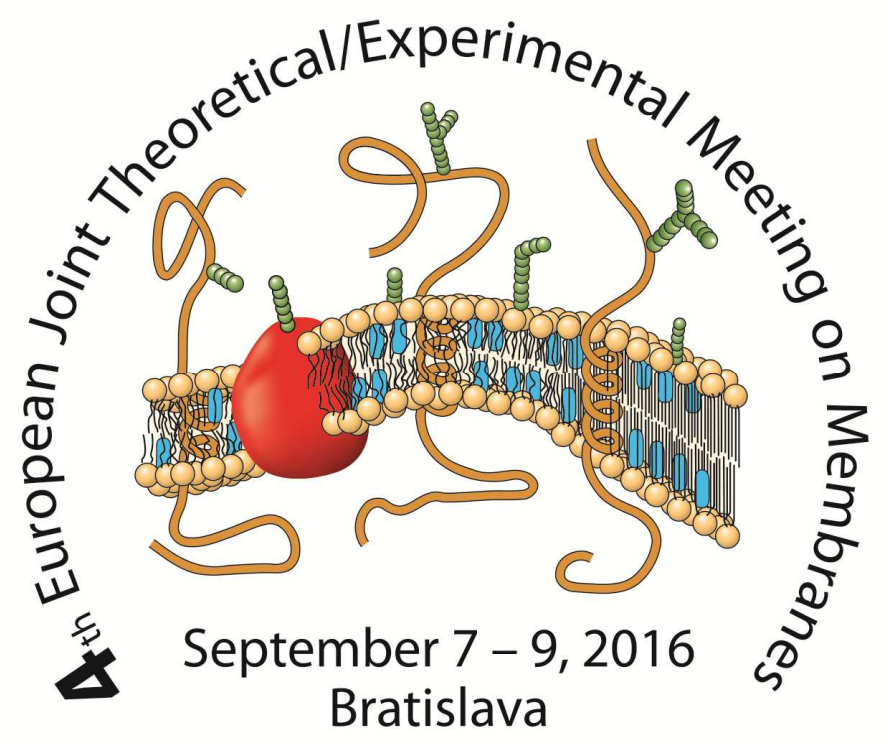

Book of Abstracts

Faculty of Pharmacy Comenius University in Bratislava

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FACULTY OF PHARMACY

4th European Joint Theoretical/Experimental Meeting on Membranes

Bratislava, September 7-9, 2016

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The Faculty of Pharmacy was founded in 1952. It has a reputable tradition, being the first pharmacy faculty in Slovakia, and is a highly respected educational and research institution in the whole European academic field. The high quality of teaching has attracted the best students from Slovakia and abroad, being one of the five most popular faculties of the Comenius University. The Faculty of Pharmacy has always been active in international collaborations. The Faculty offers an English Master in Science Pharmacy program with lecturing, laboratory practices, practical classes and research, and international universities exchange programs. The number of overseas students grows, forming a network for multi-cultural international studies.

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Wednesday, September 7

14:00 – 15:00 registration in the lobby of the Faculty of Pharmacy at Odbojárov 10

15:00 – 15:15 welcome by Norbert Kučerka and Daniela Uhríková
opening by the dean of Faculty of Pharmacy CU Pavel Mučaji

Session 1 chaired by Kateřina Vávrová
15:15 – 16:00 *invited talk*
John F. Nagle: Mechanical and structural properties of lipid bilayers

16:00 – 16:30 coffee break

16:30 – 17:00
Burkhard Bechinger: Biophysical Investigations of Histidine-rich Amphipathic Peptides with a Range of Biological Functions

17:00 – 17:30
Thomas Hauß: Lipid Membrane Structure and Dynamics in the Presence of Amyloid-ß Peptide

17:30 – 18:00
Mária Klacsová: Nonlamellar lipid structures: effect of primary alkan-1-ols

18:00 – 18:30
Olexander I. Ivankov: Small angle scattering instrument YuMO

18:30 – 20:00
Poster session + Welcome mixer
Meeting of the steering committee in parallel

Thursday, September 8

Session 2 chaired by John F. Nagle
8:30 – 9:15 *EBSA talk*
Marta Pasenkiewicz-Gierula: Profiles of the oxygen diffusion-concentration product across lipid bilayers: computer simulation *vs*. experiment

9:15 – 10:00 *EBSA student talk*
Inna Ermilova: SLipids force field: further extension and validation for polyunsaturated phospholipids

10:00 – 10:30 coffee break

10:30 – 11:00
Alex de Vries: GROMOS and MARTINI force fields for glycolipids

11:00 – 11:30
Alexander Lyubartsev: Bottom-up derived coarse-grain models for lipid bilayers simulations

11:30 – 12:00
Stefan Knippenberg: The use of DiI and Laurdan as optical probes in membranes

12:00 – 12:30
Florent Di Meo: Theoretical Elucidation of Drug-Membrane Interactions in Passive Permeation to Active Protein-Mediated Crossing

12:30 – 14:00 lunch break

Session 3 chaired by Sergio de Souza Funari

14:00 – 14:45 *EBSA talk*
Kateřina Vávrová: Models of the skin lipid barrier – from complexity to simplicity and back

14:45 – 15:15 *EBSA student competition*
Lukáš Opálka: Human Ultralong Chain Ceramides: Synthesis and Their Role in the Skin Barrier

15:15 – 15:45 *EBSA student competition*
Markéta Paloncýová: Stratum Corneum Structure – Insight from Molecular Simulations of Bilayers and Multilayers

15:45 – 16:15 coffee break

16:15 – 16:45
Karlo Komorowski: SNARE-mediated membrane fusion intermediates studied by small-angle X-ray scattering

16:45 – 17:15 *EBSA student competition*
Xiao WU: Interaction of beta-lapachone with lipids used in the formulation of liposomes for cancer therapy: experimental and theoretical approaches

17:15 – 17:45
Lukáš Sukeník: The role of membrane receptors properties in the uptake of nanoparticles

17:45 – 18:15 *EBSA student competition*
Tereza Gerguri: Membrane Nanodisc with Protein Fence

19:00 – 22:00
Banquet at hotel Junior, Drieňová 14

Friday, September 9

Session 4 chaired by Marta Pasenkiewicz-Gierula
8:30 – 9:15 *invited talk*
Sergio de Souza Funari: Additives (Urea and TMAO) on POPE and POPC membranes

9:15 – 9:45
Karel Berka: Drugs on Biological Membranes: Molecular Perspective

9:45 – 10:15
Alexander Búcsi: Kinetics of DNA condensation with DPPC

10:00 – 10:30 coffee break

10:45 – 11:15
Jana Gaburjáková: Towards predicting luminal Ca2+ binding sites on the cardiac ryanodine receptor

11:15 – 11:45
Ľubica Lacinová: Modulation of Cav3.2 T-type calcium channel permeability by asparagine-linked glycosylation

11:45 – 12:15 *EBSA student competition*
Lucia Lapínová: Grina modulates G-protein-mediated inhibition of CaV2.2 calcium channels

12:15 – 12:45
Michal Belička: Membrane domains properties influenced by their lipid architecture and ionic composition of the aqueous phase

12:45 – 13:00 closing remarks by Norbert Kučerka and Daniela Uhríková

13:00 – 14:00 farewell lunch

Throughout the biological world, cell membranes are crucial to life. Although the basic notion of the fluid mosaic model still holds true, the plasma membrane has been shown to be considerably more complex. Besides proteins playing an active role in carrying out the various functions that take place in a biological membrane, much attention has recently focused on the importance of lipids in membrane function. After all, how better to explain the diversity of lipids found in nature?

Many researchers resort for detailed simulation results, while others prefer the vigorousity of experiments in order to gain insights into the roles of individual lipid species and proteins. Fewer of them compare simulations to experiments directly. Patrick Trouillas initiated in 2013 the promotion of networking between experimental and theoretical groups to help filling such gap in European research. The emphasis of the discussions had been focused particularly on the field of biological membranes, their composition and interactions with proteins and small molecules such as drugs.

The EJTEMM 2016 is the 4th continuation of the successful series of European Joint Theoretical/Experimental Meetings on Membrane that took places in Limoges, Olomouc, and Stockholm. Bringing the symposium to the center of Europe, we feel fortunate to get a chance in extending the tradition. Please, welcome to Bratislava where we await your membrane enthusiasm and research curiosity.

Norbert Kučerka and Daniela Uhríková
for the local organizers

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SLipids force field: further extension and validation for polyunsaturated phospholipids

Inna Ermilova, Alexander Lyubartsev

Department of Materials and Environmental Chemistry, Stockholm University, Sweden

Parameters for the all-atomistic force field (FF) have been derived for polyunsaturated phosphatidylcholines (PC) with the help of the high level ab-initio calculations. Structures of *cis-3, cis-6*-nonadiene and *cis-3, cis-6*-dodecadiene were used for the derivation of the partial charges and torsional potentials for the unsaturated lipid tails.

A number of molecular dynamics (MD) simulations with different lipid bilayers have been done in order to verify the accuracy of the new FF parameters. The calculated deuterium order parameters showed a good fit with experimental ones and the temperature dependency of those parameters have been reproduced for 16:0-18:2 (*n-2*) PC. Computed areas per lipids have shown a perfect agreement with experimental areas per lipids and computed scattering form factors matched well experimental ones. Finally, 2.5 μs simulations with cholesterol molecules have been done for two different lipid bilayers consisting of 20:4-20:4 PCs and 22:6-22:6 PCs. Two interesting facts have been seen during these simulations. Firstly, cholesterol molecules are building mini-clusters in those bilayers. Secondly, cholesterol molecules were found in three different positions in simulated bilayers: the first one is the most typical position which is when the oxygen molecule “looking” towards the head group when the molecule is away from the bilayer's center, the second one is when the molecule is inverted from its most typical configuration and the third one is when the molecule is lying flat in the center of the bilayer, i.e. between layers. Two last positions have been found experimentally by Harroun and his colleagues [1]. The fact that we could see them in our calculations gives stronger arguments on validity of newly derived FF parameters.

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Additives (Urea and TMAO) on POPE and POPC membranes

Sergio S. Funari[1], *Joana Valerio*[1], *Sigrid Bernstorff*[2]

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[2]Elettra-Sincrotrone Trieste, Trieste, Italy

A cell membrane is mainly formed by lipids where a variety of functional compounds are anchored. The lipid bilayer is the support for such compounds and delimits the cell boundaries. Therefore aqueous dispersions of (phospho)lipids are the simplest way to mimic such membranes.

One easy and very informative way to understand the influence of additive substances, e.g. toxins, drugs, *etc*., on the cell is to study their influence on the structure of such dispersions. Such additives may incorporate themselves into the hydrophobic core of the membrane or remain on the surface or in the hydrophilic environment above it.

The effects can be varied, e.g. expand or contract the bilayer; modify the superficial distribution of the lipids headgroups, of the configuration of the alkyl chains in the core, etc. Depending on the kind and strength of the interactions, the additives can promote a structural phase transition that can easily be seen by Small Angle X-ray Diffraction or Differential Scanning Calorimetry. Similar phase transitions can also be induced by variation of the temperature, pressure, etc.

We shall report our results on the effect of Urea and TMAO added to dispersion of POPC and POPE. It can be seen that they exert their influence in different ways, mostly altering the temperature of the phase transitions.

These results will be compared with those from other additives, e.g. quinones with side alkyl chains in a qualitative attempt to justify the observed surface curvature on these mixtures and formation of phases not seen in dispersions with pure lipids, *e.g.* cubic phases.

Mechanical and structural properties of lipid bilayers

John F Nagle

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Properties of lipid bilayers will be reviewed with an emphasis on my work which currently focuses on mechanical properties, especially the bending modulus and the newly measured tilt modulus. Participants wishing more detail of recent work before the conference are invited to visit http://lipid.phys.cmu.edu. In addition, re-analysis that includes the tilt modulus is being performed on extensive x-ray scattering data from the past decade, and it is hoped to present adjusted values of the bending modulus and first time values of the tilt modulus for many lipids.

Profiles of the oxygen diffusion-concentration product across lipid bilayers: computer simulation vs. experiment

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Oxygen transport within and across biomembranes is of critical importance in cellular processes. As molecular oxygen (O2) dissolves in pure hexadecane about an order of magnitude better than in water, it readily partitions into the membrane. The lipid matrix of a biomembrane is inhomogeneous thus distribution and diffusion rate of O2 depend on the bilayer region. Electron paramagnetic resonance spin-labelling (EPR-SL) methods allow determination of the oxygen diffusion-concentration product as a function of the bilayer depth (profiles across the bilayer). However, separation of this product into its components is not possible with EPR-SL approaches. Conversely, molecular dynamics (MD) simulation can independently provide both the diffusion coefficient and concentration profiles across lipid bilayers.

The goal of this MD simulation study was to compare O2 distribution and diffusion in three purported domains of the phosphatidylcholine bilayer overloaded with cholesterol (Chol), and to validate the simulation data against the experimental EPR-SL data. To reach this goal, three computer models of lipid bilayers differing in 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) and cholesterol (Chol) content were used. The first (a pure Chol bilayer) modelled the cholesterol bilayer domain (CBD), whereas the second (the POPC-Chol bilayer at a 1:1 molar ratio) modelled the POPC membrane saturated with Chol in which CBD is embedded in membranes oversaturated with Chol. The third model was a pure POPC bilayer, which constituted a reference system. Each bilayer contained the same number of lipid (200) and water (6000) molecules.

Each of the three simulation boxes comprised two identical bilayers and 200 diffusing O2 molecules. In the initial structures, the O2 molecules were placed in the intra-bilayer water layer. After the systems were equilibrated, the O2 concentration profile along the normal in each system was calculated. To calculate the profile of the O2 diffusion coefficient, each of the bilayers was divided into distinct zones, and the coefficient was calculated from the linear part of the relevant 50-ps mean square displacement curve for each zone. The oxygen concentration and diffusion profiles were different for different simulation systems; however, the differences in the concentration profiles were greater than the differences in the diffusion profiles. To validate the MD simulation results against the experimental EPR-SL data, oxygen diffusion-concentration product profiles were calculated and normalized for each system. The normalization factor was calculated relative to the oxygen concentration in the water phase, ensuring that the systems were equilibrated at the same oxygen partial pressure.

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Models of the skin lipid barrier – from complexity to simplicity and back

Kateřina Vávrová

Skin Barrier Research Group, Charles University, Faculty of Pharmacy in Hradec Králové, Czech Republic

The skin barrier, which is essential for our survival on dry land, is located in the uppermost skin layer, the stratum corneum, and consists of corneocytes surrounded by multiple lipid membranes. To ensure this protective function, the composition and organization of the lipid membranes differs from common cell membranes: The major skin barrier lipids are ceramides, fatty acids and cholesterol. With over 400 molecular species of ceramide, skin barrier lipids are a highly complex mixture that complicates investigation. I will discuss examples of model membranes of skin lipid barrier in terms of their complexity and experimental usefulness because "*Essentially, all models are wrong, but some are useful"* (George E.P. Box).

The first example will be a highly complex reconstructed skin model that we used to study the acidification processes necessary for a proper lipid processing in healthy and filaggrin knock-down skin.[1] To study the effects of the individual (patho)physiological lipids in more detail than the biological tissue permits, we use simple (four to five-component) lipid membranes that mimic some of the key features of the skin lipid barrier. These models were very useful in some applications (e.g., relationships between ceramide structure, membrane permeability and microstructure)[2-4] with good correlations between the model and human skin, but failed in others. Thus, for some experiments we had to go back to find a "middle ground" in terms of model complexity by using isolated human ceramides or synthetic ceramide mixtures, e.g., in studies of the ceramide release from glucosylceramides or sphingomyelin[5] or the effects of ultra-long ceramides.

To conclude, I might well paraphrase of a famous quote attributed to Albert Einstein: "*Models should be made as simple as possible, but no simpler*"

Acknowledgements: *This work was supported by the Czech Science Foundation (projects 13-23891S and 16-2568J).*

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Biophysical Investigations of Histidine-rich Amphipathic Peptides with a Range of Biological Functions

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A family of histidine-rich peptides LAH4 was designed from general features of linear cationic peptides such as magainins and these designed peptides have been shown to also exhibit considerable antimicrobial activities. In contrast to their natural templates their membrane interactions are strongly pH dependent. Furthermore, these LAH4 peptides have been found to complex nucleic acids and to efficiently deliver DNA and siRNA into eukaryotic cells. Gene therapy/DNA transfection and small interfering RNA therapeutics are promising approaches to fight hereditary and acquired diseases, and has also opened new horizons in cancer therapy. However, the intracellular delivery of these highly charged nucleic acids across cellular barriers remains a hurdle to be overcome. The delivery of cargo by these peptides is complex, involving many steps. Therefore we investigated their structure and topology in membranes and in contact with nucleic acids using a number of other biophysical approaches. When these structural data are correlated with biological activities valuable insight into the mechanisms of antimicrobial action is obtained.

By dissecting the translocation process into individual steps and investigating each step in thermodynamic and structural detail we are able to better evaluate the optimal characteristics for complex formation, membrane interactions, transport and release processes of these non-covalent nanoparticles. Solid-state NMR measurements provide information about the peptide-lipid interactions. Furthermore, the technique reveals important details from within the transfection complexes, which have so far remained a 'black box'. Finally the peptides and derivatives thereof have been found to help lenti- and adeno associated viruses to enter the cell interior which offers interesting perspectives to clinical gene therapy applications.

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Membrane domains properties influenced by their lipid architecture and ionic composition of the aqueous phase

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Lipid bilayers form natural boundaries in living cells separating different outer- and intercellular compartments. In parallel they serve as docking platforms for a wide variety of biological macromolecules providing metabolic or signaling functions. In particular they are thought exhibit non-homogeneous lateral structures of distinct molecular composition, structure and physico-chemical properties, commonly called rafts or domains. Here we mimicked rafts using lipid-only mixtures of dioleoylphosphocholine (DOPC), distearoyl-phosphocholine (DSPC), palmitoyloleoylphosphocholine (POPC) and cholesterol (Chol), which are known to exhibit in a given range of compositions coexisting liquid-ordered (L_O) and liquid-disordered (L_D) domains. Further, such systems may, depending on lipid architecture, exhibit macroscopically large domains, which can be tuned to sizes of a few nanometers by simply exchanging POPC for DOPC. In the present work we investigated how the change in the lipid composition alters the properties of L_O and L_D domains. Additionally, we considered specific interactions of monovalent ions with lipid domains.

Drugs on Biological Membranes: Molecular Perspective

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Biological membranes form important barriers against the entrance of drugs or other xenobiotics either into the cell or into the human body. Theoretical estimation of the permeability of the drugs through the membranes is therefore important for the evaluation of their biological fate within the organism. While most drugs permeate biological membranes by passive permeation, some drugs are able to use specific transport and channel proteins. In both cases, molecule has to "dig through" the membrane via route paved by instantaneous pores and cavities which forms in the drug vicinity upon its presence.

Passive permeability of the drugs through the membrane is driven by its concentration gradient. Molecular dynamics (MD) simulations can be used in order to study passive permeation atomistically, but also to assess the drug's affinity towards the membrane. The major tool for such evaluation is a free energy profile along a normal axis to the membrane, which shows the affinity as well as penetration resistance for any given drug-membrane pair. We will discuss not only the methodology for free energy profile calculation in atomistic resolution, but also the effect of the membrane composition (DOPC as a cell-like membrane vs ceramide NS as a model of skin membrane) on the drug partitioning and penetration in comparison to experimental data. Overall, MD simulations are a useful tool for studies of biological membrane structure and permeability and provide us with the atomistic insight in sub-picosecond resolution unavailable by experiment.

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Kinetics of DNA condensation with DPPC

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Liposomes are widely used as non-viral delivery vectors in gene therapy to transport the DNA into the targeted cells. To override the negative repulsion between the lipid bilayer and the DNA, positively charged additives are mixed to the lipid namely cationic lipids, cationic surfactants or small cations.

The kinetics of the DNA condensation with liposomes from dipalmitoyl-phosphatidylcholine (DPPC) was investigated in the presence of calcium and sodium cations. The DNA condensation with cationic liposome involves a few fast reaction steps, which were studied using fluorescence spectroscopy stopped-flow technique. The influence of cations on the process of DNA complexation was analyzed from both points of view, using ethidium bromide fluorescence probe connected to DNA; and fluorescence labeled lipid. While the measurements according to the DNA revealed kinetics of subsequent reactions, we detected two independent steps of first order kinetics when using fluorescence labeled lipid. The role of calcium and sodium cations seems to be different: the calcium cations play a DNA condensation supporting role in the all studied range of its concentrations, the sodium cations themselves seem to support the DNA condensation at their low concentration, but to depress it at higher concentration. All our findings correlate with theories [1, 2] describing the DNA condensation as stepwise process with a number of reactions, with their different reaction rates, located partly on DNA and partly on the lipid bilayer. The overall process can be seen as a cumulative result of individual steps.

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GROMOS and MARTINI force fields for glycolipids

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Starting with versions 53A5 and 53A6, the united-atom GROMOS force field in 2004 [1] was the first to explicitly incorporate free energy data in its interaction parameters. The idea is that correct reproduction of partitioning free energies of simple molecules between different solvents is a major contribution to successfully build complex molecules from chemically distinct building blocks. The same philosophy was also adopted by the coarse-grained Martini model [2], which finds it origin in the same period.

Lipid molecules form fascinating structures due to their molecular architecture containing building blocks with quite different partitioning behavior and are therefore an ideal testing ground for the validity of the building-block approach. Experience by a number of groups developing a range of force fields shows that it is not trivial to properly parameterize phospholipids in such a manner that a wide range of experimentally measurable properties are correctly reproduced. Often, ad-hoc changes are adopted to improve on particular aspects of the model. It is our ambition to build up a lipid force field more carefully, assessing each building block and combination of building blocks. In our view the most comprehensive experimental data available for such an assessment are lipid phase transition data.

Here, we show how we arrived at a reasonable united-atom GROMOS-based glycolipid force field from the building blocks of alkanes, alcohols and sugars, ethers, and esters, showing results for mono- and di-glycerides as substructures of monogalactosyldiglycerides (MGDG) and digalactosyldiglycerides (DGDG). This force field served as a basis to fine-tune the Martini force field for glycolipids [3], which has been used in a number of studies, e.g. [4].

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Theoretical Elucidation of Drug-Membrane Interactions in Passive Permeation to Active Protein-Mediated Crossing

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Drug-membrane crossing is a crucial pharmacological event that affects drug action as well as toxicity. Over the past decade, molecular dynamics (MD) have become a powerful tool to rationalize such process at the atomic-scale, supporting experimental evidences. Here we comprehensively investigate this event for ganciclovir, taken as a prototypical drug for which joint theoretical-experimental studies may predict and improve drug efficacy in a clinical context. Ganciclovir is an antiviral agent widely used in kidney transplantation to prevent and/or cure viral infection associated with immusuppressant treatments.

Passive (unassisted) permeation of ganciclovir is investigated by means of *z*-constrained MD simulations. This approach provides an atomistic picture of drug-membrane crossing and the related Gibbs energy profile of crossing. It also allows sampling the diffusion process along the *z*-axis (normal to membrane surface). Such approach enables (semi-quantitative) assessment of ganciclovir passive permeation timescale. As ganciclovir influx has not been elucidated so far, an alternative route to passive permeation has been considered, namely drug-membrane crossing through solute carrier protein transporters (SLC).

Ganciclovir efficacy and toxicity also strongly depend on efflux (*i.e.*, from intracellular to extracellular compartments) by active membrane protein transporters, namely the ABCC4 (ATP binding cassette) - MRP4 (multi drug resistant protein) human transporter. The transport cycle of MRP4 has been theoretically investigated, highlighting the driving forces responsible for the large-scale conformational changes leading to drug efflux. Metadynamic simulations were used as the timescale of crossing events and MRP4 opening-closing processes are far beyond the reach of conventional MD simulations. The complete rationalization of ganciclovir efflux trough MRP4 paves the way toward an atomic understanding of any polymorphism and mutation responsible for its intracellular accumulation and of its subsequent toxicity.

Towards predicting luminal Ca^{2+} binding sites on the cardiac ryanodine receptor

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Contraction event in cardiomyocytes is tightly coupled with the transient elevation in cytosolic Ca^{2+} that is mobilized from the sarcoplasmic reticulum through the cardiac ryanodine receptor (RYR2). The main regulatory part of the RYR2 channel is a big cytosolic domain with well-defined interaction sites for various ligands. A much smaller pore-forming region embedded in the membrane holds not intensively characterized Ca^{2+} binding sites that are accessible from the RYR2 luminal face and are involved in the channel luminal regulation. Since now, localization as well as detailed functional profile of these binding sites has not been reported. The aim of our study was to advance our knowledge about the RYR2 luminal Ca^{2+} binding sites by studying the functional effects of various alkaline earth metal divalents (M^{2+}: Mg^{2+}, Sr^{2+}, Ca^{2+} or Ba^{2+}) on the channel response to cytosolic channel activator – caffeine. Native RYR2 channels were isolated from the adult rat heart and reconstituted into a planar lipid membrane. The interaction of luminal M^{2+} with the RYR2 channel was monitored by determining the EC_{50} value of caffeine dose-response curve. The RYR2 channel was the most sensitive to cytosolic caffeine when luminal Ca^{2+} was present, luminal Sr^{2+} displayed a moderate effect, and luminal Ba^{2+} and Mg^{2+} were the weakest players in this regard. Series of competition experiments, when we examined competition of luminal Ca^{2+} with Mg^{2+}, Sr^{2+} or Ba^{2+} at the RYR2 luminal face, clearly documented that the apparent heterogeneity in the RYR2 regulation by luminal M^{2+} can be explained by competitive binding of M^{2+} to the same luminal binding sites with relative binding affinities in the order: $Ca^{2+} > Sr^{2+} > Mg^{2+} \sim Ba^{2+}$. Since this order closely matched the M^{2+} affinity of the EF-hand motif, the most common sequence associated with Ca^{2+} binding in proteins, we hypothesized that RYR2 luminal face is EF-hand positive. In the helix-loop-helix topology of the EF-hand motif, only the 12 amino acid loop plays a critical role in Ca^{2+} coordination. To identify EF-hand loop in the primary sequence of protein various searching patterns have been generated. According to the membrane topology model, only three RYR2 luminal loops could harbor Ca^{2+} binding sites. To localize the EF-hand motif, we systematically compared all 12 amino acid segments derived from these RYR2 luminal loops with the advanced EF-hand signature. We considered three different attributes: (1.) how many of 12 amino acids matched the EF-hand pattern, (2.) how many of amino acids that are directly involved in Ca^{2+} coordination exactly matched the EF-hand pattern and (3.) the sequence identity at positions that show the most conservation. We identified one region in the central part of the first RYR2 luminal loop with the highest score in all three analyzed attributes that could be consistent with effective Ca^{2+} binding.

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Membrane Nanodisc with Protein Fence

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Nanodisc is a patch of phospholipid membrane stabilized by scaffold proteins such as human Apo lipoprotein or its modifications. It is a biotechnological tool that successfully provides a natural lipid environment for isolation, purification, and structure determination (both crystallographic and NMR) of transmembrane proteins. However, the nanodisc is not suitable for all proteins as the length of current scaffold protein limits its size. We used coarse-grained molecular dynamics simulations to determine orientation and packing of small amphiphilic helices, a basic building block for new scaffold protein. Our results show that the presence of aromatic residues on specific positions together with the width and length of the hydrophobic patch on the helices play crucial role in orientation on the membrane edge. Based on this information we designed new helical constructs that prefer to orient perpendicular to the membrane plane and stack together to form a protein fence around the membrane patch. These helices can be used as a repeating unit for new scaffold proteins with easily adjustable length and further protein engineering.

Lipid Membrane Structure and Dynamics in the Presence of Amyloid-ß Peptide

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A hallmark of Alzheimer's Disease (AD) are the amyloid plaques found in the brain tissue of patients died by suffering AD. These plaques are composed mainly of amyloid-β (Aβ), a 40-43 residue long peptide. According to the amyloid hypothesis, the predominantly followed concept in AD research, the accumulation of Aβ in the brain is the primary influence driving AD pathogenesis. In the last few years, more and more findings point to the fact, that the monomeric Aβ or small oligomers may be a crucial factor in neurodegeneration typical of AD. The direct interaction of the Aβ peptide with the lipid membrane causes membrane disruption and may effect signalling pathways in the brain. We investigated lipid membranes doped with amyloid-ß and fragmets of it with different scattering techniques. SANS [1] and neutron diffraction [2-4] was applied to reveal structural details. Quasi-elastic neutron scattering (QENS) was used to study the dynamical changes induces by the peptide. As a main outcome, a reduces lipid diffusion due to amyloid-β was observed [5-7].

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Small angle scattering instrument YuMO

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Main features of the modernized small-angle neutron scattering spectrometer (YuMO) [1-3] at IBR-2M pulsed reactor are described. New installations for sample environment of the spectrometer are highlighted. The modernized SANS instrument (YuMO) is equipped with two detector system which provide a unique dynamic range (Qmax/Qmin ratio is about 90). Sample environment is extended with a new sample holder for the 25 cuvettes, magnetic system (magnetic field about 2.5 Tesla), automated high pressure setup which allows simultaneous SANS and volumetric high pressure studies, light illumination system and system for mixing of two substances for the kinetics measurements. In particular, these developments led to considerable improvements of resolution of the instrument (about 1%) and opened the possibility to study anisotropic materials and perform efficient high pressure studies.

The possibilities of the BioChemLab for the sample preparation and preliminary characterization are described. Laboratory equipped with high precizion density meter, spectrophotometer, microscopes, refrigerators and other equipment for the sample preparation.

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Nonlamellar lipid structures: effect of primary alkan-1-ols

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Nonlamellar lipid structures perform a number of key roles in biological processes. They are indispensable in modification of membrane flexibility underlying the proper function of integral membrane proteins and in local formation of nonbilayer structures, e.g. during the biological processes of endo- and exocytosis and cell division. Lamellar to nonlamellar phase transition plays an important role also for application in food science and pharmacology, e.g. lipidic nanoscale systems for drug delivery, including liposomal formulations and inverted-type fluid phases and their aqueous dispersions (cubosomes and hexosomes).

Many factors influence the phase behavior of inverse hexagonal and cubic liquid crystals, such as molecular structure of lipids, hydration, temperature and/or addition of a third substance. The short talk will be focused on the occurrence and formation pathways of nonlamellar structures in lipid mixtures representing the major biomembrane lipid classes (PCs and PEs) at excess water. Based on evaluation of phase transition temperature, lattice parameters and inclination angle of the inverse hexagonal phase formation we propose a change of transition mechanism with varying PE lipid content.

Primary alkan-1-ols are substances of choice for topical drug delivery systems thanks to their transdermal penetration enhancing effect. This effect increases with alcohol chain length up to decan-1-ol and decreases for longer chains, i.e. a cut-off in the penetration enhancing potency is present. We have followed the effect of long alkan-1-ols on structural polymorphism of the PC+PE system.

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The use of DiI and Laurdan as optical probes in membranes

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The fluorescent marker Laurdan and its newer derivative, C-laurdan have been investigated in a DOPC lipid bilayer. Experimentally, the latter probe is known to have a higher sensitivity to the membrane polarity at the lipid head-group region and has higher water solubility [1]. By means of Molecular Dynamics (MD) simulations, it is shown that Laurdan is oriented with the carbonyl group towards the head of the membrane, with an angle of ca. 70-80° between the molecular backbone and the normal to the bilayer, while C-laurdan presents an angle of 50°, with opposite orientation than for Laurdan. This different orientation will reflect the difference in transition dipole moment between the two probes and, in turn, the different optical properties. The DiI-C18 probe, which can be excited in the red spectral region, has been embedded in the liquid disordered (Ld) phase of DOPC at room temperature, as well as the gel (S0) phase and Ld phases of DPPC at 298 K and 323 K, respectively, and the liquid ordered (Lo) phase of a 2:1 mixture of Sphingomyelin and Cholesterol at room temperature. In the literature however, no unambiguous answer is available about the nature of the preferred phase, which is highly determined by the chosen concentration of the various lipids [2]. To investigate it, Gibbs free energy profiles are calculated for DiI-C18 by means of the 43A1-S3 force field and the Z-constrained method. To investigate the (non-) linear absorption spectra of the probes, benchmark calculations are performed using CC2 and higher order ADC methodologies. Comparison is made with diverse TDDFT functionals, of which one is chosen for further analyses. A polarizable embedding QM/MM formalism is used to assess the optical properties in both membrane and water environment. For Laurdan and C-Laurdan, the results for one photon absorption in water show little differences, while the first hyperpolarizability b is twice as large for Laurdan. For DiI, the differences in the absorption for the different phases are rather small.

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SNARE-mediated membrane fusion intermediates studied by small-angle X-ray scattering

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Membrane fusion takes place in numerous physiological processes on the cellular and subcellular level, for instance, viral entry and synaptic transmission. For neuronal signaling, exocytosis of synaptic vesicles - induced by their fusion with the presynaptic plasma membrane to release neurotransmitters into the synaptic cleft - is mediated by the SNARE proteins synaptobrevin 2 (syb), syntaxin 1a (syx) and SNAP-25 (SN25). According to the zippering hypothesis, their N- to C-terminal assembly forms a stable four-helix bundle (SNARE complex) by binding of syb to a syx/SN25-complex, which are localized in opposing membranes, and provides the driving force to bring the two membranes into close apposition, thus initiates merger [1]. In vitro, partial zippering of the SNARE complex by using mutants of syb, which cause disruptions of the complex at certain positions, revealed membrane fusion intermediates between reconstituted proteoliposomes, in particular, a stable docking state can be arrested [2]. This way it is possible to overcome the short timescales in which the intermediates naturally occur and thus the biochemically well controlled systems are suitable for steady state small-angle X-ray scattering (SAXS) experiments.

Here we aim at the structure of the intermediates of the SNARE-mediated liposome fusion pathway, which can be partly arrested, as in particular of the two tightly docked membranes. Complementing structural studies performed previously by cryo-electron microscopy, we apply SAXS to proteoliposome suspensions for a wide range of composition and different states along the fusion pathway, including in particular the arrested fusion pathway. We also propose to extend the solution SAXS in two ways: (1) by scanning-SAXS with nano-focused beams to probe cryoimmobilized samples, which also allows a direct comparison to cryo-electron microscopy data, and (2) by SAXS in microfluidic devices, monitoring different steps of the fusion reaction (beamtime foreseen). To obtain structural parameters from the SAXS data, we make use of form factor models of lipid-bilayers based on a set of Gaussian functions in the real space [3].

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Modulation of $Ca_v3.2$ T-type calcium channel permeability by asparagine-linked glycosylation

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Low-voltage-gated T-type calcium channels are expressed throughout the nervous system where they play an essential role in shaping neuronal excitability. Currently, little is known about the cellular mechanisms controlling the expression and function of T-type channels. Asparagine (N)-linked glycosylation has recently emerged as an essential signaling pathway by which the cellular environment can control expression of T-type channels. Acute enzymatic deglycosylation of human $Ca_v3.2$ channels produces in a significant decrease of the T-type current density, and critical residues for N-linked glycosylation include asparagines N192, N1466, and N1710. In the present study, we used human $Ca_v3.2$ glycosylation-deficient channels in which critical asparagine residues were exchanged with glutamine (Q) residues (N192Q, N1446Q, N192Q + N1466Q, and N1710Q) to assess the role of N-glycosylation on the voltage-dependent gating of the channel.

While the N1710Q mutation resulted in a non-functional channel, the single N1446Q mutation, or in combination with the N192Q mutation (N192Q + N1466Q), produced a significant decrease of the mean slope conductance of the channel. In addition, the double mutation significantly decreased the coupling efficiency between the activation of the channel voltage-sensor and the pore opening. Altogether, our data demonstrate that N-linked glycosylation of $hCa_v3.2$ channels may play an important physiological role, and could also underly the alteration of T-type currents observed in disease states such as diabetic neuropathy.

Grina modulates G-protein-mediated inhibition of $Ca_V2.2$ calcium channels

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Chronic and neuropathic pain is caused by injury or disease which affects somatosensory nervous system and influence a large part of population. Neuronal voltage dependent calcium channels ($Ca_V2.2$) regulate neurotransmitter release from nerve fibers responsible for nociception and modulates synaptic plasticity of the first synapse of nociceptive pathway. Transfer of $Ca_V2.2$ channels into so-called reluctant conductance state by heterotrimeric G-proteins is well established intrinsic modulatory mechanism attenuating activity of these channels. Grina protein (glutamate ionotropic receptor NMDA type subunit associated protein 1) was recently identified using modified yeast two-hybrid assay employing a split-ubiquitin as a potential interaction partner of the $Ca_V2.2$ protein. We investigated whether Grina protein affects the modulation of the $Ca_V2.2$ channel by G-proteins.

We have used Chinese Hamster Ovary (CHO) cells permanently expressing the α1 subunit of the $Ca_V2.2$ channel together with β2a and α2δ-1 subunits. Into this cell line we transiently transfected either Grina protein, or beta and gamma subunits of G protein (Gγ2 + Gβ1), or both together. Ba^{2+} current through $Ca_V2.2$ channels was measured using whole-cell patch clamp technique.

Grina protein significantly suppressed current density, shifted voltage dependence of current activation towards more depolarized potentials and slowed a kinetics of current activation. These effects could be attributed to an enhanced channel inhibition by G-proteins. In support of this suggestion an overexpression of Gγ2 + Gβ1 mimicked these effects and G-protein-related current inhibition was further enhanced when Grina protein was coexpressed together with Gγ2 + Gβ1. Altogether, our results indicate direct involvement of Grina protein in modulation of $Ca_V2.2$ calcium channels by G proteins.

Bottom-up derived coarse-grain models for lipid bilayers simulations

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Molecular simulations of many phenomena related to biomembranes requires consideration of length scales above 10 nm and time scales longer than 1 □s, which necessitates the use of coarse-grained (low resolution) models, when each site of the model represents a group of atoms, and the solvent is often omitted. While many of coarse-grained models used in different studies in recent years relay on empirically parametrized interaction potentials (e.g. Martini force field), the systematic bottom-up coarse-graining approach is based on determination of coarse-grained potentials from atomistic (high resolution) simulations.

In this presentation a multiscale modeling approach based on the inverse Monte Carlo method is discussed [1], in which radial distribution functions (RDF) and distributions of internal degrees of freedom of molecular structure, obtained in atomistic molecular dynamics simulations, are used to reconstruct effective potentials which reproduce the same structural properties within implicit solvent coarse-grained model. Such coarse-grained models, simulated by Langevin molecular dynamics, require 2-4 order of magnitude less computer resources than atomistic model of the same size. Using this methodology we have derived coarse-grained potentials for several types of lipids and cholesterol, and used them in simulations of large-scale bilayer fragments, as well as in modeling of self-assembly of lipids into bilayer or vesicle structures.

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Human Ultralong Chain Ceramides: Synthesis and Their Role in the Skin Barrier

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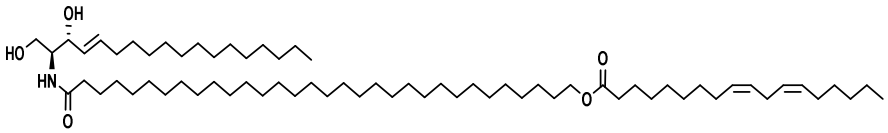
Ceramides (Cer) belong to a family of sphingolipids. They are the central molecules in sphingolipid metabolism and they participate as regulators of cellular processes as well as essential components of the skin barrier. In the skin barrier, the crucial role of these lipids is the protection against excessive water loss and against penetration of exogenous substances (such as toxins or bacteria) into the body.



The aim of my work was to synthesize and study the physiological human skin Cer with ultralong chains. These Cer are essential components of the skin barrier; any major problem in their synthesis or translocation is lethal. However, these Cer are not commercially available, which hampers further studies that would shed more light on the behavior of these lipids and their potential use in therapy of severe skin diseases.

The first efficient and scalable synthesis of all the human ultralong chain Cer subclasses was developed. The key approach involved a connection of two C16 fragments using a Wittig reaction, use of succinimidyl ester (as a protective group which also increases the solubility and activates the carboxyl for the formation of an amide bond) and Yamaguchi esterification reaction for the connection of linoleic acid. The overall yield of this 12-step synthesis was 11% in low scale and 8% in high (gram) scale.

The role of the prepared ultralong Cer was studied using model lipid membranes mimicking the human skin barrier. The long periodicity phase, which is essential for proper skin function, started to form at 10% of Cer EOS in the membrane. Then we constructed more complex models with a mixture of human Cer. The permeability of these membranes showed that ultralong Cer are essential for proper barrier function. In this work we described the concentration-dependent structure-permeability relationships for ultralong human skin Cer and the underlying mechanisms of their action on a molecular level using powder X-ray diffraction and infrared spectroscopy.

In conclusion, we developed the first scalable synthesis of human ultralong Cer and described some important characteristics of these lipids that open new possibilities in the treatment of severe diseases of the skin barrier, such as atopic dermatitis or lamellar ichthyosis.

Stratum Corneum Structure – Insight from Molecular Simulations of Bilayers and Multilayers

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Stratum corneum, the uppermost layer of the skin, forms a highly impermeable barrier on the surface of our bodies. Though an extensive experimental effort was focused on this tiny layer, its molecular structure is still not precisely known. The composition of stratum corneum includes various kinds of ceramides, free fatty acids with long saturated tails, cholesterol and cholesterol sulphate. With electron microscopy and other techniques a short and long periodicity phase was observed, however the molecular arrangement of individual lipids is still under debate. The studies of the structure of stratum corneum are challenging both by experimental and theoretical tools.

We used molecular dynamics (MD) simulations in order to investigate the stability and structure of ceramide layers.[1] We built pure ceramide bilayers in various environments and analyzed the role of water molecules on the stabilization of ceramide layers. Further, we built bilayers composed of ceramides, free fatty acids and cholesterol and by a simulated annealing we analyzed the interactions between individual lipids and their clustering behavior. And finally, we created double bilayers and in various temperatures we monitored the behavior of water molecules that penetrated through one of the bilayers in order to reach the head group region. We also analyzed the exchanges of ceramide conformations from initial hairpin to extended. And we also monitored the flip-flops of cholesterol and free fatty acids, the changes in their concentrations in various regions during the simulations. The cholesterol and free fatty acids were able to exchange fast in between the membrane leaflets. We believe that these fascinating movements of lipid molecules are able to provide a high-resolution view into the possible molecular structure of stratum corneum.

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The role of membrane receptors properties in the uptake of nanoparticles

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Cellular uptake is a crucial step in the process of drug delivery, where drugs attached to a well designed carrier can be guided into selected cells. One of the strategies in the carrier development is to target the over-expressed receptors in diseased cells to acquire cell specificity. This can be achieved via functionalization of the nanoparticle surface with proteins, antibodies or other biomolecules. However, the influence of membrane receptors properties on nanoparticle uptake is not well understood. We have investigated the process of receptor-mediated endocytosis of nanoparticles using MD simulations. In particular, we focus on the properties of membrane receptors such as flexibility, length and thickness. Generally, more flexible receptors hinder nanoparticle uptake, while rigid receptors make it easier. The effect of receptor length is profound, longer receptors require larger nanoparticles for successful uptake. Our results show that the properties of membrane receptors have a significant effect on nanoparticle uptake and should be considered in the rational design of more efficient and cell-selective carriers for drug delivery.

Interaction of beta-lapachone with lipids used in the formulation of liposomes for cancer therapy: experimental and theoretical approaches

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Beta-lapachone is an efficient drug against cancer, but it is poorly soluble in water, which limits its applications. It may be encapsulated into liposomes and due to its hydrophobicity, it is expected to intercalate between phospholipid acyl chains in the bilayers of vesicles. We have thus studied the miscibility of beta-lapachone within liposomes formulated with various lipids. Surprisingly, compression isotherms performed on a Langmuir trough did not show any effect of the presence of beta-lapachone on i) the collapse surface pressure; ii) the collapse surface area; and iii) the mean molecular area of pure phospholipids or mixtures of phospholipids with cholesterol and/or stearylamine. These results accounted for the non-incorporation of beta-lapachone into liposome bilayers. However, a thorough analysis of the data demonstrated evidence of an alteration of the compressibility moduli profiles, and compression cycles. Atomic force microscopy confirmed the alteration of lipid mixture domains in the presence of the drug. Beta-lapachone showed a tendency to structure disorganized monolayers, e.g. made of DOPC, and disorganize structured monolayers, e.g. made of DPPC or cholesterol.

Molecular dynamics simulations made on bilayers constituted of various lipids confirmed the capacity of beta-lapachone to rapidly insert into bilayers, preferentially in between the lipid tails. Larger and fast variations of the area per lipid (A_L) were observed in the presence of beta-lapachone, with respect to its absence. However, the average A_L value was similar in both cases. The organization in the bulk of the lipid tails was also slightly affected, e.g., increase of the order parameter of the saturated chain in POPC bilayers.

Structural study of multiphase lipid liquid crystalline nanoparticles with high protein upload

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Structural analysis of nonlamellar lipid/protein/PEG system was investigated by small-angle X-ray scattering (SAXS) and Cryo-TEM analyses. PEGylation lead to fragmentation of the lipid phase to nanoparticles. The nanoscale organization has influence on the loading and release efficacy of these particles. SAXS experiments were performed at the P12 BioSAXS beamline of the European Molecular Biology Laboratory (EMBL) at the storage ring PETRA III of the Deutsche Elektronen Synchrotron (DESY, Hamburg, Germany). High resolution SAXS revealed variations of the aqueous channels in single phase or multiphase cubosome nanocarriers. The obtained results confirmed that the encapsulated protein molecules, depending on their concentration and amphiphilicity, may change the curvature of the lipid assemblies or transform the internal nanostructure into a different structural organization.

Partial volumes of cholesterol and phosphatidylcholines in mixed bilayers

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Dispersions of multilamellar liposomes prepared from phosphatidylcholines and from varying amounts of cholesterol were studied by densitometry. Ideal mixing of the monounsaturated diacylphosphatidylcholines having 18–24 carbons with cholesterol in the fluid phase was observed. The temperature dependence of partial volumes of both monounsaturated phosphatidylcholines and cholesterol was determined. A slight decrease in the partial volume of cholesterol with the lengthening of the acyl chain of the host monounsaturated phosphatidylcholine was observed. The phase boundary between the solid ordered phase and the area of coexistence of the solid ordered and liquid ordered phases was detected by measuring the density of multilamellar liposomes of dinervonoylposphatidylcholine and cholesterol below the main phase transition temperature.

Dispersions of multilamellar liposomes prepared from saturated dimyristoylphosphatidylcholine and varying amounts of cholesterol were also studied below and above main phase transition but the distinguishing of the phase boundaries is questionable.

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Structural Modulation of Monoolein Cubic Phase by Amine Oxides to Enhance DNA Binding

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Monoolein is a non-toxic biodegradable polar lipid that in excess of water forms a bicontinuous cubic phase with *Pn3m* symmetry. *Pn3m* cubic phase of monoolein is formed by one congruent curved lipid bilayer extending in three dimensions, separating two congruent networks of water channels[1]. Bicontinuous nature of the cubic phase enables application of monoolein in carrier systems for drugs with various polarities[2]. The size of water channels is roughly 4-5 nm and can be modulated by incorporation of various amphiphilic molecules. Extension of water channels, enables encapsulation of large hydrophilic molecules like proteins and DNA into the cubic phase[3]. In our work we studied the effect of pH responsive surfactants *N*-alkyl-*N,N*-dimethylamine-*N*-oxides (CnNO, where n is number of carbons in alkyl chain) on structure of cubic phase of monoolein and the ability of CnNO-monoolein systems to bound DNA of different types and lengths.

At neutral pH the lattice parameter of the cubic phase rises rapidly with CnNO content. At CnNO/monoolein molar ratio = 0.2 the lattice parameter of cubic phase at 20°C rises 2-3 times compared to pure monoolein. At higher content of CnNO the swelling of the cubic phase proceeds through a phase transitions to sponge phases and further to a lamellar phase. The encapsulation of nucleic acids at neutral pH varies greatly depending on the type and length between of nucleic acid 5 to 60 %. At pH < 6 the amount of bound nucleic acids sharply increases exceeding 90%, however this drop of pH destabilize the cubic phase and supports formation of lamellar phases.

Our experiments confirmed the ability of CnNOs to modulate structural parameters of *Pn3m* cubic phase of monoolein and support encapsulation of nucleic acids. This can be used in the design of new carrier systems for nucleic acids or other biomacromolecules.

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Real-time monitoring of the interaction between surfactant N,N–dimethyl–1–dodecanamine N–oxide and giant unilamellar and multilamellar liposomes using optical microscopy.

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The effect of the surfactant *N,N*–dimethyl–1–dodecanamine *N*–oxide ($C_{12}(CH_3)_2NO$) on the giant unilamellar liposomes (GUL) composed of dioleoylphosphatidylcholine (DOPC) with/without cholesterol (CHOL) was studied in a wide concentration range up to a complete solubilization of DOPC. Optical microscopy was used for the real–time monitoring of the interaction between GUL and surfactant in a chemical microchip. At lower concentrations, surfactant $C_{12}(CH_3)_2NO$ caused a decrease of the liposome's diameter. Probably, the interaction proceeded via dissolution of the bilayer by releasing of the sub–microscopic mixed lipid–surfactant aggregates into the bulk solution while the spherical structure of the liposomes was still preserved. The effect of the GUL size on the time of complete solubilization was negligible. The solubilization time was shortened with the increase of the surfactant concentration. The increasing amount of CHOL in DOPC bilayer gradually suppressed the decrease of the liposome's diameter and extended the solubilization time.

A time dependent effect of $C_{12}(CH_3)_2NO$ on the multilamellar liposomes (MLL) from DOPC was also observed by optical microscopy. The MLL underwent different structural changes, therefore it was not possible to unify the mechanism of the solubilization.

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Effect of alkan-1-ols on the structure of DOPC model membrane

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We studied the effect of general anesthetics alkan-1-ols (CnOH, where n=10, 12, 14, 16 and 18 is the number of carbon atoms in molecule) on the structure of a model membrane system by small-angle neutron scattering (SANS) and diffraction (SAND). Extruded unilamellar liposomes for SANS measurements were prepared in excess of 100 % D_2O at the CnOH:DOPC=0.3 molar ratio. The data were evaluated by the Kratky-Porod plot $\ln[I(Q)Q^2]$ vs. Q^2 in the range of scattering vector Q corresponding to interval 0.001 $A^{-2} \leq Q^2 \leq$ 0.006 A^{-2}. The value of bilayer thickness parameter d_g has shown the trend in which the shorter alcohols (n=10, 12) decreased the thickness of DOPC bilayers while the longer ones (n=16) caused its increase. The results were corroborated with SAND measurements performed on the aligned multilayers that were utilized to eliminate the effect of bilayer curvature. Hydrated aligned fluid bilayers were prepared again from dioleoylphosphatidylcholine (DOPC) and samples at CnOH:DOPC=0.3 molar ratio were deposited on quartz plates by a „rock and roll method“. The samples were used for measurements at slightly dehydrated conditions (rel. humidity 98%, contrasts 8%, 20% and 50% D2O) for improving the resolution of obtained results. From the scattering length density profile we found the similar trend to that of SANS results. Our results support the notion of the importance of lipid bilayer thickness and the possible mechanism of its modulation by the other membrane components, while this is unaffected by the bilayer geometry nor its hydration level.

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6-Hydroxyceramide in Model Lipid Membranes - Study of Permeability and Biophysics

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Ceramides (Cer) play a significant role in many processes in living systems. In addition, they are essential in the uppermost layer of mammalian epidermis, the *stratum corneum*. There, in the *stratum corneum*, Cer act as a barrier – they participate in the maintenance of stable internal environment (preventing the penetration of unwanted substances, pathogens and the loss of body water). Cer based on 6-hydroxysphingosine have only been found in epidermis; however, the role of 6-hydroxyCer in the skin barrier homeostasis is not fully understood.

In this work, we focused on the total synthesis of 6-hydroxyCer and study of the permeability and microstructure of model lipid membranes based on 6-hydroxyCer in comparison with sphingosineCer, dihydroCer and phytoCer.

The starting substance, tridecanal, reacted with trimethylsilylacetylide to give (±)-1-trimethylsilylpentadec-1-yn-3-ol. In the next step, an enzymatic reaction with lipase was used. Lipase acetylated only the (*R*)-isomer; thus, it was easily separated from the unreacted (*S*)-alcohol in a single step. Then, the protected groups of (*R*)-isomer were deprotected and (*R*)-pentadecynol (*ee* 99%) was silylated and then as an alkynide reacted with (*S*)-Garner aldehyde. The triple bond was reduced by modified Trost's hydrosilylation/protodesilylation. The prepared 6-hydroxyCer was incorporated in model *stratum corneum* lipid membranes. Model membranes were composed of Cer/free fatty acids (C16-C24)/cholesterol/cholesteryl sulfate. Their permeability was assessed in Franz-type diffusion cells using the following permeability markers: flux of two model compounds (theophylline and indomethacin), electrical impedance and water loss through the membrane. To elucidate the mechanisms of 6-hydroxyCer effects on skin permeability, their biophysical properties were investigated by infrared spectroscopy and X-ray powder diffraction.

The results showed that the individual skin Cer classes have unique properties and changes in their structure lead to differences in barrier function of model lipid membranes. We hypothesize that this apparent heterogeneity in chemical structure, permeability and biophysical properties helps the skin lipid barrier better resist external stressors.

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Cation-induced changes to the structure of lipid membranes

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Cell membrane properties such as membrane fluidity, bending and rigidity moduli, electrostatics, and aggregation and fusion are tightly associated with ions that are prevalent in both the cytosol and the exterior of the membrane. Interestingly, the divalent metal cations were found to play a dominant role in affecting bilayer structure. For example, it is well known that Zn^{2+} plays a fundamental role in several critical cellular functions such as protein metabolism, gene expression, structural and functional integrity of biomembranes, and in metabolic processes, while Ca^{2+} was shown to alter a bacterial membrane in a manner limiting its water penetration. The cation binding depends strongly on the property of the cation and the membrane lipid head-group.

We have studied the interactions of calcium and zinc with the biomimetic membrane made of dipalmitoyl-phosphatidylcholine (DPPC). The small angle neutron diffraction (SAND) experiment on oriented multilamellar samples was employed to decouple effects due to electrostatic interactions from those of geometrical constraints found in curved vesicular bilayers. Attained results show clearly differences in the effects of the two cations. For both, a bilayer thickness increases due to divalent metal ion (Me^{2+}) binding, reaching the maximum at stoichiometry Me^{2+}:DPPC~1:7 mol/mol. However, while the further increase in Ca^{2+} results in a bilayer thinning down to the level of pure DPPC, the Zn^{2+}binding indicates the behavior of a typical isotherm, reaching a level of saturation.

Our observations compared to those obtained for curved bilayers agree well with the notion that the effect can most likely be rationalized in terms of electrostatic interactions, rather than that of geometrical constraints due to bilayer curvature, and thus reinforcing the special importance of these cations. The electronic structure of zinc is different from that of divalent alkaline earth metal ions. Zinc cation possesses a higher affinity to electronegative groups and therefore, also other electronegative moieties such as ester oxygens and/or carbonyl groups of the lipid headgroup can be directly involved in complex formation. The molecular dynamics simulations have been performed to gain more detailed information, results of which will also be discussed.

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pH sensitive N-alkyl-N,N-dimethylamine-N-oxides in gene therapy

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Gene therapy has tremendous potential for inherited and acquired diseases. Efficient delivery of plasmid DNA into cells is achieved via viral as well as nonviral vectors. Viral vectors have high transfection efficiency, but on the other side they are cytotoxic towards host cells. Consequently, nonviral transfection systems are preferred. Mixtures of N-alkyl-N,N-dimethylamine-N-oxides (CnNO, where n is the number of alkyl carbons) and DOPE (dioleoylphosphatidylethanolamine) were tested as nonviral DNA delivery vectors. CnNO with DOPE forms liposomes, which exist in a neutral or cationic form depending on the pH of aqueous solutions. At acidic pH they are protonated and are able to interact with negatively charged DNA due to electrostatic attraction. Using small angle X-ray diffraction (SAXD) we studied a structural polymorphism of pH sensitive complexes prepared from DNA, CnNO and DOPE in 150 mmol/l NaCl. We observed a condensed lamellar phase at 20°C and 37°C and condensed inverted hexagonal phase at 80°C. When the complexes were heated to 80°C and cooled down to 20°C we observed a cubic phase also. Janus Green Staining Protocol was used to investigate the cytotoxicity of C_{12}NO:DOPE for HepG2 cells (liver hepatocellular carcinoma) at neutral and acidic pH. The molar ratio of C_{12}NO:DOPE was 1:1. We found out that the viability of HepG2 cell is high after 5 as well as 24 hours. The liposomes formulation developed here shows promise as a nonviral vectors with low cytotoxicity.

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Investigation of self-assembling properties of dialkylphosphocholines

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Dialkylphosphocholines (DAPCs) represent zwitterionic gemini surfactants. These amphiphiles self-assemble into micelles, vesicles, and tubules at low concentrations in water or they form a coacervates – "sponge-like" structures [1]. Asymmetric DAPC can also form vesicles which, in turn, self-assemble into gels [2].

We investigated the self-assembling properties of 15 DAPCs (Figure) [3]. Self-assembly properties of DAPCs were investigated by measurements of surface tension, ^{31}P NMR spectroscopy and fluorescence microscopy. Measurement of surface tension enabled us to estimate their hydrophilic-lipophilic balance. ^{31}P NMR spectroscopy put some light into organization of amphiphiles in aqueous environments. We observed that the studied dialkyphosphocholines formed micelles, coacervates and vesicles. Fluorescence microscopy was used for visualization of giant aggregates. Two types of fluorescence probes were used. Amphiphilic stilbazolium alkylfosfocholine was incorporated into membranes of giants vesicles and 1,2,5-triphenylphosphol represents hydrophobic fluorescence probe which was solubilized between alkyl chains of DAPCs (Figure).

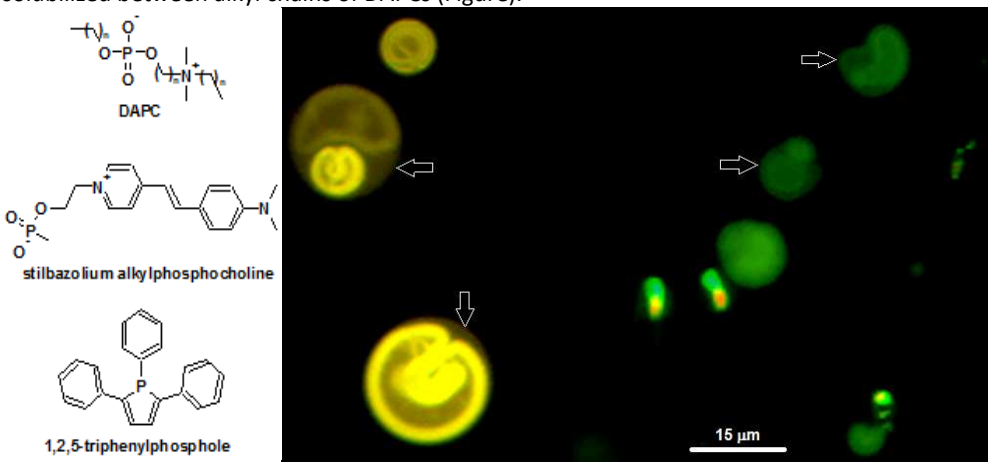

Figure: General structure of DAPCs, fluorescence probes and images of giant vesicles visualized by fluorescence micro-scopy and using stilbazolium alkylfosfocholine (yellow) and 1,2,5-triphenyphosphole (green) as fluorescence probes

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MD Study of Non-Steroidal Drug Interactions with Cyclodextrins

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The ability of cyclodextrins to form inclusion complexes with Non-Steroidal-Anti-Inflammatory-Drug (NSAID) represents one possibility how to avoid their adverse effects and how to increase the solubility of these drugs. In recent years the research area involving the structure of NSAID and interactions with cyclodextrins grows rapidly. We'd like to contribute to number of papers dealing with theoretical approaches and experimental techniques proposed in order to characterize these guest-host interactions including effect of solvent. In course of work on process of modelling the interactions of diclofenac (or diclofenac sodium salt) with beta-cyclodextrin with water molecules we make a use of classical molecular dynamics (MD) simulations performed within MS Studio modelling package. The computational details (with necessary reasoning for validity of chosen forcefield as well as with the details of MD run conditions) and analysis of the production stage trajectories by means of distribution functions and mean square displacements are briefly reported in our contribution.

Role of Membrane Composition on the Structure and Dynamics of Membrane-Attached Cytochrome P450 and Effect of Enzyme Flexibility in Ligand Access/Egress to the Active Site

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Microsomal cytochromes P450 (CYPs) are membrane attached enzymes that play indispensable roles in biotransformation of numerous endogenous and exogenous compounds. Using up to microsecond-long molecular dynamics simulations, we analyzed interaction of CYP3A4 with bilayers composed of lipids differing in their polar head groups, i.e., phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylglycerol (PG) and also in PC membranes with various amount of cholesterol [1]. In the negatively charged lipids, CYP3A4 was immersed deeper and more inclined toward the membrane because of favorable electrostatic and hydrogen bonding interactions between the CYP catalytic domain and lipid polar head groups. A similar effect of hydrogen bonding was identified also in membranes with cholesterol, where CYP also inclined towards the membrane and immersed deeper with the increasing amount of cholesterol. Even a small amount of cholesterol (3 weight %) had a significant effect on CYP's position. In larger cholesterol concentrations, CYP's structure was significantly influenced and different access and egress channels were opened, which could affect CYP's catalytic activity, which is in agreement with experiment. The identified significant role of electrostatics in CYP-membrane interactions may explain the experimentally observed preferences of individual CYP isoforms to distribute in (dis)ordered membrane microdomains.

Although the most of enzymes have buried active sites, the information about the energetics and mechanisms associated with substrate and product channeling in and out is still lacking. In this work we present a novel approach for studies of ligand permeation through enzyme channels. With MOLE 2.0 analysis we estimated the channels present in cytochrome P450 3A4 and with bias-exchange metadynamics we obtained the free energy profiles of these channels. Moreover, we were able to study also another channel that was closed in initial ligand-free simulation. Our approach based on synergy of MOLE 2.0 and bias-exchange metadynamics is fully transferable to other enzymes and provides the thermodynamic description simultaneously with a dynamic overview of the permeation.

Acknowledgement: *IGA_Prf_2016_028*

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Modeling B-Glucocerebrosidase Deficiency In Epidermis: Effects Of Glucosylcermaides/Ceramides Ratio On Barrier Properties Of Model Stratum Corneum Lipid Membranes

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Ceramides (Cer) are released from their precursors – glucosylceramides (GCer) - by enzyme β-glucocerebrosidase (GCerase). This step is crucial for formation of the permeability barrier in the stratum corneum (SC).[1] Decrease in GCerase activity or its complete loss of function leads to accumulation of GCer and decrease in Cer content in SC. This results in impairment of skin barrier which was described in several skin disorders. In our work we studied how the presence of GCer and/or lack of Cer influences permeability barrier properties of model SC lipid membranes simulating GCerase deficiency.

The SC model membranes were prepared as an equimolar mixture of Cer or GCer in different ratios, Chol, FFA and 5% of CholS. We used the full spectrum of isolated human SC Cer (hCer). Also membranes with decreased hCer fraction were prepared. To determine the permeability of prepared membranes, four permeability markers – water loss through the membrane (TEWL), electrical impedance (opposition of the membrane to alternating current) and steady state fluxes of theophylline and indomethacin, were evaluated. The membrane microstructure was characterized using X-ray powder diffraction.

The replacement of 5 – 25% of hCer by GCer led to impairment of the permeability of the prepared membranes to all 4 permeability markers. At these concentrations, the presence of GCer is a stronger contributor to this disturbance than a lack of hCer. The reduction of hCer to 50 or 0% showed that the lack of hCer disturbs the barrier, while the larger GCer/hCer ratio or complete replacement of hCer by GCer has no negative effects on permeability.

In conclusion, we confirmed that the accumulation of free GCer associated with their incomplete processing contributes to altered permeability barrier properties in skin disorders. However, this barrier perturbation by free GCer seems to be concentration-dependent. Although we should keep in mind that our model has a limitation of being devoid of covalent lipids, these results raise a question what actually happens at complete GCerase deficiency.

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Cytochrome P450 Reductase Simulations: Conformation changes and Cytochrome P450 complex

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The Cytochrome P450 Reductase (CPR) is large 680 amino acids long microsomal multidomain enzyme responsible for electron donation to its redox partner cytochrome P450 (CYP) involved in drug metabolism. Electron transfer (ET) chain is mediated by two riboflavin-based cofactors – flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) within their respective domains and nicotinamide adenine dinucleotide phosphate (NADPH). During this electron transfer CPR undergoes several structural changes in open and closed state with domains in different degree of contact. In spite of the fact that CYP-CPR complexes play a key role in drug metabolism, the atomistic mechanism of structural rearrangements during complex electron transfers is still lacking.

Here, we present the results of our study on structural changes during CPR multidomain complex movement between individual electron transfers using classical molecular dynamics (MD) and metadynamics (MTD) simulations with cofactors of NADPH, FAD and FMN in resting state. Homology model of human CPR in both conformations (open and closed) were embedded into pure dioleoylphosphatidylcholine (DOPC) bilayer. After systems equilibration structural changes of protein, anchor and cofactor movement were studied. We were able to select possible CPR-membrane orientation which would allow interaction with cytochrome P450. In addition, MTD simulations describing closing mechanism were performed pointing out for so called to be the most flexible part during conformation changes. At least, we successfully created model of CPR with its redox partner cytochrome P450 3A4 both embedded in membrane. CPR-CYP model was used for prediction of amino acid residues responsible for interprotein electron transfer.

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Application of AFM for morphological, mechanical and adhesive characterisation of microbial biofilms

or

Studying morphology, mechanics, and adhesion of microbial biofilms by AFM

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Last three decades have established the atomic force microscope (AFM) as an indispensable high-resolution tool for analysis of morphology, mechanics, as well as adhesion profiles of specimens ranging from single molecules to complex biological systems, in combination to commercially available optical microscopy.

A number of important research issues in the field of biomedicine, are directly related to the increased antimicrobial resistance of various biofilms to commonly prescribed drugs, as well as, understanding adhesion, as the leading factor for biofilm formation, colony progression, and pathogenesis of microbial agents. The very high sensitivity of novel AFM systems have led to the development and customisation of particular techniques, that enable the study of single-molecule forces and adhesion profiles at the cell/cell or cell/substrate interface.

We will give examples of the application of a new force tool “Quantitative Imaging” (QI™) based on fast force mapping which offers nanotopographical resolution with the opportunity of obtaining mechanical properties from various bacterial strains with pN-resolution. The “entire force distance curve behind every pixel” philosophy enables a full-range user-customised topographic and force spectroscopy analysis, including contact point (“zero-force”) images, Young´s modulus maps, as well as recognition events. The nanoscale mechanical compliance of bacterial walls can therefore be directly correlated to existing/hypothetic models of their structure. We will also discuss the application of novel fast scanning AFMs for the characterisation of dynamic biofilms with high spatiotemporal resolution reaching seconds per frame.

Structural polymorphism of pH responsive C_{12}NO/DOPE/DNA complexes

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The interaction of DNA polyanion with a dispersion of cationic liposomes result in formation of colloid particles of regular inner microstructure. They attract attention as delivery vectors for genetic material. Despite the fact that cationic liposomes have been used for transfection, and commercial lipid formulations are available, their efficiency needs to be improved. We will discuss structural diversity and binding capacity for DNA of complexes prepared from neutral phospholipid dioleoylphosphatidylethanolamine (DOPE) and N-dodecyl-N,N-dimethylamine-N-oxide (C_{12}NO). C_{12}NO is non-ionic surfactant in aqueous solutions at neutral pH. However, strong polar N–O bond with a high electron density on the oxygen results in the protonation of molecules ($C12N^{+}OH$) at acidic pH (pK~5). Polymorphic behaviour of the complexes was studied using small angle X-ray diffraction (SAXD). The system shows a large variety of liquid-crystalline phases, for example: at pH ~ 7, lamellar (L) and hexagonal (H_{II}) were detected phases by C_{12}NO/ DOPE mixture. Incorporation of DNA into the mixture results to the formation of additional condensed $L\alpha^{C}$ and H_{II}^{C} phases; at acidic conditions the H_{II}^{C} to $L\alpha^{C}$ phase transition can be modulated through C_{12}NO/DOPE molar ratio. Complexes of C_{12}NO/DOPE/DNA at C_{12}NO/DOPE~ 0.4 and pH ~7 have shown a cubic phase of *Pn3m* space group after the heating to 80^{o}C and cooling back to 20 ^{o}C. The newly formed *Pn3m* cubic phase tested for several days in a wide temperature range has shown excellent stability. UV-vis experiments revealed that both the complexes composition and pH modulate their binding capacity for DNA reaching up to ~95 % of total DNA volume in the sample at acidic condition. The detected rich structural polymorphism of C_{12}NO/DOPE/DNA complexes modulated through pH and composition suggests their use as promising drug delivery system.

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The effect of primary aliphatic alkanols on phospholipid bilayer properties

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Changes in structural properties of the membrane bilayer are thought to play a significant role in modification of the protein-bilayer hydrophobic interactions leading to anesthesia. The effect of primary alcohols (CmOH; m = 8, 12, 16), as simple models of general anesthetics, on volumetric properties and bilayer thickness in synthetic membranes prepared from homologous series of monunsaturated phospholipids (diCn:1PC; n = 14-24) was studied using densitometry and small-angle neutron scattering (SANS), respectively.

In the presence of anesthetically active C8OH, the sample specific volume v_s does not differ from reference pure lipid membranes, while a slight decrease of the bilayer thickness in all diCn:1PC membranes studied is observed. On the other hand, anesthetically non-active alcohols, C12OH and C16OH, increase the value of v_s, this increase being more significant for higher diCn:1PC chain lengths. Hereby, an increase of the bilayer thickness in presence of anesthetically non-active alcohols is observed at all diCn:1PC chain length. These effects became more pronounced at increased temperature and correlate with the position of the double bond in diCn:1PC.

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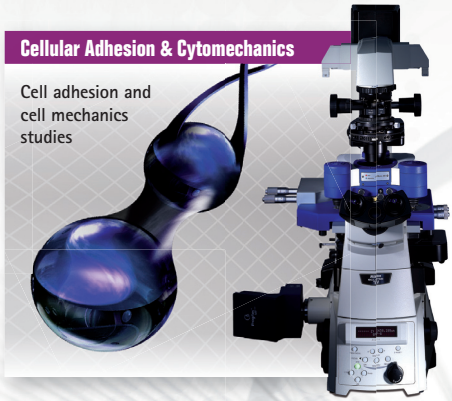

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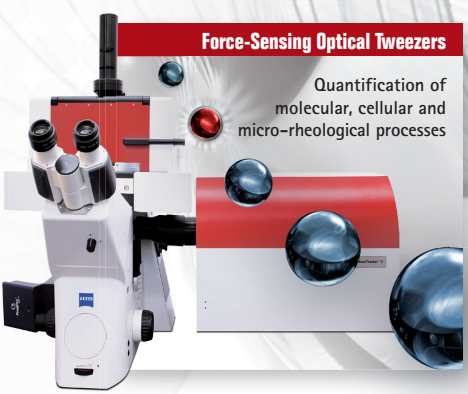

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