

## P19 – Force Spectroscopy on Model Membranes to Predict the Mechanical Properties of Biological Cell Membranes

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Mechanical properties of biomembranes define several biological processes, for example osmotic shrinkage and swelling of cell volume or cell membrane deformation during endo- and exocytosis or cell migration. Therefore the determination of these properties is highly relevant to understand the above described cell processes. Using atomic force microscopy (AFM) membrane elasticity (Young modulus), bending stiffness or area compression modulus can be determined gently pressing a cantilever onto the membrane surface and simultaneously recording the restoring force vs. deflection (Fig.1). In the literature numerous models are known to interpret these force distance curves to calculate the mechanical parameters of the membrane. Here, we used a model where shallow spherical shells deform under point loads [1], [2], [3]. As shells spherically closed phospholipid bilayers (unilamellar vesicles) coated by surface proteins were used. These serve for adequate cell membrane model systems based on their composition, structure and size. Moreover, they are filled with buffer instead of biological polymers and yield information exclusively on the mechanical properties of the membrane and not on the whole cell. The fact that our results are in good agreement with other values from the literature demonstrates that AFM is an appropriate technique to study mechanical biomembrane properties. Simultaneously, protein coated unilamellar vesicles proved to be a suitable model system for cell membranes from the mechanical point of view.

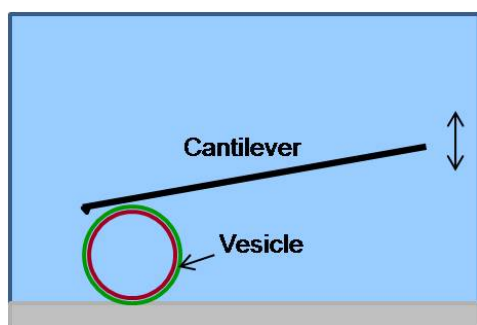


Fig.1 Scheme of the AFM set-up with vesicle as sample.

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# Force spectroscopy on model membranes to predict the mechanical properties of biological cell membranes

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Mechanical properties of biomembranes determine a number of biological processes like cell division or locomotion. Therefore, examination of these characteristics is of tremendous importance. Protein coated giant unilamellar vesicles are optimally suited to mimic biological cell membranes. They have substantial similarity in composition, structure and size with cells. We used AFM force spectroscopy to provide quantitative data like effective spring constant, Young modulus and bending stiffness of biomembranes. The applied model describes the deformation of a spherical shell under point loads, using force spectroscopy we could distinguish different coating proteins in aqueous solution.

## Structure of a coated giant unilamellar vesicle (GUV)

Giant unilamellar vesicles (GUVs) are composed of a spherical closed phospholipid bilayer. Some of these phospholipids are labeled with Lissamine Rhodamine dye. A streptavidin or avidin layer is specifically bound to lipids via cap Biotin linkers. The protein layer is labeled by green fluorescent Alexa 488.



- protein
- phospholipid, SOPC
- capBiotin
- Alexa 488
- Lissamine Rhodamine

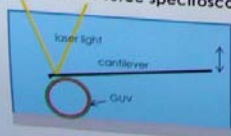
Size of GUVs 10-20 µm,  
Thickness of the lipid bilayer ~ 4 nm,  
Thickness of the protein layer ~ 4-5 nm<sup>2,3</sup>

## Top view of the force spectroscopy experiment



Fluorescence image of the labeled vesicle during cantilever approach. Fluorescence benefits the control of the force experiment in respect to the quality of vesicle composition and cantilever contamination by proteins.

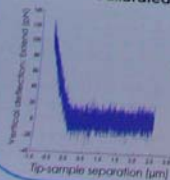
## Schematic side view of the force spectroscopy experiment



The cantilever hit the vesicle on top with its blank bar. The signal of the reflected laser spot was recorded vs. the piezo movement, and was in the range of 1% of its diameter.

- outer coated green labeled protein layer
- inner red labeled lipid bilayer

## Calibrated force-distance curves



Tip-sample-separation of a force spectroscopy experiment, where a single vesicle was gently deformed by a cantilever. Only the approaching curve is presented. The response of the vesicle occurred in the range of several pH. The used silicon cantilever had a nominal spring constant of 6 pN/nm.

## Effective spring constant of the vesicle

The measured force-distance curve contains the combination of the spring constant of the cantilever and the sample, here the vesicle<sup>4</sup>.

$$k_{\text{eff}} = \frac{k_{\text{cant}} \cdot s}{1 - s}$$

- $k_{\text{cant}}$  spring constant of the vesicle
- $k_{\text{cant}}$  spring constant of the cantilever
- $s$  slope of the force curve

## Young modulus and bending stiffness of the vesicle

$$d = \frac{RF\sqrt{3(1-\nu^2)}}{4Eh^2}$$

- $R$  radius of the shell
- $F$  force
- $\nu$  Poisson ratio
- $E$  Young modulus
- $h$  thickness of the shell

$$\kappa = \frac{Eh^3}{12(1-\nu^2)}$$

$\kappa$  bending stiffness of the vesicle

To obtain intrinsic mechanical material parameters like Young modulus and bending stiffness, we used a model, where a shell was pressed between two plates (here glass slide and cantilever bar). More precisely, a shell was deformed under point loads<sup>5,6,7</sup>. To obtain a linear dependency, the evaluated deformation was in the range of 1% of the vesicle diameter. The resulting quantity is  $Eh^2$ . To obtain the Young modulus,  $Eh^2$  was divided by  $h^2$ . The value of  $h$  was taken from<sup>2,3</sup>. To derive the bending stiffness  $\kappa$ , the value of  $Eh^2$  was multiplied by  $h$ . The Poisson ratio  $\nu$  was assumed as 0.5.

## Table containing the mechanical properties of protein coated GUVs

sample	Effective spring constant (N/m)	$Eh^2$ (Pa m <sup>2</sup> )	Young modulus $E$ (Pa)	bending stiffness $\kappa$ (J)
Streptavidin-coated vesicle	$8.6 \times 10^{-4}$	$2.5 \times 10^{-9}$	$1.1 \times 10^6$	$1.3 \times 10^{-18}$
	$7.4 \times 10^{-4}$ (29)	$2 \times 10^{-9}$ (29)	$0.9 \times 10^6$ (29)	$1.1 \times 10^{-18}$ (29)
Avidin-coated vesicle	$2.8 \times 10^{-4}$	$9.8 \times 10^{-10}$	$5.2 \times 10^5$	$4.7 \times 10^{-19}$
	$1.5 \times 10^{-4}$ (26)	$5.5 \times 10^{-10}$ (26)	$2.9 \times 10^5$ (26)	$2.6 \times 10^{-19}$ (26)

Values are shown as mean ± sd. The numbers in parenthesis represent the sample of recorded vesicles.

## Outlook

- Characterization of other surface proteins, e.g. 5-layers
- Combination with Reflection Interference Contrast Microscopy (RICM)
- Comparison of different models

## References

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