UNIVERSITÄT LEIPZIG

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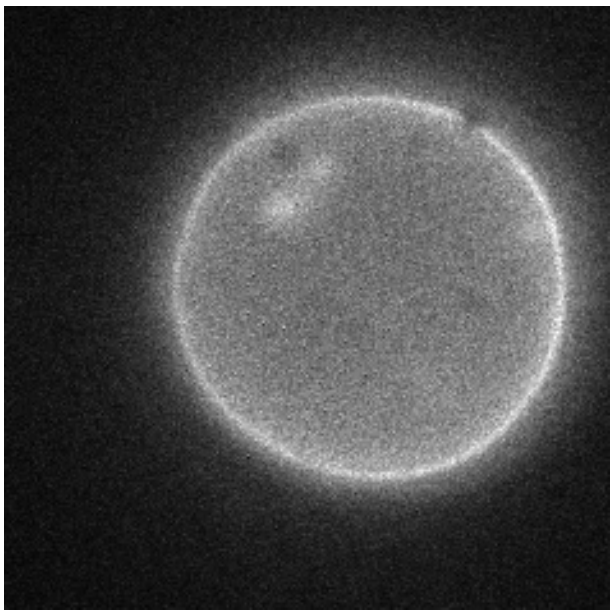
Vesicle Fluctuation and Membrane Elasticity

Soft Matter Physics Lecture - Experiment 3



Leipzig, May 2010

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Chapter 1

Introduction

Lipid bilayer vesicles fascinate biophysicists because they exhibit typical mechanical and rheological features of cell membranes. They provide insight in cellular transport, stability and morphology of cells or diffusion processes across the membrane. The aim of this experiment is to calculate the bending elasticity of the membrane of giant unilamellar vesicles (GUV). The measurements are made using vesicle fluctuation analysis, a non-intrusive method of statistical physics correlating the shape distributions of the vesicle to its elasticity and spontaneous bending.

1.1 The Phospholipid Bilayer

Phospholipids form the main building block of membranes. Unlike storage lipids (fats), which consist of a glycerol and three fatty acid chains, phospholipids only contain two fatty acids. They are derived from either glycerol or sphingosine. Phosphoglycerides (glycerophospho-lipids) contain a phosphatidate, i.e. they have two fatty acids esterifed to two carbons of the glycerol and a phosphoric acid group esterifed to the third carbon. The phosphate group in turn is esterifed to an alcohol, like ethanolamine, choline, serine or inositol. Sphingomyelin is based on sphingosine, which in contrast to glycerol already contains a long hydrocarbon chain. The second hydrocarbon chain is again provided by an ester-linked fatty acid. For the headgroup, the sphingosine is esterified to a phosphoric acid group, which is in turn ester-linked to a choline.

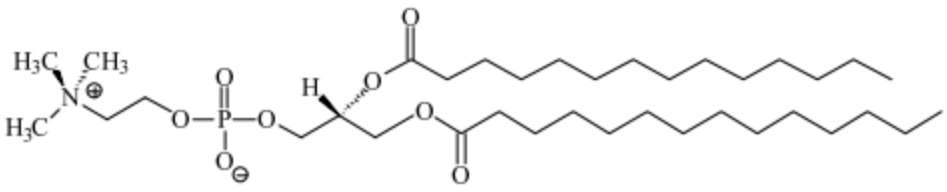

Figure 1.1: Dimyristoylphosphatidylcholine(DMPC) from ([1])

1.1.1 Vesicles

Vesicles are approximately spherical organelles in the cytoplasm of eukaryotic cells. Their functions include the isolated transport of agents through the cytosol in intracellular compartments or in between membranes and intracellular compartments.
Vesicles are model like, i.e. they have no membrane proteins, can be created synthetically and are used to study biological membranes.

1.1.2 Mechanics of Lipid Bilayers

Fluid lipid membranes undergo thermally induced shape fluctuations which can be investigated to determine the bending elasticity. Helfrich ([2])demonstrated in 1973 that the mechanical state of a membrane element can be completely defined by its area and principal curvatures.
The fluctuation analysis was developed by Schneider et al. in 1984 ([3]). The model is based on the undulations of the membrane which are captured as a contour in the equatorial plane of the quasi-spherical object in two dimensions. Spherical harmonics are used in a projection algorithm to correlate to the three dimensional theory.

Chapter 2

Experiment

To accomplish the experiment an adequate theoretical background knowledge about the main topics is mandatory. Thus, questions may be ask to test the applicability of the experimenter. Furthermore, every student has to be familiar with the safety provisions.

2.1 Preview

For an adequate preparation the experimenter has to present the following points in the preview section:

- Fluorescence Microscopy
- Giant Unilamellar Vesicles Preparation
- Bending Elasticity
- Phospholipid Bilayers
- Effect of Cholesterol to Membranes
- Helfrich Hamiltonian
- Vesicle Fluctuation Analysis
- *other possible Methods to determine Bending elasticity

2.2 Experimental procedure

The vesicle preparation is a process over several hours. It will be done for the students prior to the experiment due to time constraints. The used electroformation method is similar to the one described in ([4])

The aim during the lab time is to get several records (around 500 frames) of Dimyristoyrolphosphocholine (DMPC) GUVs (Ø 10-30μm) with two different Cholesterol concentrations.
For the accomplishment, the following equipment will be available:

- Fluorescence microscope
- DMPC + Cholesterol vesicles in 0.06M sucrose solution
- Glucose solution
- Petri dishes applicable with the microscope
- Petri dish heater
- Pipettes

2.3 Data analysis

The collected data should be used to determine several properties like:

- Bending rigidity κ of vesicles
- Comparison between different cholesterol concentrations

Additionally, the protocol should explain the method of contour detection (including an overlay image of contour and vesicle for each vesicle) and explain in detail the applied fluctuation analysis. A discussion of errors is also obligatory.

Chapter 3

Safety provisions for workers

Some general rules concerning safety at work

- before start: design of experiments, preparation, check of used equipment and chemicals
- knowledge about potential danger of the chemicals and adequate precaution (see safety data sheets)
- wearing of appropriate protective clothing
- doors and windows must be closed during the experiments
- no food, no drink, no smoke
- freezers for chemicals are not for food
- keep the labs clean, dispose wrapping immediately
- experimental equipment is signed clearly (person in charge, time of experiments)
- after finishing the experiments cleaning up, disposing waste
- scalpels, cannulas and glass waste in the concerning signed boxes
- only authorized persons have entrance in the labs

Storage, transport and handling of chemicals

- store chemicals in the original package (clear labeling, safety and danger advice)
- when using other boxes attend to clear and durable labeling
- when decanting of chemicals use adequate device (funnels ,pipettes...)
- when chemicals toxic or corrosive use extractor hood

- no needless supply inventory of chemicals
- transport of chemicals (glass bottles) in racks or buckets - possible risk of breakage

Handling with liquid nitrogen

- wear safety glasses
- the filled liquid nitrogen tank must be transported in the elevator by oneself (risk of suffocation in emergency case!)

Some Special Rules S1 / S2 Labs

S1 and S2 are safety levels for genetic engineering labs and defined in the Gentechnikgesetz as:

S1 - no risk for human health and environment due to genetically modified organisms

S2 - minor risk for human health and environment due to genetically modified organisms

(the classification is resulted from Zentrale Kommission für biologische Sicherheit
http://www.bvl.bund.de/DE)
S1 labs are 131a/b (AFM), 309 (bong lab) and 310 (sample prep.) S2 labs are 116 (cell culture lab) and 311 (stretcher lab)

In principle

- apply the instructions on how to do genetical operations (S1/S2 level)
- follow the instructions of the hygiene plan

(both are published in every lab)

General

- wear protective clothing
- protective clothing must not be worn outside the labs
- workings of the following kind require nitril-protective gloves:
- passaging of transfected or virus-infected cell lines

- preparation with potentially infectious cells or tissue from animals and when dealing with human blood or tissue
- caustic, poisonous or mutagene material and material combinations
- mouth-pipetting is strictly prohibited
- syringes and canulaes should only be used if it cannot be avoided
- after every single working step or when leaving the S1/S2 lab, hands are to be washed and disinfected (see hygiene plan)
- disinfect work spaces after working with genetically modified organisms or pathogenic germs
- all equipment that had contact with genetically modified organisms has to be disinfected or has to be autoclaved
- lock the door when leaving the lab

Waste disposal

- collect contaminated material separately, non-fluid and liquid lab waste in different boxes
- waste that could contain genetically modified orgamisms or pathogenic germs must be autoclaved before disposing
- collect organic solvents and poisonous substances separately

In case of an accident

- if an accidental release of genetically modified organisms has happend, please inform all employees and superior authorities immediately (Prof. Käs, Undine)
- spilled biological material must be adsorbed at once and disinfected according to the rules of the hygiene plan
- if an extensive contamination of equipment or working spaces cannot be avoided, please turn off the device, ensure that nobody gets close to the site of accident and decontaminate the space carefully
- contaminated protective clothing must be taken off and be put into the autoclave
- contaminated skin must be medicated with a special disinfectant
- injuries should bleed properly (give off dangerous substances)
- after contamination of the mucous membranes - clean with water (eye shower room 310, 178)
- every injury / accident has to be register

Disinfectants

- equipment: Descosept, 80% ethyl alcohol
- tables: Descosept, 80% ethyl alcohol
- hands: Sterilum

Bibliography

[1] Erik Brückner. *Solubilisierung lipophiler Substanzen durch Phospholipidvesikel.* PhD thesis, Universität Gesamthochschule Essen, Essen, 2000. In german.

[2] W. Helfrich. *Elastic properties of lipid bilayers: theory and possible experiments.* Z Naturforsch C., 28:693–703, 1973.

[3] M. B. Schneider, J. T. Jenkins, and W. W. Webb. *Thermal fluctuations of large quasi-spherical bimolecular phospholipid vesicles.* J. Physique, 45:1457–1472, 1984.

[4] Philippe Meleard, Claire Gerbeaud, Tanja Pott, Laurent Fernandez-Puente, Isak Bivas, Marin D. Mitov, Jean Dufourcq, and Pierre Bothorel. *Bending Elasticities of Model Membranes: Influences of Temperature and Sterol Content.* Biophysical Journal, 72:2616–2629, 1997.