Structure, shape and dynamics of biological membranes.

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CHAPTER 1

INTRODUCTION

Biophysics is the study of the physics behind biological processes. Biophysicists therefore want to know both *how* things happen in living systems and *why* they happen. The terrain where both disciplines overlap, and both these questions can be asked, is mainly a single cell. A cell is large enough to be a living system in its own right, and thanks to recent advances in observational techniques the different processes within the cell that make up its life cycle can now be studied in great detail. It would be a gross exaggeration to state that a single cell is simple enough to be within the reach of a description in terms of elementary physics, however, in some simplified cases, these same cellular processes can also be studied from a physicists point of view. Living systems have to abide by the laws of physics every bit as much as billiard balls and planets do, and even though they are much more complicated, the basic rules remain the same. Nonetheless, like it is an impossible task to describe the flow of water based on a microscopic description of the motion of all individual molecules, it is not realistic to expect the processes in a much more complicated, living system to be any more tractable. Like in hydrodynamics we have to resort to effective and often phenomenological models based on statistical physics. Even in cases where single-molecule descriptions are possible, we still have to invoke statistics to be able to come up with predictions on the scales available to experiment. Biophysics therefore is mainly statistical physics, and like for example the turbulent flow of water, which has remained a puzzle over the many centuries since it was first studied by Leonardo da Vinci, figuring out the physics behind the fundamental processes that regulate the cell will remain a challenge for many years - if not centuries - to come.

1.1 Cells

Cells are the building blocks of all living creatures. The vast majority of the species that we know of are unicellular. However, the majority of the organisms that we can actually see with the naked eye, like plants and animals, are multicellular. Unicellular organisms were first described by Anthonie van Leeuwenhoek, who used his newly developed microscope to study many different samples in the second half of the 17th century. Simultaneously, Robert Hooke, also known for the law of elasticity named after him, discovered that cork was made of many chamber-like subunits, for which he coined the term cell in 1665. Later on, Hooke also discovered cells in the tissue of living plants. Nonetheless, it took till 1839 for the general cell theory, stating that all living organisms are composed of cells, to be developed by Matthias Schleiden and Theodor Schwann. The general cell theory in its modern form encompasses more that just the statement that cells are the basic subunits of life. It also states that all cells come from preexisting cells, and that they pass on hereditary information from one generation to the next. This information is stored in the genetic code of the DNA molecule found in all cells, which also encodes for all its characteristic properties and functions.

All cells share some common features. They are well-defined compartments bound by a plasma membrane, a bilayer of lipid molecules (see section 1.2). The cells of some groups of organisms (*e.g.* plants) also have a more rigid cell wall outside the plasma membrane, whereas others (*e.g.* animals) do not. The inside of the cell is an aqueous solution containing many chemicals. Genetic information is stored in DNA, a polymer with a double-helix structure. The building blocks of DNA are four both chemically and structurally different bases, which provide four 'letters' in which the genetic information is written. Parts of the DNA known as genes encode for the production of proteins, which are themselves polymers made of amino acids. There are 22 different amino acids known, each of which is coded for by 3 bases in the DNA. Proteins occur in a wide range of types and with a large variety in function. Some are basic structural subunits, while others play important roles in processes like chemotaxis, signaling, transport, cell division and apoptosis (cell death). Proteins are produced from the DNA code in two steps. First, a part of the code (the gene, or a part of it) is transcribed from DNA to an RNA molecule, another biopolymer which also has four different subunits, corresponding to the bases of the DNA. The RNA subsequently gets translated into a protein. This at first sight rather cumbersome method allows for a physical separation between the DNA containing the valuable genetic code, and the many other processes in a cell.

In eukaryotic cells the DNA is stored in a separate compartment called the nucleus. The region outside the nucleus is called the cytoplasm. DNA transcription onto RNA takes place inside the nucleus. After the transcription, the RNA is transported outside the nucleus and translation takes place in the cy-

toplasm. Most eukaryotes have another specialized subunit called the endoplasmic reticulum where RNA translation and protein production takes place. Cells without a nucleus are known as prokaryotes. The division between pro- and eukaryotes is the first that can be made when classifying organisms. All multicellular organisms, including animals, plants and fungi, but also some unicellular ones like yeast, are eukaryotes; all bacteria are prokaryotes. Although it is an exaggeration to state that prokaryotes have no internal structure at all, they do not contain internal membranes separating different parts of the cells in functional units called organelles, like eukaryotes do.

The cells of many eukaryotic species have a high level of internal organization. As indicated above, the boundary between the inside of the cell and the outside world is a lipid bilayer membrane, known as the plasma membrane of the cell. Inside, there are many organelles, subunits with specific functions that themselves are also bounded by lipid bilayer membranes. Examples are the endoplasmic reticulum, the Golgi apparatus (which functions as the distribution center of the cell) and mitochondria (which even have a double membrane and function as the power plant of the cell, converting sugar into the biological fuel ATP).

Apart from being organized into subunits, eukaryotic cells also possess a cytoskeleton. The cytoskeleton is a network of biopolymers which enhances the structure of the cell and provides a network for transportation within the cell. Three types of biopolymers make up the cytoskeleton: microtubules, actin filaments and intermediate filaments. Both microtubules and actin filaments are polar, while intermediate filaments are apolar. Actin filaments are the most flexible part of the cytoskeleton and are predominantly found in the cell cortex, just beneath the plasma membrane. Actin has an important function in cell motility, a process in which cells extend protrusions such as lamellipodia and filopodia, but also in adhesion in mature, stationary cells. Intermediate filaments are found throughout the cell, where they provide it with mechanical strength. Moreover, also intermediate filaments play an important role in cell adhesion, in particular in sheets of epithelial cells (found for instance at the inside surface of all cavities of the body), where they are crucial factors in relaxing mechanical stress. Microtubules are the stiffest component of the cytoskeleton with a persistence length much larger than the size of a cell. They are predominantly radially organized in the cell, with one end usually attached to an organizing center (called the centrosome) close to the nucleus and the other end close to the plasma membrane at the cell periphery. Because they are polar, both microtubules and actin provide platforms for motion of molecular motors through the cell. These motors are responsible for the various transport processes within the cell, as well as for separating the cell into two parts during division. Motors are further introduced in section 1.3.

Cells are by no means static. Even in mature organisms, cells grow and divide to replace dead cells continuously. We call the period that leads from the birth of a cell to the point that it divides the cell cycle. Given the right environ-

mental conditions, the cell cycle of a bacterium can be as short as 20 minutes, whereas that of a cell in a mature multicellular organism can take up to a year. The cycle of an eukaryotic cell can be divided into two clear phases: the interphase or growth phase, and the M or dividing phase. Duplication of the DNA already takes place during the growth phase. During the M phase, first the nucleus is split in two (each containing a complete copy of the DNA), a process known as mitosis. The cell subsequently divides in two separate daughter cells. Prokaryotes have a similar cycle, in which first the DNA is duplicated, after which it is pulled to opposing ends of the cell and the cell divides. In both systems, the proper separation of the DNA is a crucial step, in which molecular motors (and in the case of eukaryotes, also the cytoskeleton) play a vital role.

1.2 Membranes

Biological membranes consist of a double layer of lipid molecules. Lipids are a class of molecules with the common feature that each of them has a hydrophilic (polar) head and up to three hydrophobic tails. There are many different kinds of head groups, exhibiting a rich variation in characteristics like charge and size. The tails always consist of hydrocarbon chains which are mostly saturated, although a single tail may have some unsaturated bonds. Common examples of lipids include cholesterol, fatty acids, phospholipids, sphingolipids and vitamins.

Molecules that contain both hydrophilic and hydrophobic parts are known as amphiphiles and may self-organize in an aqueous environment. The self-organization is driven by the reduction in free energy that can be achieved by shielding the hydrophobic part from the water molecules. If the lipids are roughly wedge-shaped, the shielding can effectively be done by organizing the lipids into micelles, in which the tails are all directed towards a common center point and the head groups face outward (see figure 1.1a). The conical shape is found in lipids which have a large head group and typically only a single hydrocarbon tail. For more cylindrical-shaped lipids the micelle organization is not as advantageous, because the head groups will not be able to completely shield the tails from the (much smaller) water molecules. Such lipids therefore rather organize into lipid sheets which have no intrinsic curvature. To separate the hydrophilic and hydrophobic parts such a sheet should be a bilayer of lipids, with the tails pointing inwards and the heads once again on the outside (see figure 1.1b). In an aqueous environment a lipid bilayer will close up on itself to create a shape without exposed edges, resulting what is known as a liposome or vesicle. The two lipid layers in a vesicle are called the inner and outer leaflet of the membrane. Vesicles have a small curvature, which means that the cylindrically shaped lipids will incur a small bending energy penalty (measured by the bending modulus κ of the lipid bilayer membrane). Moreover, in biological systems the inner and outer leaflet may have different compositions, and the

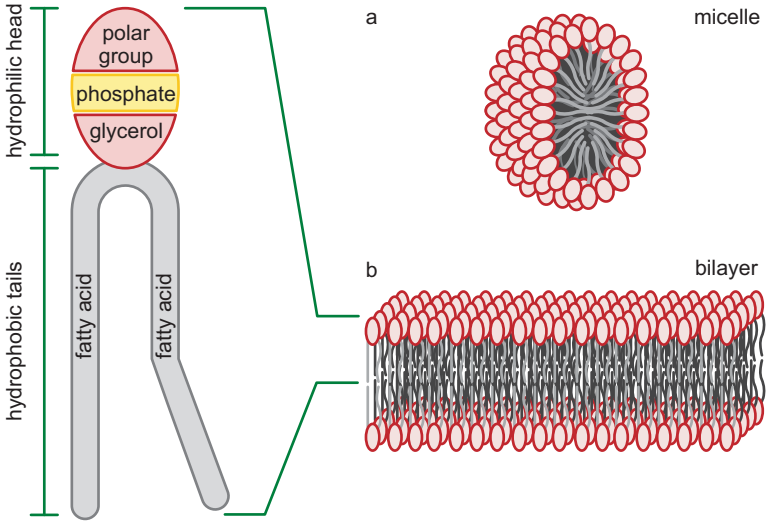

Figure 1.1: Biological membranes are bilayers of lipid molecules. A schematic image of a lipid is shown on the left. Lipids consist of a hydrophilic (polar) head group and one or more hydrophobic tails, which are long fatty acids. In aqueous solution, lipids self-organize into larger structures. Lipids which are cone-like in shape may organize in a micelle (a), more cylindrically-shaped lipids typically organize into bilayers (b). In water, bilayers close up on themselves, creating a vesicle or liposome with aqueous solutions both inside and outside.

inside and the outside of a vesicle may contain different solutions. All biological membranes are lipid bilayer sheets.

Although the biological significance of the head groups is large, in the biomimetic membranes we will focus on in this thesis their role is small. Head groups are used in experiments to specifically attach fluorescent markers, but do not influence the organization of the membrane itself. In contrast, there is an important property of the tail groups of the lipids used which directly influences the membrane structure [1]. Some of the hydrocarbon tail chains are completely saturated, whereas others have a single unsaturated bond. Such an unsaturated bond acts as a kink in the hydrocarbon chain, which has the important consequence that the lipid will have some part of one (or both) of its tails stick out in a random (and variable) orientation. If we build a membrane out of a single species of lipids which has unsaturated tails, we get a structure which is different from a membrane consisting of lipids with saturated tails. The difference can be characterized by looking at the correlation of tail orientations. For unsaturated tails, with parts sticking out in random directions,

these correlations are small; such a membrane is said to be in a liquid disordered (L_d) phase. Saturated tails on the other hand are not as free to choose their orientation and the correlations are much stronger, resulting in what is known as a liquid ordered (L_o) phase. Both phases are fluid, which means that there is no long-range order between the positions of the lipids themselves; the difference is in the ordering of the tails. Especially for saturated-tail lipids it is possible to make a transition to a gel phase, in which also the lipid positions are ordered; however this only occurs at low temperatures and is not a phase that has been observed in living cells.

In a membrane containing both lipids with saturated and unsaturated tails, it is experimentally found that these lipids do not mix well. Especially in the presence of the much smaller cholesterol as a third component, the membrane exhibits demixing into separate L_d and L_o domains below a critical temperature [2–7] (see figures 1.2 and 1.3). The cholesterol is mostly found in the L_o phase (which is rich in saturated-tail lipids), where it 'fills up the gaps' between the straight tails. In chapter 3 we study the properties of the phase diagrams of such ternary systems.

Typically lipids with fully saturated tails are effectively slightly longer than those with unsaturated tails. To keep a closed front of hydrophilic head groups lipids will therefore have to be stretched or compressed close to a domain boundary (see figure 1.2d), resulting in an energy penalty associated with the presence of such a boundary [8–10]. A membrane consisting of multiple lipid types will therefore want to minimize the total domain boundary length, which is the driving force for the separation into domains and the growth of such domains. In chapter 4 we study the effects of this segregation into domains on the vesicle shape.

Biological membranes do not only contain lipids, but also many kinds of proteins. Like lipids, most proteins have both hydrophilic and hydrophobic parts, and in order to shield the hydrophobic parts from the water in a cell's cytoplasm they are embedded in a membrane. Proteins are typically much larger than lipids, and a recent study shows that up to approximately half the mass of the plasma membranes is concentrated in proteins [11]. Proteins which are not cylindrical in shape will locally deform the membrane, and communicate with each other through such membrane deformations. A well-known example is the clathrin-coated pit, where clathrin proteins get recruited to a part of the membrane that is to be budded off. Because of their conical shape the clathrins force the membrane to assume a strongly bent shape, which is subsequently pinched off by another protein called dynamin, creating a small membrane vesicle [12, 13]. Individual proteins are too small to be observed in light microscopes, but lipid domains are not, and we will use them as a probe to study membrane-mediated interactions in detail in chapters 5 and 6.

Although the biomimetic vesicles we study clearly exhibit separation into domains, it is not known whether similar processes also occur in biological membranes in living cells [1]. Simons and Ikonen [14] proposed the existence

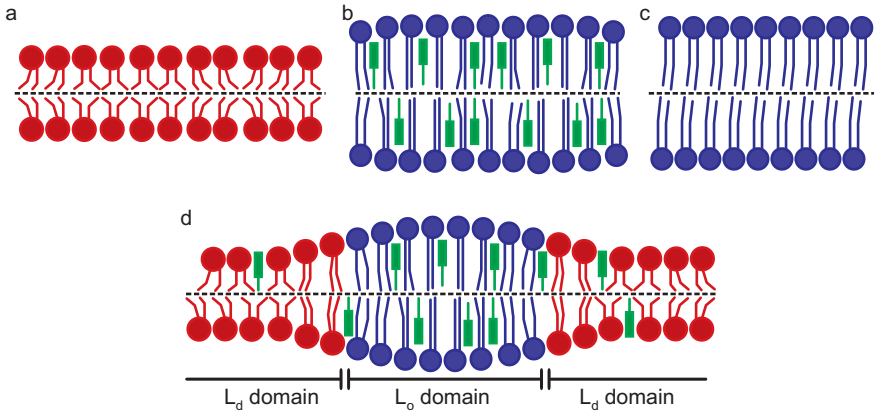

Figure 1.2: Structures of lipid bilayer membranes exhibiting different phases. (a) Liquid disordered (L_d) phase, mostly found in lipids which have one or more unsaturated carbon bonds in their tails. (b) Liquid ordered (L_o) phase, a combination of lipids with saturated tails (blue, two tails) and cholesterol (green, one tail). (c) Gel phase, found for all lipids below their critical temperature. (d) Lipid bilayer with coexisting L_d and L_o domains. Please note that cholesterol is present in both phases, although its concentration in the L_o phase is higher than in the L_d phase.

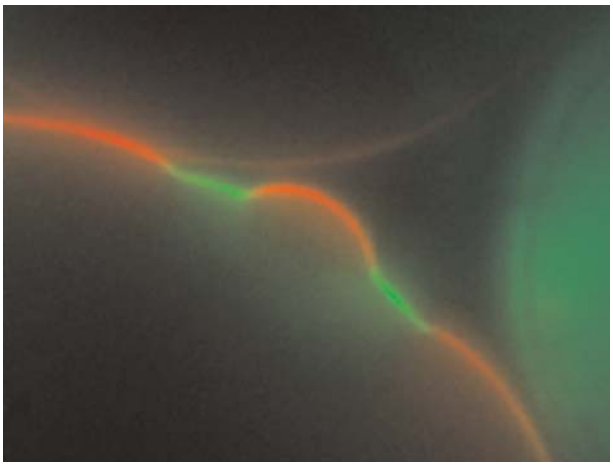

Figure 1.3: Image of a tricomponent membrane exhibiting coexistence of a L_d phase (green) and an L_o phase (red). The line tension between the domains of the two coexisting phases causes the membrane to deform locally, resulting in different curvatures for the different domains. The line tension arises as an energy penalty for phase coexistence within a single membrane. In section 3.4 we calculate the value of the line tension from an expression of the Gibbs free energy for ternary mixtures. In chapter 4, we study the effect the line tension has on the membrane shape.

of such functional domains (or 'rafts') in the plasma membrane as an alternative to the existing fluid mosaic model. The fluid mosaic model [15] states that the plasma membrane is essentially a uniform mixture of many types of lipids and numerous transmembrane and membrane-associated proteins. In the picture sketched by Simons and Ikonen a raft would be an environment enriched in certain types of lipids, creating a preferential environment for some proteins whilst effectively excluding others. Rafts have not been directly observed to date, which means their maximal size is limited to the maximal resolution of optical microscopy, about $100\,\mathrm{nm}$ [16]. Based on the measurement of certain membrane parameters in biomimetic vesicles, and models for domain growth and membrane recycling in living cells, we predict in chapter 4 that rafts in fact can only have a size of about $10\,\mathrm{nm}$. Such small rafts would still be able to organize proteins, but only in very small numbers. Moreover, in this scenario one should ask the question whether the rafts recruit the proteins or the proteins organize their local environment into lipid rafts. For a more thorough introduction into that discussion, see the recent reviews by Lee [17] and Sackmann [18].

1.3 Molecular motors

Molecular motors are the workers of the cell. They convert chemical energy into mechanical work. Because they use energy, motors are able to perform such tasks as creating directed motion and producing concentration gradients. There is a rich variety of types of motors. Examples are the dynamin that pinches off membrane vesicles, DNA and RNA polymerase that read DNA and produce DNA copies or RNA transcriptions, ion pumps that transport sodium or potassium ions across a membrane, and cytoskeletal motors that transport materials through the cell. Molecular motors are proteins, more specifically mechanoenzymes, and depending on their function can have transmembrane or membrane-associated domains. Their fuel is typically ATP (though motors that run on GTP also exist), molecules produced by the mitochondria from the oxidation of sugar, and containing three phosphate groups (indicated by T for three and P for phosphate). Hydrolization of such a molecule (in which it reacts with water and releases one of its phosphate groups, producing ADP and phosphate) releases energy [12]. Motor proteins act as catalysts for this reaction, and during a single cycle (in which they for instance transcribe a single DNA-base) they have different configurations in their free, ATP-bound, and ADP-bound states. The combination of an energy consuming step with a polar polymer to walk on, ensures unidirectionality in the motion of molecular motors.

The motors we study in chapter 7 are microtubule-walkers, which transport membrane vesicles through the cell along the microtubule network. Microtubules are part of the cytoskeleton, and as such already introduced in section 1.1. Microtubules are hollow tubes, made of many copies of polar tubulin dimers, which consist of an α and a β tubulin protein. Typically a microtubule has thirteen subfilaments, in which case the filaments are straight within the microtubule tube. Microtubules with less or more than 13 subfilaments also exist, resulting in a tube with winding subunits. In living cells microtubules constantly grow and shrink at their plus end, and sometimes dissociate from the centrosome and subsequently also shrink from the minus end [19]. They are extremely stiff, with a persistence length of the order of millimeters [20], much larger than the size of a typical cell ($10 - 100\ \mu\mathrm{m}$). Apart from their role as structural components within cells, microtubules are also involved in many dynamic processes, such as mitosis, cytokinesis, and vesicular transport.

Molecular motors occur in two types: processive and nonprocessive ones. Processive motors can take many steps on the polymer they walk on before detaching. Nonprocessive motors can only take a single step. Both types occur frequently: DNA and RNA polymerase are examples of processive motors with high processivity, whereas the myosin motors in muscle cells are strictly nonprocessive. We study both types of motors, and particularly focus on the differences between them.

The motors we study walk on microtubules using one or two active parts,

which are usually called heads. The precise dynamics of walking are subject of intensive study. Recent results show that processive motors walk in a head-over-head fashion: when both heads are bound to the microtubule, the trailing one unbinds, then the leading one makes a power stroke, putting the now free second head in a position such that it can bind in front of the bound motor [21]. Nonprocessive motors can have only one head (as is the case for some actin-walking Myosin motors, *e.g.* Myosin-I), or two heads of which one is either disfunctional or unable to reach the polymer due to structural constraints. Nonprocessive motors can still bind their active head to their associated polymer, and the head can also make a power stroke to push the entire motor forward, but it has to release afterwards because there is no second head that can bind [22, 23]. Nonprocessive motors therefore necessarily need to work cooperatively to exert a continuous force. Processive motors also often exhibit collective behavior, because the force generated by an individual motor is not large enough to perform a required task, and both efficiency and processivity can be increased in a system containing multiple motors. The collective effects of molecular motors have therefore been studied intensively in recent years [24–32], and are the main subject of chapter 7.