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UNDERSTANDING VESICLE ORIGAMI – REASONS FOR ARTIFICIAL PHOSPHOLIPID MEMBRANES FORMING NON-SPHERICAL STRUCTURES

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Vesicles or lipsosomes are spherical is a commonly accepted statement, correct for most vesicles and liposomes under standard conditions. However, changes in the molecular structure of the lipids forming the vesicles can result in non-spherical vesicles with unique properties, like mechanoresponsiveness. The problem is we do not understand in detail why these non-spherical shapes occur. To investigate the fundamental forces, which cause the faceting of non-spherical vesicles, different analytical techniques can be used. A selection of these techniques are further introduced and explained in this work.

This thesis consists of four publications highlighting different aspects of this field of research. In the first publication highly facetted, cuboid vesicles formed by the synthetic phospholipid Pad-Pad-PC are described. Intermolecular hydrogen bonds are shown to be the main driving force for the faceting, due to an exceptionally tight subgel herringbone packing of the lipid. This tight packing results in a stiffening of the membrane making high curvatures impossible. Minimization of the structures energy leads to a maximization of bilayer faces over edges, thus resulting in cuboid structures.

Besides obvious shape-changes it is a complex task to quantify a membrane's surface tension. The existing methods are laborious and have low statistical yield. The correlation of a monolayer's surface pressure to the fluorescence signal of a lateral pressure sensitive membrane probe is described in the second publication. This is the proof-of-principle for the quantification of the probe's fluorescence/lateral pressure relation, as the probe has so far only been used for qualitative analytics.

Publications three and four show iterative improvement of a formerly discovered formulation, which is under investigation as a potential first-line treatment after a heart attack. Increasing the fatty acyl chain length of a 1,3-diamidophospholipid, known to formulate mechanoresponsive vesicles, from a C16 to a C17 chain increases the membrane's main phase transition temperature from 37 °C to 45 °C making the lipid Rad-PC-Rad a perfect candidate for further *in-vitro* and *in-vivo* studies. To study the influence of intermolecular hydrogen bonds in a membrane in more detail, the diurea-analogeous phospholipid Sur-PC-Sur was synthesised. The capabilities to form hydrogen bonds is more pronounced for urea containing compounds compared to amides. However, the lipid shows exceptional monolayer properties. In surface pressure/area per molecule isotherms, Sur-PC-Sur has a phase transition with a negative entropical change as well as one with a positive change. Ultimately the results show that the assumption of a two-dimensional Clausius-Clapeyron equation being correct for all monolayer studies is flawed. Due to a strong change in monolayer thickness between the different phase states in a Sur-PC-Sur monolayer, originated in a large difference in the lipid acyl chain tilt angles, it is necessary to take the membrane thickness into account for thermodynamical calculations.

The publications presented in this thesis give insights into the forces at play in a bilayer membrane to understand the mechanism of the formation of non-spherical vesicles. In the future this could lead to a new generation of drug delivery vehicles.

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