Of Stalks and Diamonds. Simulation Studies of Membrane Fusion and the Role of Fusion Peptides
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Chapter 2

Background and theory

Nothing exists in itself.
Herman Melville

Before we can go into the actual discussion of my work, it is necessary to establish the context it has to be seen in. To that end, this chapter will introduce the general background and theoretical framework of my thesis.

2.1 Molecular dynamics

With the possible exception of Chapter 6, little knowledge of molecular dynamics is needed to understand the work presented in this thesis, and the method will quietly remain in the background, leaving the stage to what it served to bring about. Nevertheless, or maybe rather because of that, I feel it necessary to give a brief introduction to how molecular dynamics accomplishes what it does. While the aim is to keep this part general, it is primarily an introduction of the methods used in this thesis and as such necessarily a selection1.

2.1.1 Basic molecular dynamics

Molecular dynamics is a method for the simulation of molecules with the help of computers. The molecules are described as a number of particles or interaction sites, which in the most straightforward case represent the atoms comprising the molecules in a one-to-one correspondence. Every particle is associated with three unchanging parameters defining its mass, charge and type, and two variable vectors describing its position \( \mathbf{x} \) and velocity \( \mathbf{v} \).

Interactions between particles fall into two categories: non-bonded and bonded. Non-bonded interactions connect all possible groupings of particles up to a certain group size, \( e.g. \) every pair, triple, \( etc. \) with at least one potential. A common choice is to use two pair potentials, a Coulomb potential reflecting charge-charge

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1For a broader perspective on molecular dynamics, the reader may consult [1, 2]. Other than that, no references will be given in this section. Original publications and further information will be given in the methods sections of the respective chapters.
interactions and a Lennard-Jones (12-6) potential, mimicking long-range van-der-Waals attractions and short-range Pauli repulsion, whose parameters are listed for every possible pair of particle-types. As such, non-bonded interactions describe the interactions that can be systematically ascribed to particle properties alone. Bonded interactions, on the other hand, describe the more individual restrictions imposed on the relative position of particles that belong to the same molecule and have to be explicitly defined for every restriction. To that effect, potentials depending on the positions of two, three or four particles are introduced, representing the molecular “skeleton” as bonds, angles and dihedrals, respectively.

The actual simulation, that is the propagation of the particles’ positions through time, cannot be accomplished in a smooth, continuous fashion for any but the most simple systems due to the interdependency of the particles’ motion. One therefore has to settle for a numerical, stepwise solution, calculating the approximate positions after a timestep $\Delta t$ from values known from previous steps. Expressing the positions at $t + \Delta t$ and $t - \Delta t$ as (infinite) Taylor expansions of $x(t \pm \Delta t)$, it is possible to eliminate all terms of uneven order by adding the two expressions. Ignoring terms of order $O(\Delta t^4)$ and higher, the only value needed for the computation of $x(t + \Delta t)$ not immediately available from previous steps is $\ddot{x}(t) –$ the acceleration. However, since the potentials connecting the particles are known, the resulting forces and therefore the accelerations can be calculated. In addition, interpolated velocities at half timesteps $t + \Delta t/2$ can be introduced as the difference between the positions at $t + \Delta t$ and $t$ divided by $\Delta t$. With these relations, it is possible to calculate new velocities at $t + \Delta t/2$ using velocities at $t - \Delta t/2$ and accelerations at $t$, and new positions at $t + \Delta t$ using positions at $t$ and velocities at $t + \Delta t/2$, iteratively propagating the simulation in a leapfrog fashion along the way.

While it is desirable to use a large timestep in order to cover the most time with every step, the permitted stepsize is limited by the complexity of the model used, since the region in which the introduced approximations are valid is smaller, the more rugged the potential landscape is. A too large timestep will carry the simulation into unrealistic regions of phase space. However, even with a small timestep, the sheer amount of necessary calculations will lead to an accumulation of neglected higher order terms and numerical error and cause global properties of the system, i.e. pressure and temperature, that ought to be conserved to gradually drift over time. While more subtle than the effects of using a too large timestep, this effect nevertheless needs to be avoided, which can be accomplished by coupling the drifting properties to a reference value by scaling the positions and velocities in a direction that drives the coupled property towards the default. An example is the weak coupling scheme introduced by Berendsen, in which the scaling is performed so that deviations from the default decay exponentially with time.

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2meaning the space whose coordinates are given by the total of the particles’ position and momentum vectors
3at least at equilibrium
2.1. MOLECULAR DYNAMICS

2.1.2 Efficiency enhancements

What has been described so far is in principle sufficient to run a basic molecular dynamics simulation, but it is not very economic. The most time-consuming step is the calculation of the forces, making it attractive to omit some of the interactions defined in the model. While it is not possible to ignore any of the bonded potentials, many of the non-bonded interactions are so weak that they contribute little to the outcome of a simulation. Since both the Coulomb and Lennard-Jones potential decay at larger distances, only including non-bonded interactions within a certain cut-off can greatly enhance the performance of a simulation, while resulting artifacts can be minimized by shifting the potentials to smoothly decay to zero at the cut-off and introducing a correction for charged particles mimicking the force such particles experience due to the polarization they induce in their surroundings. In addition, the distance particles travel in a single timestep is so small, that only the interactions between particles in very close proximity differ significantly between consecutive timesteps, especially since those close to the cut-off are comparatively weak in the first place. It is therefore possible to introduce a second, smaller cut-off, beyond which non-bonded interactions are only calculated for the first of a number of steps and reused in the following steps until the next calculation.

Another possible improvement in computational efficiency stems from the fact that the permitted size of the timestep is not only limited by the ruggedness of the potential landscape, but also by the velocity of the particles. If there are some particles in the system that have a much lower mass than the rest, e.g. hydrogens, their movements will usually be the fastest in the system. At the same time, however, these movements will have little effect on the dynamics of the particles of higher mass and can be regarded as decoupled. Unless one is specifically interested in them, it is therefore safe to constrain these degrees of freedom to their equilibrium value, trading some extra calculations for the possibility to use a larger timestep.

2.1.3 Modeling strategies

It is common to refer to the set of potentials describing the interactions between particles as model, representation or forcefield, and to separate them from the parameters used for the simulation. However, the simulation parameters will affect the behavior of the model, and are therefore in practice inseparable from it.

When determining the particle types and interactions used for a model, the aim is usually to study some aspect of reality the underlying relations of which cannot be solved analytically. One approach is therefore to create a system in which all interactions are known in order to study the resulting behavior. For this purpose, it is most meaningful to create a model that is either so simple that the behavior can indeed be understood as consequence of its interactions or to create a model whose interactions mimic known principles as accurately as possible in the hope of gaining insight into an aspect of reality that does not lend itself to direct observation.
Alternatively, it is possible to attribute less meaning to the model itself, seeing the defined interactions as little more than means to the end of effecting a certain behavior. In this approach, the model is tuned to display a number of basic properties, studying what complex behavior can be observed as a consequence and superposition of the simple input-properties.

Another distinction between models can be made with regard to the level of detail of the representation. Since molecular dynamics is based on classical mechanics, an all-atom representation in which the interaction sites have a one-to-one correspondence to atoms is the natural upper limit to the possible resolution a model can have. However, many of the degrees of freedom contained in such a representation are of little relevance to a large number of questions. For these, choosing a model with fewer degrees of freedom will greatly increase the speed at which the phase space is sampled, making coarse-grained models in which more than one atom are mapped to a single interaction site attractive.

An example is the coarse-grained MARTINI model which has been used for most of the work presented in this thesis. In this model, the interaction sites represent on average four atoms and are assigned a type based on their polarity. This type governs the strength of a Lennard-Jones (12-6) pair potential, the interaction-levels of which have been parametrized to effect the proper partitioning behavior of polar and apolar particles, and realistic densities and relative diffusion rates. In addition, the particles are assigned a charge by which they interact through a Coulomb potential, and are connected by bonded potentials describing bonds, angles and dihedrals.\footnote{A detailed description of the MARTINI model can be found in the Appendix of this thesis.}

While much can be gained by choosing the proper model for a given question, purely fine- and coarse-grained models both have limitations that cannot be overcome. A combination of multiple levels of representation, on the other hand, might open new possibilities. To this end, new methods are presently being developed, enabling the smooth switching between different representations and their simultaneous use in single simulations, both in a temporal and spatial sense.

\subsection{Geometrical concepts}

For the discussion of lipid polymorphism, the main topic throughout this thesis, a precise concept of shape is indispensable. This section will therefore present curvature as a measure of the local shape of interfaces, and morphology as a characterization of the global properties of the regions of space enclosed by these interfaces.
2.2. GEOMETRICAL CONCEPTS

Figure 2.1: Illustration showing how to obtain the curvature of a planar curve. The unit normal vectors corresponding to the points passed during progressing a distance $\Delta s$ along the curve are mapped to the unit circle. The length of the dimensionless arc swept out by this mapping is the change of direction $\Delta \theta$.

2.2.1 Curvature

Planar curves

For a planar curve, its curvature $k$ at a given point is defined as the rate with which its direction changes while progressing an infinitesimal distance $ds$ on the curve:

$$k = \frac{d\theta}{ds} = \lim_{\Delta s \to 0} \frac{\Delta \theta}{\Delta s}.$$  \hfill (2.1)

The change of direction $\Delta \theta$ can be obtained as the length of the dimensionless arc the unit normal vector\(^5\) sweeps out when the vectors for all points passed on the curve are mapped to the unit circle, as illustrated in Fig. 2.1. The results are independent of the direction of progress along the curve, with the curvature’s sign depending on the choice of orientation for the unit normal vector.

Surfaces

In analogy to the definition for planar curves, the curvature $K$ of a surface at a point $P$ can be defined by the area $\Delta G$ swept out by the projection of the unit normal vectors for every point within a connected area patch containing $P$ onto the unit sphere, divided by the corresponding area $\Delta F$ on the original surface in the limit $\Delta F \to 0$:

$$K = \lim_{\Delta F \to 0} \frac{\Delta G}{\Delta F}.$$  \hfill (2.2)

\(^5\)or alternatively the unit tangent vector
The curvature $K$ defined in this way is known as the Gaussian or total curvature. Unlike the curvature $k$ for planar curves, however, the Gaussian curvature does not uniquely define the shape of the underlying surface at that point.

For a full characterization of the local shape, it is necessary to introduce the concept of principal curvatures. To that end, we note that the local shape of a surface at a point $P$ is completely determined by the entirety of the curvatures of the geodesics running through $P$, that is the planar curves that are defined as the intersections of the surface with all planes containing the normal vector at $P$, as illustrated in Fig. 2.2. Due to the local symmetry of these curvatures around the normal vector, however, only the minimum and maximum values $k_1$ and $k_2$\(^\dagger\) are needed to uniquely define the shape of a regular surface at any point.

It can be shown from analytical considerations that the product of the principal curvatures is equal to the Gaussian curvature:

$$K = k_1 k_2.$$  \hspace{1cm} (2.3)

\(^\dagger\)which lie in orthogonal directions
Introducing the mean curvature $H$ as the average of the principal curvatures,

$$H = \frac{k_1 + k_2}{2},$$

(2.4)

$H$ and $K$ together unambiguously describe the local curvature of a surface.

**Positive and negative curvature**

Only for the Gaussian curvature, the sign of the curvature is unambiguous and allows the distinction of hyperbolic ($K < 0$), parabolic ($K = 0$) and elliptic ($K > 0$) regions. For the curvature of curves and consequently principal and mean curvature, there is no clear meaning attached to negative and positive values, except that they are distinct.

If we use curvature to describe physical systems, however, we usually deal with surfaces that separate clearly distinguishable regions. In these cases, it is customary to make the choice of sign for the principal curvatures dependent on which region the corresponding geodesic embraces at that particular point.

### 2.2.2 Morphology

In the previous section, we have described the local shape of surfaces in terms of curvature. What is missing is a characterization of the more global properties of geometrical objects in the sense of their overall shape and connectivity, which is referred to as morphology.

**Minkowski functionals**

Examples of measures $\phi$ characterizing the shape of geometrical objects are area and perimeter in two dimensions, or surface area and volume in three dimensions. All of these share two properties: motion-invariance and additivity. The first implies that these measures do not depend on the object’s position and orientation in space and therefore have identical values for congruent objects. The second demands that the measure of any object $P$ is the sum of the measures of the non-overlapping subsets for any partition of $P$, which is equivalent to demanding that

$$\phi(P_1 \cup P_2) = \phi(P_1) + \phi(P_2) - \phi(P_1 \cap P_2)$$

(2.5)

for any two objects $P_1$ and $P_2$.

For the special case of geometrical objects that belong to the convex ring $\mathcal{R}$, that is the family of subsets of Euclidean space that can be expressed as finite unions of compact convex sets, it has been shown that there is only a limited number of independent measures that have these two properties. For $d$ dimensions, any motion-invariant additive functional on $\mathcal{R}$ can be written as a linear combination of $d+1$ so-called quermassintegrals or Minkowski functionals [3, 4]. These Minkowski functionals correspond to basic geometrical aspects of the system. For three dimensions, they are proportional to the occupied volume,
the surface of that volume, the integrated mean curvature of that surface and the object’s Euler characteristic\textsuperscript{7}.

However, while the Minkowski functionals allow a systematic characterization of important properties of geometrical objects, the morphological description given is not complete, and it is not generally possible to reconstruct the underlying object from the values of the Minkowski functionals alone.

**Morphological image analysis**

A $d$-dimensional black and white digital image is a set of pixels, each of which has the same dimensionality as the image. The pixels have edges of uniform length that are aligned with the orthogonal dimensions. If one focuses on what is depicted, that is the positive (e.g. the black) pixels, this can be seen as a region of $d$-dimensional Euclidean space that possesses a distinct shape defined by the number of positive pixels and their relative position.

As such, digital black and white images are a special case of objects belonging to $\mathcal{R}$. For these, the extraction of the Minkowski functionals is especially easy and can be accomplished by simply counting the number of pixels and pixel-components\textsuperscript{8} comprising the image, where pixel-components shared by multiple pixels are counted only once [4]. For a three-dimensional image one therefore only needs the number of pixels, which in three dimensions are called voxels, and the number of faces, edges and vertices these consist of. The exact relation between these and the geometrical properties corresponding to the Minkowski functionals is given in Chapter 5, Table I.

2.3 **Lipid polymorphism**

Lipids are a class of chemical compounds that possess a low solubility in polar solvents. Most lipids are amphiphilic, implying they have both hydrophobic and hydrophilic parts, the classic example being phospho- and sphingolipids which possess one or two flexible hydrocarbon tails covalently bound to a polar, often charged or zwitterionic headgroup.

This section will introduce the nature of lipid aggregates and describe two successful models used to reduce the complex phenomena encountered in the study of mixtures of lipids and water to simple underlying principles\textsuperscript{9}.

2.3.1 **Lyotropic phases**

Lipids and other amphiphiles have the unique ability to form lyotropic phases when mixed with water. These phases possess both liquid- and solid-like qualities

\textsuperscript{7}the latter is an integer number that is a measure of the volume’s connectivity and proportional to the integrated Gaussian curvature of its surface

\textsuperscript{8}i.e. the sequence of pixels of lower dimensionality down to the vertices of dimensionality zero

\textsuperscript{9}A general introduction to this topic can be found in [5].
2.3. **LIPID POLYMORPHISM**

Figure 2.3: Illustrations of typical motifs encountered in lyotropic phases adopted by lipids. Shown are a regular micelle (A), three cylinders of an inverted hexagonal phase (B), one unit cell of an inverted double cubic bicontinuous phase corresponding to the Schwarz P surface (C), a stalk (D), and two lamellae of a lamellar phase (E). Exclamation marks indicate regions of unfavorable packing.

...in the sense that they show no short-range order in the arrangement of molecules but still maintain a crystal-like long-range order.

As liquid crystals, lyotropic phases are characterized by their symmetry. The main feature used to order the different phases, however, is the mean curvature of the interface between polar and apolar components. As such, one can state a clear sequence from spherical to cylindrical to planar configurations, where both regular and inverted forms exist for the spherical and cylindrical systems.

If we adopt the convention of assigning positive mean curvature to a sphere filled with apolar moieties in a polar environment, the highest mean curvature is encountered in micellar phases in which the lipids form spherical aggregates with the headgroups lining the surface of the sphere and the hydrophobic tails towards the center (Fig. 2.3 A). Consistently, the highest negative mean curvature is encountered in inverted micellar phases, where spherical cavities of water are surrounded by lipid headgroups and the hydrocarbon tails fill the remaining space. Both regular and inverted micellar phases can possess several symmetries, depending on which packing is optimal for the given conditions.

Cylindrical phases, in which linear tunnels filled with either polar or apolar moieties run through a matrix of the other moieties with the lipid headgroups...
lining the interface are typically encountered with hexagonal symmetry. The regular hexagonal phase where the lipid tails fill the cylindrical tunnels has positive mean curvature, and the inverted hexagonal phase where the tunnels are filled by water has negative mean curvature (Fig. 2.3 B).

Finally, the planar configurations have zero mean curvature. These are called lamellar phases and consist of stacked lipid bilayers, in which parallel planar interfaces are lined with lipid headgroups, alternatingly separated by hydrocarbon tails and water (Fig. 2.3 E).

In addition, several intermediate phases with non-zero mean curvature have been reported. These correspond to less homogeneous geometries than the micellar, cylindrical and planar phases. These are the doubly periodic mesh phases and the triply periodic cubic phases.

The mesh-phases can best be described in terms of stacked bilayers similar to the lamellar phase. However, either the lipid bilayers or the water layers have holes filled with the moieties from the other layer. The arrangement of the holes determines the symmetry of the phase, rhombohedral as well as tetragonal and monoclinic configurations are possible. As before, regular and inverted phases are distinguished. Regular phases have holes penetrating the lipid bilayers (pores) and display positive mean curvature, whereas negative phases have lipid bridges (stalks, Fig. 2.3 D) crossing the water layers and display negative mean curvature. In both pores and stalks the interface is regarded as lined with lipid headgroups.

The triply periodic cubic phases are bicontinuous phases in which both the polar and the apolar moieties are continuous in all three dimensions. These phases are believed to generally correspond to certain triply periodic minimal surfaces that have the peculiar property of having zero mean curvature at every point of the surface. So far, bicontinuous cubic phases corresponding to the Schwarz (P) (Fig. 2.3 C), the diamond (D) and the gyroid (G) surface have been reported, in which the minimal surface corresponds to the midplane of a (curved) lipid bilayer. The topology of these phases can be described as two separate but interwoven networks of water channels, isolated by the lipid bilayer, making them double phases, since two isolated compartments exist for at least one of the components. For the P, D and G surfaces, those two compartments are congruent, although this is not a formal requirement of a double phase. Both regular and inverted phases are defined, even though only inverted phases like the ones described above have been reported for lipids. The hypothetical regular phases correspond to tubular networks of lipids running through a matrix of water.

2.3.2 Theoretical aspects

The general driving force behind the formation of phases is the energetic cost of mixing moieties of different polarity. Simply put, a phase is the attempt to isolate the lipid tails from the aqueous environment. The result is an interface, lined by

\[ \text{\footnotesize This name is not used consistently throughout the published literature. In this book, we adopt the notion of mesh phases referring only to the doubly periodic, non-cubic stalk- and pore-phases.} \]
the lipid headgroups.

**Surfactant parameter**

Assuming that there is a fixed amount of matter in a system, and given the low compressibility of liquids, a simple characterization of a system can be given as the surface-to-volume ratio of the lipid aggregate. While seemingly crude, this characterization is surprisingly powerful if analyzed systematically. Since the lipids in the aggregate can be seen as individual building blocks each of which contributes an amount of area to the interface corresponding to the size of its headgroup and occupies a specific volume with its tails, it is clear that the composition of the system directly gives rise to a preferred surface-to-volume ratio of the aggregate.\(^\text{11}\)

In addition, even though the lipids are relatively flexible molecules especially in the tail region, for energetic as well as entropic reasons it is favorable for all lipids to assume roughly similar shapes in the aggregate, allowing the building blocks to be treated as possessing an approximate shape. This assumption imposes further restrictions on the shape of the interface, demanding a certain amount of homogeneity to account for the shape of the building blocks without leaving empty space.

A simple way to describe the shape of a lipid is the dimensionless surfactant parameter \(N_s\) introduced by Israelachvili [6] as

\[
N_s = \frac{v}{al},
\]

where \(v\) is the actual volume taken up by the lipid tails, \(a\) is the preferred area per headgroup and \(l\) is the optimal length of the tails. \(N_s\) compares the actual volume of a lipid to that of a cylinder with a height corresponding to the tail length and a cross-section identical to that of the headgroup. Comparing the surfactant parameter of a lipid to that of known geometrical bodies like (inverted) cones or cylinders, simple considerations can give an idea of which phase is most suitable for a given lipid. As an example, the volume of a cone is \(\frac{1}{3}\) the volume of a cylinder with the same height, suggesting a micellar phase for lipids with a surfactant parameter of \(\frac{1}{3}\), while a value of 1 would suggest a lamellar phase.

It is possible to refine this approach further by introducing a relation to the curvature of the interface as demonstrated by Hyde [7]. It is known from differential geometry that the area \(a(d)\) of parallel surfaces is connected via the Gaussian and mean curvature \((K\text{ and } H)\) by

\[
 a(d) = a(0) \left(1 + 2Hd + Kd^2\right),
\]

where \(d\) is the distance between the surfaces. The volume between parallel surfaces can be obtained as the cumulative “sum” of the areas of all the parallel surfaces

\(^{11}\)This behavior is strongly affected by the conditions the system is under, like for example temperature and pH. These effects will be treated in the next section. For now, the conditions and therefore the properties of the lipids are assumed to be constant.
in-between by integrating Eq. 2.7. Doing this for a lipid monolayer of thickness \( l \) (as given by the tail length) yields a volume of

\[ v = a(0) \left( l + Hl^2 + \frac{Kl^3}{3} \right). \]  

(2.8)

Setting \( a = a(0) \), this expression can be related to the surfactant parameter, defining a restriction of the allowed curvatures for a given lipid shape by

\[ N_s = \frac{v}{al} = 1 + Hl + \frac{Kl^2}{3}. \]  

(2.9)

Eq. 2.9 has to be satisfied for a surface composed of lipids with a certain surfactant parameter, but does not in general specify a unique solution and describes only monolayers. Taking into account that only certain relative arrangements of monolayers are possible leads to additional boundary conditions. As a consequence, bilayers have to satisfy Eq. 2.9 for both monolayers simultaneously, requiring that the midplane has zero mean curvature.

Altogether, it is found that in order to fulfill all requirements, a surface has to have uniform mean and Gaussian curvature over its whole area [7]. Due to the approximate nature of the description, however, this is not a strict condition, and should rather be read in the sense that for a given surface-to-volume ratio, the most homogeneous solution to Eq. 2.9 is realized. Since the surface-to-volume ratio depends mainly on the topology of the aggregate and is at the same time imposed by the composition of the system, one can conclude that the composition determines the topology of the adopted phase, and the exact shape is then tuned to be as homogeneous as possible. This is, however, an overly simplified picture, since there is no guarantee that the required surface-to-volume ratio can be realized with a curvature satisfying Eq. 2.9, potentially leading to intrinsically frustrated systems.

Looking at the phases described above it is possible to rationalize them within this description. The micellar, cylindrical and planar phases possess constant uniform curvature. In addition, surfaces of constant mean and nearly homogeneous Gaussian curvature exist parallel to the triply periodic minimal surfaces of the cubic bicontinuous phases as well as around the lipid aggregates of the mesh-phases [7].

**Helfrich energy of curvature**

A different model has been introduced by Helfrich for the bending energy of monolayers [8]. In this model, the monolayers are regarded as elastic sheets giving rise to

\[ g = \frac{k_m}{2} (H - c_0)^2 + k_g K \]  

(2.10)

as the expression for the elastic energy of curvature per unit area \( g \) and therefore

\[ G = \frac{k_m}{2} \int_A (H - c_0)^2 dA + k_g \int_A K dA \]  

(2.11)
for the total energy of curvature $G$, where $k_m$ is the bending rigidity and $k_g$ the elastic modulus of the Gaussian curvature. The spontaneous curvature $c_0$ takes the shape of different lipids into account as a propensity for a certain amount of either positive or negative mean curvature.

Like the surfactant parameter, the Helfrich energy of bending can be used to rationalize the different lipid phases in terms of their curvature, predicting phases with uniform mean curvature of a value close to $c_0$, since any deviation from that value will raise the energy. The Gaussian curvature receives less attention in this prediction since its contribution only depends on the topology and is invariant under mere stretching and bending. In addition, little experimental data is available on the Gaussian curvature elastic modulus. Recent findings [9] suggest, however, that the energetic contribution of Gaussian curvature can be significant and the topology of a phase therefore has more implications than satisfying the required surface-to-volume ratio (see above).

While the Helfrich model allows the prediction of phases as those with surfaces of low energy of curvature, it neglects the packing efficiency of the different phases (cf. Fig. 2.3). Since inefficient packing will lead to deviations from the ideal curvature in a real system, the predicted energy of a system is likely too low, possibly making phases with higher predicted energy but more efficient packing favorable. In addition, these two contributions to energy may be hard to minimize simultaneously, again giving rise to intrinsically frustrated systems.

### 2.3.3 Phase transitions

So far we have discussed lipid polymorphism from a static perspective. However, the favored phase depends on the prevailing conditions and changes in the environment will result in dynamic behavior of the lipid aggregates. For example, high temperature or dehydration can trigger a transition from the lamellar to the inverted hexagonal phase, while a low pH will cause the opposite, and the addition of linear alkanes is found to stabilize the inverted hexagonal phase. This can be rationalized using the presented theories as a dependence of the surfactant parameter $N_s$, spontaneous curvature $c_0$ and packing on the conditions:

- **High temperature** Due to increased conformational chain isomerization, a rise in temperature will increase the volume occupied by the lipid tails and lower their length in accordance. This corresponds to an increase of $N_s$ and a decrease of $c_0$, both of which explain the change towards more negative mean curvature.

- **Low pH** A drop in pH and ensuing protonation of the lipids’ headgroups will lead to an increase of the repulsion between the headgroups\(^\text{12}\). The larger area required per headgroup gives rise to a decrease of $N_s$ and an increase of $c_0$, rationalizing a change towards positive mean curvature.

\(^{12}\)except for anionic lipids
• **Dehydration**  Dehydration will lower the area per lipid headgroup, in consequence increasing $N_s$ and decreasing $c_0$, rationalizing a transition towards more negative mean curvature.

• **Addition of linear alkanes**  Linear alkanes partition to the hydrophobic tail region of lipid aggregates. If a phase possesses potentially void areas in that region, which is the case for the inverted hexagonal phase, these voids can be filled by the alkanes, reducing the energetic costs due to unfavorable packing.

Most phases are topologically distinct from each other and therefore cannot be interconverted by only stretching and bending, requiring parts of the surface to be broken and joined. Since these transitions usually involve intermediate states of high energy, lipid phases have a high degree of metastability far beyond their preferred range of conditions, making the transitions very slow and often hysteretic. In addition, multiple seeds are typically required at different locations before a transition can take place, which can turn out to be a hindrance when initial and final phase are topologically very different, making especially the formation of bicontinuous cubic phases difficult to observe.

### 2.4 Fusion and fusion peptides

Cellular life relies on the separation of an in- from an outside. In addition, compartmentalization of the intracellular space into organelles is crucial for many vital cellular functions. All of this is accomplished with the help of lipid bilayers. While a certain amount of stability is required for these layers, many processes demand a significant degree of plasticity. The way this plasticity is enabled and controlled will be the subject of this section.

#### 2.4.1 Biological membranes

The majority of lipids found in the membranes of eukaryotic cells are phospholipids, glycolipids and steroids. In those lipids that possess fatty acids donating hydrocarbon chains, these are mainly palmitic acid (16:0)\(^\text{13}\), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and arachidonic acid (22:4), while typical lipid headgroups contain choline, ethanolamine, serine and inositol. In addition, most biological membranes have a large amount of protein, contributing around 50% of the membrane’s mass. While the lipid composition differs between different organisms, cell types and cellular compartments, it is specific for its location and rigorously controlled to maintain the properties required for its function.

The basic arrangement of the lipids is that of a bilayer similar to a single lamella of the lamellar phase described in the previous section. It is however worth noticing that the lamellar phase is not necessarily the favored phase for the

\(^{13}\text{the notation (X:Y) denotes a fatty acid containing X carbon atoms and Y unsaturated bonds}\)
composition of the membranes. In fact, most biological membranes have a large amount of lipids that favor inverted phases. While this affects various properties of the membranes and has been suggested to regulate the permeability (e.g. Cullis et al. [10]) and to enable certain functions of membrane proteins (e.g. Hui [11]), it is generally accepted that one of the main reasons for this tendency is to supply the membrane with the needed plasticity. Since fusion and fission can be compared to local phase transitions and involve intermediate stages that resemble inverted phases (see Section 2.4.2), keeping the lipid composition close to mixtures that have phase transitions under physiological conditions can help to overcome the high metastability of lipid aggregates. Biological membranes can therefore be seen as a compromise between the stability needed to avoid spontaneous rupture caused by fluctuations within the physiological conditions and the plasticity needed to allow (controlled) rearrangement.

The role of the lipids favoring inverted phases is thereby twofold. By keeping the energy of bilayers high, energetic barriers are more likely to be overcome. At the same time, these barriers are lowered due to the intermediate states' inverted character.

2.4.2 The fusion pathway

Fusion is the process of merging two separate regions of space both of which are bordered by lipid bilayers into one connected region bordered by a single bilayer. Both theoretical consideration of the topological requirements of merging separated bilayers as well as strong experimental evidence point towards a pathway known as the stalk-pore model which is generally accepted as an accurate description of fusion. The following stages are distinguished (cf. Fig. 2.4):

- The first state of fusion is known as stalk. This is the initial, very localized contact between the two opposing membranes in which the cis\textsuperscript{14} monolayers are joined in a symmetrical hourglass shaped connection. The stalk is characterized by an overall negative mean curvature and two potentially void areas immediately above and below the connection that might be a source of high packing energy. Theoretical models demonstrate that due to the opposing signs of the principal curvatures encountered it is possible for the stalk to adopt a stress-free surface for most of the neck region [12]. In addition, the energetic costs of unfavorable packing associated with the void regions can be reduced by introducing a gradient in the tilt angles of neighboring lipids, allowing locally non-smooth surfaces and therefore enabling the lipids of the trans monolayers to fill the void regions by slightly dipping towards the stalk [13]. The Gaussian curvature of stalks is high and gives a significant contribution to the energy [14].

The occurrence of stalks is not limited to fusion, but is believed to be the first step in most phase transitions from lamellar to inverted phases.

\textsuperscript{14}The two monolayers of the bilayers facing each other are known as the cis monolayers, while the two monolayers facing away from each other are called the trans monolayers.
• The next stage is usually referred to as the hemifused state and is characterized by a single bilayer, the hemifusion diaphragm, separating the interiors of the compartments to be fused. This is predicted to be achieved via radial expansion of the stalk and simultaneous approaching of the trans monolayers. This process is believed to be driven by negative spontaneous curvature of the merged cis monolayer which can increase the negative mean curvature of its surface by increasing the curved area via expansion. However, the expansion and approach of the trans monolayers creates a circular void region around the hemifusion diaphragm, which limits the expansion [15].

• Finally, the hemifusion diaphragm has to rupture to form the fusion pore. This step is believed to be driven by the accumulation of unfavorable packing and curvature energy in the small region of the hemifusion diaphragm together with an asymmetry of surface area of the cis and trans monolayers caused by the expansion of the stalk [15, 16]. This stage bears significant resemblance to the double cubic bicontinuous phases described in Section 2.3.1. In fact, these phases can be seen as a periodic arrangement of fusion pores.

With the formation of the fusion pore the topological changes are complete and the fused aggregate is free to assume its final shape via bending without further rupture or merging of membranes.

While the intermediate states of the fusion process are also required steps of inverted phase formation, fusion usually does not lead to these transitions even with lipid compositions close to the edge of bilayer instability. This is again due to the high metastability of the lipid aggregates, which allows the formation of stalks only when there is close contact between bilayers. Since the formation of inverted phases requires the formation of an array of multiple stalks, these changes are possible in the closely stacked bilayers of the lamellar phase\textsuperscript{15} but very unlikely to happen in systems that do not offer the opportunity of multiple inter-membrane contacts like vesicles in solution\textsuperscript{16}.

\textsuperscript{15}And even there the formation of cubic phases is not easy to trigger.
\textsuperscript{16}An exception are vesicles under extreme curvature stress caused by their composition or size. While stable at high hydration, contact between vesicles at lower hydration and subsequent stalk formation leads to their rupture and the assumption of different phases.
2.4.3 Controlled fusion and proteins

Fusion is not normally a spontaneous event. For it to occur by itself, the membranes in the system must be under significant stress or artificially made attractive to each other. Experimental techniques to study fusion therefore employ membranes, most often vesicles, that have been prepared to be prone to fusion without external intervention. In these experiments, close contact between the membranes is achieved by either raising the chemical potential of the water in the vesicles’ hydration shells via addition of polyethylene glycol (PEG-induced fusion, e.g. [17]), effectively driving the vesicles together, or by mixing vesicles containing anionic lipids with vesicles containing cationic lipids (e.g. [18]). Fusion under physiological conditions, that is controlled fusion of otherwise stable membranes, has to be mediated by the presence of additional molecules, most notably proteins.

There are three principal mechanisms in which proteins can affect membranes to facilitate fusion:

- **Enzymatic alteration of the lipid composition** This category includes enzymes that cleave lipids, removing either the headgroup or a tail, thereby altering the behavior of the membrane by introducing lipids with different properties. Examples are phospholipases and sphingomyelinase.

- **Mechanical work** This category includes proteins that form a scaffold with a preferred curvature around the membrane providing energy for the formation of fusion intermediates and membrane-anchored proteins that impose curvature or disrupt the bilayer by undergoing conformational changes. The scaffold mechanism has been suggested to be important for influenza HA mediated fusion [19], while SNARE\(^{17}\) mediated fusion is an example of proteins located in opposing membranes changing conformations upon binding to each other, supplying energy for the fusion (e.g. [20]).

- **Influencing the energies associated with geometry and packing** This category consists of proteins and peptides that alter the lipids’ behavior by their binding alone. While possessing no enzymatic activity or potential for mechanical work, they can influence the preferred shape of bilayers simply by taking space of their own and interacting with the surrounding lipids, changing the favored conformation or reducing the costs of unfavorable packing. These proteins can be subdivided into two groups depending on whether they penetrate into the membrane or merely sit on the interface. Examples are the fusion peptides described in the next section.

All of these can work both by shifting the equilibrium state of the system towards the desired change, and by affecting transition states and thereby removing kinetics barriers of the fusion process.

\(^{17}\)soluble N-ethylmaleimide-sensitive factor attachment protein receptor
2.4.4 Viral fusion proteins and peptides

Enveloped viruses need proteins to fuse their membrane with the membrane of their host cells. These proteins possess a short helix spanning the viral membrane and an ectodomain containing a fusion peptide. While other parts of the fusion proteins are considered to be important (e.g. in the scaffold mechanism of influenza HA mentioned above or for the association of the fusion proteins into multimers), the investigation of virus induced fusion has concentrated mainly on the membrane anchor and fusion peptide, which are representatives of the third category of fusion related proteins introduced in the previous section. As an example of the structure of fusion proteins, a sketch showing the relevant subunit of influenza HA is given in Fig. 2.5.

This section will give a brief introduction to the assumed role of the transmembrane domain and then focus on the fusion peptides, which are most important for the work presented in this thesis.

Transmembrane anchors

The membrane anchors have been shown to be involved in the fusion activity, as e.g. for influenza HA [22]. Since no specific amino acid sequences appear to be required for this effect as long as the membrane-spanning domain has a certain length, it has been suggested that this activity is due to the influence of hydrophobic mismatch\textsuperscript{18} on membrane thickness. This is partially supported

\textsuperscript{18}i.e. the discrepancy between the length of the hydrophobic part of a transmembrane domain and the membrane thickness
2.4. **FUSION AND FUSION PEPTIDES**

by studies using WALP peptides\(^{19}\), in which peptides with both positive and negative mismatch reduced the temperature at which cubic phases were observed \(^{23}\), rationalizing a stabilization of fusion pores.

**Fusion peptides**

Fusion peptides are short peptides of normally less than 25 residues, often comprising the N-terminus of viral fusion proteins, and have an amphipathic nature and bind to the lipid/water interface. They are believed to be the first part of the viral fusion proteins to be in close contact with the target membrane and are consequently likely to be involved in at least the early steps of the fusion process.

In some cases, this involvement could be directly demonstrated. Addition of the influenza HA fusion peptide at low pH caused hemolysis of chicken erythrocytes and vesicle fusion \(^{24}\), albeit at lower efficiency than the full protein. However, this approach was not successful for all fusion peptides and does not shed much light on the underlying mechanism. For these reasons, many studies focus on the effect of the fusion peptides on the lipid phase behavior, reasoning that the ability to induce or stabilize hexagonal and cubic phases is directly related to the fusogenicity and indicates an effect on the preferred curvature as the mechanism.

Findings of these studies show that the fusion peptides of several viruses lower the temperature \(T_\text{Q}\) at which cubic phases are observed (see \([25, 26, 27, 28]\) for fusion peptides from measles, influenza HA, SIV and FLV, respectively\(^{20}\)) as well as the lamellar-to-inverted phase transition temperature \(T_\text{H}\) (see \([29, 30, 31]\) for fusion peptides from HIV, canine distemper virus and influenza HA, respectively). In some cases \([28]\), a cubic phase was even observed in a system that did not display this phase in the absence of fusion peptides.

The correlation found for the fusogenicity of the fusion peptides and their effects on the phase transition temperatures is, however, not without ambiguity. In one study, the fusion peptides lowered \(T_\text{H}\) only at very low concentrations, while they raised it at the concentration corresponding to the conditions expected \textit{in vivo} \([28]\). Moreover, studies involving mutants found the fusogenicity and the effect on \(T_\text{Q}\) to be not necessarily proportional \([32]\), with at least one case of a non-fusogenic mutant lowering \(T_\text{Q}\) by a higher amount than the wildtype \([33]\).

In addition, the assumed correspondence of the ability to stabilize inverted hexagonal and cubic phases to a direct effect on the spontaneous curvature \(c_0\) has been cast into doubt by x-ray diffraction measurements. Using the lattice constant of the inverted hexagonal phase in excess water to estimate \(c_0\), no significant decrease relative to the pure lipid could be found in the presence of fusion peptides from influenza and SIV \([34, 35, 33]\), with high concentrations even inducing an increase of spontaneous curvature \([34]\). In the theoretical framework developed in Section 2.3.2, this would suggest an effect on the energy of the Gaussian curvature.

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\(^{19}\) short synthetic peptides consisting of a variable number of hydrophobic repeats of alanine-leucine flanked by a hydrophilic tryptophane at each end

\(^{20}\) The unabbreviated names of the viruses are: simian influenza virus (SIV), feline leukemia virus (FLV), human immunodeficiency virus (HIV).
rather than the mean curvature as a putative mode of action for the fusion peptides [9, 14].

2.5 Computer simulation of lipids

While conformations of lipid aggregates can be studied by spectroscopic experimental methods if they are stable for a sufficiently long time, short-lived intermediate morphologies are difficult to observe in experiments. In addition, specific details like the conformation of individual lipids or the exact position of peptides in lipid aggregates cannot normally be extracted from experimental data, making computer simulation of lipids an attractive option.

Historically, the simulation of lipids started approximately twenty years ago with picosecond simulations of small membrane patches [36]. Over the years, collective phenomena like bilayer undulations and self-assembly [37, 38] became accessible to simulation. Recently, the effects that proteins and lipids have on each other have become the focus of attention of several studies, simulating events like poration of bilayers by antimicrobial peptides [39] and the self-assembly of membrane proteins like rhodopsin [40]. For a detailed review, the reader may consult [41].

2.5.1 Specific versus generic models

One option for the simulation of lipids is to use atomistic models in which the potentials describing the particles’ interactions are typically obtained by fitting to the results of quantum chemical calculations for small molecular fragments. These potentials often are modified to better match experimental findings, a prominent example being the parameters derived by Berger et al. [42]. Based on these atomistic models, it is possible to obtain coarse-grained models, e.g. by using a reverse Monte Carlo approach [43] or force matching [44].

However, the very general nature of the theoretical models for the description of lipid aggregates presented in Section 2.3.2 suggests that this bottom-up approach is not the only way to design a forcefield for lipids and that a large part of the behavior of lipid aggregates should be universal to a broad range of models. It is therefore also possible to make use of this universality and directly construct generic (coarse-grained) models by defining a representation including interactions suitable to bring about the phenomenon that is to be studied. In this approach, one typically chooses non-bonded interactions to reproduce thermodynamic data obtained for the lipids and bonded interactions to reproduce structural data. A detailed overview of the different levels of coarse-graining applied to the simulation of lipids can be found in [45].

For the work presented in this thesis we mostly used the MARTINI model [46], which is an example of the second category of models described above. Details about this model are given in the Appendix.