Physics implications of shape on biological function

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Summary

Schrödingers book 'What is life', which he wrote in 1944, is often considered to be the start of the field of biological physics. Yet the term and the research field of biophysics existed already before, probably starting with the experiments of Luigi Galvani on the electrical stimulation of frog-muscle already in 1780. Physics and biology were established research fields with extensive history at that time. It is the confluence of those two fields, that has lead to unforeseen discoveries in the last century, such as the identification of DNA as the origin of heredity (discussed in Schrödingers book), the map of the human genome in the 1990's, our current knowledge of the structure of a large part of the proteome, and our emergent understanding of cellular control and self-organization by chemical and physical cues. Many of those successes were tied to technical developments in microscopy, one of the main tools of biophysicists. Advances in microscopy were awarded three Nobel prizes. The scanning electron microscope developed by Ernst Ruska was awarded the Nobel prize in physics in 1986, the 2014 Nobel prize in chemistry was awarded to biophysicists for the development of optical super-resolved fluorescence microscopy. And recently, in 2017, the Nobel prize in chemistry was awarded to biophysicists that dramatically pushed the capabilities of the electron-microscope for determination of life protein structures.

The work described in this thesis makes heavy use of high-resolution, ultrasensitive fluorescent microscopy, mostly for observation of dead or living samples. Yet in chapter 3 the microscope is also used for the locally controlled initiation of a biology-inspired process itself. Similarly, in chapter 2, the microscope is used in building the structure needed for the experiment at hand. In chapters 4 and 5, at which three-dimensional objects are investigated, a special form of microscopy called confocal microscopy is used to form extra clear images.

The field of biological physics studies biological phenomena at a vast range of length-scales. From the individual water molecule that permeates through a lipid membrane, to processes that occur on read-out of the genetic information from DNA, to mechanical squeezing of a cell through narrow pores in tissue, to flight navigation of a fly in a very agile manner, to even the effect of the global environment on organisms and populations. Overarching in all those studies from the smallest molecule to the biggest organism is that shape plays an immense role in defining the function. In this thesis, I touch upon the topic of shape and function in biology, selecting a few exciting examples around cellular scale and below.

In **chapter 2** I explored the influence of shape and morphology on the function of dendritic spines. Dendritic spines are important structures of the brain involved in memory and learning. On the dendritic spine, one neuron connects to another. The connection is called the 'synapse', forming the contact to transfer signals between the two. The axon terminal of the neuron on the sending side is opposite to the dendritic spine, usually a mushroom-shaped protrusion, of the other neuron on the receiving end. The signal is transmitted by the release of small molecules, the neurotransmitters, by the axon terminal. These neurotransmitters are sensed by specialized receptors on the membrane of the dendritic spine. The number of receptors on the dendritic spine thereby determines the strength of the signal.

The experiment described in chapter 2 involves mimicking the dendritic spine. The artificial dendritic spine I constructed consists of a giant unilamellar vesicle, from which I pulled a few-hundred-nanometer-thin membrane tube. In this way, the mushroom shape is mimicked. The role of receptors in my mimetic system is played by quantum dots attached to lipids in the membrane. The quant-dot labeled lipids were able to move freely within the membrane. Using this system I was able to underline the influence of the shape of the dendritic spine on the retainment of receptors in the dendritic spine.

In **chapter 3** I investigated a system on a smaller scale, where I show the influence of local differences in mechanics in the membrane of a cell. The plasma membrane defines a cell by separating the inside of a cell from the outside. Other membranes are used inside the cell to form compartments with different functions. The largest of which, in eukaryotic cells, is the nucleus. Just like any material, lipids, of which membranes are made, can be in different states of matter. For most substances, those are the solid, fluid and gaseous phases. Lipid membranes exhibit a richer phase-space. There is a gel phase in which lipids are locked in place, like in a solid. There are two liquid phases, commonly labeled 'liquid-ordered' and 'liquid-disordered' in which lipids are free to move

within the membrane, yet the tail-regions are oriented in a preferred direction. The temperature at which lipids will undergo a phase-change depends on the lipid type. Hence, at a given temperature different lipids have preferences for different phases. Because of that they can separate and form homogeneous areas, much like the separation of oil from water.

This process called phase separation, I induced in giant unilamellar vesicles, using light. During this process the membrane changes significantly, more surface area becomes available and, as a result, the membrane tension drops. In this way, the shape of a membrane is affected on a small scale. It has been suggested that such separation of lipid phases influences the binding of proteins and leads to structural organization of the cell membrane.

I changed gears a bit in **chapter 4**. In this chapter, I looked at the shape of cells as a whole. The shape of a cell is largely determined by its cytoskeleton. I used a confocal microscope to image actin, a major component of the cytoskeleton. Actin, together with bundles of motor proteins in muscle cells, is the basis of the contractility of the muscle. In general, actin is ubiquitous to any cells and is a major structural component. I looked at the organization of actin inside the cell and correlated its organization with the shape of the cell.

Cells on a flat substrate usually attach at discrete points along the edge of the cell. In between these points, the edge of a cell is smooth and curved in a characteristic way. It turns out that the particular shape of these edges tells about the anisotropy of the cytoskeleton inside the cell. The anisotropy in the cell is due to aligned actin stress fibers, long strands of bundled actin spanning the whole cell. I discovered that the edge of a cell follows an elliptical curve, which is characteristic for each cell. I developed a model that predicted that all ellipses fitted to a single cell have the same size and aspect ratio. Moreover, the long axis of these ellipses pointed in the direction in which the stress fibers were pointing.

I continued this story in **chapter 5**. In this chapter, I actively influenced the shape of cells by adding a drug (ROCK-inhibitor). The protein ROCK normally promotes the activity of myosin motors, which leads to the contraction of actin fibers. Further, it promotes the formation of long strands of actin, resulting in so-called stress fibers. The ROCK-inhibitor blocks this activity, hence makes the stress fibers shorter and less contractile. As a result, the actin cytoskeleton became isotropic. I discovered, as predicted by the model developed in chapter 4, that the shape of the cell edges assumed a circular shape after addition of the drug.

In summary, this thesis applied physical principles to biology, by considering the influence of the shape of structures on biological systems. Ideas have been applied at the cellular level and below. By developing physical models, I was able to quantify important parameters of the systems I was looking at. This enabled me to qualitatively assert that cell shape is an important parameter for the biological function.