation of momentum, while the lower part represents mass conservation and is referred to as an incompressibility condition.

A standard approach to solve partial differential equations such as Eq. (24) is to use various discretization techniques (e.g., finite difference, finite volume, finite element) in combination with proper initial and boundary conditions. This class of numerical methods is often referred to as computational fluid dynamics and represents rather well-established numerical techniques. However, in continuum approaches the inclusion of features present at the micro- and meso-scale (e.g., thermal fluctuations) is a non-trivial task.

Another class of efficient numerical approaches for modeling fluid dynamics includes particle-based Lagrangian methods such as molecular dynamics (MD) [25], dissipative particle dynamics (DPD) [99, 100], multi-particle collision dynamics (MPCD) [101, 102], and smoothed particle hydrodynamics (SPH) [103, 104]. Microscopic modeling at the atomistic scale is often performed using MD, while the other aforementioned methods correspond to mesoscopic approaches. In these approaches, a fluid is represented by a number of particles which interact with each other through specified forces (in MD, DPD, and SPH) or collisions (in MPC). Through the conservation of local and global quantities such as mass and momentum, all these methods provide proper hydrodynamic interactions at large enough length scales. Even though particle-based approaches are generally more expensive computationally than continuum techniques, they often allow a rather straightforward incorporation of desired micro- and mesoscopic features. This advantage often favors the use of particle-based methods in modeling complex fluids at the micro- and meso-scale over conventional computational fluid dynamics.

Due to the importance of particle-based approaches for simulations of the (hydro)dynamics of vesicles, we briefly describe the basic algorithms of two hydrodynamics techniques (DPD and MPCD) in a little more detail. In DPD, the conservative forces between different particles i and j are assumed to be of the form

$$\mathbf{F}_{ij}^C = a_{ij} (1 - r_{ij}/r_0) \hat{\mathbf{r}}_{ij} \quad (25)$$

for distances $r_{ij} < r_0$ and zero otherwise. This guarantees that potentials are smooth, and relatively large time steps can be used in the integration of the equations of motion. Similarly, the dissipative friction forces are taken to be

$$\mathbf{F}_{ij}^D = \gamma_{ij} (1 - r_{ij}/r_0)^2 (\hat{\mathbf{r}}_{ij} \cdot \mathbf{v}_{ij}) \hat{\mathbf{r}}_{ij} \quad (26)$$

for distances $r_{ij} < r_0$ and zero otherwise, where $\mathbf{v}_{ij} = \mathbf{v}_i - \mathbf{v}_j$ is their relative velocity. Finally, there are thermal random forces which follow from the fluctuation-dissipation theorem [100].

In MPCD, the fluid consists of point particles which have *no* conservative interactions. The dynamics proceeds in two alternating steps. In the streaming step, particles move ballistically for a time interval δt . Then, all particles are sorted into the cells of a cubic lattice, which defines the collision environment. All fluid particles within one collision cell exchange momentum, for example by a random exchange of momentum increments, such that the total momentum of each cell is conserved. The fluid particles interact with the membrane in two ways. First, the membrane vertices are included in the MPC collision procedure. Second, for triangulated surfaces, the fluid particles are scattered with a bounce-back rule from membrane triangles. These interactions together ensure that the fluid satisfies a no-slip boundary condition on the membrane.

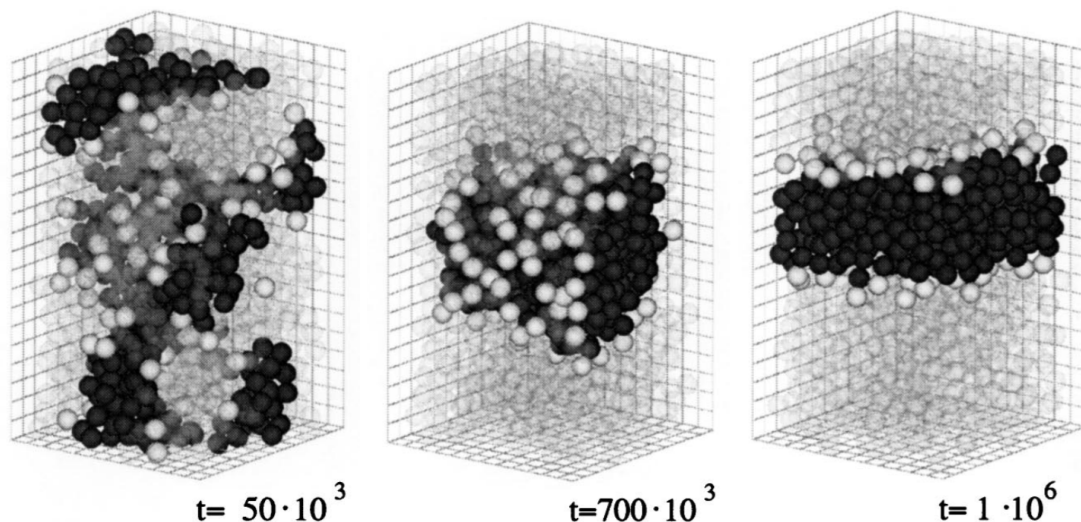


Fig. 8: Self-assembly of a bilayer membrane in a mixture of HT_4 amphiphiles and solvent particles. The solvent particles are nearly transparent. The initial configuration, which is not shown, consists of a random mixture of 100 amphiphiles and 840 solvent particles. The configurations are snapshots which illustrate the time evolution of the structure. After about 10^5 molecular dynamics (MD) time steps, the amphiphiles form a cylindrical micelle, which spans the simulation box horizontally. This state is metastable for some time, before it transforms into a stable bilayer structure. From Ref. [7].

3 Applications

3.1 Self-Assembly of Micelles and Bilayers

The self-assembly of amphiphilic molecules in aqueous solution into a large variety of different structures is their most important property [43, 105]. The type of structures found depends very much on the amphiphile concentration, but also on the amphiphile architecture and environmental conditions, such as temperature, salt concentration, etc.

At very small amphiphile concentrations, the amphiphiles are molecularly dispersed, since the translational entropy dominates over any interaction energy. Only when a minimal concentration — the *critical micelle concentration* (CMC) is exceeded, the amphiphiles aggregate into small droplets called *micelles*, in which the hydrocarbon tails are shielded from water contact by a layer of head groups. The typical size of a spherical micelle is therefore determined by the length of the amphiphilic molecules. In some systems, when the size of the head group is larger than the tail, micelles can grow into long cylindrical rods which are called cylindrical micelles. On the other hand, when head and tail of the amphiphiles have roughly the same size, micelles can grow into two-dimensional bilayer patches. This can happen at still small amphiphile concentrations (above the CMC). In this case, the patch does not grow indefinitely in the lateral directions, because the rim of the patch is energetically less favorable than the interior. This can be understood as a line tension of the rim. Since the rim energy grows linearly with the radius of the patch, at some point the flat bilayer becomes less favorable than a closed membrane shape or a vesicle, see Sec. 3.2 below. In contrast to micelles, vesicles can be much larger than the length of an amphiphile. At considerably higher amphiphile concentrations, micelles, bilayers,

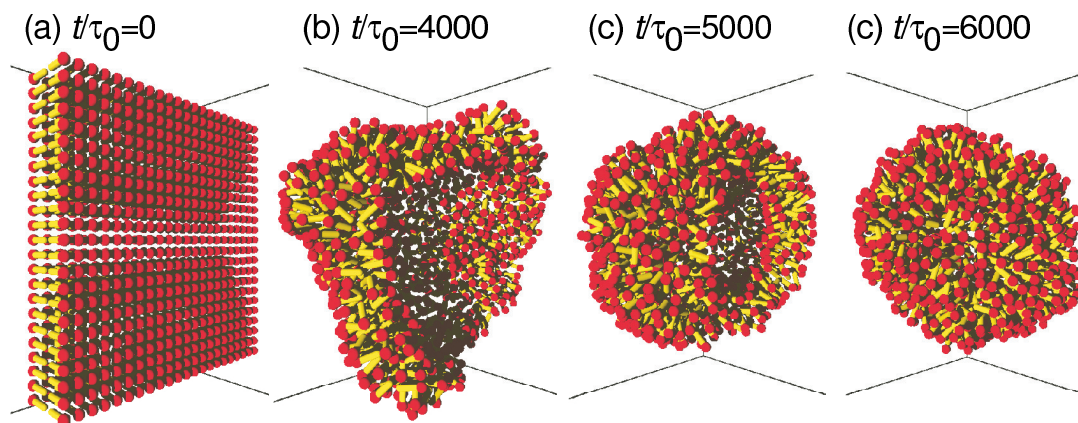


Fig. 9: Snapshots of vesicle formation from an initially planar membrane patch, for $k_B T/\varepsilon = 0.5$ and $N = 1000$. Time is measured in units of $\tau_0 = \zeta \sigma^2/\varepsilon$, where the mass $m = 1$ and the friction constant $\zeta = 1$ of segments are fixed. Red spheres and yellow cylinders represent the hydrophilic and hydrophobic segments of amphiphilic molecules, respectively.

or vesicles can pack together to form three-dimensional order phases, such as cubic micellar crystals, or lamellar phases in which bilayers form a stack in one direction.

Many aspects of this self-assembly process have been studied by simulations. For example, the formation of a bilayer from an initially random mixture of amphiphiles and water, as obtained from MD simulations of the coarse-grained Lennard-Jones model in Sec. 2.1, is shown in Fig. 8. It shows the formation of a transient cylindrical micelle structure, which transforms after some time into a stable bilayer state. Note that due to the finite box size, the amphiphile concentration is rather large, so that this bilayer should be considered as a part of a lamellar phase.

3.2 Vesicle Formation

Amphiphilic molecules spontaneously form vesicles at $N = 1000$ and $k_B T/\varepsilon \leq 0.9$. When the initial state is a random distribution of molecules, amphiphiles aggregate into spherical or disk-shaped micelles, which assemble and reform into vesicles. When the initial state is a flat bilayer membrane, the membrane undulates by thermal fluctuations, and then bends into a vesicle through a bowl-shaped conformation to reduce the length of the membrane edge (see Fig.9). The closed-bilayer vesicles are equilibrium states under these conditions.

The vesicle exhibits a clear bilayer structure (see Fig. 9) and is in a fluid phase. Molecules in vesicles diffuse laterally: the lateral diffusion constant is 0.004 at $k_B T/\varepsilon = 0.2$. The unit length σ corresponds to $\sim 1\text{nm}$. The unit time step τ_0 corresponds to $\sim 1\text{ns}$ when the lateral diffusion constant is assumed to correspond to that of phospholipid at 30°C , $\sim 10^{-7}\text{cm}^2/\text{s}$. The area per molecule in membranes is $2\sigma^2$ and is larger than the experimental data for lipid molecules: $0.4 \sim 0.8\text{nm}^2$. A few lipid molecules are coarse-grained to one rigid molecule.

This model is designed for simulations of fluid membranes, since it has a wide temperature range where the fluid phase is stable, and a very low critical micelle concentration (CMC). The membrane properties can be varied easily by a modification of the model parameters and functional forms of the potentials. Other solvent-free models, with pair-interactions only, have been used, for example, to study gel and crystalline phases [21, 38]. Thus, the solvent-free model can be adjusted depending on the type of physical problem under investigation.

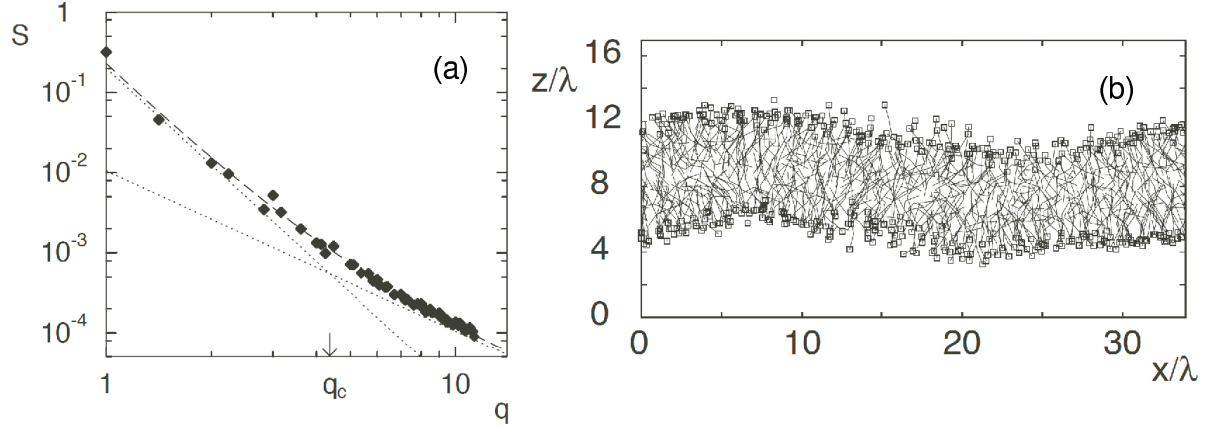


Fig. 10: Fluctuation modes of thermally excited membrane deformations. (a) Fluctuation spectrum $S = \langle |h(\mathbf{q})|^2 \rangle$ as a function of the dimensionless wave number q . The largest wave number is determined by the box size and corresponds to $q = 1$. The dotted lines show the expected power-law behavior due to undulations (small q) and protrusions (large q), respectively. (b) Typical configuration of a bilayer membrane composed of 1152 HT_4 amphiphiles. At small scales, individual molecules protrude from the bilayer. At large scales, the bilayer looks like an elastic, smoothly curved sheet. The basic length scale λ represents the range of the Lennard-Jones potential. From Ref. [7].

3.3 Thermal Membrane Fluctuations

Thermal fluctuations of the lipid molecules in a membrane lead to two types of thermal excitations, see Fig. 10b. On short length scales, the lipid molecules are not perfectly aligned and do not have their heads all in the same plane, but there are small vertical displacements between neighbors. These thermal motions are called *protrusion modes*. On length scales much larger than the bilayer thickness, there is a collective excitation where the whole membrane displays a wave-like deformation, which is called an *undulation mode*. The amplitudes are accessible experimentally, for example by scattering techniques.

In order to determine the spectrum of fluctuation modes of a membrane in simulations, a scalar height variable $h(\mathbf{r})$ is introduced, which measures the deviation of the *local* position of the amphiphile head from a planar reference state (Monge parametrization). The fluctuation spectrum is then obtained from the correlation function

$$S(\mathbf{q}) \equiv \langle |h(\mathbf{q})|^2 \rangle, \quad (27)$$

where

$$h(\mathbf{q}) = \frac{1}{N} \sum_{i=1}^N h(\mathbf{r}_i) \exp(i\mathbf{q} \cdot \mathbf{r}_i), \quad (28)$$

is the two-dimensional Fourier-transform of the height-field $h(\mathbf{r})$ with N being the number of amphiphiles.

The results are shown in Fig. 10 (a). For small wave numbers q , the spectrum shows a q^{-4} decay, which is characteristic for surfaces which are governed by the *curvature elasticity*. The amplitude of this power law is the (inverse) bending rigidity, which can thereby be extracted from the simulations. This behavior should be compared with the spectrum of a surface governed by the

surface tension (as the air-water interface), where the spectrum decays as q^{-2} for small wave numbers. The spectrum for large wave numbers, on the other hand, follows a q^{-2} power-law. It is no coincidence that this is the same power law as for surfaces with surface tension, since the energy of the protrusion modes is proportional to the hydrophobic area exposed to the water when the amphiphiles “stick their head out” of the bilayer.

Measurements of the undulation spectrum of quasi-spherical vesicles is one of the standard experimental approaches to determine the bending rigidity of bilayers. In this case, the radial membrane displacements (from the center of the vesicle) are expanded in spherical harmonics $Y_{lm}(\Omega)$ as

$$r(\Omega) = R_0 \left[1 + \sum_{l=0}^{l_M} \sum_{m=-l}^l u_{lm} Y_{lm}(\Omega) \right] \quad (29)$$

at the solid angle $\Omega = (\theta, \varphi)$. The spectrum of undulation modes is predicted to be

$$\langle |u_{lm}|^2 \rangle = \frac{k_B T}{\kappa} \frac{1}{(l+2)(l-1)[l(l+1) + Q]} \quad (30)$$

where $Q = 2(C_0 R_0)^2 - 4C_0 R_0 + \gamma R_0^2 / \kappa$, with spontaneous curvature C_0 . This result implies that

- the spectrum is governed by the bending rigidity for large l , and decays like $\kappa^{-1} l^{-4}$
- the spectrum is governed by the “membrane tension” for small l , and decays like σl^{-2}
- the spontaneous curvature C_0 cannot be measured in this approach, because it only appears in combination with the membrane tension.

However, spontaneous curvature plays the key role in determining the morphology of biomembranes, lipid vesicles, and polymersomes; it is crucial for maintaining the spatial organization of, and traffic between, cellular organelles and the plasma membrane; and finally, it is believed that it controls the functional state of certain integral membrane proteins and membrane fusion competence. Therefore, it is very important to have a simple, straightforward procedure for the direct determination of the spontaneous curvature and bending modulus. Flicker spectroscopy of *non-spherical* vesicles avoids the shortcomings of analysis of undulations of quasi-spherical vesicles discussed above, by utilizing results of Monte Carlo simulations of dynamically triangulated vesicles for a wide range of reduced volumes and spontaneous curvatures to extract the elastic parameters of the membrane from experimental flicker spectroscopy data [106].

In experiments, fluctuating prolate vesicles are stabilized by gravity - due to a small density difference of the solvent inside and outside the vesicle - on the bottom of a microchamber. The focal plane of a microscope is adjusted to include the long axis of the vesicle, and shape contours are recorded [107], see Fig. 11 (a). Choosing a coordinate system in which the x coordinate lies along the long axis of the vesicle, the 2D contours are then represented in polar coordinates as

$$r(\varphi) = r_0 \left[1 + \sum_n a_n \cos(n\varphi) + b_n \sin(n\varphi) \right]. \quad (31)$$

The mean values $\langle a_n \rangle$ describe the mean vesicle shape; for the oriented contours, $\langle b_n \rangle = 0$. The mean-square amplitudes $\langle (a_n - \langle a_n \rangle)^2 \rangle$ measure the thermal fluctuations of the vesicles about their mean shape.

The simulated vesicles are analyzed in the same way as the vesicles in experiments, see Fig. 11 (b). With increasing $\bar{c}_0$, a transition from an oblate to a prolate shape is observed, which leads to a pronounced increase of $\langle a_2 \rangle$ and $\langle a_4 \rangle$. The oblate-to-prolate transition is reflected in a sharp peak of the fluctuations in a_2 , see Fig. 11 (c). By fitting the experimental data for the averages and variances of a_2 , a_3 , a_4 , and a_5 to the simulation results, the parameters κ , $\bar{c}_0$, and v can be extracted simultaneously for a single vesicle [106].

This method has been employed to measure electrostatically induced spontaneous curvature of SOPC vesicles. It has been suggested that a change in pH induces membrane curvature via the association of hydroxyl ions with the trimethyl-ammonium group of the phosphatidylcholine molecule [108]. The results of the flicker analysis, as described above, are shown in Fig. 11(D). A strong change in spontaneous curvature at a constant bending modulus $\kappa = 32k_B T$ is obtained. Indeed, the bending modulus of SOPC should not change considerably since electrostatic contributions to the elastic modulus are expected to be small. Note that the reduced volume of the vesicle is also found to be constant, as it should be. The large increase in the spontaneous curvature can be understood by considering the balance of the electro-static free energy and the intrinsic bending energy of the membrane.

3.4 High-Genus Vesicles and Gaussian Saddle-Splay Modulus

Usually, vesicles with genus $g = 0$ that have the topology of a spherical vesicle are investigated. However, also toroidal vesicles with genus $g = 1$ are experimentally observed, as well as vesicles with high genus and many handles. Also, in biological cells organelles with high genus membranes exist. For example, the nuclear membrane and the endoplasmic reticulum (ER) are connected and together form complicated shapes. The nucleus is wrapped by two lipid bilayer membranes connected by many lipid pores, and the ER can have a sponge-like structure. The genus of the vesicle is controlled by the Gaussian saddle-splay modulus of the membrane, high values for $\bar{\kappa}$ favour formation of high-genus vesicles. For a fixed genus, computer simulations of vesicles do not have to take into account the value of $\bar{\kappa}$ because of the Gauss-Bonnet theorem: the integral over the Gaussian curvature of a closed vesicle depends only on its topology. Shape fluctuations, however, are controlled by the bending rigidity and the area difference between the monolayers of the lipid bilayer. Fig. 12 shows a collection of experimental and simulation figures for vesicles with genus $g \geq 1$ and finite values of the area-difference elasticity; all computer simulations have been performed using Monte Carlo simulations and triangulated membranes [109, 110].

Because the Gaussian saddle splay modulus of a membrane only affects the vesicle topology and not its shape fluctuations, its value is often not well known. Other systems besides vesicles with topology changes for which $\bar{\kappa}$ is important are phase-separated multi-component membranes [111, 112] and membranes with open edges [113, 114]. Such systems can therefore be used to determine the value of $\bar{\kappa}$ using experiments or computer simulations. Recently, molecular dynamics simulations for partially-bent circular membrane patches have been performed using coarse-grained lipid models [113, 114]. If membrane patch sizes are chosen such that an energy barrier between the initial patch and a closed vesicle exists, from multiple simulations the probability for closing can be measured and used to determine $\xi = \gamma R / (2\kappa + \bar{\kappa})$, where R is the initial radius and γ is the edge tension of the patch. In case all other parameters are known for a particular system, e. g. κ from analyzing the fluctuations as described in Sec. 3.3 or from simulations of membrane tethers [115, 116] and the edge tension from pore formation [117, 118], $\bar{\kappa}$ can be extracted from the measurement of ξ . For DMPC membranes

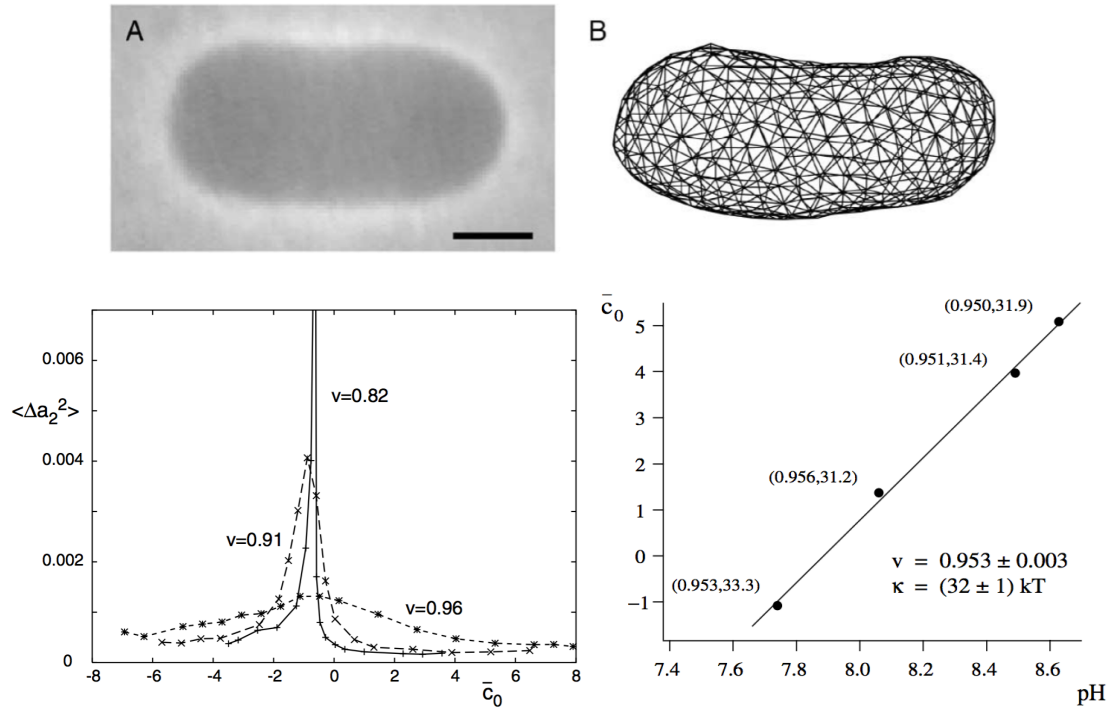


Fig. 11: (A) Phase contrast micrograph of a vesicle ($v = 0.828$) sedimented on a glass substrate. The scale bar corresponds to $5\mu\text{m}$. (B) Simulation snapshot ($v = 0.825$, $\bar{c}_0 = -0.28$). (C) Simulated mean-square amplitude $\langle \Delta a_2^2 \rangle$ of shape fluctuations as a function of the effective spontaneous curvature $\bar{c}_0$. Note the peak at the prolate-to-oblate transition. Three different values of the reduced volume v are shown, as indicated. The other parameters in the simulations are $\kappa/k_B T = 25$, $k_{ADE} = 0.9$, and $g = 0.37$. [??? Gerhard: please check!] (D) Spontaneous curvature $\bar{c}_0$ for $g = 0.8$. Note that the reduced volume and bending modulus, which are given in brackets, (v, κ) , remain constant. v , c_0 , and κ are obtained simultaneously via comparison of the experimental data to the Monte Carlo simulations. Reprinted with permission from Ref. [106].

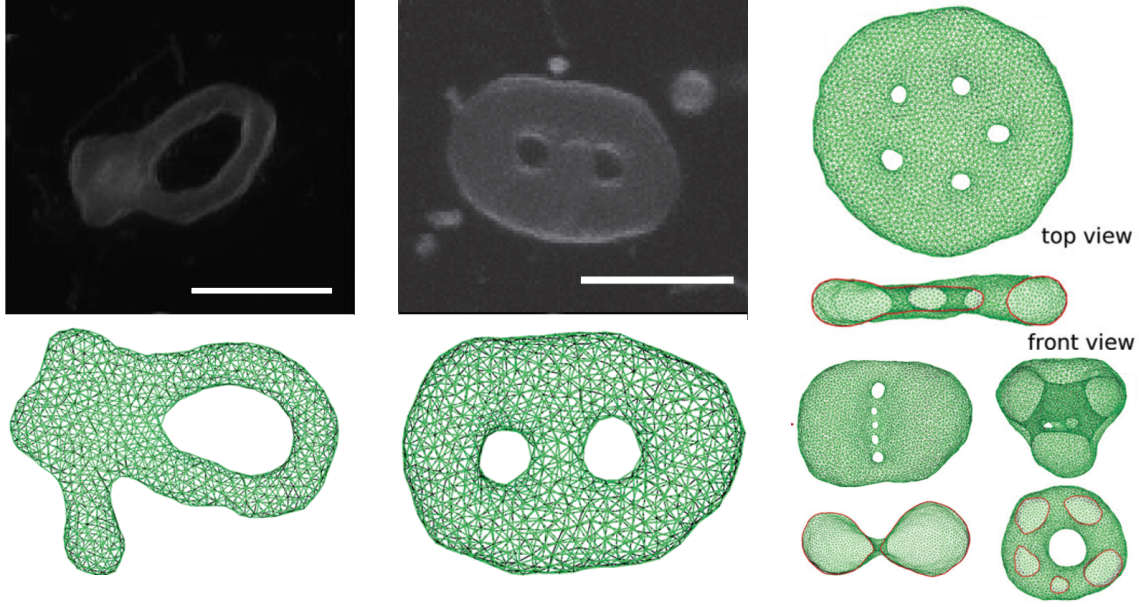


Fig. 12: *High-genus vesicles. Microscopy images and simulation snapshots for vesicles with (a) $g = 1$ and (b) $g = 2$. The lengths of the scale bars are equal to $10 \mu\text{m}$. Reprinted with permission from Ref. [109]. (c) Simulation snapshots for $g = 5$ vesicles. Reprinted with permission from Ref. [110].*

modeled using the MARTINI force field, $\gamma = 40.49 \pm 0.34 \text{ pN}$, $\kappa = (16.6 \pm 0.5)10^{-20} \text{ J}$, $\bar{\kappa} = (-17.3 \pm 1.0)10^{-20} \text{ J}$, and therefore $\bar{\kappa}/\kappa = -1/04 \pm 0.03$ have been determined using simulation data [114].

3.5 Complex Membranes, Inclusions, and Bud Formation

Membranes of giant vesicles do not have to be homogeneous lipid-bilayer membranes, but can be multi-component membranes that are more complex. For instance, curvature-inducing inclusions—e.g. proteins or spherical caps of viruses [119–121], or polymers anchored only to one monolayer [122–125]—induce an effective spontaneous curvature of the membrane. While it is well known that curved inclusions on a planar membrane mutually repel each other [126, 127], an effective attraction is found for curved inclusions on a vesicle. The optimal vesicle radius is [120]

$$R \approx (\cos \alpha) / (\pi \sigma \sin^2 \alpha) (1/r_i) \quad (32)$$

for a vesicle with

$$n \approx (4 \cos^2 \alpha) / (\pi \sigma \sin^4 \alpha) (1/r_i^2) \quad (33)$$

inclusions. Here, r_i is the curvature radius of the inclusion, α is the opening angle of the spherical cap, and σ is the number density of inclusions on the total membrane area. This optimal radius corresponds to an effective spontaneous curvature for the inclusion-decorated membrane of $c_0 = 1/R$. The reasoning is that around each curved inclusion a catenoidal membrane patch with vanishing curvature energy forms. The energy of a vesicle covered with curved inclusions at low density is therefore $\mathcal{E} = 8\pi\kappa(1 - S_{\text{cat}}/S_{\text{sph}})$, where $S_{\text{cat}}/S_{\text{sph}}$ is the area fraction of the vesicle which is covered with inclusions and catenoidal patches, see Fig.13 (b). At optimal inclusion density, the vesicle is entirely covered by curved inclusions with their catenoidal

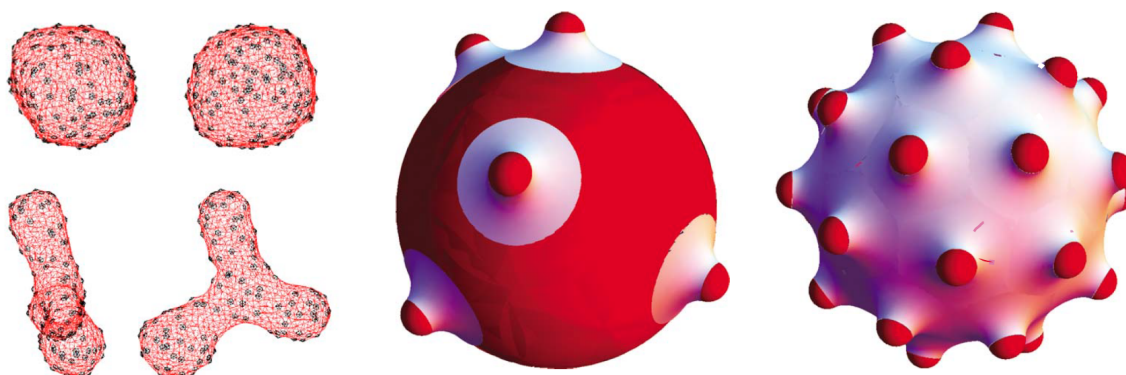


Fig. 13: (a) A spherical vesicle with curved proteins modeled by a triangulated membrane and simulated using the Monte Carlo technique. The proteins are described by specific angle between the membrane normal and the adjacent edges of a triangle, the base length of these edges, and the angles between them. An initially spherical vesicle with 120 proteins (top) relaxes to a three branched tubular structure. Reprinted with permission from Ref. [119]. (b) and (c): Vesicle decorated with curved inclusions at (b) low and (c) optimal inclusion density. Around each inclusion, the membrane can be modeled by segments of the catenoid minimal surface (white). At optimal density the bending energy of the vesicle vanishes. Reprinted with permission from Ref. [120].

patches, see Fig. 13 (c). Computer simulations of coarse-grained lipid bilayer membranes with spherical caps impressively show the dynamics of bud formation starting with planar membrane patches [121]. Using Monte Carlo simulations with triangulated membranes, the deformation of an initially spherical vesicle into a three-armed star-shaped vesicle has been simulated, see Fig. 13 (a) and Ref. [119].

Giant vesicles decorated with curved proteins are a special case for giant vesicles with complex membranes. The large range of relevant length scales from several nanometers for the components of the complex membrane to several micrometers for the giant vesicles makes such systems extremely challenging to be studied using a single simulation. Instead, a two-step approach can be applied where both length scales are decoupled. In a first step, effective curvature elastic properties of complex membrane are calculated using analytical calculations or computer simulations for small model systems, e.g. membrane patches. In a second step, these effective curvature-elastic properties are used to simulate giant vesicles with the help of triangulated membranes that are well suited for the micrometer scale. Common examples for complex membranes are biological membranes that usually contain a mixture of charged and uncharged lipids and proteins and that may be decorated by a glycocalix. In particular membranes decorated with polymers have been investigated in various studies [122–125, 128–135], see Fig. 14 (a) and (b). While the absolute value of the induced changes of the curvature-elastic constants due to certain components in complex membranes can often be tuned by adjusting their density, how they affect bending rigidity and Gaussian saddle splay modulus in a coupled way is specific for each mechanism. Fig. 14 (c) shows the ratios $\Delta\kappa/\Delta\bar{\kappa}$ extracted for added charges [134], polymers embedded into the membrane [129], membrane-grafted ideal and self-avoiding linear polymer chains, polymer brushes, and star polymers [122, 123, 123–125, 128, 130–133, 135]. In particular star polymers allow to change κ and $\bar{\kappa}$ in a different way depending on the functionality of the

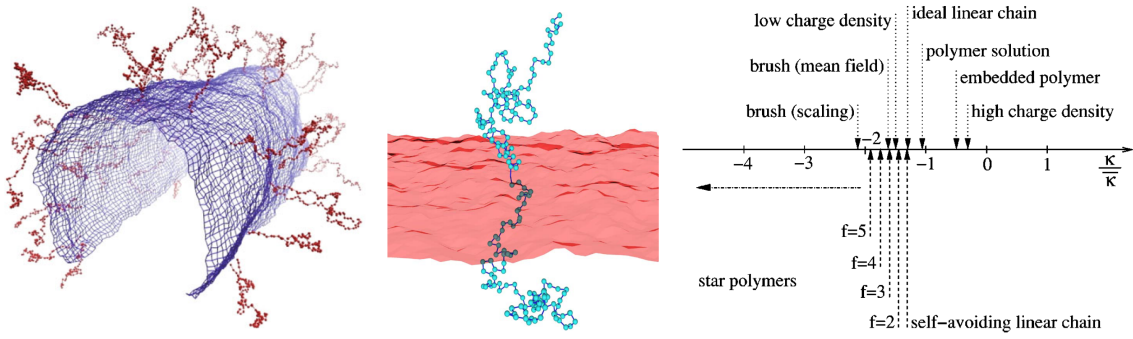


Fig. 14: (a) Simulation snapshot of a tethered membrane with fixed connectivity decorated with end-grafted linear polymers with 64 monomers. Reprinted with permission from Ref. [128]. (b) Simulation snapshot of a symmetric diblock-copolymer attached to a fluid membrane. Reprinted with permission from Ref. [123]. (c) Various modifications change the elastic constants of a lipid bilayer membranes, κ and $\bar{\kappa}$. The ratio of the changes $\Delta\kappa/\Delta\bar{\kappa}$ is specific for each mechanism [122, 129–134]. As indicated by the horizontal arrow, using star polymers a wide range of ratios $\kappa/\bar{\kappa}$ can be accessed. Reprinted with permission from Ref. [122].

star.

3.6 Vesicles in Capillary Flow

The deformability and dynamics of vesicles under flow through narrow channels and capillaries plays an important role in determining their behavior in microfluidic devices. Vesicles can be simulated in capillary flow using a combination of the dynamically-triangulated surface model (see Sec. 2.3) and mesoscale hydrodynamics simulation techniques (see Sec. 2.6). The triangulated-network model has to be slightly modified in order to combine it with one of mesoscale hydrodynamics simulation techniques. Since the temporal evolution of the positions of the membrane vertices is determined by Newton’s equation of motion, soft pairwise potentials have to be employed for the tether-bond and excluded volume. The volume V and surface area S of a vesicle are kept constant by constraint potentials. The membrane viscosity can be varied by changing the bond-flip rate, where the membrane viscosity increases with decreasing number of bond-flips per time step [74, 75].

Simulation results for fluid and elastic (polymerized) vesicles in a cylindrical channel are displayed in Fig. 15 for two different flow velocities [76]. Simulations are performed for discocyte shapes (at rest) and the reduced volume $V^* = V/(4\pi R_s^3/3) = 0.59$, where $R_s = \sqrt{S/4\pi}$ is the effective vesicle radius. At this reduced volume, a biconcave discocyte is the equilibrium vesicle shape, and a prolate ellipsoid and stomatocyte are metastable in the absence of flow [136]; therefore, vesicle shapes should be very sensitive to flow at this particular reduced volume.

Under typical experimental conditions of capillary flows, the Reynolds number $\text{Re} = \rho v_{\text{ves}} R_s / \eta_0$ is very small, typically $\text{Re} \simeq 10^{-2}$, where v_{ves} is the mean velocity of the vesicle. Therefore, parameters are chosen such that $\text{Re} < 1$ in all simulations. Both fluid and elastic vesicles retain their discoidal shapes in slow capillary flows (see Fig. 15(a)). The vesicles align the longest axis of the gyration tensor with the flow direction, even if their initial conformations are coaxial with the capillary. The discoidal shape is elongated in the flow direction and its front-rear symmetry is broken, but the biconcave dimples and the mirror symmetry with respect to the

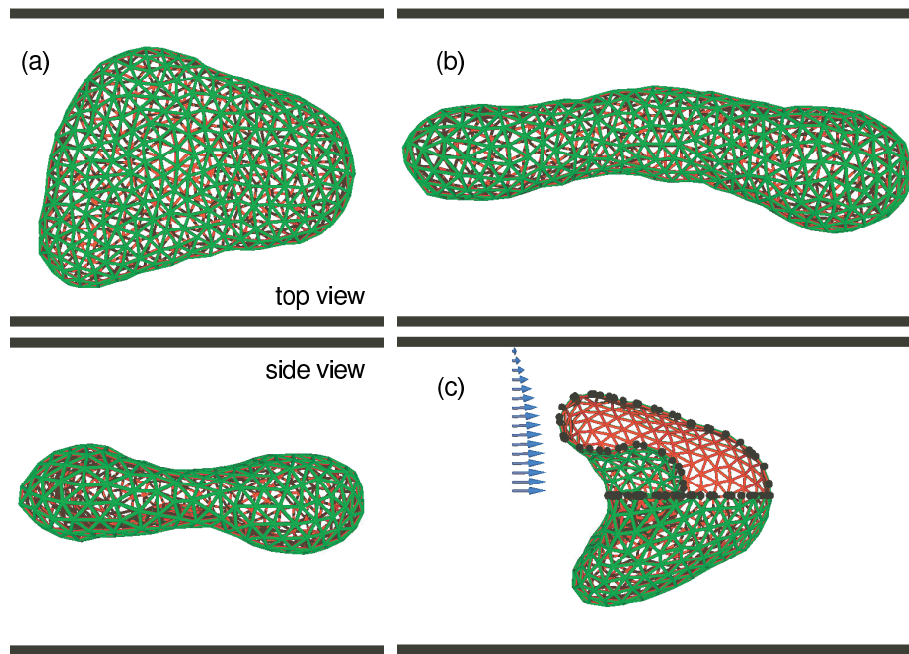


Fig. 15: Snapshots of vesicles in capillary flow (with bending rigidity $\kappa/k_B T = 20$), for a capillary radius of $R_{\text{cap}} = 1.4R_0$. (a) Fluid vesicle with a discoidal shape at the mean fluid velocity $v_m \tau / R_{\text{cap}} = 41$, both side and top views. The results are scaled with the intrinsic relaxation time $\tau = \eta_0 R_{\text{cap}}^3 / k_B T$. (b) Fluid vesicle with a prolate shape at $v_m \tau / R_{\text{cap}} = 69$. (c) Elastic vesicle (RBC model) with a parachute shape at $v_m \tau / R_{\text{cap}} = 218$ (with shear modulus $\mu R_0^2 / k_B T = 110$). The membrane consists of $N_{\text{mb}} = 500$ vertices. The blue arrows represent the velocity field of the solvent. The upper front quarter of the vesicle in (c) is removed to allow a look into the interior; the black circles indicate the lines where the membrane has been cut in this procedure. Thick black lines indicate the walls of the cylindrical capillary.

plane determined by the two eigenvectors of the gyration tensor with the largest eigenvalues are retained. For larger mean fluid velocity, a fluid vesicle transits into a prolate ellipsoidal shape (see Fig. 15(b)), since this shape change reduces the flow resistance. An elastic vesicle transits into a parachute shape, (see Fig. 15(c)), since the shear elasticity of the membrane prevents the elongation of the vesicle into a prolate shape in this case.

Even more interesting in comparison to homogeneous cylindrical (or rectangular) channels are structured channels, in which the channel cross-section varies periodically along the channel. In such structured channels the vesicle shape is no longer stationary, so that the internal dynamics of a vesicle can be probed. This allows to explore the behavior of vesicles in more complex flow geometries, as it can be realized quite easily in modern microfluidic devices.

Snapshots of vesicles under flow through zig-zag channels are shown in Fig. 16(A). These snapshots already demonstrate that the vesicles deform periodically, as they move from wide to narrow regions of channel, and back [77]. The reason is that the flow velocity is fast in the narrow parts, and slow in the wide parts of the channel. Therefore, the vesicle becomes elongated as it approaches the narrow parts, and shortened as it enters the wide parts. However, this behavior is only observed for flexible vesicles, with a small bending rigidity $\kappa^* \ll 1$, with $\kappa^* = \kappa L_y / (\eta R_V^3 v_m)$, where v_m is the mean flow velocity (and R_V is the effective vesicle radius related to the vesicle volume). For stiff vesicles, with $\kappa^* \gg 1$, flow forces are too small to overcome the deformation energy costs. In this case, the vesicle adjusts to the compressional force when it enters the wide region by *tilting* its long axis away from the channel axis, as shown in Fig. 16(B). These results indicate that a lot of unexpected behaviors are to be discovered in complex flow geometries.

3.7 Red Blood Cells – Membrane Fluctuations

Red blood cells take the concept of vesicles as model systems one step further towards complex biological cells. These cells are still comparatively simple with a well-defined polymerized membrane attached to the lipid bilayer, the cortical spectrin cytoskeleton. Similarly to vesicles, red blood cells are easy abundant and much easier to handle than cells with a 3D cytoskeleton. A healthy human red blood cell (RBC) has a biconcave shape with an average diameter of about $8 \mu m$ [137]. Its membrane consists of a lipid bilayer with an attached cytoskeleton formed by a network of the protein spectrin linked by short filaments of actin. The membrane's shear elasticity supplied by the spectrin network constitutes the main difference between RBCs and giant vesicles. The presence of shear elasticity in a RBC membrane significantly affects its behavior and response to various external fields in comparison to vesicles.

One of the interesting measurements for RBCs or vesicles is membrane fluctuations, since they should be directly associated with the membrane characteristics and properties of cytosol and suspending media. RBC membrane fluctuations have been measured in a number of experiments including RBC edge flicker microscopy [138, 139] and tracking of beads attached to the RBC [140, 141]. In contrast to fluctuation measurements on vesicles, see section 3.3, the interpretation of these measurements for RBCs often leads to rather disparate outcomes. For instance, the results of measuring RBC edge fluctuations [138] have suggested a vanishing (or nearly negligible) effect of membrane shear elasticity, while experiments on RBC deformation with optical tweezers [142, 143] clearly identify a finite shear elasticity. Furthermore, the interpretation of fluctuation measurements in Ref. [141] has resulted in very high (unrealistic) values for the effective viscosity of the fluid. These differences are likely to originate from the approximations used in analytical models which are mainly derived for planar lipid bilayer

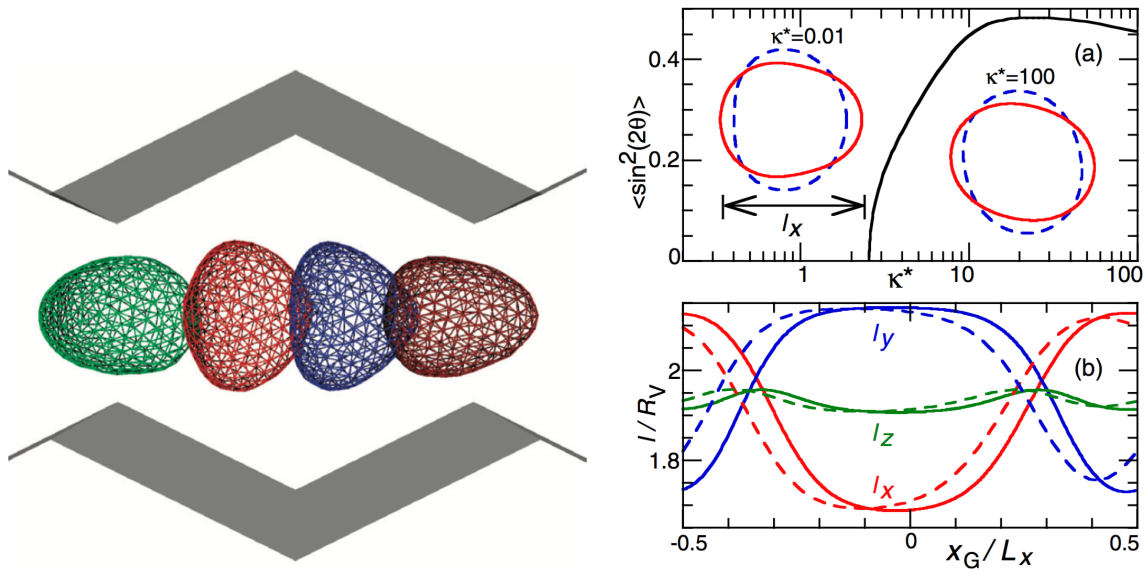


Fig. 16: (A) Sequence of snapshots (at equal time intervals) of vesicles with reduced volume $v = 0.96$ and $R_S/L_y = 0.21$ (where R_S is the effective vesicle radius related to the membrane area) moving through a structured microchannel, where L_y is the average channel width. (B) Dynamics of a quasi-spherical vesicle at reduced volume $v = 0.988$. Dependence of the tilt angle θ of a vesicle on κ^* , for $L_x/L_y = 4$ (where L_x is the periodicity length along the channel) and $a_y = 0.5$ (which is the amplitude of the wall sawtooth, such that the maxima and minima are located at $y = (1 \pm a_y)L_y/2$). Here, $\langle \sin^2(2\theta) \rangle$ describes the deviation from symmetric shape. The insets show sliced snapshots of vesicles in the xy -plane for $\kappa^* = 0.01$ and $\kappa^* = 50$. Solid and dashed lines indicate shapes of extremal elongation or tilt. Also, maximum vesicle lengths in the x , y , and z directions as a function of the center-of-mass position x_G , for $\kappa^* = 0.01$ and $a_y = 0.5$. Solid and dashed lines represent results for $L_x/L_y = 4$ and $L_x/L_y = 16$, respectively. From Ref. [77].

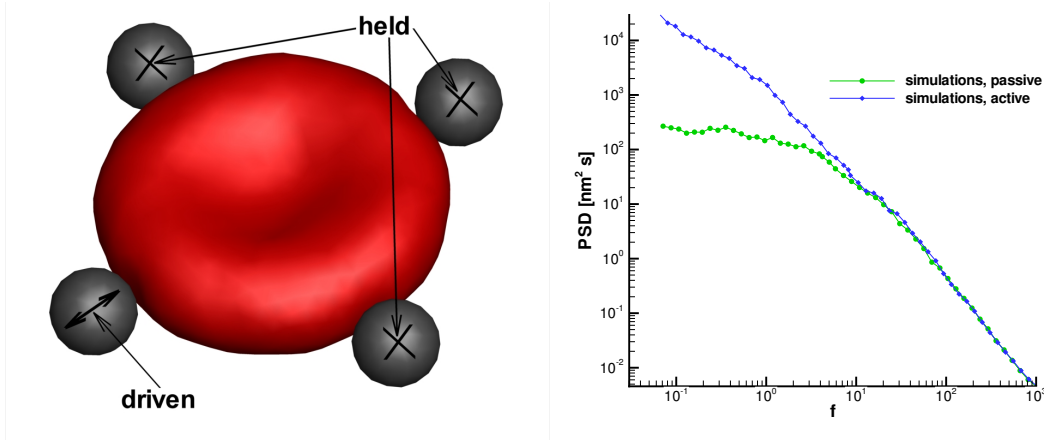


Fig. 17: Simulations of RBC membrane fluctuations. (a) Simulation set-up mimicking the experimental conditions with a red blood cell and four beads attached. Three beads (marked by crosses) are used as handles via a harmonic potential, whereas the fourth probe bead (marked by a two-headed arrow) is either sinusoidally driven to measure the mechanical response or left free to monitor its fluctuations. (b) Comparison of the power spectral density (PSD) of a system with passive thermal fluctuations and the corresponding system with an activity generated by random kicks at the membrane.

membranes such as the model in Eq. (30).

Recently, it has been recognized that cell activity (e.g., metabolic) through the consumption of adenosine triphosphate (ATP) contributes to measured flickering for RBCs. The effect of ATP on membrane fluctuations has been investigated in a number of experiments [141, 144–148] with contradicting outcomes. RBC fluctuations have been reported to depend on the viscosity of suspending media [144], which points toward out-of-equilibrium contributions. The studies with ATP depletion [141, 146] have shown that membrane fluctuations decrease; however, during the ATP depletion process there is no guarantee that RBCs are not subject to changes in membrane elasticity. In contrast, other investigations [145, 147] have questioned the effect of ATP on measured flickering. Recent work [148] has provided compelling evidence for cell activity by testing directly the fluctuation-dissipation relation, which is valid for any system in equilibrium. A violation of the fluctuation-dissipation relation has been shown using a setup illustrated in Fig. 17(a). In this setup, the three handle beads are held by a harmonic potential in simulations mimicking optical tweezers in experiments, whereas the probe bead is moved sinusoidally to determine the mechanical response function. The free fluctuations of the probe bead are measured separately in simulations and experiments. Simulations have closely mimicked experimental conditions [148] and were used to quantitatively extract RBC membrane properties including shear elasticity, bending rigidity, and membrane viscosity. To simulate active processes, random active forces acting normally on membrane vertices were added. Figure ??(b) shows the power spectral density (PSD) of a passive system, where thermal fluctuations of the probe bead were monitored, and the corresponding system with the activity model. PSD is the Fourier transform of the trajectory of the probe bead. Clearly, certain frequencies are enhanced in case of an active process, which is in quantitative agreement with the experimental observations [148]. The combination of experiments, simulations, and theory has allowed the quantification of active fluctuations and the characterization of properties and kinetics of cell's

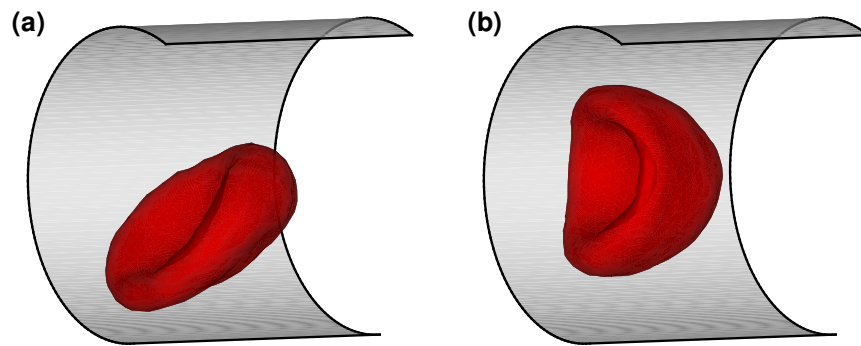


Fig. 18: Simulation snapshots of a RBC in tube flow (from left to right). (a) an off-center slipper cell shape and (b) a parachute shape. From Ref. [97].

activity [148].

3.8 Red Blood Cells in Flow

The behavior of RBCs in microcirculation plays an important role in blood flow resistance and in the cell partitioning within a microvascular network. Therefore, a number of investigations have focused on the understanding of RBC deformation and dynamics in simple flows such as shear and Poiseuille flow. RBCs are very similar by construction to polymerized vesicles. Most of available experiments with single RBCs were performed in suspending fluids with a relatively high viscosity, often much larger than that of blood plasma. This allows the imposition of high enough fluid stresses on RBCs, while keeping the corresponding shear rates under a certain limit, which is often imposed by used experimental instruments (e.g., rheometer). However, such conditions are very different from physiological conditions and may not properly reflect the behavior of RBCs in blood. Below, we will briefly review the dynamics of RBCs in shear and Poiseuille flows and emphasize the differences in RBC behavior in suspensions with different viscosities.

RBCs suspended in a relatively high-viscosity fluid (greater than about 5 times viscosity of water) show tumbling dynamics at low shear rates and tank-treading at high shear rates in Couette flow [149–151]. The existence of the tumbling-to-tank-treading transition is attributed to a RBC minimum energy state, such that a certain energy barrier has to be exceeded for a RBC to start the tank-treading motion. In the tank-treading state, a RBC also oscillates around the preferred inclination angle of tank-treading with a certain frequency and amplitude [151–154]. Recent experiments [155] have identified another dynamics, RBC rolling, which occurs within the range of shear rates between RBC tumbling and tank-treading states.

Note that all these studies have been performed under the conditions where the viscosity of suspending media was larger than that of the RBC cytosol. However, under physiological conditions blood plasma has a viscosity about five times smaller than that of a RBC cytosol. Using a similar viscosity ratio, recent experiments [156] and simulations [157] have shown that a large enough viscosity contrast between inner and outer fluids suppresses the tank-treading motion of RBCs, leading to the preference for RBC tumbling. This behavior is qualitatively consistent with that for vesicles, where a transition from tank-treading to tumbling can be triggered by an increase in the viscosity contrast [158]. This indicates that membrane shear elasticity may play a secondary role for RBC dynamics at high enough viscosity contrast.

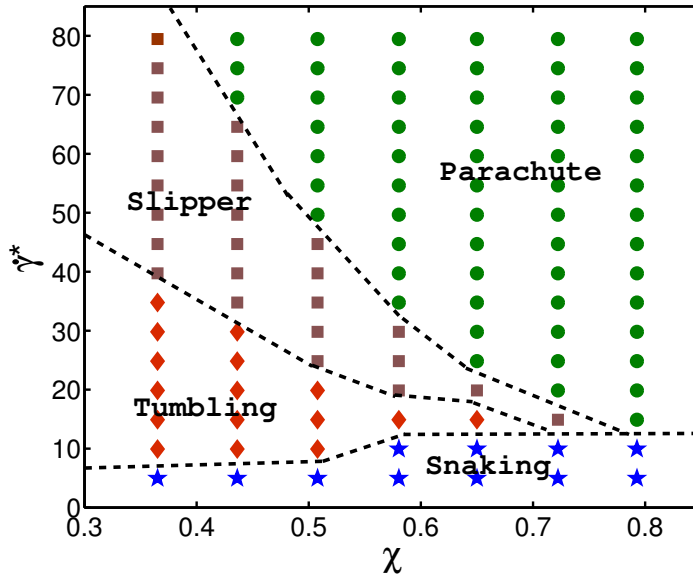


Fig. 19: A phase diagram of RBC shapes in tube flow for the average membrane properties of a healthy RBC with a Föppl-von-K’arm’ number $\Gamma = 2652$. Different dynamic states, depending on the flow strength characterized by a non-dimensional flow rate $\dot{\gamma}^*$ and the confinement χ , are depicted by symbols: parachute (green circles), slipper (brown squares), tumbling (red diamonds) and snaking (blue stars) discocyte. The phase-boundary lines are drawn approximately to guide the eye. From Ref. [97].

Similar to vesicles, RBCs in Poiseuille flow show a rich behavior, characterized by various shapes including parachutes and slippers [97, 159–163], as illustrated in Fig. 18. Parachutes are characterized by a symmetric shape similar to a semi-spherical cap and they flow in the center of a tube without significant membrane motion. In contrast, slippers are non-symmetric RBC shapes, where the membrane is subjected to a tank-treading motion. Thus, slippers are mainly differentiated from parachutes by an asymmetric shape and the membrane motion.

Recent 2D simulations [162, 163] have led to a phase diagram of various shapes including parachute, slipper, and a snaking dynamics, as function of RBC confinement and flow strength. The snaking dynamics is characterized by a wiggling motion of a discocyte shape near the tube center. 3D simulations [97] have been used to generate a similar diagram of RBC shapes in tube flow, which is qualitatively similar to the diagram in 2D. Figure 19 shows the RBC shape diagram in 3D for different flow rates and confinements. The flow rate is characterized by a non-dimensional shear rate $\dot{\gamma}^*$, which is a product of the average shear rate (or pseudo-shear rate) and the characteristic relaxation time $\tau = \eta R^3 / \kappa$ of a RBC [97]. The confinement χ is the ratio of an effective RBC diameter and the tube diameter. At strong confinements and high flow rates, parachutes are mainly found, while low confinements lead predominantly to off-center slippers. When the flow rate is small enough, off-center tumbling RBCs are found, which can be explained by the existence of the tumbling-to-tank-treading transition mentioned above for RBCs in shear flow. In contrast to the 3D model results, this region is absent in 2D simulations [162, 163], since this transition cannot be captured by a 2D model. Another prominent difference between the phase diagrams in Fig. 19 and in 2D simulations [162, 163] is the existence of the “confined slipper” in 2D at high confinements which is absent in 3D. Slippers at high confinements in 3D are hindered due to the cylindrical shape of a tube, which makes

a confined slipper configuration energetically unfavorable, since it would have to conform the wall curvature. In microcapillary flow, changes in RBC membrane properties lead to a shift of boundaries between different RBC shapes and dynamics illustrated in Fig. 19 [97]. Consequently, it should be possible to detect such changes based on the observation of RBCs in flow and simulations can provide the basis for a quantitative interpretation of these observations.

4 Conclusions and Outlook

Models and simulation techniques for the entire range of length scales relevant for giant vesicles – from atomistic models for single lipids via coarse-grained molecular models for self-assembly and lipid organization in a membrane, to discretized continuum models for vesicle shapes – have been developed and applied in recent years. Developments of these techniques have extended the applicability domain for vesicle simulations towards more complex many-component systems that cannot be studied easily by analytical calculations. On the molecular scale, coarse-grained molecular models with chemical specificity have become available – independently from the increase in computational speed – for much larger systems. The combination of discretized continuum membrane models with mesoscopic hydrodynamic simulation techniques is nowadays successfully used to simulate vesicles and simple cells in flow, such as vesicle deformation in structured channels and blood flow in small capillaries. Finally, a combination of continuum membrane models with models for membrane proteins, the simulation of coupled fluid and polymerized membranes, and modeling of the interaction of membranes with cytoskeletal filaments allow the extension of bare vesicle simulations to biomimetic and biological systems. In combination with hydrodynamics, also the dynamics of such biomimetic systems can be accessed. This provides versatile tools and opens up exciting possibilities toward studying equilibrium and dynamic properties of multi-component vesicular systems with passive and active components.

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