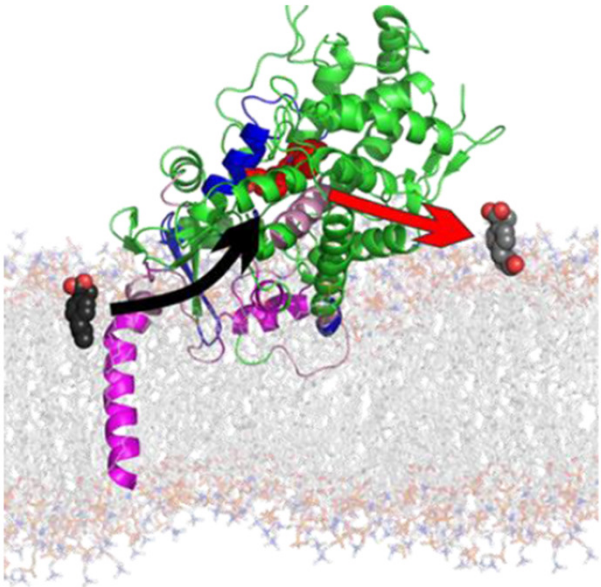
3rd European Joint Theoretical/Experimental Meeting on Membranes

Stockholm

30 September – 02 October 2015

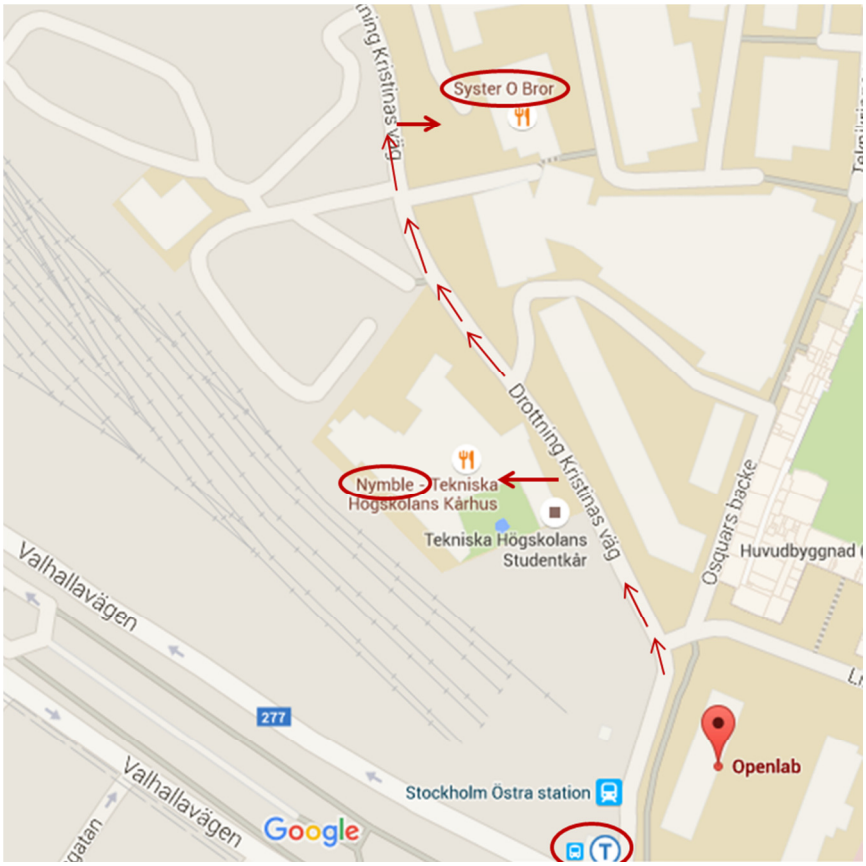


Book of Abstracts

In 2013, Dr. Patrick Trouillas organized the first European Joint Theoretical Experimental Meetings on Membranes in Limoges (France). It promoted profound networking between experimental and theoretical groups working in the field of biological membranes, their composition and interactions with proteins and small molecules. The lively discussions covered a wide range from theoretical investigations to real-world applications e.g. in the pharmaceutical/medical area. In 2014, Prof. Michal Otyepka and Dr. Karel Berka followed the spirit of the first EJTEMM meeting and set up the second one in Olomouc (Czech Republic). Willing to continue this successful series of conferences, we are happy to welcome you to Sweden and Stockholm. We look forward to fruitful discussions and hope that you can benefit from newly established contacts and may work out fresh ideas.

Stefan Knippenberg
Alexander Lyubartsev
Olle Edholm

Venue



The conference takes place in *Openlab* (Valhallavägen 79, 11427 Stockholm). It is located in the direct vicinity of the T-Bana *Tekniska Högskolan*, in the building in front of *Stockholm Östra Station*. Please be aware that the entrance to Openlab is situated at the opposite site of the building.

The introductory dinner on Wednesday as well as the fare-well lunch at Friday is organized inside *Openlab* – in that part of the building which is conveniently called *Opencafé*.

The lunch of Thursday will be taken in restaurant *Nymble*, while the conference dinner is booked in *Syster O Bror*. Both restaurants are accessible via the *Drottning Kristinas väg*.

More information about the accessibility of the conference site with respect to e.g. the local airports can be found at the webpage of the meeting: http://www.theochem.kth.se/ejtemm15/

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Program

Wednesday 30 September

[Arrival]

18h00: Registration
19h00:Dinner – *OpenLab*, Valhallavägen 79

Thursday 01 October

(*OpenLab*, Valhallavägen 79)

08h00: Registration
08h30-08h45: Opening: S. Knippenberg

Session 1, Chair: O. Edholm
08h45-09h20, Keynote 1:
N. Kučerka, "Lipid Structural Diversity: from SIMulation to EXPeriment and back"

09h20-09h55, Keynote 2:
I. Vattulainen, "The influential cholesterol – how membranes sense changes in cholesterol structure"

09h55-10h15:
A. Rabinovich, "Molecular dynamics simulations of membranes: bond orientation properties in lipid molecules"

10h15-10h35:
M. Lindén, "Anisotropic membrane curvature sensing by antibacterial peptides"

10h35-10h55: Coffee

Session 2, Chair: A. Girard-Egrot
10h55-11h30, Keynote 3:
B. C. Lagerholm, "Recent results of experimental measurements of lateral diffusion of phospholipids in live cells by Single Particle Tracking and Stimulated Emission Depletion Fluorescence Correlation Spectroscopy"

11h30-11h50:
R. Vácha, "Influence of ligand distribution on nanoparticle uptake efficiency"

11h50-12h10:
B. Bechinger, "Histidine-rich designer peptides with PH-dependent membrane topology, antimicrobial, nucleic acid transfection and viral transduction capabilities"

12h10-13h30: Lunch
(*Nymble*, Drottning Kristinas väg 15)

Session 3, Chair: I. Parmryd
13h30-14h05, Keynote 4:
O. G. Mouritsen, "Active membranes"

14h05-14h40, Keynote 5:
P. Trouillas, "Molecular dynamic simulation of biological membranes: towards in silico pharmacology"

14h40-15h00:
N. Awasthi, "On the transition region of trans-membrane pores"

15h00-15h20:
R. Šachl, "The oxidized phospholipids PazePC and PoxnoPC change Bax ability to induce pores in mitochondrial membranes"

15h20-15h40: Coffee

Session 4, Chair: O. G. Mouritsen
15h40-16h15, Keynote 6:
I. Parmryd, "The role of actin filaments in creating order in the plasma membrane"

16h15-16h35:
G. Fabre, "Effect of membrane composition on the interaction of small molecules with bilayer membranes"

16h35-16h55:
L. Cwiklik, "Molecular-level organization of the tear film lipid layer"

16h55-17h15:
M. Paloncýová, "Estimation of skin permeability – simulation approaches"

17h15-18h30: Posters

19h00: Conference Dinner
(*Syster O Bror*, Drottning Kristinas Väg 24)

Friday 02 October
(*OpenLab*, Valhallavägen 79)

Session 5, Chair: B. Bechinger
08h30-09h05, Keynote 7:
M. Hof, "Sphingomyelin specific triggering of in-membrane oligomerization of β-amyloid(1-40) and the inhibitory effect of monosialoganglioside GM1"

09h05-09h25:
A. Girard-Egrot, "New simple & versatile tethered phospholipid bilayers for membrane protein reincorporation"

09h25-09h45:
J. Kapla, "Molecular dynamics simulations and NMR spectroscopy studies of trehalose – lipid bilayer Systems"

09h45-10h05:
B. Chantemargue, "Human ABC transporter MRP4: construction of a model, transport cycle and drug efflux"

10h05-10h25:
R. Covino, "mga2 senses lipid saturation in yeast ER membranes by using a rotation-based mechanism"

10h25-10h45: Coffee

Session 6, Chair: A. Lyubartsev
10h45-11h20, Keynote 8:
M. Otyepka, "Positioning of microsomal CYPs and drugs in lipid bilayers"

11h20-11h40:
A. A. Gurtovenko, "Calcium-triggered adsorption of DNA on zwitterionic phospholipid membranes: Insight from MD simulations"

11h40-12h00:
N. A. Murugan, "Multiscale modeling approach for membrane probes"

12h00-12h10: closing remarks

12h10-13h30: Lunch
(*OpenLab*, Valhallavägen 79)

[Departure]

Invited lectures

Lipid Structural Diversity: from SIMulation to EXPeriment and back

Norbert Kučerka
Frank Laboratory of Neutron Physics at Joint Institute for Nuclear Research in Dubna, Russia
and
Faculty of Pharmacy at Comenius University in Bratislava, Slovakia

Throughout the biological world cell membranes are crucial to life, with lipids being one of their major components. Although the basic notion of the fluid mosaic model still holds true, the plasma membrane has been shown to be considerably more complex, especially with regard to the diversity and function of lipids. 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? In order to gain insight into the roles of individual lipid species, we have developed a hybrid experimental-simulation approach and determined the structures of commonly used PC, PG, PS, and PE bilayers. Each designed parsing scheme was based on extensive MD simulations, and was utilized in the SDP analysis of both X-ray and neutron scattering measurements. Obtained experimental scattering form factors were directly compared to the simulation results, serving as a benchmark for future development of force fields. Among the evaluated structural parameters, the bilayer response to changing temperature was found similar to all studied bilayers with different headgroups. On the other hand, the reduced hydration of PE headgroups, as well as the strong hydrogen bonding between PE headgroups, dramatically affects lateral packing within the bilayer. Despite sharing the same glycerol backbone, a markedly smaller area per lipid distinguishes PE from other bilayers studied to date. Overall, our data are consistent with the notion that lipid headgroups govern bilayer packing, while hydrocarbon chains dominate the bilayer's response to temperature changes.

The influential cholesterol – how membranes sense changes in cholesterol structure

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Cholesterol is one of the vital components in regulating the physical properties of animal cell membranes. Its effects on lipid membranes are quite exceptional given that other sterols are not as effective as cholesterol in modulating membrane properties such as order and elasticity, yet it remains unclear what renders cholesterol so unique. The complexity of cholesterol is further stressed by the fact that there are membrane proteins whose function is dependent on cholesterol, and it seems evident that other sterols are not able to replace cholesterol in maintaining protein function. In this talk, we discuss how membranes sense changes in cholesterol structure. We consider synthetic sterols non-existent in nature to elucidate the roles of cholesterol's individual structural elements. We also discuss how oxysterols based on oxidation of cholesterol affect membrane properties in a manner that is distinct from cholesterol. The data emerged from atomistic molecular dynamics simulations as well as experiments provide a basis to better understand why cholesterol is indeed unique in modulating membrane properties and membrane protein function, and can possibly be used to predict new synthetic sterols with applications in biotechnology.

Recent results of experimental measurements of lateral diffusion of phospholipids in live cells by Single Particle Tracking and Stimulated Emission Depletion Fluorescence Correlation Spectroscopy

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Abstract:

Single particle tracking (SPT) studies of phospholipids and membrane proteins in the plasma membrane of live cells with large (i.e. 40 nm diameter) gold particle probes at image acquisition rates of up to 50 kHz has been reported. The results of these studies have been interpreted as evidence that lateral mobility of even phospholipids in the plasma membrane is severally constrained by the cortical actin cytoskeleton such that phospholipids are freely mobile at short time-scales of ~ ≤ 1-5 ms within cortical actin stabilized corrals of ~40-240 nm diameter but that long range mobility is suppressed by a factor of 10-20 (i.e. at short time scales D≈5-10 $\mu m^2/s$ while at long time scales D=0.5-1 $\mu m^2/s$). These results have however neither been validated by a smaller probe nor by a different experimental method.

The motivation of our work has been to attempt to validate these results both by SPT with smaller Quantum dot (Qdots) probes, and by using a different method, namely stimulated emission depletion fluorescence correlation spectroscopy (STED-FCS) with an even smaller fluorescent lipid analogue. Qdots are photostable, brightly fluorescent nanocrystals with a 20 nm diameter ($1/8^{th}$ the volume of the utilized gold particles) which can be specifically conjugated to phospholipids and which we have shown can be imaged at image acquisition rates of ≈ 1.8 kHz with a minimal localization error, $\delta_{x,y}$, of ≈20 nm. STED-FCS in contrast enable systematic lateral dynamic measurements of even fluorescent dye labelled (in our case Atto647N) phospholipid analogues over a size range of 40 to 240 nm, corresponding to typical mean transit times through the focal volume of 0.5 to 35 ms. I will discuss our experimental results and further show how these results compare to previous studies using SPT with large gold particles. Finally, I will present simple simulations and theoretical arguments that consolidate all these results into a single unifying result that suggests that phospholipids are indeed constrained by the cortical actin cytoskeleton but that the confinement is much weaker than has previously been suggested.

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Active membranes

Ole G. Mouritsen

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Interaction between membrane-active proteins and the lipid-bilayer component of biological membranes is expected to mutually influence the proteins and the membrane. Two specific cases are discussed: i) peripheral enzymes (phospholipases) and ii) integral membrane proteins (ion pumps). In the case of secretory phospholipase A_2 it is shown that on the one hand the activity of the enzyme is extremely sensitive to the physical properties of the lipid-bilayer substrate and on the other hand the hydrolysis products tend to remodel the membrane structure in ways that can be described by curvature stress. In the case of the integral membrane protein, Na^+,K^+-ATPase, which is an important ion pump, we find quantitative evidence of manifestation of the lipid-protein interactions in liposomal membranes, reconstituted with actively pumping proteins, in terms of non-equilibrium large-scale *macroscopic* shape fluctuations characterized by a relaxation phenomenon that reflects the intrinsic *microscopic/molecular* reaction-cycle time.

Some recent references

1. Bagatolli, L. A., J. H. Ipsen, A. C. Simonsen, and O. G. Mouritsen. A new outlook on organization of lipids in membranes: searching for a realistic connection with the organization of biological membranes. *Prog. Lip. Res.* **49**, 378-389 (2010).
2. Mouritsen, O. G. Model answers to membrane questions. *Cold Spring Harb. Perspect. Biol.* **3(9)**, 33-47 (2011).
3. Arouri, A., and O. G. Mouritsen. Phospholipase A2-susceptible liposomes of anticancer double lipid prodrugs. *Eur. J. Pharm. Sci.* **45**, 408-420 (2012).
4. Bouvrais, H., F. Cornelius, J. H. Ipsen, and O. G. Mouritsen. Intrinsic reaction-cycle time scale of Na^+,K^+-ATPase manifests itself in the lipid-protein interactions of non-equilibrium membranes *Proc. Natl. Acad. Sci. USA* **109**, 18442-18446 (2012).
5. Arouri, A., A. H. Hansen, T. E. Rasmussen, and O. G. Mouritsen. Lipases, liposomes and lipid-prodrugs. *Curr. Opin. Colloid Int. Sci.* **18**, 419-430 (2013).
6. Arouri, A. and O. G. Mouritsen. Membrane-perturbing effect of fatty acids and lysolipids. *Prog. Lip. Res.* **52**, 130-140 (2013).
7. Mouritsen, O. G. Thermodynamics of lipid interactions In *Encyclopedia of Biophysics* (G. C. K. Roberts, Ed. in Chief) Springer, pp. 2606-2613 (2013).
8. Mouritsen, O. G. Physical chemistry of curvature and curvature stress in membranes. *Curr. Phys. Chem.* **3**, 17-26 (2013).

Molecular dynamic simulation of biological membranes: towards *in silico* pharmacology

Gabin Fabre, Tahani Ossman, Benjamin Chantemargue, Karel Berka, Marketa Paloncýová, Veronika Navrátilová, Michal Biler, Pierre-André Billat, Tahani Ossman, Nicolas Picard, Pierre Marquet, Michal Otyepka, Patrick Trouillas[a,b]

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Molecular dynamics of membrane and antioxidants. Membrane penetration / accumulation / crossing / positioning play a crucial role in antioxidant delivery, metabolism and action in the human body. Over the past decade, *in silico* membrane models and molecular dynamics (MD) simulations have appeared much promising, complementary to experimental measurements, to predict antioxidant-membrane interaction. Theoretical MD simulations have been performed to provide an accurate picture of the intermolecular interaction between antioxidants and lipid bilayer membranes, thus predicting location, orientation and partitioning. Collaborative effects between antioxidants to inhibit lipid peroxidation have been highlighted from joint theoretical and experimental approaches. We really aim at using advanced molecular modeling methods for an applicative purpose to develop new cocktails of antioxidants e.g., in food and cosmetic industries. The predictive character of these methods allows building molecular guidelines for a better and safer use of antioxidants.

Molecular dynamics of membrane and pharmacology. We aim at building *in silico* biomembrane models to get in-depth insight into the mechanisms of drug delivery. The construction of an efflux transporter MRP4 (ABCC4) model encompassed in lipid bilayer will rationalize translocation of drugs used in transplantation (e.g. ganciclovir, valganciclovir and tenofovir; cyclosporine and tacrolimus). Building the human membrane transporter and evaluating all conformational changes (namely, MRP4 inward to outward facing motions) related to drug efflux are a major challenge. Nowadays, all-atom MD simulations allow undertake this task, then to support the fragmented knowledge on drug influx/efflux and (collaborative, competitive and inhibiting) drug interactions as well as the role of MRP4 polymorphisms on drug efflux and on patient outcome. *In silico* models of drug transfer through cell membranes will bridge the gap between drug blood concentrations and pharmacodynamics.

References

1) Košinová, P. *et al. J Phys Chem B*, **2012**, 116, 1309; 2) Poudloucka, P. *et al. J Phys Chem B*, **2013**, 117(17), 5043; 3) Paloncýová, M. *et al.* JCTC, **2014**, 10(9), 4143; 4) Fabre, G. *et al. Chemical Communications*, **2015**, 51, 7713.

The role of actin filaments in creating order in the plasma membrane

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My group study the relationship between ordered plasma membrane nanodomains, known as lipid rafts, and actin filaments using the membrane dyes di-4-ANEPPDHQ and laurdan together with reagents that affect actin filament dynamics in live Jurkat and primary T cells. The degree of lipid packing is quantified using polarity sensitive membrane dyes that display a red shift in their emission peaks for membranes in ld phase relative to lo phase. Laurdan is uncharged and therefore readily flips between the two leaflets of the plasma membrane and we have shown that it distributes equally between the two leaflets whereas the positively charged di-4-ANEPPDHQ does not flip between the two leaflets.

Stabilizing actin filaments using jasplakinolide increased the fraction of ordered membrane domains in the plasma membrane while disrupting actin polymerization using latrunculin B had the opposite effect [1]. Importantly, altering the dynamics of intracellular actin filaments affects the lipid packing of the exoplasmic leaflet of the plasma membrane. Membrane blebs, plasma membrane regions with no connections to actin filaments, had a higher fraction of disordered domains than the bulk plasma membrane. Decreasing the level of phosphoinositides, which lowers the number of potential attachment points for actin filaments in the plasma membrane, also led to an increased proportion of disordered membrane domains. Moreover GM1, a ganglioside that serves as a marker for ordered domains, could not be aggregated in membrane blebs i.e in the absence of links to actin filaments. Aggregation of either lipid raft or non-lipid raft markers generally increased the fraction of ordered domains and this increase was strongly correlated with an increase of polymerized actin filaments just beneath the plasma membrane coinciding with the patches. However, aggregation of the T cell receptor (TCR) resulted in the reorganisation, not the formation, of ordered domains which is consistent with the receptor in resting T cells residing in ordered domains [2]. In addition, the TCR colocalized with actin filaments at the plasma membrane in unstimulated Jurkat T cells, consistent with it being localised to ordered membrane domains that form upon attachment of actin filaments to the plasma membrane.

In summary, our results suggest that actin filaments attachment to the plasma membrane leads to the formation of ordered domains and which downplays lipid-lipid interactions as the main driving force behind the formation of ordered membrane domains *in vivo*, giving a prominent role to lipid-protein interactions.

References

[1] J. Dinic, P. Ashrafzadeh, I. Parmryd, *Biochim. Biophys. Acta* **1828** (2013) 1102-1111.
[2] J. Dinic, A. Riehl, J. Adler, I. Parmryd, *Scientific reports* **5** (2015) 10082.

Sphingomyelin specific triggering of in-membrane oligomerization of β-amyloid(1-40) and the inhibitory effect of monosialoganglioside GM1

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Oligomers of the β-amyloid (Aβ) peptide are thought to be implicated in Alzheimer's disease. The plasma membrane of neurons may mediate the oligomerization of Aβ present in brain. Using the single-molecule sensitivity of fluorescence, we address the oligomerization of Aβ monomers on lipid bilayers containing essential components of the neuronal plasma membrane. We find that Sphingomyelin triggers the oligomerization of Aβ and that physiological levels of GM1, organized in nanodomains, do not seed oligomerization. Moreover, GM1 prevents oligomerization of Aβ counteracting the effect of Sphingomyelin. We discuss the molecular explanation for the above observations using (still ongoing) all-atom molecular dynamics simulations. Our results establish a preventive role of GM1 in the oligomerization of Aβ suggesting that decreasing levels of GM1 in brain, e.g. due to aging, could lead to reduced protection from the oligomerization of Aβ and contribute to Alzheimer's onset.

Positioning of Microsomal CYPs and Drugs in Lipid Bilayers

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Drugs enter cells via active and passive transport processes through lipid membranes and inside cells both processes significantly contribute to the final drug disposition. In humans, the majority of marketed drugs undergo biotransformation processes catalyzed by cytochrome P450 (CYP) enzymes, which are attached to membranes of endoplasmic reticulum and mitochondria. In recent years, molecular dynamics simulations provided useful insights into role of membrane in CYP anchoring and substrate binding. CYP catalytic domain flows on the lipid bilayer being partially immersed to the membrane interior and anchored by N-terminal helix. N-terminal part and F/G loop are immersed to the non-polar membrane interior. The deeply buried active site is positioned above the membrane surface and accessible through a network of access channels. Analysis of access channels by MOLE 2.0 [1] showed that openings of access channels are positioned inside the membrane and the solvent channel exit points towards the water/membrane interface. The individual CYP forms (CYP1A2, 2A6, 2C9, 2D6, 2E1, and 3A4) show rather small differences in their membrane positioning, which affect locations of the channel openings with respect to the membrane [2]. Membrane composition also influences orientation of CYP.

Most of the marketed drugs are amphiphilic compounds and they have a large potential to accumulate in lipid bilayers [3]. CYP catalyzes the monoxygenase reaction as the prototypical reaction. Formally, an oxygen atom is inserted into a substrate in the monoxygenase reaction, and the product is typically more hydrophilic than the respective substrate. Comparison of affinities of drugs and their respective CYP metabolites to membranes shows that substrates have higher affinities for the membranes and are positioned deeper in the membrane structure than their respective metabolites [3, 4]. The drugs are typically localized inside the lipid bilayers just below the polar head group region and the positions of CYP access active site channels openings are in the same membrane layer. Membrane positions of metabolites correspond to the solvent channel exit, which points towards the water/membrane interface. Based on these findings, one may hypothesize that the drugs penetrate from the membrane to the CYP active sites. They are oxidized and released to the cytosol via the solvent channel.

References

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Oral contributions

Molecular dynamics simulations of membranes: bond orientation properties in lipid molecules

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The most commonly occurring hydrocarbon chains of phospholipid molecules of biological membranes have 12 to 24 carbons and may contain 1 - 6 carbon - carbon double bonds (as a rule, of the *cis*-configuration) in different positions along the chain. The polyunsaturated hydrocarbon chains are of great importance in structure and functioning of biological membranes. Nevertheless, full understanding of the effects of lipid unsaturation on various physical properties of membranes at the molecular level, affecting their functioning, is not yet achieved. Computer simulations of various lipid membranes allow elucidating the detailed relations between the chemical structure and physical properties of various lipids.

Atomistic molecular dynamics simulations have been carried out for 16 different fully hydrated phosphatidylcholine bilayers, having 16 or 18 carbons in fully saturated *sn*−1 chain and from 18 to 22 carbons in *sn*−2 chain with different degree (from 1 to 6) of unsaturation, with the purpose to investigate the effect of unsaturation on physical properties of lipid bilayers. The simulation boxes were filled by 64 lipid molecules per monolayer and 30 water molecules per lipid. The two hydrocarbon tails, the glycerol section and the head group of the lipid molecules were treated in accordance with their known chemical structure. All hydrogen atoms were explicitly included in the computations. The 16 unsaturated pure bilayer systems were coupled to an external temperature bath of 303 K and a pressure bath of 1 atm. 100 ns trajectories were calculated for all bilayer systems: after 20 ns relaxation trajectories the MD production runs of 80 ns were executed.

Different equilibrium structural and dynamic properties of the bilayers were calculated, such as profiles of C-C and C-H bond order parameters of lipid molecules with respect to the bilayer normal, fluctuations of orientations of the bond vectors (probability density distributions of C-C and C-H bond orientations), the root mean square values of fluctuations of the locations of all lipid atoms relative to the average atomic coordinates, etc. It was shown that study of the degree of anisotropy of probability distributions of the bond orientations allows distinguishing extended regions with various types of angular fluctuations of bonds in a membrane formed by lipid molecules with unsaturated chains. Thus, besides of computations of the conventional order parameter we suggested to characterize the bonds according to a newly introduced ‘anisotropy coefficient’ computed from the angular distributions between the bond and the bilayer normal. We have demonstrated that such an analysis allows one to get deeper insight into ordering and orientation behavior of various bonds and to separate these two effects. The computed properties were compared with the available experimental data and discussed in relation to their possible role in the biological functioning of membranes.

This work has been supported by Swedish Institute Visby programme (grant 00675/2009), Vetenskapsrådet (grant 621-2007-5256), by grants for leading research schools of Russian Federation (1642.2012.4, 1410.2014.4). The authors thank Swedish National Infrastructure for Computing (SNIC) for granting computer facilities.

Anisotropic membrane curvature sensing by antibacterial peptides

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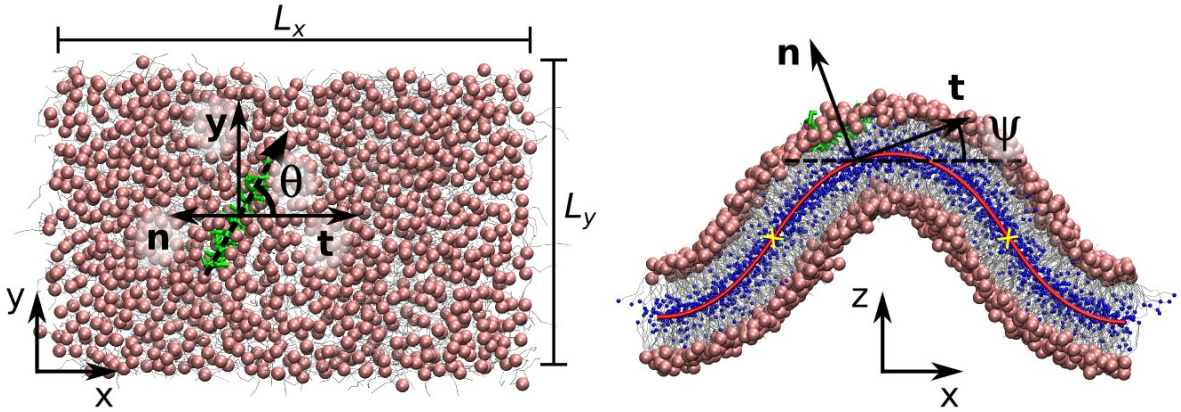
Many proteins and peptides have an intrinsic capacity to sense and induce membrane curvature, and play crucial roles for organizing and remodeling cell membranes. However, the molecular driving forces behind these processes are not well understood. Here, we describe a new approach to study curvature sensing, by simulating the direction-dependent interactions of single molecules with a buckled lipid bilayer. We analyze three antimicrobial peptides, a class of membrane-associated molecules that specifically target and destabilize bacterial membranes, and find qualitatively different sensing characteristics that would be difficult to resolve with other methods. These findings provide new insights into the microscopic mechanisms of antimicrobial peptides, and challenge existing theories of curvature sensing by amphipatic helices. Our approach is generally applicable to a wide range of curvature sensing molecules, and provide strong motivation to develop new experimental methods to track the position and orientation of membrane proteins.

(Preprint: arXiv:1412.2371.)



Influence of Ligand Distribution on Nanoparticle Uptake Efficiency

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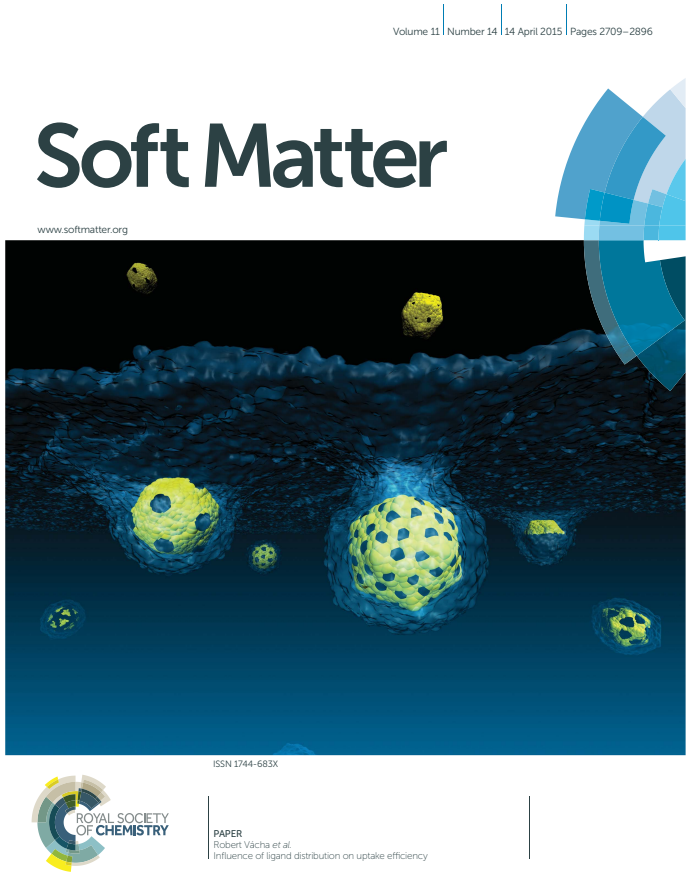
Cellular uptake is a crucial process for nanotoxicity and drug delivery. One of the general uptake paths is a receptor-mediated endocytosis, where ligand coated nanoparticles can cross a phospholipid membrane due to the ligand-receptor interaction. The nanoparticles can have very different surfaces covered with ligands in different stable or mobile patterns. However, it is not know how the ligand pattern/distribution affect the efficiency/speed of the uptake. Using computer simulations, we found that the fastest are the nanoparticles with the most homogeneous ligand distribution, where ligands are spread almost evenly on the surface. The slowest were the nanoparticles with freely diffusing ligands, which became very inhomogeneous upon interaction with the membrane. Those even prevented the uptake unless almost the whole (about 80 %) of the nanoparticle was covered. The efficiency can be understood in terms of necessary activation energy during the nanoparticles wrapping, which decreases with decreasing distance between ligands. Our results demonstrate how the uptake rate can be influenced by the nanoparticle surface. This insight shall to be used for rational redesign of the nanoparticles used in nanomedicine.



HISTIDINE-RICH DESIGNER PEPTIDES WITH PH-DEPENDENT MEMBRANE TOPOLOGY, ANTIMICROBIAL, NUCLEIC ACID TRANSFECTION AND VIRAL TRANSDUCTION CAPABILITIES.

Christopher Aisenbrey, Philippe Bertani, Andrea Farrotti, David Fenard, Anne Galy, Elise Glattard, Arnaud Marquette, Evgeniy S. Salnikov, Joachim Seelig, Lorenzo Stella, Jesus Raya, Louic Vermeer, Nathaliia Voievoda, Burkhard Bechinger

The synthetic LAH4 peptides were designed investigate the interactions that determine the membrane topology of helical peptides (*1*). Their core consist of alanines, leucine and four histidines arranged to form an amphipathic helix, as well as two lysines at each terminus. Through protonation of its histidines (pKs between 5.4 and 6.0) the alignment of the helices is transmembrane at neutral pH and in-plane at pH <5.5 (*1*).

The LAH4 peptides exhibit membrane pore-formation and antimicrobial action at both neutral and at acidic pH including against clinical isolates where the low pH configuration is more active (*2*). The LAH4 peptides have been found to also exhibit potent DNA and siRNA transfection activities (*3*). LAH4 can therefore act as a non-viral vector and has indeed been used for the delivery of quantum dots or protein-based vaccines. Furthermore, transduction by adeno-associated viruses or lentiviruses is enhanced by LAH4 peptides (*4*). Ongoing biophysical and structural investigations will be reported which aim to understand these activities at atomic resolution (*5-8*).

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On the transition region of trans-membrane pores.

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Trans-membrane pores are transient structures that play an important role in translocation of small molecules and peptides across cell membranes. Cell-penetrating and anti-microbial peptides act via pore-mediated mechanisms. Characterizing the free energy landscape of pore-formation is thus required for understanding membrane permeation or for designing antimicrobial peptides.

We calculated free energies for trans-membrane pores from atomistic molecular dynamics simulations using the umbrella sampling method. Three reaction coordinates were used for the umbrella sampling simulations; a) The radial collective reaction coordinate by Wohlert et al [1], b) distance of a single lipid phosphate from the center of the membrane [2], and c) water density-based reaction coordinate [3]. Ideally, a reaction coordinate should efficiently sample the transition state of pore formation. However, none of the three reaction coordinates [1-3] is capable of sampling the transition region efficiently. For instance, we observe large differences in the number of water molecules inside the pore, between opening and closing simulations, even after long simulations times, indicative of strong hysteresis effects (Fig. 1 for the collective radial coordinate [1]). Closer inspection of the simulations shows that the transition state of a pore formation is characterized by the formation and disruption of a continuous channel of water.

Our work provides molecular insight into the transition state of pore formation, and reports the limitations of the existing reaction coordinates used for potential of mean force (PMF) calculations for trans-membrane pores.

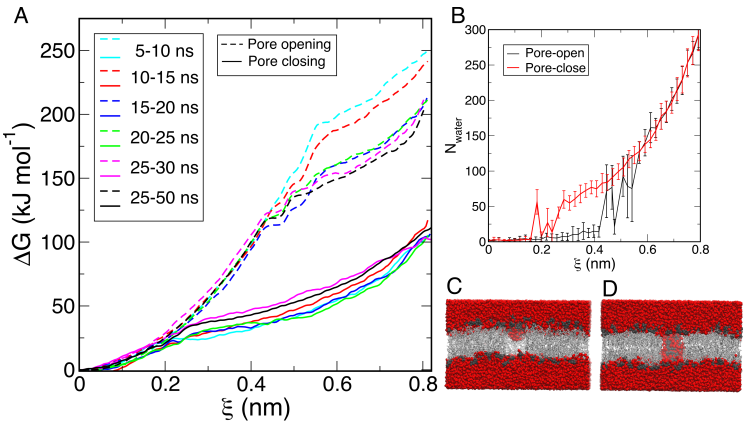

Fig 1. The transition region of a trans-membrane pore for DMPC lipids. A) PMFs for pore opening (dashed lines) and pore closing (solid lines) simulations as a function of the collective radial reaction coordinate, ξ [1]. B) Number of water molecules, N_{water}, as a function of ξ, for pore-opening and pore-closing simulations, showing strong hysteresis effect. N_{water} was calculated using a cylinder function [4]. Snapshots for pore-opening (C) and pore-closing (D) simulations at ξ=0.4, showing different water content (red spheres) in the respective pores. Lipid tails: silver lines, head groups: dark grey spheres.

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The oxidized phospholipids PazePC and PoxnoPC change Bax ability to induce pores in mitochondrial membranes.

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Apoptosis (programmed cell death) is crucial is embryogenesis, regulation of tissue homeostasis and removal of harmful cells in higher organisms. However, failing of this cell suicide program can trigger a range of severe pathological disorders, including neurodegenerative diseases (neuronal cell death) and cancer (cell survival). Mitochondria – the cell's powerhouse play there a crucial role via the intrinsic apoptotic pathway. They initiate cell death upon cytotoxic stress by undergoing a permeabilization process of their mitochondrial outer membrane (MOM), followed by the release of apoptotic factors such as cytochrome c and the irreversible loss of mitochondrial function. Key regulator of this process is the Bcl-2 protein family whose opposing (apoptotic versus survival) members meet at the MOM and arbitrate a life or death (membrane permeabilization) decision there. The main protein causing permeabilization is the apoptotic Bax protein which upon stress-induced activation translocate to the MOM, inhibits there the pro-survival Bcl-2 protein, and induces homo-oligomeric pores (1).

However, the underlying mechanism controlling this membrane-associated Bax action is still not elucidated. Recently it was found, that the mitochondrial membrane system seems to play an active and crucial role with its lipids directly involved. It was namely found that under oxidative stress conditions oxidized lipids can be generated that are directly involved in the onset and proceeding of mitochondrial apoptosis (2). Addition of oxidized phospholipid PazePC (carboxylgroup at *sn-2* chain end) into MOM mimicking model lipid bilayer significantly enhanced membrane affinity and partial penetration of full length Bax (3). Based on these first studies we used here a combined biophysical approach to address the questions i) if Bax can induce pore formation in membranes containing oxidized lipids (OxPls) without the requirement of further mediator proteins such as tBid, ii) if this process is OxPls specific and iii) if there is a threshold in OxPls concentration in the membrane for Bax to carry out its membrane permeabilization. Using fluorescence leakage studies of GUVs and LUVs we found that Bax induced leakage which was sensitive to the type and concentration of OxPL in the membrane. And the leakage rate was correlated to the Bax level. Additional solid state NMR studies confirmed that the incorporation of OxPls distorts the membrane organization, which is essential for the Bax to penetrate into the membrane without a high energy penalty. Further calorimetric experiments support the NMR results, since the incorporation of Bax into these lipid vesicles caused an ordering for the lipid chains and more cooperative phase behaviour.

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Effect of membrane composition on the interaction of small molecules with bilayer membranes

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In the past few years, molecular dynamics (MD) simulations have been successfully used to help rationalize the interaction of drugs and small compounds with membranes. [1–3] However, membrane models have mainly been confined to pure phosphatidylcholine lipid bilayers. This may explain some discrepancies regularly observed between the theoretical prediction and the experimental observations. [1]

Membrane biophysical properties depend on lipid composition (lipid chain length and unsaturation, presence of sterols or sphingolipids) as well as temperature or pressure. Based on these parameters, bilayers can adopt various phases that are known experimentally to influence the behaviour and partitioning of xenobiotics in membranes. To approach as best as possible the real-world conditions and so to better support experimental data, MD simulations must be carried out on more complex bilayers. The *in silico* bilayers, sufficiently relaxed by MD simulations, have to reproduce all lipid bilayer properties according to composition, temperature and pressure (*e.g.*, phase transition).

Here, we explore the interaction of two potential drugs used to treat Alzheimer's disease and two fluorescent dyes with various membrane compositions and temperatures. We clearly predict that membrane composition and temperature influence the positions, orientations and partitioning of these molecules in lipid bilayers.

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Molecular-Level Organization of the Tear Film Lipid Layer.

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The ocular surface of the human eye is protected by the tear film, an aqueous multilayered structure. The interface between the tear film and air is covered by the tear film lipid layer (TFLL), a relatively thin layer of various lipids . Its main function is reduction of the surface tension of the tear film/air interface. Moreover, the presence of the TFLL prevents rapid evaporation of water from the underlying water aqueous phase. TFLL deficiencies lead to evaporative dry eye syndrome, one of the commonly reported eye ailments.

The aqueous phase that covers the cornea contains several classes of proteins. Among them, lysozyme is the most abundant. The main biological function of lysozyme in the tear film is anti-microbial activity against pathogens. Noteworthy, lysozyme concentration is decreased in patients with dry eye. This can be somewhat related to the recent observation that tear liquid proteins play a role in maintaining stability of the tear film. Namely, it was experimentally demonstrated that tear proteins, including lysozyme, lower the surface tension of tears and hence increase its stability. It is also notable that lysozyme upon its interaction with TFLL maintains its enzymatic activity.

Recently, we introduced a computational model of the tear film lipid layer and employed it in coarse grain MD simulations focused on structural properties of TFLL under varying lateral pressure [1]. Here, we extend this model to include lysozyme molecules in order to study interactions and accumulation of the protein in the TFLL. We investigate how lysozyme which resides in the water sub-phase interacts with the TFLL and how this behavior depends on the TFLL structure induced by tear film dynamics.

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Estimation of Skin Permeability – Simulation Approaches

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Despite high effort from scientists all over the world, theoretical estimation of skin permeability is still limited in many situations of our interest. Potts&Guy equation [1,2], Kasting model [3] and other statistical approaches [4] work sufficiently well for some molecules (usually small and hydrophilic) and a healthy skin, but the estimation of permeability for lipophilic molecules, steroids or for a diseased skin can be biased by a high error. Permeability of the drugs is driven by the diffusion and the free energy profile showing the partitioning and penetration barrier in lipid membrane. We employed molecular dynamics (MD) simulations in order to estimate a free energy profile along a normal axis to the gel ceramide membrane and observed much higher energy barriers for permeation when compared to fluid dioleoylphosphatidylcholine (DOPC) membranes [5], as expected from experiments. We also constructed bilayer models of ceramide 2 with various acyl tail lengths both in a pure state and in a mixture with cholesterol and free fatty acid [6] in order to mimic a previous experimental study on stratum corneum substitutes [7]. In a pure membrane we observed a spontaneous permeation of water molecules in direct correlation with experimental permeabilities. Moreover, we analyzed the structural details causing this behavior and we observed increased fraction of L-shaped ceramide molecules in short ceramides in contrast with strict hairpin conformation of long ceramides. The short acyl tails placed in the head group region destabilize the whole membrane, decrease the order parameters and change the phase behavior of the membranes upon heating. Overall, MD simulations appeared to be a useful tool for studies of skin structure and permeability and provide us with the atomistic insight in sub-picosecond resolution unavailable by experiment.

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New simple & versatile tethered phospholipid bilayers for membrane protein reincorporation

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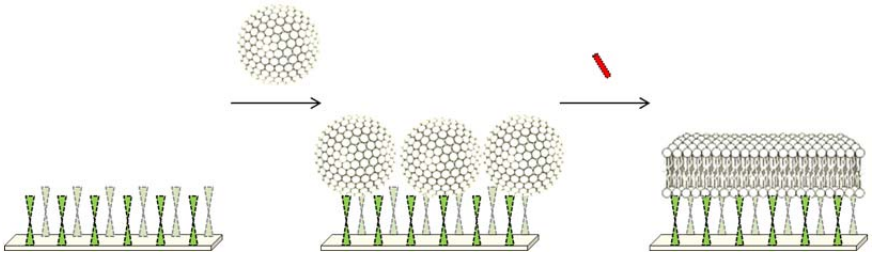
Biomimetic membranes are ideally suited to investigate many issues in membrane biology like membrane/protein interactions. Several systems such as Langmuir monolayers or supported lipid bilayers (SLB) are now widely accepted as reference methods for such investigations.

Beside these studies, biomimetic membranes are appropriate supports for protein interaction screening biochips. To that end, we develop a new simple and versatile technique for creating tethered Bilayer Lipid Membranes (tBLM), an attractive platform thanks to its quasi-natural assembly adapted for membrane protein reinsertion. In this approach, the lipid bilayers are achieved by small unilamellar vesicles fusion triggered by a synthetic fusogenic peptide to obtain tBLM on peptide spacers grafted on Au surface. Surface Plasmon Resonance (SPR) and Atomic Force Microscopy (AFM) have been used to characterize and to visualize the formation process, as well as the interaction between proteins and these membrane models.

The main original feature of this simple methodology for forming tBLM on gold surface is that the lipid composition of the bilayer can be easily tuned. Several compositions have been tested which can mimic biological membranes as desired. Hence, it is adapted for (trans)membrane protein reinsertion since we have shown that fusion of proteoliposomes on peptide tethers can be efficiently achieved.

This new approach is a versatile tool for investigating membrane/protein interactions and membrane protein reincorporation.



Scheme of tBLM formation on Au-peptides surface by SUVs fusion triggered by a synthetic fusogenic peptide [1]. The biomimetic membranes obtained could be used for studying membrane protein interactions with SPR or reincorporating (trans)membrane proteins.

References

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Molecular Dynamics Simulations and NMR Spectroscopy Studies of Trehalose - Lipid Bilayer Systems

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Carbohydrate molecules are amazingly diverse, and can be found in our everyday lives in food and wooden products, as well as in the actual processes that define life. We see their presence in the biological cells as glycolipids in the cell membrane, as building blocks of larger proteins or free in solution. A small, but yet intriguing, molecule in this perspective is the disaccharide trehalose (TRH). It is well known that this small molecule is enriched at the interface, close to the cell membranes. This phenomenon occurs in certain plants and bacteria, and results in a protecting behavior against freezing or dehydration. Different schemes for trehalose-membrane interactions have been proposed, but none of these could be identified as exclusive.

Focusing on TRH/bilayer systems (DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine), we try to reveal underlying mechanisms of TRH interacting at the bilayer/water interface. Employing the Molecular Dynamics (MD) simulations and free energy calculations a simple two state model, for the binding of TRH to the bilayer surface, is suggested[1], showing results very close to existing experimental studies[2]. The force fields used in the simulations are validated by comparing NMR dipolar couplings derived from the simulations, with couplings determined by i) conventional high resolution NMR spectroscopy on TRH in a weakly ordered solvent (bicelles) and ii) solid state NMR in multilamellar vesicles (MLVs) formed by DMPC. The conformational behavior of TRH (crucial for the analysis of the residual dipolar couplings, RDCs, in TRH), indicates that the general assumptions in the analyses of RDCs are well founded [3].

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Human ABC transporter MRP4: construction of a model, transport cycle and drug efflux

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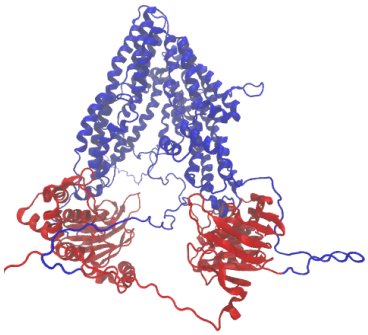
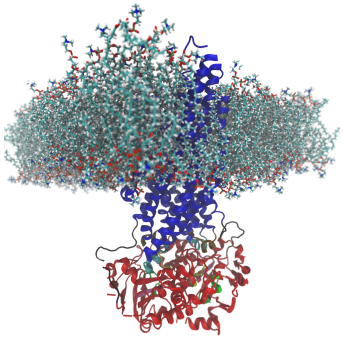
ABC transporters are membrane proteins and have a key role in the efflux (inside-to-outside) of drugs through membranes, with clear evidences observed on patients. Numerous an antiviral drugs are widely use for the treatment against infection by cytomegalovirus, especially ganciclovir (GCV). The GCV accumulation in cells has been shown responsible for cytotoxicity and subsequently treatment interruption[1]. It has been demonstrated *in-vitro* that the human ABCC4-MRP4 protein has a very important role in the efflux of GCV[1], similarly for other nucleoside-based antiviral drugs[2]. The complete mechanism of the transport cycle for ABC transporters has not yet been fully elucidated, but two models of transport mechanism have been suggested, namely the alternating-access model[3], and the ATP-switch model[4]. Based on those models of transport, the human MRP4 model has to be constructed in a configuration, which allows having a proper transport cycle description. The study of homologous protein models were embedded in DOPC bilayers and subjected to molecular dynamics simulations. The structures were successfully relaxed but no movments related to efflux were observed. Further metadynamics simulations have been assessed to force the movements responsible for protein opening and closing. These simulations have indeed allowed observing periplasmic opening of the transporter, which is responsible for efflux. The complete transport cycle should be observed with further sampling.





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mga2 senses lipid saturation in yeast ER membranes by using a rotation-based mechanism

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Biological membranes are highly complex systems, characterised in particular by a heterogeneous lipid environment, changing in space and time. Far from being just passive barriers, membranes actively shape crucial biochemical pathways, and also dynamically respond to changes in the environment. In this regard, lipid composition is a key determinant, and regulatory mechanisms exist that communicate to the rest of the cell such a composition and maintain its identity. However, such molecular sensing mechanisms are often poorly understood.
By using a systematic interdisciplinary approach, we investigate mga2, an endoplasmatic reticulum protein in yeast, and determine its role and mechanism as a intra-membrane lipid-saturation sensor. We obtain evidence from yeast genetics, systematic electron paramagnetic resonance screening in reconstituted *in vitro* systems, and multi-scale molecular dynamics simulations. We show that individual dimers of the transmembrane domain of mga2 respond to variations in the saturation of the lipid environment, by sampling different interfaces via rotation of the protomers.

Calcium-triggered adsorption of DNA on zwitterionic phospholipid membranes: Insight from MD simulations

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Interaction of DNA with zwitterionic phospholipid membranes is an important problem in the field of liposome-based gene delivery. Experimentally, it is well-established that divalent cations can trigger formation of stable DNA–phospholipid complexes. However, the underlying molecular mechanism remains largely unknown. Here we employ atomistic molecular dynamics simulations to gain insight into the kinetics of calcium-triggered adsorption of DNA on zwitterionic phosphatidylcholine membranes as well as into the structure and stability of the resulting complexes. Overall, our findings show that binding of divalent cations to the lipid–water interface turns the surface of the zwitterionic membrane positively charged, promoting thereby the electrostatic attraction of a polyanionic DNA molecule [1]. Furthermore, we show that calcium ions are crucial for stabilizing the DNA–lipid membrane complex as they bridge together phosphate groups of DNA and lipid molecules [1]. Interestingly, we demonstrate that direct interactions between choline groups of phospholipids and DNA phosphates play only a rudimentary role as they are relatively short-lived and unstable: Typical residence times for such interactions are two orders of magnitude smaller than those for Ca-mediated bridges between DNA and lipid phosphate groups. All in all, the findings of our study can serve as a basis for a deeper understanding of molecular mechanisms behind noncovalent binding of DNA and DNA-based nanodevices to complex surfaces such as cell membranes.

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Multiscale Modeling Approach for Membrane Probes

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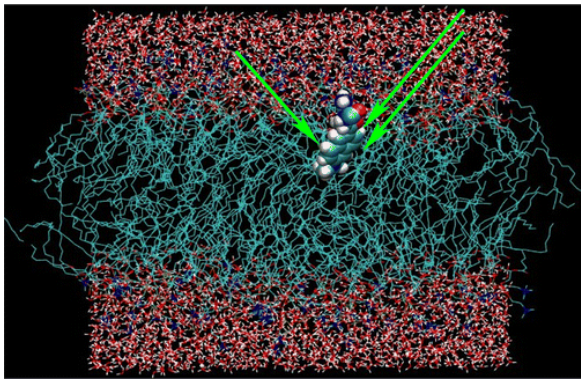

Membranes and membrane proteins are considered to be key targets for the treatment of a number of diseases and many of the currently available drugs aim to target these biostructures [1]. The disruptions from usual structure and conformational changes of these biostructures have been reported to be responsible for many diseases and so it is essential to monitor their structure to identify the normal or diseased stage of human body. A number of molecular probes have been developed that can be used for in vitro sensing of membranes. However, when it comes to in vivo imaging in the living subjects many of the probes fail and so there is a need for developing probes with specific spectroscopic properties and improved binding affinity. This requires the understanding about the binding mechanism of membrane probes and their spectroscopic properties in their membrane bound states. In this presentation, I will present an integrated approach based modeling [2,3] to understand the membrane association mechanism of a probe and its linear and non-linear optical properties in its membrane bound state [4].

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Poster presentations

Lipid membrane simulation with an all atom model as a tool for liposome based delivery system development

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The technique of simulating a section of phospholipid membrane with periodic boundary conditions has become a mature methodology for the investigation of the properties of biomembranes. We have transferred this methodology to the field of liposome based drug delivery systems (LDS). We have gained insight, not accessible through experimental methodologies, into their structure and interaction with the bloodstream plasma that has, in many cases, directly assisted in their development as clinical drug delivery vectors. The LDS represents the most mature application of *nanomedicine*, the development of nanoscale drug carriers. As an enclosed sack composed of a phospholipid membrane, the LDS is an extremely versatile cattier; it is capable of carrying hydrophobic drugs within the membrane or hydrophilic drugs in the internal cavity. Properties can be fine tuned through formulation. Lipids functionalized with a protective polymer can be included to form a protective corona. The current gold standard for this is lipids functionalized with poly(ethylene) glycol (PEG) and the inclusion of such a PEG corona in the LDS is known as "PEGylation". Additionally, it is possible to incorporate cosurfactant molecules, e.g. cholesterol, to alter the properties of the liposome membrane. It is also possible to attach targeting ligands to direct drug delivery to the targeted tissue (active targeting). While many avenues are available to manipulate the properties of the LDS, the investigation of the effect of formulation on the surface structure remains limited, hence, a role for Molecular dynamics simulation to provide this insight. Our research group has, over the past five years, investigated a number of issues relating to LDS structure and function using precisely this methodology. A specific focus has been PEGylation, where we have determined the mechanism through which PEGylation inhibits calcium induced liposome fusion, the reason for the optimum PEGylated lipid density of ~5 molar % and the cause of failure of a new hydrophobic targeting ligand. More recently we have studied the effect of inclusion of cholesterol into the LDS and the interaction between cholesterol and PEG. We have also studied an LDS formulation composed of novel synthetic lipids and novel liposomes designed to treat liver cancer. The presentation covers work described in a number of publications[1-8].

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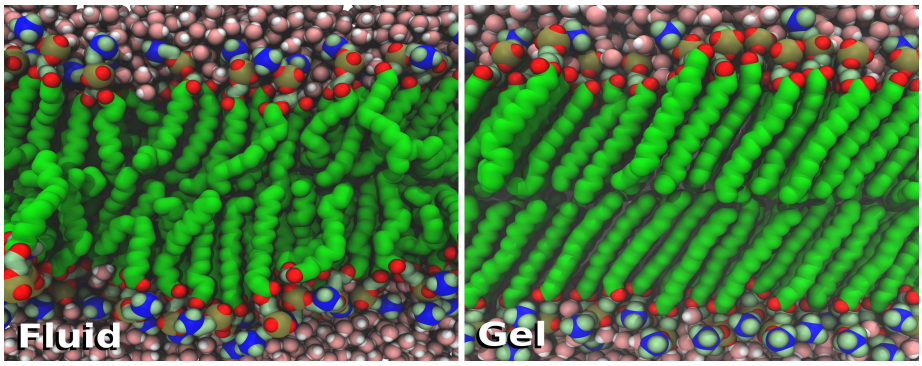

Atomistic Simulations of Gel and Fluid Phase Lipid Bilayers

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A re-parametrized united atom force field that can describe the gel as well as the fluid (liquid crystalline) phase with reasonable accuracy has been constructed. It is based on new quantum mechanically determined (using CPMD calculations on sets of representative molecular conformations) fractional charges and tail dihedral potentials. For the Lennard-Jones parameters of the tail hydrocarbons we have chosen a set that has been fitted to give a good description of liquid hydrocarbons. Experimental data as area per lipid, x-ray and neutron scattering structure factors and NMR order parameters are well reproduced for both phases. The tilt angle of the gel phase is also reproduced. The experimental phase transition enthalpy is obtained with good accuracy. Still, it is noted that even better accuracy with experiment can be obtained by doing a separate parametrization for the two phases.

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Metadynamics simulations of curvature sensing in a buckled membrane

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We present a novel computational method to analyze the curvature preference of probe molecules (peptides, proteins, lipids) on a curved lipid bilayer. The outset is to buckle a flat bilayer by compressing it along its longest axis, and subsequently collect statistics of the probe molecule, as it diffuses on the buckled membrane. Advantageous features of the approach include probing a wide range of curvatures (in contrast to vesicle-tubule assays), and the modest size of the simulated membrane patch (conventional simulations of full vesicles require thousands of lipid molecules and exhaustive equilibration of the inside and outside solute). Our analysis method counterpoises the random noise on the buckled shape by strong thermal fluctuations (particularly prominent as phase fluctuations) by fitting and aligning each simulation frame to a theoretical buckled shape.

Sufficient sampling of the curvature energy landscape can be prohibitive since the simulation time is limited by the diffusivity of the probe molecule. To enhance sampling, we have implemented our method within the framework of metadynamics, by introducing a biasing potential that discourages the system from being trapped in local energy minima. The coarse-graining of the free energy landscape is achieved by selecting a small number of so-called collective variables (CVs), which describe all the slow events in the system. Determining proper collective variables is critical for the efficiency of metadynamics. We introduce the arc-length position of the probe molecule along the buckled bilayer as a novel collective variable. The arc-length position is not an explicit function of the system coordinates, but implicitly defined from fitting the membrane to the theoretical buckled shape. We present an efficient and robust implementation of the arc-length CV and show how it can be used, as a first example, to determine the curvature preference of lipids in membrane models.

Parametrization of the Forcefield for Polyunsaturated Phosphatidylcholines

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Abstract

Polyunsaturated phosphocholines, or polyunsaturated phospholipids, (PUPCs) are important components of biological systems and processes. Modern nutritionists are suggesting to use for medical purposes PUPCs which are known as ω -3 and ω -6 lipids. Positive effects of PUPCs have been observed in treatment of such diseases like cancer, atherosclerosis, Parkinson, Alzheimer, diabetes etc. Molecular mechanisms behind these effects are largerly unknown, and molecular simulations can contribute significantly to this point. During recent years our group has been involved in development of force field for simulation of lipid bilayers which resulted in force field Slipids [1], and the aim of the current work in extending further the Slipids force field for PUPCs. High-level ab initio calculations were used in order to parametrize partial atomic charges and torsion potentials. Parameters for lipid tails have been further optimized based on knowledge of experimental data for dienes and polyunsaturated lipids. The new parameters of the FF were tested for a number of unsaturated lipids: 1-stearoyl-2-docosapentaenoyl -sn -glycero-3-phosphocholine (18:0-22:6 PC), 1-octadecanoyl-2-docosapentaenoyl -sn -glycero-3-phosphocholine (18:0-22:5 PC), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (16:0-22:6 PC), 1-palmitoyl-2-isolenoleoyl -sn -glycero-3-phosphocholine (16:0-18:2 PC), 1-stearoyl-2-linoleoyl -sn -glycero-3- phosphocholine (18:0-18:3 PC), 1-stearoyl-2-arahidonoyl -sn -glycero-3- phosphocholine (18:0-20:4 PC), 1-stearoyl-2-eicosapentaenoyl -sn -glycero-3- phosphocholine (18:0-20:5 PC), 1-stearoyl-2-oleoyl -sn -glycero-3- phosphocholine (18:0-18:1 PC). Deuterium order parameters , scattering form factors, areas per lipid were investigated for bilayers composed from these lipids. A good agreement of computational results with experimental data for different temperatures of bilayers was found that validates the developed force field making it suitable for applications in biology and biochemistry.

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Specific ion effects in lipid membranes using TDFS and MD. Increasing model complexity.

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It is an open question, to what extend specific interactions of ions with model membranes are significant for biological systems. We have been studying such effects using fluorescence techniques (mainly Time-Dependent Fluorescence Shift method) and molecular dynamics simulations [1-5]. The results obtained in simple models – lipid bilayers consisted of either single or two lipid species – gave rather clear picture of specific interactions of cations [1, 3, 5] and anions [2, 4] with phospholipids. We proposed that Hofmeister ordering is a manifestation of number of molecular mechanisms involved in ion-counterion-lipid-water interactions [2]. Here we summarize those results and present our recent attempts to incorporate into our model additional lipid components commonly present in biological membranes. New results obtained at various $CaCl_2$ concentrations for POPC/POPS bilayers containing cholesterol and sphingomyelin are discussed. We compare our model studies with experients performed on plasma membranes isolated from Human Embryonic Kidney 293 cells [5].

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Membrane bound COMT isoform is an interfacial enzyme: general mechanism, new drug design paradigm

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In bitopic proteins catalytic domain anchored to a membrane through a single transmembrane α-helix, is the dominant form of membrane protein yet their mechanism of interaction remains poorly understood With the combined application of molecular dynamics (MD) simulation and quartz crystal microbalance (QCM) experiments, our study represents the first direct evidence of interfacial catalysis in a bitopic enzyme. The catechol-O-methyltransferase has two isoform soluble (S-COMT) and membrane bound (MB-COMT). Of these MB-COMT only is a selective drug target due to its role in neurological function. While the two isoforms share a common catalytic domain, their enzyme kinetics differs. We show that this difference in affinity arises from interaction of substrates with membrane bilayer specifically, there distribution and orientation in membrane bilayer. We show that: 1) the membrane bound isoform of catechol-O-methyltransferase, MB-COMT, is an interfacial enzyme, 2) binding of the ADOMET cofactor induces association of the catalytic surface of the protein with the membrane, where the substrates and Mg2+ ions required for catalysis, are found and 3) substrates with preferential affinity for MB-COMT over the water soluble isoform, S-COMT, orient in the membrane in a fashion that promotes catalysis. Further data mining of the protein sequence database reveals over 1000 reported bitopic enzymes that require a cofactor and more than 600 with a water soluble isoform which can have same mechanism of action. Of the 156 reported catalytic transmembrane proteins that are, according to the DrugBank database, drug targets, 65 have both bitopic and water soluble isoforms suggesting the similar mechanism of action.

Water/membrane interface of a lipid A bilayer

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Lipid A is considered as the most conserved part of lipopolysaccharide (LPS), which is the main constituent of the outer membrane of Gram-negative (G−) bacteria. In aqueous solutions, lipid A alone adopts either non-lamellar or lamellar phases depending primarily on the number of acyl chains attached to the diglucosamine backbone, temperature, hydration level, and the presence of particular cations. *Escherichia coli*-specific hexa-acyl lipid-A (ECLA) forms stable bilayers in the presence of either sodium or magnesium cations, and its main phase transition temperature is 44°C [1].

Here, we report results of 800 ns molecular dynamics simulations of a fully hydrated bilayer made of ECLA at 50°C in presence of sodium ions [2]. The simulations employed refined OPLS-AA parameters [3]. We validated our simulations of the lipid A bilayer against scarce experimental data including surface area per lipid, the membrane width, and known conformations of the β1 → 6 glycosidic linkage in carbohydrates showing structural similarity to the diglucosamine part of lipid A. Our study reveals strong inter-lipid links and numerous H-bonds with water in the interfacial region of the ECLA bilayer. One can expect that these interactions reduce water penetration across the lipid matrix of the bacterial outer membrane. At the interface, also sodium ions are readily bound to the polar groups of ECLA, which most likely hinders their passive diffusion across the matrix.

Altogether, the lipid A bilayer emerges as a solid barrier, which provides a bacterial cell with reliable protection against hazardous environmental factors. These protective features of the lipid A bilayer can only be strengthened by the presence of densely packed long polysaccharide chains of LPS in the outer bacterial membranes.

The work was supported by the Polish National Science Center under grant no. 2011/01/B/NZ1/00081.

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DRUG/MEMBRANE INTERACTION VIA MOLECULAR MODELING.

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One of the most important questions in biology nowadays is how drugs cross membranes. Drug-membrane interactions play a crucial role in drug delivery and metabolism in the human body. Over the past years, molecular dynamics (MD) of lipid bilayer membranes have appeared a new and complementary tool to figuring out drug/membrane interaction.

We studied the membrane crossing of several classes of compounds (antiviral, immunosuppressant, diuretic, antioxidant …) for their capacity to penetrate and cross lipid bilayers. pH effect and influence of substituent has been rationalized for these compounds at an atomic resolution. Their location and orientations in lipid bilayer (DOPC) were determined via MD simulations.
The simulations of most of drugs showed that the neutral forms of molecules approach the surface of the lipid bilayer and then penetrate the bilayer. As expected, the hydrophobic groups orient towards the membrane center whereas polar groups interact with the polar head region. The simulations clearly showed that location and orientation of drugs in membranes are driven by the number and position of polar groups, H-bonding and charge groups. The anionic forms are located outside the bilayer in interaction with the head group region and water molecules.
The energetic profiles of penetration and passive crossing were evaluated using COSMOmic (e.g. figure 1). Again the structure property relationships are better documented and support the pharmacological knowledge for many drugs.

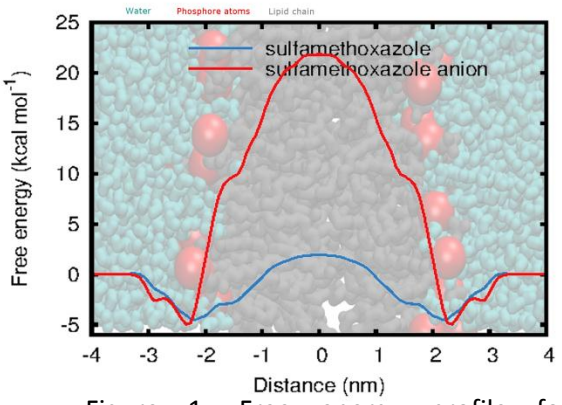

Figure 1: Free energy profile for penetration of neutral and anionic forms sulfamethoxazole in DOPC membrane.

Effects of Procaine and Tetracaine on the DMPC Lipid Bilayer Structure: A Molecular Dynamics Simulation Study.

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The molecular mechanism of pharmacological action of local anesthetic (LA) used to relieve localized pain has remained elusive despite the use of anesthetics over 150 years [1]. In this article we have examined the effect of charged and uncharged form of two frequently used amino-ester type anesthetics, procaine and tetracaine, on the properties of the lipid DMPC bilayer. For this purpose, four different simulations have been carried out consisting of 128-lipid DMPC bilayer and 12 charged procaine, 12 uncharged procaine, 12 charged tetracaine, 12 uncharged tetracaine which were initially positioned in the water phase. Each MD simulation was performed for 100 ns using the GROMACS simulation package, version 4.5.4 [2].

The average area per lipid for simulated system was shown in table 1. It can be seen that simulated systems have somewhat larger area per lipid than in the reference system. For the system with charged procaine the average area per lipid is 1.02 $Å^2$ larger than in the system with the uncharged procaine which has similar area per lipid to the reference system. In contrary, system with charged tetracaine has the average area per lipid 1.77 $Å^2$ smaller than the system with the uncharged tetracaine. So, the behavior of charge and uncharged forms of procaine and tetracaine in the lipid bilayer are completely different.

Table 1. Summary of simulation results discussed.

System	Charged procaine	Uncharged procaine	Charged tetracaine	Uncharged tetracaine	Pure DMPC
A ($Å^2$)	62.52	61.5	61.78	63.55	61.67
H (Å)	33.55	34.43	34.21	33.92	33.84
$S_{N\text{-}N}$	-0.24	0.26	0.12	-0.25	-

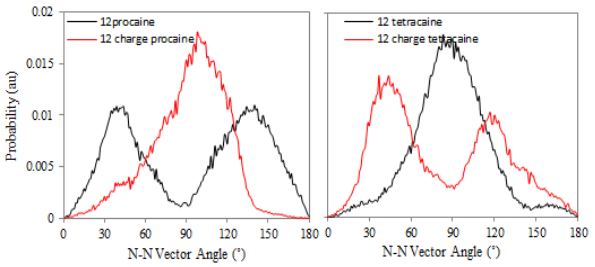

Figure 1. Angular distribution of the N-N vector of drug molecules.

The bilayer thickness of the DMPC lipid bilayer was calculated as the distance between the center of mass of phosphate atoms in two different monolayers which was shown in the Table 1. As can be seen, two different forms of procaine and tetracaine molecules show opposite effects on the thickness of the lipid bilayer. It may be attributed to the difference in molecular orientation and positing of drugs in the lipid bilayer.

The orientational order of drug molecule can be calculated as: $S = \frac{1}{2} < 3cos^2\theta - 1 >$, where θ is the angle between the molecular vector which was considered N-N and the bilayer normal which is assumed to be parallel to the z-axis. From the angular distribution of N-N vector as well as the order parameter of drug molecule in Table 1, it can be seen that the charged procaine molecules which have negative order value (-0.24) tend to be oriented perpendicular to the bilayer normal (the N-N angle relative to the bilayer normal is 90˚), so they increase the area per lipid of bilayer, but the procaine molecules tend to be oriented parallel to the bilayer normal and increase the thickness of the bilayer.

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Hydrocarbon chains of phospholipids in liquid crystalline bilayers and in unperturbed state: computer simulations study

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Hydrocarbon chains (saturated and unsaturated) are examples of the most important natural molecules; they play the key role in various processes of biological membranes as constituents of phospholipid molecules. One of the topical problems is revealing the relations between the chemical structure and physical properties of such molecules. Computer simulations of various hydrocarbon chains and lipid bilayers were carried out in this work. Unperturbed linear hydrocarbon chains 16:0, 18:0, 18:1(n-9)*cis*, 18:2(n-6)*cis*, 18:3(n-3)*cis*, 18:4(n-3)*cis*, 18:5(n-3)*cis*, 20:4(n-6)*cis*, 20:5(n-3)*cis*, 22:6(n-3)*cis* were studied by Monte Carlo simulations: the chains contained 16, 18, 20, 22 carbons and 0, 1, 2,…, 6 methylene-interrupted *cis* double bonds. Conformational energy of a hydrocarbon chain was represented as sum of energies $U_m(\varphi_\gamma, \varphi_{\gamma+1}, \varphi_{\gamma+2})$ of *m* structural units, where φ_γ, $\varphi_{\gamma+1}$, $\varphi_{\gamma+2}$ are torsion angles, i.e., a scheme of interdependence of each three torsions along the chain was used. The units reproduced precisely the structure of various chain fragments. An importance sampling technique was developed for the efficient generation of chain conformations, with continuous variation of all single C-C bond torsions within (0, 360°) range considered; ~10^{12} conformations of each chain were generated at temperature *T*=303K to calculate average characteristics. Several fully hydrated homogeneous phosphatidylcholine (PC) bilayers were studied by molecular dynamics simulations at the same temperature and force field; *sn*−1 chain in each PC molecule was fully saturated (16:0 or 18:0), and *sn*−2 chain was one of the above-listed unsaturated chains. The simulation boxes contained 64 PCs per monolayer and 30 H_2O per lipid; 100 ns trajectories were calculated for each bilayer 20 ns of which used for relaxation. Mean end-to-end distances <h> of all chains were calculated. It is shown that while the conformations of the chains in the unperturbed state are not the same as those in liquid crystalline bilayers, the relative difference in <h> was found to be moderate. In the bilayer regions near PC head groups, the hydrocarbon chains are drawn by the neighboring chains, resulting in somewhat higher <h> values than those in the unperturbed state. The relative differences were found to be about 9 – 10% for 16: 0, 18: 0 chains; ~11 – 18% for unsaturated 18-carbon chains and ~23% for polyunsaturated 22:6(n-3)*cis* chain. These values can be considered as assessments of the effect of long-range interactions in a chain and the interactions with neighboring chains and PC head groups of lipid bilayer, on the chain end-to-end distance <h>, compared with the effect on the <h> of only short-range interactions (which completely determine the conformations of the unperturbed chain).

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Computational analysis of the electroporation process- network Monte Carlo model.

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Electroporation is one of the commonly used techniques to transport substances into cells. After crossing the critical value of the electric field applied across the membrane, permeability increase due to the pores formation. Thereby additional path opens for desired substances to be introduced into the cell. Nevertheless, the success of this method depends on the size and stability of the formed pore which depends on the proper selection of parameters of the applied electrical field: the amplitude of the voltage, frequency, duration of action and the shape of the signal. Despite the development of experimental techniques, relatively little is known about the molecular mechanisms behind the process of pore formation during electroporation, i.e. the behavior of lipid molecules [1]. To improve our understanding of the pore formation molecular mechanisms we developed mathematical model based on the Monte Carlo (MC) technique. It allows us to identify electroporation conditions under which the formation of the pore is feasible and determine the minimum and maximum pore diameters under various environmental and physico-chemical conditions.

Developed author's model is based on Pink's model, which provides analysis only of the alkyl chains. Here, polar lipid heads were additionally taken into account. In author's model the lipid membrane is presented as triangular lattice consisting of two parallel layers. Each node is occupied by a hydrocarbon chain, and every two hydrocarbon chains have one polar part - a dipole. The Hamiltonian of the studied system consists of four terms: I) the energy of van der Waals interactions, II) the conformational energy, III) the energy of electrostatic interactions between the lipid heads and IV) the energy which describes the polar part interactions between the polar parts of lipid molecule (heads) and the electric field. To find the maximum size of stable pores in certain ambient conditions we have performed MC simulations for DPPC membrane in gel and fluid phase, surrounded by 10 and 100 mmol NaCl solution, exposed to the electric field from 10^5 to 10^8 V/m. The system was equilibrated for the 200 - steps per node in 320 K (fluid phase), then 1000 steps per site were performed to find the average energy of the studied system.

We have found that if the electric field intensity is 10^6V/m potential energy of the studied system is positive and rising with the hypothetical pore diameter. When we added the positive energy of van der Waals interaction of lipid chains, we found that in such conditions the pore is not stable. By increasing the electric intensity to 10^7 V/m the energy of polar part of a pore is negative from d_a=3.68 nm and for smaller pores the energy becomes positive. For larger values of the electric field (10^8 V/m) the energy of dipoles is negative and decreases with increasing in pore size. If the considered chains are all in fluid conformation, for which the van der Waals and conformational energy are positive, despite of the negative energy of the polar part, the appearing pore could be stable only in small size.

References

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