Conference

“Biologically Relevant Membrane and Monolayer Related Processes”

aka

“Levi IV”

May 23-26, 2018

Nové Hrady, Czech Republic

Organizers

Dr. Hector Martinez-Seara

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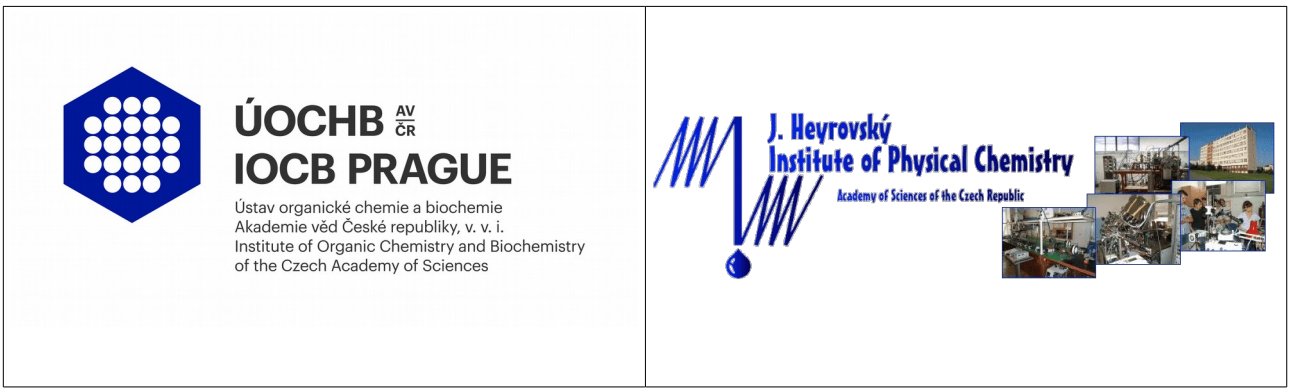

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Program: Biologically Relevant Membrane and Monolayer Related Processes

Nové Hrady, Czech Republic

May 23-26, 2018

23/05			24/05			25/05			26/05		
			08:00-09:00	Breakfast		08:00-09:00	Breakfast		08:00-09:00	Breakfast	
			Session 4	Membrane homeostasis		**Session 8**	Biological interfaces		**Session 12**	Membrane fusion & curvature	
			09:00-09:50	K9	A.K. Menon	09:00-09:50	K20	T. Viitala	09:00-09:30	T31	P. Jungwirth
13:00-15:30	Registration		09:50-10:20	T10	U. Coskun	09:50-10:20	T21	A. Olzynska	09:30-10:00	T32	A. Magarkar
15:00-15:10	Opening		10:20-10:35	S11	D. Bonhenry	10:20-10:35	S22	P. Delcroix	10:00-10:30	S33a-S33b	J. Kadlec & D. Holý
			10:35-11:00	Coffee		10:35-11:00	Coffee		10:30-11:00	Coffee	
Session1	Glycocalyx		**Session 5**	Role of lipids in cellular processes		**Session 9**	Biomolecules-membrane interaction (II		**Session 13**	Lipid phases & methods	
15:10-16:00	K1	B. J. Kirby	11:00-11:30	T12	A. Nadler	11:00-11:30	T23	A. Bunker	11:00-11:30	T34	M. Pasenkiewicz-Gierula
16:00-16:30	T2	M. Ingr	11:30-12:00	T13	M. Potocký	11:30-12:00	T24	T. Róg	11:30-12:00	T35	G. Enkavi
16:30-16:45	S3a	V. Palivec	12:00-12:15	S14a	F. Lolicato	12:00-12:15	S25a	I. Kabelka	12:00-12:15	S36a	R. Nencini
16:45-17:00	S3b	J. Vuorio	12:15-12:30	S14b	S. Lautala	12:15-12:30	S25b	P. Parkkila	12:15-12:30	S36b	P. Khoroshyy
			12:30-17:00	Lunch + leasure		12:30-17:00	Lunch + leasure		12:30-14:00	Lunch	
Session 2	Oxidated lipids		**Session 6**	Ions-membrane interaction		**Session 10**	Effect of ions in membranes & signaling		14:30	Departure	
17:00-17:30	T4	M. Vazdar	17:00-17:30	T15	S. Ollila	17:00-17:30	T26	C. Allolio			
17:30-17:45	S5a	W. Kulig	17:30-17:45	S16a	E. Duboué-Dijon	17:30-17:45	S27a	O. Ticháček			
17:45-18:00	S5b	P. Jurkiewicz	17:45-18:00	S16b	J. Melcr	17:45-18:00	S27b	A. Melcrova			
18:00-18:30	Coffee		18:00-18:30	Coffee		18:00-18:30	Coffee				
Session 3	Membrane proteins		**Session 7**	Biomolecules-membrane interaction (		**session 11**	Immunology				
18:30-19:00	T6	R. Ettrich	18:30-19:00	T17	R. Vácha	18:30-19:00	T28	J. Bernardino de la Serna			
19:00-19:30	T7	J. Lazar	19:00-19:30	T18	R. Pleskot	19:00-19:30	T29	M. Cebecauer			
19:30-19:45	S8a	X.P.Anthony Raj	19:30-19:45	S19a	M. Girych	19:30-19:45	S30a	K. Riedlova			
19:45-20:00	S8b	I. Kishko	19:45-20:00	Free time		19:45-20:00	Free time				
20:00-21:00	Dinner		20:00-21:00	Dinner		20:00-21:00	Dinner				
21:00-23:00	Discussion		21:00-23:00	Discussion		21:00-23:00	Discussion				

(Key=K= 35 + 15 min; Talk=T= 20 + 10 min; Short Talk=S=10 + 5 min)

Table of Titles

Table of Authors

Talk 1- Key

The role of metabolism and drug resistance in vesicle secretion and uptake in cancer cells

Brian Kirby[1]

[1]*Cornell University - College of Engineering*

Our group, in conjunction with collaborators at Cornell, is striving to understand the role of vesicle secretion and uptake in cancer cells. In particular, we study the transport processes associated with vesicle transport and measurement and engineer tools to measure these processes, and we directly study vesicular secretion and uptake processes in an attempt to link membrane biophysics to vesiculogenesis to cell phenotype. Our core expertise is in fluid mechanics, electrostatics at interfaces, and microdevice design.

I will show several aspects of our work. Specifically we have shown that vesicle secretion can be suppressed with metabolic inhibitors, which identifies several metabolic processes as potential targets for therapy. Because the metabolic inhibitors suppress glutaminase metabolism and glycoprotein expression, we are studying the electrostatic aspects that link glycoprotein expression to membrane bending and vesiculogenesis. We have developed microfluidic devices to isolate nanoparticles of various sizes to separate vesicles by subtype, and I will show results linking vesicle uptake to phenotype in the context of epithelial-to-mesenchymal transition and resistance to antimetabolite chemotherapy.

Talk 2 – Normal

Binding of hyaluronan and its neutral analog by TSG-6 protein studied by molecular dynamics.

Roman Witasek[1], Eva Kutálková[1], Josef Hrnčiřík[1] and Marek Ingr[1,2]

[1]Tomas Bata University in Zlín, Faculty of Technology, Department of Physics and Materials Engineering, Nám. T.G. Masaryka 5555, 76001 Zlín, Czech Republic
[2]Charles University, Faculty of Science, Department of Biochemistry, Hlavova 8/2030, 12843 Praha 2, Czech Republic

Hyaluronic acid (HA, hyaluronan), an alternating co-polymer of glucuronic acid and N-acetylglucosamine ([4)-β-D-Glc*p*A-(1 3)-β-D-Glc*p*NAc-(1]$_n$), is a major component of extracellular matrix of animal connective tissues. Besides being only a passive construction material, it also plays roles in numerous signaling cascades and is thus involved in inflammation, progression of various diseases including cancer, and wound healing. In these processes HA interacts with several membrane-bound and soluble protein receptors called hyaladherins. The former group contains mainly proteins CD44, LYVE-1 and RHAMM, the latter e.g. TSG-6. In spite of several decades of research, many of the signaling cascades exploiting HA are still not fully understood[1]. Molecular-dynamics simulations can therefore help us to explain the structural features of the HA-hyaladherin complexes and the relations between their structure and function. TSG-6 is an intensively studied molecule whose structure is known from numerous NMR experiments that indicate the interaction of this protein with HA as well as another glycosaminoglycan, heparin and chondroitinsulfate[2]. In this study we simulated the binding of HA oligosaccharides by TSG-6 and we identified two binding sites of this molecule. One of them is identical with the sites described previously[3], but the other one, so far unknown, partially overlaps with the less stable binding site of heparin[4] and also for chondroitinsulfate[2]. In order to evaluate the specificity of the binding sites for HA and charged oligosaccharides in general, we carried out analogous simulations with the neutral HA analog containing the glucuronic acid residue instead of glucose[5]. They showed that this molecule can be bound by both these sites. However, the stabilization Gibbs energy determined by the umbrella sampling method that in the first site the analog binds much more weakly than HA, while in the other site the analog binding is even more stable than that of HA. The results thus show that, while the first binding site is quite specific for HA, the second one is able of binding various oligosaccharides and the binding is independent of the negative charge of the ligand. It indicates the possibility of designing artificial ligands of hyaladherins with a potential pharmaceutical application.

Acknowledgments:
Access to computing and storage facilities owned by parties and projects contributing to the National Grid Infrastructure MetaCentrum provided under the program "Projects of Large Research, Development, and Innovations Infrastructures" (CESNET LM2015042), is greatly appreciated. This work was supported by The Ministry of Education, Youth and Sports from the Large Infrastructures for Research, Experimental Development and Innovations project „IT4Innovations National Supercomputing Center – LM2015070". RW was supported by the Internal Funding Agency of Tomas Bata University in Zlín, projects IGA/FT/2016/011, IGA/FT/2017/009 and IGA/FT/2018/010.

Talk 3a – Short

Towards better understanding of sugars behaviour with help of Raman spectroscopy – where experiment and computer modeling meet

Vladimir Palivec[1], Petr Bouř[1], Pavel Jungwirth[1], Jakub Kaminský[1], and Hector Martinez-Seara Monne[1]

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Sugars play a key role in all organisms with functions spanning from structural, e.g. cellulose or chitin, to storage, e.g. starch or glycogen. They are scattered across all cell compartments, one of which we are greatly interested – glycocalyx – the external coating on the surface of plasma membranes. In glycocalyx they are mainly in form of glycosaminoglycans (GAGs), e.g. hyaluronic acid, hepran sulphate, or chondroitin sulphate. These molecules are modified polydisperse polysaccharides which mediate interactions between plasma membrane and proteins and form the polymeric network in the bulk region of glycocalyx. Although the importance of knowledge of precise structure is clear, a little is known about the microscopic structure of glycocalyx. Interestingly, even structure of GAGs alone remain elusive. While typical methods of probing structure often fails, Raman scattering and Raman Optical Activity seems like a viable option. Coupling of ab initio methods and molecular dynamics can allow full interpretation of experimental spectra, which alone provides rather limited information. Understanding origin of the spectral features can give us valuable information about the structure of polysaccharides. However, before tackling large and complex polysaccharides a reliable approach how to simulate Raman/ROA spectra has to be developed. To this end, we have have been able to develop computational approach which successfully interprets Raman/ROA spectra of monosaccharides. A practical example of valuable information provided by this joined experimental/modeling approach is showed on decomposition of spectra to major present structures or calculation of anomeric ratio. Furthermore we are exploring interpretation of spectral features that comes with effects of local environment, sugars concentration, rotation around glycosidic bond, or ring puckering. We are gradually increasing system complexity, with the ultimate goal interpreting spectra of long polysaccharides, which could enormously help with understanding of glycocalyx bulk environment.

Talk 3b - Short

ATOMISTIC FINGERPRINT OF CD44—HYALURONAN INTERPLAY

Joni Vuorio[1,2], Ilpo Vattulainen[1,2], Hector Martinez-Seara[3,1]

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CD44 is a receptor protein present on the plasma membrane of several mammalian cell types. Its primary ligand is hyaluronan, a megadalton-scale polysaccharide abundant in both extra and pericellular matrices. Through its interaction with hyaluronan, CD44 is involved in cell-cell interactions, migration, and adhesion. Mutagenesis studies on CD44 have identified multiple widespread residues to be responsible for its recognition of hyaluronan. In contrast, the structural characterization of CD44 has revealed a single binding mode associated with interactions that involve just a fraction of these residues.

In this study, we employ atomistic molecular dynamics simulations to show how CD44 can bind hyaluronan with three topographically different binding modes, collectively defining an interaction fingerprint. Aligning the contact regions of these three binding modes, we identify common interactions involving the majority of the residues previously identified as the epitopes of the CD44—hyaluronan interaction. Hence, our results provide a plausible explanation for the apparent disagreement between the mutagenesis and structural studies. Our measurements also confirm that the previously-characterized crystallographic binding mode has the highest affinity among the three modes. The other two binding modes represent metastable configurations readily available in the initial stages of the binding. These lower-affinity modes allow the CD44 to form transient interactions with hyaluronan. The transient interactions again allow the attached CD44 proteins to diffuse along a hyaluronan strand – a feature that could facilitate the aggregation of CD44 on the plasma membrane. Importantly, CD44 aggregation has been suggested to be a possible mechanism in CD44-mediated signaling.

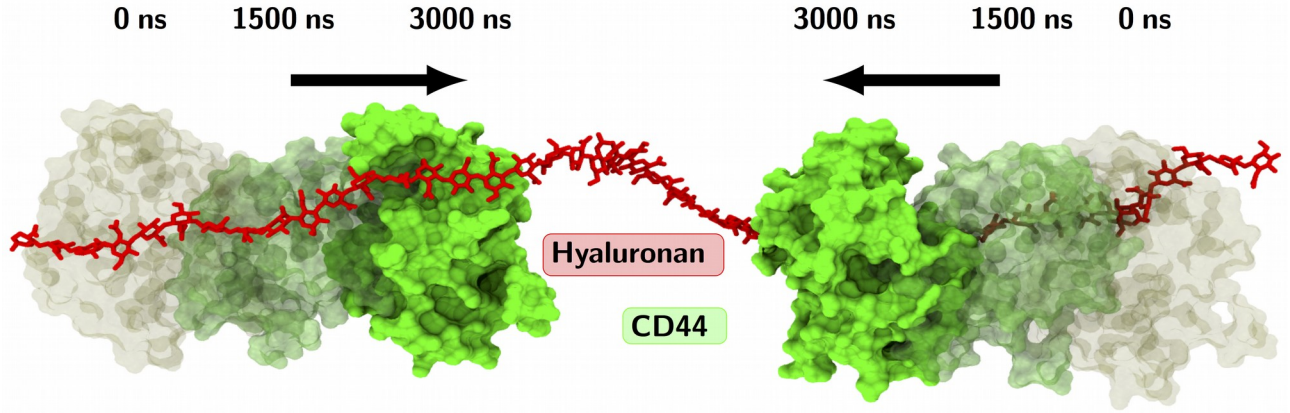

Figure 1. CD44 (hyaluronan binding domain, green) diffusing along a strand of hyaluronan (red) with an indication of the related timescales.

Talk 4 - Normal

Chemical Reactions in Phospholipid Bilayers and Their Relevance to Membrane Biophysics

Mario Vazdar[1]

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Reactive aldehydes, such as 4-hydroxynonenal (4-HNE) and 4-oxononenal (4-ONE), are formed as a consequence of radical peroxidative reactions of polyunsaturated fatty acids in cellular membranes during oxidative stress. Due to the presence of several reactive sites, these compounds can readily react with membrane proteins [1] and phosphoethanolamine (PE) lipids [2, 3] thus impairing the membrane protein function.

In this talk, molecular dynamics (MD) simulations of bilayers composed of mixed phosphatidylcholine (PC) lipids and various PE lipid adducts will be presented. MD simulations will be supported with mechanistic details of chemical reactions in model biological systems described with highly detailed quantum (QM) chemical calculations and different experimental techniques, such as nuclear magnetic resonance (NMR) and liquid chromatography tandem mass spectrometry (LC-MS). Using this synergistic approach, we will describe how specific chemical reactions of 4-HNE with PE lipids resulting in complex PE adducts induce a drastic increase of Na^+ transport across model biological membranes, in contrast to other simpler PE adducts which do not significantly alter membrane biophysical properties.

References:

[1] G. Poli, R. J. Schaur, W. G. Siems, and G. Leonarduzzi, Medicinal Research Reviews, 2008, 28, 569-631.
[2] O. Jovanovic, A. A. Pashkovskaya, A. Annibal, M. Vazdar, N. Burchardt, A. Sansone, L. Gille, M. Fedorova, C. Ferreri and E. E. Pohl, Free Radicals Biology and Medicine, 2015, 89, 1067-1076.
[3] K. Vazdar, D. Vojta, D. Margetić, M. Vazdar, Chemical Research in Toxicology, 2017, 30, 840−850.

Talk 5a - Short

Translocation of Oxysterols in Biological Membranes

W. Kulig[1,2], H. Mikkolainen[1], A. Olżyńska[3], P. Jurkiewicz[3], L. Cwiklik[3,4], T. Rog[1,2], M. Hof[3], I. Vattulainen[1,2,5], P. Jungwirth[4,1]

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[5]MEMPHYS-Center for Biomembrane Physics

Cholesterol is a key molecule that regulates properties of animal cell membranes. In particular, cholesterol affects membrane thickness and elastic properties, controls its phase state, and influences the dynamics of membrane components. One of biologically most important cholesterol functions is regulation of bilayer permeability. Among about eighty steroids known in nature only several, including cholesterol and ergosterol, are crucial components of lipid membranes. Most of the remaining ones are metabolites or signalling molecules, while few others occur as products of oxidation, e.g., due to oxidative stress. Oxysterols occur both as products of enzymatic and spontaneous (involving reactive oxygen species) oxidation. They differ from cholesterol by the presence of additional polar groups that are typically hydroxyl, keto, hydroperoxy, epoxy, or carboxyl moieties. Like cholesterol, many oxysterols are hydrophobic and hence confined to cell membranes. Although biophysical studies provided an extensive characterization of oxysterol effects on lipid bilayer properties, little is known about their ability to modulate membrane permeability. Using several experimental techniques, including dynamic light scattering and time-resolved fluorescence spectroscopy, together with atomistic molecular dynamics simulations, we characterized the behaviour of oxysterols in phospholipid membranes and compared the resulting data to that of cholesterol. We found that permeability of lipid bilayers changes drastically (as compared to cholesterol) when tail-oxidized sterols are present, meanwhile this effect was not observed in systems containing ring-oxidized sterols. Here, we rationalize the different behaviour of various oxysterol classes based on both experimental data and molecular dynamics simulation.

Talk 5b – Short

Coaction of Oxysterols and Oxidized Phospholipids in Model Lipid Membranes

Sylwia Cyboran-Mikołajczyk[1], Pauline Delcroix[2,3], Anastasiia Stepanchuk[2], Lukasz Cwiklik[2,3], Martin Hof[2], Piotr Jurkiewicz[2]

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Oxidation of cholesterol and membrane lipids occurs to a very limited extent under physiological conditions but escalates in various pathologies accompanied by oxidative stress and inflammation. While the role of oxysterols and oxidized phospholipids (oxPLs) gains recognition in cancer, neurodegenerative diseases, diabetes type 2, cardiovascular disorders and atherosclerosis, little is known how the coexistence of oxysterols and oxPLs influence the course of a particular disease. Herein we focus on the effect of 27- and 7- hydroxycholesterols and truncated oxPLs: palmitoyl-glutaryl-phosphocholine (PGPC) and palmitoyl-oxovaleroyl-phosphocholine (POVPC) on the physical properties of lipid bilayers including lipid mobility, membrane hydration and permeability. The results indicate a synergy between 27-hydroxycholesterol and PGPC. We try to address the question about the molecular mechanism of this synergy and also speculate on the possible biological consequences.

Talk 6 – Normal

Modulation of human ORAI1 channels: modeling and simulations

Daniel Bonhenry[1], Saurabh Kumar Pandey[1], Lydie Plackova[1], David Reha[1], Irene Frischauf[3], Isabella Derler[3], Rainer Schindl[2], Christoph Romanin[3] and Rudiger H. Ettrich[1]

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[2]*Institute for Biophysics, Medical University of Graz, Graz A-8010, Austria*
[3]*Institute of Biophysics, Johannes Kepler University of Linz, Gruberstrasse 40, 4020 Linz, Austria,*

Orai1 is a calcium-selective channel located in the plasma membrane, and belongs to the family of calcium release activated channels (CRAC) [1]. Orai1, as component of store-operated calcium entry (SOCE), is activated by the second component of SOCE, STIM1, when intracellular calcium stores are depleted. STIM1, located at the endoplasmatic reticulum (ER), senses levels of calcium in the ER and is activated by calcium store depletion. In turn, calcium influx via Orai1 channel refills calcium levels in the endoplasmic reticulum [2]. Based on the *Drosophila melanogaster* Orai crystal structure [3] a homology model of human Orai1was prepared that includes extracellular and intracellular loops existing only in the human isoform [4]. The sequence and architecture of Orai channels is unique among other ion channels and suggests a novel gating mechanism. The selectivity filter is formed by a ring of six glutamate residues followed by a hydrophobic and consequent basic region further down the pore. The pore extends into cytosol by approximately 20 Å. Using combined experimental and theoretical approaches this study focuses on the central ion pore to investigate the gating mechanism of this unique channel including altered gating of Orai1 mutants occurring in tumor cells [6], the communication between the intracellular loop and the N-terminus [7], or its interaction with STIM1.

References:

[1] M. G. Matias et al “Animal Ca2+ release-Activated Ca2+ (CRAC) Channels Appear to Be Homologous to and Derived from the Ubiquitous Cation Diffusion Facilitators” *BMC Research Notes* **2010,** *3,* 158
[2] S. Feske “CRAC channelopathies” *Pflugers Archiv : European journal of physiology* **2010,** *460 (2)*,417-435
[3] X. Hou et al “Crystal Structure of the Calcium Release-Activated Calcium Channel Orai” *Science* **2012**, *3389 (6112)*, 1308–1313
[4] I. Frischauf et al “A calcium-accumulating region, CAR, in the channel Orai1 enhances Ca(2+) permeation and SOCE-induced gene transcription” *Science Signaling* **2015**, *8*, ra131
[5] S. Feske et al “A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function” *Nature* **2006**, 441, 179-185
[6] Frischauf et al “Transmembrane helix connectivity in Orai1 controls two gates for calcium dependent transcription”, *Science Signaling* **2017**, 10 (507): eaao0358..
[7] Fahrner et al “Communication between N-terminus and Loop2 tunes Orai activation” The Journal of Biologicali Chemistry **2018**, 293: 1271-1285.

Talk 7 – Normal

2P or not 2P: using single- and two-photon polarization microscopy to gain insights into protein structure

Josef Lazar[1,2]

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[2]Institute of Microbiology of the Czech Academy of Sciences

Our recent advances in understanding of optical properties of fluorescent proteins, along with developments in image analysis, allow obtaining more detailed information about membrane protein structure in living cells.

Talk 8a – Short

Proton translocation mechanism in mitochondrial ATP synthase

Xavier Prasanna Aanthony Raj[1]

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Adenosine triphosphate (ATP), the biochemical energy currency of the cell, is generated by a class of multi-subunit molecular motors called ATP synthase. In eukaryotic cells, mitochondria serves as the storehouse for ATP production where ATP synthase employs the transmembrane proton motive force to drive the ATP synthase rotor. This rotatory motion is coupled to ATP generation from ADP and Pi. However molecular details of this mechanism remains largely unresolved. Several suggestions have been proposed based on the recently determined cryo-electronmicroscopy structures of ATP synthase. In this study, we investigate these hypotheses by examining water dynamics to identify a possible proton translocation pathway through mitochondrial ATP (mATP) synthase. Our preliminary results reveal the presence of two half water channels: one originating from the periplasmic space, extending to the interface between subunit c and subunit a; and the other extending from an offset site on another subunit c protomer and opening out into the cytoplasmic space. These findings suggest that protons migrate to a critical negatively charged residue at the subunit c-subunit a interface and neutralises it, resulting in a brownian rotational motion of subunit c and consequent release of a proton from an upstream residue to the cytoplasmic site. Furthermore we have also investigated the mechanism of proton translocation in mATP synthase with pathological mutations in subunit a.

Talk 8b – Short

Polarity and mobility of the enzyme active cavity as potential factors affecting the enzymatic activity – time dependent fluorescence shift study

Iryna Kishko[1], Jan Sykora[1], Veronika Dockalova[2], Zbynek Prokop[2, 3], Avisek Ghose[4], Jiri Damborsky[2, 3]

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Enzymes are effective biological catalysts with exceptional specificity and selectivity. Enzymatic reactions take place in the active sites, often hidden inside the protein core. The active sites are connected to the bulk solvent through one or more transport tunnels. Beyond the complementarity between the substrate and the binding site, tunnels offer another level of resolution for the molecules entering and leaving the enzyme active cavity. The tunnels discriminate preferred substrates or cofactors, reduce the access of solvents which may disturb chemical reactions, or prevent the escape of reactive intermediates, potentially harmful to the cells [1]. The hydration and mobility of these access pathways towards the active sites are believed to profoundly affect their function [2]. However, only a few approaches for monitoring these characteristics within the relevant protein regions are available. Here we apply a fluorescence method for the site-specific analysis of the extent of hydration in enzyme Haloalkane Dehalogenase. This approach is based on recording „time dependent fluorescence shift“ (TDFS) [3] placing the dye in the various positions along the tunnel of this enzyme [4,5]. The hydration monitored within the biologically relevant regions of the dehalogenase enzymes is then compared with their enzyme kinetics of various mutants, which can bring the deeper insight into the functioning of these enzymes.

References:

[1] Prokop, Z., Gora, A., Brezovsky, J., Chaloupkova, R., Stepankova, V., Damborsky, J., 2012: Engineering of Protein Tunnels: Keyhole-lock-key Model for Catalysis by the Enzymes with Buried Active Sites. In: Lutz, S., Bornscheuer, U.T. (Eds.), Protein Engineering Handbook, Wiley-VCH, Weinheim, pp. 421-464.

[2]Levy, Y.; Onuchic, J. N. Annu. Rev. Biophys. Biomolec. Struct. 2006, 35, 389.

[3]Horng, M. L.; Gardecki, J. A.; Papazyan, A.; Maroncelli, M. J. Phys. Chem. 1995, 99, 17311.

Acknowledgements:

Authors greatly acknowledge the Czech Science Foundation (project GACR 16-06096S)

Talk 9 – Key

Endoplasmic reticulum-plasma membrane contact sites integrate sterol and phospholipid regulation

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Tether proteins attach the endoplasmic reticulum (ER) to other cellular membranes, thereby creating contact sites that are proposed to form platforms for regulating lipid homeostasis and facilitating non-vesicular lipid exchange. Sterols are synthesized in the ER and transported by non-vesicular mechanisms to the plasma membrane (PM) where they represent almost half of all PM lipids and contribute critically to the barrier function of the PM. To determine whether contact sites are important for both sterol exchange between the ER and PM and inter-membrane regulation of lipid metabolism, we generated Δ-super-tether (Δ-s-tether) yeast cells that lack six previously identified tethering proteins (yeast extended synatotagmin (E-Syt), VAP, and TMEM16-anoctamin homologs) as well as the presumptive tether Ice2. Despite the lack of ER-PM contacts in these cells, ER-PM sterol exchange is robust, indicating that the sterol transport machinery is either absent from or not uniquely located at contact sites. Unexpectedly, we found that the transport of exogenously supplied sterol to the ER occurs more slowly in Δ-s-tether cells than in wild-type cells. We pinpointed this defect to changes in sterol organization and transbilayer movement within the PM bilayer caused by phospholipid dysregulation, evinced by changes in the abundance and organization of PM lipids. Indeed, deletion of either *OSH4*, which encodes a sterol/phosphatidylinositol-4-phosphate (PI4P) exchange protein, or *SAC1* which encodes a PI4P phosphatase, caused synthetic lethality in Δ-s-tether cells due to disruptions in redundant PI4P and phospholipid regulatory pathways. The growth defect of Δ-s-tether cells was rescued with an artificial "ER-PM staple," a tether assembled from unrelated non-yeast protein domains, indicating that endogenous tether proteins play non-specific bridging functions. Finally, we discovered that sterols play a role in regulating ER-PM contact site formation. In sterol-depleted cells, levels of the yeast E-Syt tether Tcb3 were induced and ER-PM contact increased dramatically. These results support a model in which ER-PM contact sites provide a nexus for co-ordinating the complex inter-relationship between sterols, sphingolipids, and phospholipids that maintain PM composition and integrity.

Talk 10 – Normal

Why would anyone in the clinics care about our Prague/ Levy/Dresden lipid perspective?

Ünal Coskun[1]

[1]*Paul Langerhans Institute Dresden — TU Dresden*

From the clinical perspective, the contribution of dietary fat, for instance to insulin resistance and cancer, is well accepted. From the basic research point of view, however, dietary fats extend far beyond serum cholesterol and triglyceride levels. Cellular membranes contain thousands of distinct lipid species and we have only begun appreciating their implications in cellular functions, such as signaling. One aspect of lipid function is the building of biological membranes; thus, lipids are key determinants of membrane physicochemical properties. The initial steps of receptor signaling occur at the plasma membrane: adaptor and downstream effector proteins need to directly attach to the site of action at the membrane to accomplish their function. From an objective perspective, it is entirely not understood how cell signaling outcomes can be controlled at all, considering the fact the myriad of membrane receptors signal though commonly shared signaling effector proteins. Are membrane lipid-protein interactions key to ultimately understanding cell signaling? In my presentation I will give an overview of how dietary lipid directly contribute to the remodeling of membrane lipidomes at the organ level, and how these changes directly impact on the structure and function of the human EGF and Insulin receptors and their downstream singling effector proteins. My talk will highlight some of the key questions that our Prague/Levy/Dresden community needs to answer in order to make a change in biochemical research and clinical application thereof.

Selected Publications:

[1] Gutmann, T., Kim, K.H., Grzybek, M., Walz, T., Coskun, Ü. Visualization of ligand-induced transmembrane signaling in the full-length human insulin receptor. J. Cell Biol., JCB (2018)

[2] Bilkova, E., Pleskot, R., Rissanen, S., Sun, S., Czogalla, A., Cwiklik, L., Róg, T., Vattulainen, I., Cremer, P.S., Jungwirth, P., Coskun, Ü. Calcium directly regulates phosphatidylinositol 4,5-Bbsphosphate headgroup conformation and recognition. J. Am. Chem. Soc. 139, 4019-4024 (2017)

[3] Kaszuba, K., Grzybek, M., Orłowski, A., Danne, R., Róg, T., Simons, K., Coskun, Ü., Vattulainen, I. N-Glycosylation as determinant of epidermal growth factor receptor conformation in membranes. Proc. Natl. Acad. Sci. U.S.A. 112, 4334–4339 (2015)

[4] Coskun, Ü., Grzybek, M., Drechsel, D., Simons, K. Regulation of human EGF receptor by lipids. Proc. Natl. Acad. Sci. U.S.A. 108, 9044-9048 (2011)

[5] Coskun, Ü., Simons, K. Cell membranes: The lipid perspective. Structure 19, 1543-1548 (2011)

Talk 11 – Short

Simulation of a calcium sensor – STIM

Lydie Plačkova[1], Daniel Bonhenry[1], David Řeha[1] and Rüdiger H. Ettrich[1]

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The stromal interaction molecule (STIM) is a protein located at the level of the membrane of the cellular calcium stores, namely the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR). This protein is able to sense the level of calcium ions within the stores and is able to trigger the activation of calcium channels located in the plasma membrane (PM) upon depletion of calcium[1].

STIM is a single-pass transmembrane protein. Its N-terminal is located within the luminal part of the ER while the C-terminal is bathing in the cytosol. The N-terminal has the ability to probe the calcium level within the ER thanks to EF-hand calcium binding motifs[2]. On the other side of the membrane, the cytosolic part contains domain able to bind and activate[3] target calcium-channels in the plasma membrane depending on the calcium level within the ER.

In non-excitable cells, such as lymphocytes, mutations impairing the proper function of this protein lead to immunodeficiency, autoimmune disease or myopathy[4].

Molecular dynamics simulations of wild type phenotype as well as mutants are used to explore how this protein is sensitive to luminal Ca^{2+} levels and able to initiate a cascade of events leading to the refilling of the calcium stores.

References:

[1] Liou, J. *et al.* STIM Is a Ca2+ Sensor Essential for Ca2+-Store-Depletion-Triggered Ca2+ Influx. *Curr. Biol.* 15, 1235–1241 (2005).
[2] Stathopulos, P. B. *et al.* Structural and Mechanistic Insights into STIM1-Mediated Initiation of Store-Operated Calcium Entry. *Cell* 135, 110–122 (2008).
[3] Fahrner, M. *et al.* A Coiled-coil Clamp Controls Both Conformation and Clustering of Stromal Interaction Molecule 1 (STIM1). *J. Biol. Chem.* 289, 33231–33244 (2014).
[4] Feske, S. et al. Immunodeficiency due to mutations in ORAI1 and STIM1. Clin.Immunol. 135, 169–182 (2010).

Talk 12 – Normal

Organelle specific & quantitative lipid biochemistry in living cells.

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New approaches for analyzing lipid dynamics and lipid-protein interactions are needed to understand the biological consequences of the molecular complexity of the cellular lipidome. We employ a combination of photo-caged lipid probes and mathematical modelling to extract quantitative kinetic parameters for diacylglycerol transbilayer movement and turnover and to determine binding constants of diacylglycerol-protein interactions at the plasma membrane of living cells. Affinities and kinetic parameters vary by orders of magnitude due to diacylglycerol acyl chain diversity and these differences are sufficient to explain fundamentally different recruitment patterns of diacylglycerol binding proteins. Our findings suggest that both lipid-protein interactions and lipid dynamics on the species level constitute molecular mechanisms for determining signaling outcome at cellular membranes. We propose that a key reason for lipid diversity is to provide cells with a repertoire of signaling lipid species that differ in dynamic properties and affinity for effector proteins to enable precise interpretation of signaling events.

Talk 13 – Normal

Good cops or bad cops? Distinct roles of anionic phospholipids in plant membrane identity and vesicular traffic

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Minor signalling lipids at the plasma membrane (PM) have been implicated in the regulation of many cellular events, including cytoskeletal dynamics, membrane trafficking and stress responses. In plants, signalling lipids typically show rapid turnover but detailed information about their spatio-temporal distribution and downstream targets is still largely missing. Using tip-growing plant cells expressing genetically-encoded lipid markers as experimental model, we show that specific localization of anionic lipids define functionally distinct domains of plasma membrane. In particular, phosphatidic acid (PA) localization coincides with the domain enriched with endocytic recycling. By combining microscopic, biochemical and computational approaches, we present evidence that PA is involved in targeting and regulation of components of plant clathrin-meditated endocytic machinery.

Acknowledgements:
This work was supported by the Czech Science Foundation (grant no. 17-27477S) and Ministry of Education Youth and Sport of the Czech Republic (project no. NPUI LO1417).

Talk 14a – Short

INITIAL STEPS IN THE BASIC FIBROBLAST GROWTH FACTOR OLIGOMERIZATION

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[3]Paul Langerhans Institute Dresden, Dresden, Germany
[4]Heidelberg University Biochemistry Center, Heidelberg, Germany

The majority of protein in the mammalian cells are transported to the extracellular space by the endoplasmic reticulum (ER)/Golgi-dependent secretory pathway. Many proteins, however, can be secreted independently bypassing the ER-Golgi complex. Fibroblast Growth Factor II (FGF2) is one of those. The unconventional secretory pathway process secreting FGF2 from cells depends on the interactions of FGF2 with $PI(4,5)P_2$ lipids and heparan sulfate proteoglycans at the inner and outer leaflet of the plasma membrane, in respective order. Here, we used atomistic molecular dynamics simulations to shed light on how $PI(4,5)P_2$ triggers the oligomerization of FGF2 on the membrane surface. We demonstrated that the amino acids K127, R128, and K133 that in experimental studies have been found to be part of a binding pocket interacting with the head group of $PI(4,5)P_2$ are not the only ones able to recruit $PI(4,5)P_2$. Instead, simulations brought up that FGF2 monomers can simultaneously interact with several $PI(4,5)P_2$ molecules through additional residues (K34, K137, K143) that contribute to the binding process. These interactions gave rise to the high-affinity orientation of FGF2 that fosters its dimerization through the formation of intermolecular disulfide bridges. Extensive simulations confirmed dimerization through the C95-C95 bridge, in agreement with experimental data. The importance of ion pairs in the stabilization of the dimer interface was also evident. The findings highlight that the FGF2 dimers containing the C95-C95 disulfide bridge represent the initial step of PI(4,5)P2 dependent FGF2 oligomerization.

Talk 14b – Short

Does interaction with the inner plasma membrane effect the binding of isophthalic acid derivatives to PKC C1 domain?

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The enzyme protein kinase C (PKC) has a plethora of roles in human physiology, and thus plays also a significant role in pathologies of many diseases, such as cancer and Alzheimer's disease [1, 2]. This enzyme is activated when its C1 domain interacts with an intracellular membrane that incorporates the lipid second messenger diacylglycerol (DAG) [3]. In cancer research PKC was previously thought to be an oncoprotein, but after PKC inhibitory drugs failed to show positive results, it was discovered, that PKC associated cancer activity is in fact associated with loss-of-function mutations in PKC, and therefore PKC could actually be a tumor suppressor [4]. The PKC enzyme has a role in controlling learning and memory as well - its activation is involved in cholinergic muscarinic receptor mediated effects in the brain, which is not being properly activated in Alzheimer's disease, and therefore drugs that activate PKC could be used as drugs for it as well [5].

In a study by Boije af Gennäs & Talman et al. several potential drug molecules to target PKC in the aforementioned way were tested. These drugs are designed to be partial agonists since too strong activation leads to PKC being vulnerable to loss of function [4]. Three particularly promising drug molecules showed similar binding affinities *in vitro*, but their potency varied *in vivo* [2]. This behavior is not completely explained by just the fit of the molecule to the binding site of the PKC C1 domain, and thus we hypothesized that the interaction of the drug molecules and the membrane that binds PKC and DAG is of importance and improved efficacy in PKC targeted drugs can be achieved, if the developed drug not only binds to the C1 domain well, but also mimics the orientation of the DAG headgroup at the membrane surface. Properly therapeutically activating of PKC would thus be a combination of the drug mimicking DAG in both binding and orientation.

We are currently studying the mentioned three drug and DAG molecules in physiological membrane environment using molecular dynamics simulations and in the process of comparing their interactions and orientation to each other. The information gained here will give good guidelines how to better target PKC in the molecular level.

References:
[1] V. Talman, A. Pascale, M. Jäntti, M. Amadio, and R. K. Tuominen, Basic & Clinical Pharmacology & Toxicology, vol. 119, no. 2, pp. 149–160 (2016).
[2] G. Boije af Gennäs, V. Talman, O. Aitio, E. Ekokoski, M. Finel, R. K. Tuominen, and J. Yli-Kauhaluoma, Journal of Medicinal Chemistry, vol. 52, no. 13, pp. 3969–3981 (2009).
[3] A. C. Newton, M. D. Bootman, and J. D. Scott, Cold Spring Harbor Perspectives in Biology, vol. 8, no. 8 (2016).

Acknowledgments:
This work was supported by the Orion Foundation sr and by the Doctoral Programme of Drug Research doctoral school.

Talk 15 – Normal

Understanding ion binding to lipid bilayers using Open Collaboration

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Chemical details of lipid headgroups and their interactions with ions are of paramount importance in biological signaling processes. NMRlipids (nmrlipids.blogspot.fi) is an Open Collaboration project to understand lipid headgroup structures and ion binding to lipid bilayers with unpresendent detail by combining NMR data and molecular dynamics simulations. The massive amount data of data collected using the Open Collaboration method revealed that none of the available MD simulation model correctly reproduced the lipid headgroup structures or cation binding details, the main artefact being the overestimated cation binding affinity to lipid bilayers [1,2].

Recently we were able to correctly reproduce the cation binding details to PC lipid bilayers by implicitly including the electronic polarizability into the MD simulation models using the electronic continuum correction [3]. In this model, calcium ions bind to the phosphate groups of 1-3 PC lipids and sodium ions bind with lower affinity than water molecules. Similar studies for negatively charged PS lipids are ongoing within the NMRlipids IV project (http://nmrlipids.blogspot.cz/).

References:

[1] Botan et al. J. Phys. Chem. B 2015, 119, 15075–15088
[2] Catte et al. Phys. Chem. Chem. Phys. 2016,18, 32560-32569
[3] J. Melcr, H. Martinez-Seara, R. Nencini, J. Kolafa, P. Jungwirth, *and O.H.S. Ollila J. Phys. Chem. B, DOI: 10.1021/acs.jpcb.7b12510*

Talk 16a – Short

Getting ion-biomolecule interactions right in simulations

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Interactions between ions and biomolecules are ubiquitous in Nature: they play a central role in a number of fundamental biological processes, ranging from calcium signaling to insulin storage. However, ion pairing properties of biological cations, especially multivalent cations, are known to be poorly reproduced by standard empirical force fields, because of polarization and charge transfer effects. Our goal is to improve the description of divalent cations in molecular dynamics (MD) simulations and subsequently use it to tackle biologically relevant problems.

Our strategy is to first combine neutron scattering data experiments performed on concentrated electrolytes with *ab initio* MD simulations in order to characterize at the molecular level the interactions of non-transition metal divalent cations (zinc, calcium, magnesium) with relevant protein groups (acetate, histidine). These data are then used to develop a scaled charge empirical description of the ions, which takes into account electronic polarization in a mean field way [1-3]. This allows us to design an accurate and computationally cheap method for the description of these divalent cations. We show that our new approach provides a qualitatively accurate picture of ion binding to proteins [4] at physiological conditions, which opens the way for simulation of important ion-mediated biological processes.

References:

[1] Leontyev I., Stuchebrukhov A., Phys.Chem.Chem.Phys., 2011, 13, 2613-2626
[2] Duboué-Dijon E., Mason P.E., Fischer H., Jungwirth P., J. Phys. Chem. B, 2017, 122, 3296-3306
[3] Martinek T., Duboué-Dijon E., Timr S., Mason P.E., Baxova K., Fischer H., Pluharova E., Jungwirth P., J. Chem. Phys., 2018, 148, 222813
[4] Duboué-Dijon E., Delcroix P., Martinez-Seara H., Hladilkova J., Coufal P., Krizek T., Jungwirth P., J. Phys. Chem. B, 2018, doi:10.1021/acs.jpcb.7b12097

Talk 16a – Short

Binding of cations to neutral and negatively charged membranes in (virtual) reality

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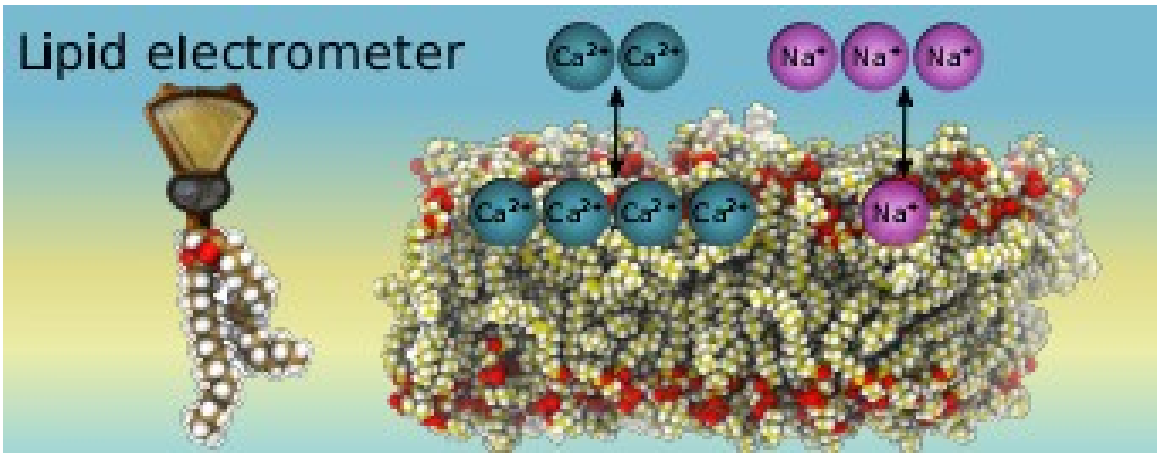
Binding affinities and stoichiometries of Na^+ and Ca^{2+} ions to phospholipid bilayers are of paramount significance in the properties and functionality of cellular membranes. However, interactions of cations with phospholipid bilayers in classical MD simulations are currently overestimated in their strength in almost all state-of-the-art models. We have recently shown on the case of a POPC bilayer that accounting for polarizability on both the cations and the lipids improves the description of the cation binding details dramatically. In order to reach a quantitative agreement with available experimental data from solid state NMR and x-ray scattering, it was sufficient to use a simple implicit model of polarizability termed Electronic Continuum Correction (ECC).

I will provide a peek under the lid of our current progress on how we cook up models for also other lipids including PE and PS. Major obstacles and a small “free lunch” will be shown and served in the short presentation.



Talk 17 – Normal

Formation of membrane pores by amphiphilic peptides

Robert Vácha[1]

[1]*Masaryk University*

Amphiphilic peptides can kill pathogens by disrupting their membrane integrity via formation of membrane pores. However, the peptide properties that are advantageous for pore formation remain elusive. Using multi-scale computer simulations supported fluorescence leakage experiments we investigated the formation of various membrane pores. Our simulations were able to reproduce the stabilization of the well-known barrel-stave and toroidal pores. We proposed a new collective variable for pore formation in Martini or all-atom simulations. Finally, few crucial peptide properties responsible for given pore structures we identified. The identified properties can be utilized in the rational peptide-based drug design with the controlled pore formation that could serve as a new generation of antibacterial agents.

Talk 18 – Normal

The initiation of clathrin-mediated endocytosis in plants

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Clathrin-mediated endocytosis (CME) is one of the main mechanisms for plasma membrane-based proteome and lipidome turn-over in eukaryotic cells. In plants, CME is essential for physiology and development, but many aspects of CME initiation and regulation are still unknown. Recently, our laboratory showed that an eight-subunit complex, the TPLATE complex, is a major adaptor module involved in the CME initiation and cargo recognition in plants. In this work, we focus on structural arrangement of the TPLATE complex and its interactions with the plasma membrane. By combination of computational modelling with biochemical methods together with live cell imaging, we dissected several subunits of the TPLATE complex. We modelled a structure of the TPLATE complex based on its shared evolutionary origin with other adaptor complexes with experimentally solved structures. We have initiated a model evaluation by cross-linking mass spectrometry as a part of integrative modelling approach. Moreover, we identified domains of the TPLATE complex, which are responsible for direct interaction with anionic phospholipids of the plasma membrane thus shedding light on first steps of CME in plant cells.

Talk 19a – Short

Role of Glycans in EGFR Conformation and Interaction with Glycolipids

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Over the past decade, protein glycosylation attracts ever-growing attention in a variety of research areas, from biomedicine to biotechnology. The abnormal glycosylation of specific proteins proved to be associated with a number of pathological conditions, including neurological diseases and malignant transformation. Accumulating evidence indicates that interfaces formed by cellular membranes play a crucial role in interaction with protein glycans in vivo and mediate of activity of glycosylated membrane proteins. Epidermal growth factor receptor (EGFR) is a glycosylated transmembrane receptor that plays a pivotal role in a vast majority of cellular processes and involved in the development of several forms of cancer. Human EGFR, a receptor tyrosine kinase with complex glycosylation pattern, is the first discovered oncogene receptor and a prevalent defected protein in the majority of breast and lung cancers and glioblastomas. Moreover, EGFR is a primary target for pharmaceutical and antibody-based therapy for cancer treatment. Despite the increase in understanding of EGFR structure and function, the limitations of experimental biochemical and biophysical approaches didn't allow to elucidate the details of EGFR mechanisms.

It was recently demonstrated that EGFR activity is mediated by association with membrane glycolipid GM3 through carbohydrate-to-carbohydrate interactions. However, the exact molecular mechanism of GM3 interaction with differentially glycosylated EGFR remains unexplored. Recently, the full sequence of EGFR glycosylation was experimentally determined. Accordingly, the new model of EGFR with long glycans is expected to give fruitful results of GM3-mediated regulation of the protein.

Here we are going to investigate the mechanisms behind the regulation of EGFR activity by GM3 glycolipid using an extensive all-atom Molecular Dynamics (MD) simulations of fully glycosylated EGFR. The main focus of the study is on the role of cancer-specific glycan modifications in EGFR GM3-mediated regulation.

Talk 20 – Key

Real-time label-free cell based platforms as novel analytical tools for drug and nanoparticle targeting studies

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In vitro biochemical and cell culture assays forms the cornerstone in life sciences for building a sound understanding of fundamental inter- and intracellular mechanisms, as well as to identify molecular mechanisms of human disease and develop novel therapeutics. The majorities of current functional *in vitro* biochemical and cell culture assays used in pharmaceutical research are static and often only shed light on the endpoint equilibrium. They often rely on labelled-materials and require secondary detection techniques. These static functional assays omit important, dynamic events taking place on the way to the equilibrium, and the use of labels can affect drug or nanoparticle properties or influence cell behaviour. This may be one of the main reasons that promising *in vitro* results in drug development are often not always followed by respective success *in vivo*. Hence, there is a clear need for complementary real-time label-free cell based *in vitro* tools that could be used to quantify biological activity and distinguish between biochemical mechanisms consequently facilitating a better understanding of cellular mechanisms, as well as for careful screening and faster development of novel therapeutic solutions at the pre-*in vivo* level.

We have developed novel cell-based analytical platforms for studying drug and nanoparticle interactions and targeting with cell membranes and living cells. Our analytical platforms combines either surface plasmon resonance (SPR) detection with living cells [1, 2] or a quartz crystal microbalance (QCM) in combination with supported lipid bilayers containing extracted cell membranes [3]. Both SPR and QCM techniques are highly surface-sensitive techniques that enable real-time label-free monitoring of interactions taking place at interfaces. Herein, we present how we have successfully been able to utilize these platforms for 1) quantifying the biological activity of drugs targeting G-protein coupled receptors (GPCR), 2) measuring various GPCR signals and differentiating the SPR responses for different receptor types and 3) determining the targeting efficacy and cell uptake kinetics of liver targeted liposomes.

Collectively, our results show that the real-time label-free cell based platforms we have developed have high potential to become an integral part of the toolbox of life science researchers for studying cellular mechanisms and drug/nanoparticle interactions with cells.

References:

[1] Tapani Viitala*, Niko Granqvist, Susanna Hallila, Manuela Raviña, Marjo Yliperttula: Elucidating the signal responses of multi-parametric surface plasmon resonance living cell sensing: a comparison between optical modeling and drug–MDCKII cell interaction measurements. PlosOne 8, e72192 (2013)
[2] Teemu Suutari, Tiina Silen, Heikki Saari, Diti Desai, Erja Kerkelä, Saara Laitinen, Martina Hanzlikova, Jessica M Rosenholm, Marjo Yliperttula, Tapani Viitala*: Real-Time Label-Free Monitoring of Nanoparticle Cell Uptake. Small 12, 6289-6300 (2016)

[3] Mohammed Elmowafy, Alma Kartal-Hodzic, Timo Oksanen, Marjo Yliperttula, Tapani Viitala*: Real-time label-free in vitro assessment of targeting and drug delivery efficacy of liver targeted liposomes. Unpublished data.

Talk 21 – Normal

Tear Film Lipid Layer (TFLL) – from a healthy eye model system to a polar lipid deficient TFLL stabilized by two drug molecules – in vitro and in silico studies

Agnieszka Olżyńska[1] , Lukasz Cwiklik[1]

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An aqueous thin tear film (TF), with tear film lipid layer (TFLL) as it outermost part, is a very dynamic system covering and protecting the surface of cornea. Its structure is constantly perturbed and restored with the cyclic blinking of eye lids. However, in Dry Eye Disease (DED), the stability of the TF is diminished leading to enhanced film breakup. It is assumed that TFLL is altered in DED but the details of its structure and behavior are not fully understood.

We apply a multi-level approach to investigate basic properties of TF and the role played by TFLL. We experimentally characterize TFLL-mimicking systems by Langmuir balance combined with fluorescence microscopy. In parallel, we employ in silico modeling using large-scale coarse grain molecular dynamics simulations of TF models at the molecular level. Additionally, these studies of TFLL mimics are supported by experimental evaluation of the behavior of human TF extracts. The combination of these three levels of description, spanning from behavior of individual molecules to collective macroscopic properties of human tear extracts, allows us for reliable in-depth description of basic tear film properties.

We introduced this multi-level approach to investigate the role of two drug molecules, cetalkonium chloride (CKC) and poloxamer 188 (P188), as stabilizers of TF. We demonstrated that deficiency of polar lipids in TFLL, which is observed in DED, induces destabilization of TF by enhanced water-nonpolar lipids contacts. We try to explain how molecular-level interactions of CKC and P188 with TF can prevent these disruptions.

Talk 22 – Short

Towards realistic models of lung surfactant - MD simulations with improved water and ion force fields

Pauline Delcroix[1], Agnieszka Olzynska[1] and Lukasz Cwiklik[1]

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Lung surfactant lines the gas-exchange interface in the lungs and reduces the surface tension at the air-liquid interface to minimize the work of breathing. The lung surfactant consists mainly of lipids with a small amount of proteins and forms a monolayer at the air-water interface connected to bilayer reservoirs. The composition of the lung surfactant and the border conditions of normal human breathing are relevant to characterize the interfacial behavior of pulmonary layers.

In this work, we focused on the composition of the lung surfactant. In a first step, we performed classical molecular dynamics simulations at an atomistic resolution of a new more realistic model of the monolayer. The lipid composition is such as each leaflet contains 128 lipids distributed between DPPC, POPC, POPG and Cholesterol. Simulations were performed with the Slipids force field and TIP3p parameters for water at a microsecond timescale. The role and behaviour of the ions were analyzed.

In a second step, scaled parameters for the ions and the new OPC water force field have been used. The results were compared to the ones obtained with standard parameters.

In a series of successive steps, the role of oxidized lipids, the role of cholesterol, the so-called “Cholesterol Mystery” and the role of oxysterols were investigated. The effect of the addition of the oxidized species and the effect of cholesterol removal on the properties and behaviour of the monolayer were investigated. The results were compared to those obtained in non-oxidized conditions and in presence of cholesterol.

Finally, for each system composition, the whole isotherm will be explored (i.e: Area Per Lipid= 50, 60, 70, 80, 90, 100, 100) and the influence of each parameter on the behaviour of the isotherm will be investigated.

In parallel, Langmuir trough experiments are conducted with similar compositions as in the simulations to obtain macroscopic properties of the monolayer. In combination with microscopic properties obtained from simulations, it will allow us to analyze the effects caused by the presence or absence of different lipids on lung surfactant properties.

Talk 23 – Normal

The catalytic mechanism of MB-COMT: a story a decade in the making

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alex.bunker2@gmail.com

We summarize work that has been carried out over the past decade, using computational molecular modelling in synergistic combination with a wide range of experimental methodologies, on the pharmaceutically important enzyme: catechol-O-methyltransferase (COMT) [1]. The enzyme has both a water soluble (S-COMT) and a membrane bound (MB-COMT) isoform; MB-COMT is a drug target in relation to the treatment of Parkinson's disease: selective targeting of this isoform is desirable. This should also be possible since, while S-COMT and MB-COMT have identical catalytic domains, their substrate profiles differ[2].

Starting from our initial publication regarding COMT[2] through our study of the trans membrane helix and linker of MB-COMT[3] and our study of the interaction with different relevant cell membranes of COMT substrates[4] and, finally, to our determination of the catalytic mechanism of MB-COMT [5] that differentiates it from S-COMT, and ultimately opens the door to the possibility of selective targeting, this talk describes a ten year arc of research culminating in the recently published cover article in Chemical communications.

Our hypothesis was that the substrate differentiation mechanism was interaction with the membrane of both substrates or potential inhibitors and the catalytic domain of MB-COMT; we thus determined the catalytic mechanism specific to MB-COMT. We found that (1) substrates with preferred affinity for MB-COMT over S-COMT orient in the membrane in a fashion conducive to catalysis from the membrane surface and (2) binding of COMT to its cofactor ADOMET induces conformational change that drives the catalytic surface of the protein to the membrane surface, where the substrates and Mg^{2+} ions, required for catalysis, are found. Through Bioinformatics analysis we found evidence of this mechanism in other proteins, including several existing drug targets.

References:

[1] P. T. Männistö and S. Kaakkola, Pharmacol. Rev., **51**, 593–628, (1999).
[2] R. G. Robinson *et al*. ACS Chem. Neurosci., **3**, 129–140, (2012).
[3] A. Bunker, P.T. Männistö, J.-F. St. Pierre, T Róg, P. Pomorski, L. Stimson and M. Karttunen, SAR and QSAR in Env. Res., **119**, 149–160 (2008).
[4] A. Orłowski, J.-F. St. Pierre, A. Magarkar, A. Bunker, M. Pasenkiewicz-Gierula, I. Vattulainen and T. Róg, J. Phys. Chem. B, **115**, 13541−13550 (2011).

[5] A. Orłowski, M. Grzybek, A. Bunker, M. Pasenkiewicz-Gierula, I. Vattulainen, P. T. Männistö and T. Róg, J. Neurochem. **122**, 612–690 (2012).

Talk 24 – Normal

Glycosylation determines orientation of extracellular domains of bitopic membrane proteins

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Bitopic membrane proteins with a single transmembrane helix are the largest group of membrane proteins, as approximately half of the integral membrane proteins belong to this group. Typically, extracellular domain of bitopic proteins is heavily glycosylated. In this study we performed molecular dynamics simulations of three proteins: epidermal growth factor receptor (EGFR), T-cell surface antigen CD2, and tool like receptor 4 (TLR4) in glycosylated and non-glycosylated form. For the case of EGFR and CD2 extracellular domain tends to collapse at the membrane surface while in the presence of glycosylation upright orientation is adopted. This is due to steric effects and due to shielding of patches of positively charged residues at the protein surface, which promote interactions of proteins with lipids. For the case of TLR4 we observed large tilting of the extracellular domain for the case of glycosylated form of the protein in active state while upright position was observed when carbohydrates branches were removed. To summarize, glycosylation is one of important determinants of the orientation of the extracellular domains of bitopic membrane proteins.

Talk 25a – Short

Optimizing Peptide Properties For Translocation Across Lipid Membranes

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Cell-penetrating and some antimicrobial peptides can translocate across lipid bilayers without significantly perturbing the membrane structure. However, the molecular properties required for efficient translocation are not fully understood. We employed Metropolis Monte Carlo method together with coarse-grained models to systematically investigate free energy landscapes associated with the peptide translocation. We studied alpha-helical peptides with different length, amphiphilicity, and distribution of hydrophobic content and found common translocation path consisting of adsorption, tilting, and insertion. In the adsorbed state the peptides are parallel to the membrane plane, while in the inserted state the peptides are always perpendicular to the membrane. Our simulations demonstrate that, for all tested peptides, there is an optimal ratio of hydrophilic/hydrophobic content, at which is the translocation free energy minimal and peptides cross the membrane the easiest. These results provide general guidence to optimize peptides for use as carriers of molecular cargos or as therapeutics themselves.

Talk 25b – Short

Supported lipid bilayers – application to biointeraction analysis

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Supported lipid bilayers (SLBs) have long been a model membrane of choice as biomimetic platforms, mainly due to their relative ease of *in situ* preparation from small unilamellar vesicle dispersions. Here, novel uses of these platforms in biointeraction analysis, are discussed.

Catalytic mechanism of cathecol-O-methyltransferase [1]

Cathecol-O-methyltransferase (COMT) is an important enzyme responsible for methylation of catecholamines. Membrane-bound isoform of COMT, abundant in brain, is the preferable target of drugs when treating Parkinson's disease. Combination of surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) techniques revealed the increase in affinity of soluble COMT in its holo form (ligand bound) to the supported lipid bilayer. This, together with MD simulations, suggests interfacial mechanism of catalysis for membrane-bound isoform of COMT. Also, COMT inhibitors specific to the membrane-bound isoform were found to orientate in the lipid bilayer in a favorable manner for catalysis. Recently, bilayer binding of COMT inhibitors, tolcapone and entacapone, and COMT substrate, dopamine, were investigated. Tolcapone, an inhibitor specific to the membrane-bound isoform, had preferential affinity towards the SLBs mimicking the endoplasmic reticulum membrane, native environment for MB-COMT. This supports the hypothesis of membrane-mediated catalytic mechanism.

Cofactor mechanism of APO-A1 [2]

Apolipoprotein A1 is the major protein component of HDL particles in plasma. Mutations in the APO-A1 gene may lead to metabolic disease conditions. Lecithin-cholesterol acyltransferase (LCAT) is an enzyme responsible for the formation of cholesteryl esters from cholesterol and phospholipid molecules in HDL particles. Using binding studies in QCM, it was found that the APO-A1 derived peptides from LCAT activating region bind to LCAT and promote its lipid surface interaction, although some of these peptides do not bind lipids alone. This effect was found to disappear in the presence of a point mutation in the peptide corresponding to the amino acids 150-170 of the APO-A1. The results have implications regarding the cofactor mechanism of APO-A1.

References:

[1] A. Magarkar, P. Parkkila, T.Viitala, T. Lajunen, E. Mobarak, G. Licari, O. Cramariuc, E. Vauthey, T. Róg, A. Bunker: Membrane bound COMT isoform is an interfacial enzyme: general mechanism, new drug design paradigm. *Chemical Communications* **54**, 3440-3443 (2018)
[2] M. G. Casteleijn, P. Parkkila, T. Viitala, A. Koivuniemi: Interaction of lecithin-cholesterol acyltransferase with lipid surfaces and apolipoprotein A-I derived peptides. *Journal of Lipid Research* **59**(4), 670-683 (2018)

Talk 26 – Normal

The Effect of Ions on Membrane Elasticity - From Curvature Generation to Vesicle Fusion

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Ions and charged peptides can have dramatic effects on the properties of membranes. A case in point is Calcium and its crucial role in vesicle fusion.[1] We simulated cation-mediated vesicle fusion using All-atom molecular dynamics[2] and described the effect of Calcium on membrane curvature.[3] With the help of a new approach to determining membrane elastic properties[4] we gained new insights into the mechanism of membrane fusion and curvature generation by ions. Furthermore, we apply our methodology to a host of other ions and membrane compositions in order to explore the molecular origins of curvature generation and membrane stiffening by charged adsorbates. Another example of prominent membrane deformations caused by ions is the internalization of arginine rich cell penetrating peptides. We present an investigation of the role of ion pairing[5] and a new mechanism for entry driven by curvature elasticity.[1]

References:

[1] E. Neher, R. Schneggenburger, *Nature* 406, 889–893 **2000**
[2] C. Allolio, A. Magarkar, P. Jurkiewicz, K. Baxova, R. Šachl, M. Hof, D. Horinek,V. Heinz, R. Rachel, C. Ziegler, P. Jungwirth, *submitted* **2018**
[3] A. Magarkar, P. Jurkiewicz, C. Allolio, M. Hof and P. Jungwirth, *J. Phys. Chem. Lett*., 8, 518-523 **2017**
[4] C. Allolio, A. Haluts, D. Harries, *Chem. Phys.* ***2018*** *(in press)*
[5] C. Allolio, K. Baxova, M. Vazdar, P. Jungwirth J. Phys. Chem B., 120, 143-153 **2016**

Talk 27a – Short

Role of receptor potential in hair cells in the cochlea

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The cochlea is a part of the inner ear, functioning as a mechano-electrical transducer. Inbound acoustic stimuli mechanically excite sensory cells (the so-called hair cells) which induce action potentials in the auditory nerve. Our interest in this system origins from our long-term goal – developing a computational model of the auditory periphery using a functional approach. We aim to simulate processes along the auditory pathway up to the generation of action potentials in the auditory nerve.

The extracellular environment of a hair cell is not uniform along the cell. The apical part of the cell is surrounded by a fluid called endolymph (~ 150 mM K^+, 13 mM Na^+), the basilar part is surrounded by a fluid called perilymph (~ 4 mM K^+, 140 mM Na^+). The difference in ionic concentration between the two extracellular environments induces an electric potential of –85 mV over the whole hair cell. We will refer to the partial potential drop over the membrane in the basilar part as the receptor potential. The value of the receptor potential depends on the intracellular ionic concentration, which changes in response to acoustic stimuli. The receptor potential plays an important role: it's modulating the conductance of ion channels located in the plasma membrane - for instance, the Cav1.3 calcium channel regulating the synaptic activity of the hair cell.

The electric potential over the cell can be measured in isolated cells quite easily via the patch-clamp technique. However, isolated hair cells are no longer situated in the natural setting of two extracellular environments, which is essential for their function. Measurements in vivo via the patch clamp are nearly impossible. We hypothesize, that the receptor potential is not uniform across the basilar part of the cell membrane as a result of a heterogeneous response in the apical part of the membrane. We think, that the effect is more pronounced due to the rapid mechanic stimulation (e.g. up to 20 kHz in humans). A proper estimation of the receptor potential in the hair cells is essential for precise quantization of the response.

In the present model, ion currents through cells and the respective potentials are modeled via a linear electric circuit -- cell membranes are represented as capacitors, ion channels as resistors. By setting the values of these representative elements (measured experimentally), we can estimate the receptor potential of hair cells in steady state as well as in response to sound stimuli. Since we simulate the cells as if they were in their natural environment (i.e. not extracted like in patch-clamp), we can estimate the in-vivo receptor potential, that is inaccessible by experimental techniques.

Besides the model of ionic currents and receptor potential in the cochlear partition, the present model includes also a model of the cochlear mechanics with an active nonlinear amplification, a multi-step model of neurotransmitter dynamics within inner hair cells, and a model of the afferent nerve fibers. Consequently, we can find correlations between the receptor potential in hair cells and other processes along the auditory pathway. Since all parameters in the model are physiologically well-justified, there is also a potential for "in-silico" experiments of specifically damaged hearing.

Talk 27b – Short

Influence of a transmembrane domain on calcium-membrane interaction

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Calcium plays a significant role in various cellular processes including cell signaling or membrane fusion. Calcium ions have strong interactions with the negatively charged inner leaflet of plasma membrane (PM) where are the target molecules of many signaling pathways. Changes in biophysical properties of phospholipid membranes upon calcium binding were recently investigated (1). We took another step in further understanding the calcium binding to biologically relevant model of PM by incorporating a transmembrane peptide. We designed the peptide as a single transmembrane helix with either zero total charge or with a mild amount of positive charge. The positively charged peptide follows the so called positive-inside rule stating that the excess positive charge of helical TM proteins resides at the cytoplasmic side of the PM. The positive charge is however expected to repel the calcium ions. We use time-resolved emission spectroscopy of lipid vesicles accompanied by zeta potential measurements. The fluorescence technique gives us information on hydration and mobility of molecules in the vicinity of a fluorescent probe whereas the zeta potential shows trends in surface charge on the studied membranes. We found out that both transmembrane peptides indeed rigidify phospholipid bilayers. The positive charge only strenghtens this effect. Surprisingly, the positive charge of the peptide has no influence on calcium binding to membranes which is the same regardless the peptide content.

References:

[1] Melcrova et al. The complex nature of calcium cation interactions with phospholipid bilayers. Scientific Reports. 2016;6(1):38035. Available from: doi: 10.1038/srep38035.

Talk 28 – Normal

Membrane Sensing and Remodelling during the immune synapse.

A spatiotemporal modulation tale of lipid packing, mobility, and collective assembly.

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Lymphocyte T cells are responsible for cell-mediated adaptive immune responses, involving transient interactions of the T-cell receptor (TCR) with peptides presented by MHC proteins. A productive interaction triggers the T-cell signaling forming the immunological synapse (IS). Initially, Lck, a membrane-anchored tyrosine kinase, phosphorylate the TCR, ultimately producing basal plasma membrane microclusters and calcium release. For this purpose, the preferred imaging method to unravel nanoclustering at surface-membrane close contact zones during in-vitro T cell activation is TIRF-based super-resolution imaging (i.e., dSTORM and PALM). Classically, clustering characterisation of the resting (non-activated) state relied in observing T cell onto surfaces coated with Poly-L-lysine; whereas T cells, onto functionalised surfaces coated with anti-CD3, and -CD28 antibodies, resembled the T cell activation during the IS. In the last years, some super-resolution studies suggested protein nanoclustering already at resting state, which contradicts the consensus picture of protein aggregation upon activation. Recently, results from T cells in controlled suspension (immersed in a hydrogel gradient) and later on following its activation highlighted that unnatural cell membrane interactions might hinder our understanding of early T cell activation and the subsequent IS. Whereas these studies focused in the protein clustering and its relation to cortical actin dynamics, they overlooked at the role of the lipids membranes in the early activation and during the IS. Typically, protein clustering is assumed to be coordinated with a higher lateral lipid packing at the membranes. We aim at gaining some knowledge on how lipids would behave in these highly dense localised protein aggregation conditions. For this purpose, to better understand how t cell membranes sense and remodel during the immune synapse, we employ fluorescence imaging correlation spectroscopy based methods and reveal the membrane lipid spatiotemporal localisation, diffusion, and collective motion at the plasma membrane.

Talk 29 – Normal

3D Single-Molecule Localization-Microscopy reveals the nanoscale topology of CD4 monomers on resting T cells

Zuzana Kvíčalová[1], Daniela Glatzová[1,2], Christian Franke[3,4], Tomáš Lukeš[5], Tomáš Chum[1], Theo Lasser[5], Tomáš Brdička[2], Sebastian van de Linde[6] & Marek Cebecauer[1]

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The surface glycoprotein CD4 is essential for maturation of T lymphocytes in thymus and is involved in their activation in peripheral tissues. CD4 enhances T cell responses to suboptimal antigens by stabilising TCR-MHC complex but was also suggested to function as adhesive molecule. We were interested about its nanoscopic localisation on the surface of T cells. Our recently developed quantitative cluster analysis based on SOFI super-resolution imaging demonstrates an intricate nanometer organization of CD4 on resting T cells.[1] The clustering depends on the intact extracellular domain and palmitoylation sites of CD4, since mutants lacking these structural elements exhibit random distribution. To further elucidate CD4 clusters in three-dimensional (3D) space, we developed an advanced version of the recently reported photometric 3D super-resolution method called temporal, radial-aperture-based intensity estimation (TRABI).[2] Utilising a biplane detection scheme and directly measured single-molecule emitter intensities from both axial channels, we were able to map protein distribution on ruffled plasma membrane with 3D nanometer resolution.

We demonstrate that native CD4 accumulates in high density regions which represent 'tips' in the nano-topology of membrane ruffles and microvilli. A mutant of CD4 which cannot be post-translationally palmitoylated randomly covers the ruffled surface of resting T cells.[3] Using fluorescence anisotropy measurements we also demonstrate prevalently monomeric character of CD4 in these structures. Altogether, our data suggests that, similar to TCR [4], monomeric CD4 accumulates on microvilli of resting T lymphocytes, a process which depends on palmitoylation of this co-receptor.

References:

[1] Lukeš T, et al. Quantifying protein densities on cell membranes using super-resolution optical fluctuation imaging. *Nat. Commun.* **8**. 1731 (2017)

[2] Franke C., et al. Photometry unlocks 3D information from 2D localization microscopy data. *Nat. Methods* **14,** 41–44 (2017)

[3] Franke C., …. Cebecauer M. *in preparation* (2018)

[4] Jung et al. Three-dimensional localization of T-cell receptors in relation to microvilli using a combination of superresolution microscopies. *Proc Natl Acad Sci USA* 113, E5916-E5924 (2016)

Talk 30a – Short

Pore-forming colicins behavior in model lipid membranes

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Colicins are protein toxins produced by Escherichia coli which at the same time are toxic to the related strains E. coli. Some of these toxins exhibit their cytotoxic effect while their C-terminal pore-forming domain (CTD) is embedded in bacterial inner lipid membrane. In our work, we study behavior of individual helices from CTD of colicin U embedded in selected lipid bilayers (POPC, POPG, POPE) at the atomistic level. We perform the all-atom molecular dynamics (MD) simulations. Our simulations include also other pore-forming colicins (A, S4, B, N, E1, Ia) and longer segments of pore-forming CTDs as well as the whole CTDs. We focus on an embedding mechanism and stability of the protein fragments in the selected lipid bilayers. Furthermore, pore-forming properties and modulation of lipid bilayer permeability are investigated in atomistic detail. MD simulations are supported by experimental permeability measurements employing Black Lipid Membranes.

Talk 31 – Normal

Cell Penetration and Membrane Fusion: Two Sides of the Same Coin

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Cell penetrating peptides have a unique potential for targeted drug delivery, therefore, mechanistic understanding of their membrane action has been sought since their discovery over 20 years ago. While ATP-driven endocytosis is known to play a major role in their internalization, there has been also ample evidence for the importance of passive translocation for which the direct mechanism, where the peptide is thought to directly pass through the membrane via a temporary pore, has been widely advocated. In this talk, I will question this view and demonstrate that arginine-rich cell penetrating peptides can instead enter vesicles and cells by inducing multilamellarity and fusion, analogously to the action of calcium ions. The molecular picture of this penetration mode, which differs qualitatively from the previously proposed direct mechanism, is provided by molecular dynamics simulations. In addition, the kinetics of vesicle agglomeration and fusion by nonaarginine, nonalysine, and calcium ions are documented in real time by fluorescence techniques and the induction of multilamellar phases in vesicles and cells is revealed both via electron microscopy and fluorescence spectroscopy. We thus show that the newly identified passive cell penetration mechanism is analoguous to vesicle fusion induced by calcium ions, demonstrating that the two processes are of a common mechanistic origin.

Talk 32 – Normal

Bend it like SNAREs: The role of SNARE proteins in the membrane fusion.

Aniket Magarkar[1], Cristoph Allolio[1], Daniel Holy[1], and Pavel Jungwirth[1]

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SNAREs are the core machinery mediating membrane fusion. In this review, we provide an update on the recent progress on SNAREs regulating membrane fusion events, especially the more detailed fusion processes dissected by well-developed biophysical methods and in vitro single molecule analysis approaches. SNAREs are the core machinery mediating membrane fusion. In this review, we provide an update on the recent progress on SNAREs regulating membrane fusion events, especially themore detailed fusion processes dissected by well-developed biophysical methods and in vitro single molecule analysis approaches.

SNAREs protein complex is the core machinery mediating membrane fusion as it is known from in vitro studies. In the present study, we have investigated explicit role of SNAREs complex in membrane remodeling. We have carried out extensive Molecular Dynamics (MD) simulations of SNAREs complex with model cell membrane bilayers. Our simulations reveal that, 1) SNAREs complex induce membrane curvature, which brings together target cell membrane and vesicle membrane, where fusion initiation site is formed. 2) Further novel lipid-nanodisc setup allowed us to investigate the kinetics of the membrane fusion process which shows that membrane fusion occurs significantly faster rate in presence of calcium ions. 3) With step-wise deletion and mutation analysis it was possible to identity the specific amino acids responsible for causing the membrane curvature. Namely there are nine charged amino acid residues which are present at the end of the SNAP-25 of the SNAREs protein complex; once these mutated to alanine, the SNAREs protein complex is no longer able to cause the membrane curvature.

Talk 33a – Short

Energetics of myristoyl insertion and of electrostatic interaction of recoverin and membrane; membrane curvature

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The protein recoverin is a neuronal calcium sensor involved in vision adaptation. Recoverin reversibly associates with cellular membranes via its calcium-activated myristoyl switch. This reversible interaction is vastly dependent on a concentration of calcium ions in the environment and on recoverin's conformation. By using methods of MD simulations and free energy profiles calculations we examine the energetics of myristoyl insertion into a membrane bilayer and electrostatic interactions of non-myristoylated recoverin with a membrane.

The second part of this talk will be focusing on membrane curvature caused by either lipid composition or peptides.

Talk 33b – Short

Does curvature matter? Ion binding at flat and curved lipid bilayers

Daniel Holy[1] and Pavel Jugwirth[1]

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Cells interact with the environment through plasma membranes. Because of their complexity simpler lipid bilayers are use as models to study their properties. An important component which modulates plasma membranes is the calcium ion which is for example involved in a number of processes, such as neurotransmitter release and membrane fusion. Calcium is also known to modulate the physical properties of plasma membranes and to accelerate the process of membrane fusion. Previous research suggests that calcium binds more strongly to positively curved bilayers than to flat ones.

We build on this finding and extend the investigations by using an improved lipid force field for POPC in our simulations. Using this ECC-POPC force filed, which provides a better description of ion-lipid interaction, we characterize the influence that the lipid bilayer shape has on the binding of the calcium ions to the bilayer. To describe the way calcium binds to the bilayer, we first carry out molecular dynamics simulations of flat POPC bilayers at different calcium concentrations and analyze the calcium density distributions in a manner which can be extended to curved systems. After adequately describing the flat bilayer system, we apply the same methodology to bilayers of varying curvatures. We also compare the results of this approach to the calcium density profiles calculated using the instantaneous liquid interface approach. The results of this project will allow us to comment on the mechanisms the cells use to regulate calcium concentration related to curvature of the membrane.

Talk 34 – Normal

Lamellar and inverted hexagonal phases of polyunsaturated monogalactolipid assembly: computer models

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1,2-di-O-acyl-3-O-β-D-galactopyranosyl-sn-glycerol (MGDG) with α-linolenoyl (di-18:3, *cis*) acyl chains is the main lipid component of thylakoid membranes of higher plants and algae. MGDG is a non-bilayer lipid but actually it can form both lamellar and an inverted hexagonal (H_{II}) phases. It is postulated that a transient H_{II} phase in the thylakoid membrane is required for reversible de-epoxidation of violaxanthin to zeaxanthin in one of the xanthophyll cycles.

In the presented study, molecular dynamics (MD) simulations of the fully hydrated di-18:3 MGDG bilayer in the lamellar phase were carried out for 450 ns at 295 K using the GROMACS 4 software package and OPLS-AA force field. The main aim of this study was to validate the computer model of di-18:3 MGDG in the bilayer and also to assess the properties of the bilayer to compare them with those of the inverted hexagonal phase. As the direct validation of the MGDG bilayer was not entirely feasible, an indirect approach was also used. Conformations of the torsion angles in the glycosidic link of the MGDG molecule in the bilayer agreed well with those determined experimentally. The predicted properties of the MGDG bilayer such as A_L, D_{RR} and the shape of S_{CD} profiles were close to those obtained experimentally. Also, the value of the bending rigidity modulus, κ^b, for the MGDG bilayer followed the predicted trend. Thus, we believe that the generated computer model of the MGDG molecule is faithful and can be used to model both the lamellar and non-lamellar phases.

Talk 35 – Normal

How does membrane composition modulate cholesterol carrier protein NPC2?

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Cholesterol efflux, a vital step in its metabolism, is mediated by Niemann-Pick Protein C2 (NPC2) in the late endosomes/lysosomes. Mutations in NPC2 result in Niemann-Pick C disease, in which accumulation of lipids, especially unesterified cholesterol, leads to neuronal degeneration and death. Specific lipids found in the lysosome/late endosomes were shown to affect NPC2-mediated cholesterol transport, but the molecular mechanism of such activity modulation remains elusive. We performed an extensive set of atomistic molecular dynamics simulations and free energy calculations to investigate the effect of relevant lipids on NPC2-membrane binding. Our simulations reveal two distinct membrane binding modes, in which the cholesterol binding pocket faces the membrane (cholesterol-exchange mode) or faces the aqueous phase (idle mode). We show that while anionic lipids can drive unspecific membrane association, the unique anionic lysosomal/late endosomal lipid BMP is required for specific binding in the “cholesterol exchange mode”. Moreover, addition of sphingomyelin shifts the preference towards the “idle mode” even in the presence of BMP. These findings suggest that BMP and sphingomyelin modulates NPC2-mediated cholesterol transport by favoring one of the membrane binding modes.

Talk 36a – Short

Properties of membrane models using electronic continuum correction.

R.Nencini[1], J. Melcer[1], D.Holý[1], S.Ollila[1,2],H. Martinez-Seara[1], P.Jungwirth[1]

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Cell membranes are critical for life as they enable cells to communicate with the environment [1]. Phosphatidilcholines together with cholesterol are the most abundant type of lipids in membranes. For this reason, they are often used to model biological membranes both in experiments and in simulations [2]. One aspect of keen interest to us regarding membranes is the interaction of cations with phospholipids which is of extraordinary physiological relevance. To study such processes using computational methods, we require models which accurately describe binding affinities. The accuracy of binding affinities can be measured by validating computational data of the binding against NMR experiments utilizing electrometer concept [3]. After testing, a number of force-fields it was observed that none of them achieved quantitative and often qualitative agreement with experimental values [3]. We believe that the source of such discrepancy arises for the lack of electronic polarizability in classical molecular models. Interestingly, a mean field correction that accounts for the missing electronic polarizability, named electronic continuum correction (ECC), can be readily applied to biomolecules [4]. During the past years, we have systematically applied ECC to ions [5] and recently to lipids which led to significant improvement of binding affinities of cations to POPC membranes [6].

In this work, we check thoroughly the performance of some structural properties of our modified POPC force field. We extend our approach to another phosphatidylcholine moiety, i.e., DPPC. Our preliminary results, show promising agreement with NMR and scattering experiments. Still, we also find that some reported differences using NMR between DPPC and POPC membranes when binding to cations are not reproduced by our force fields, calling for further refinements.

References:
[1] J. Lombard, Biology Direct, **9**, 32 (2014).
[2] J. P. Jämbeck, A. P. Lyubartsev, Journal of Chemical Theory and Computation, **9**, 774-784 (2012).
[3] A. Catte, M. Girych, et al., Physical Chemistry Chemical Physics, **18**, 32560-32569 (2016).
[4] I. Leontyev, A. Stuchebtukhov, Physical Chemistry Chemical Physics, **13**, 2613-2626 (2011)
[5] T. Martínek, E. Duboué-Dijon, et. al., The Journal of Chemical Physics, 148, 222813, (2013)
[6] J. Melcr, H. Martinez-Seara, et al., The Journal of Physical Chemistry. B, in press (2018)

Talk 36b – Short

Novel implementation of fluorescence anisotropy measurements and its application to studies of membrane proteins in living cells

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Fluorescence microscopy techniques are widely used to study protein-protein and protein-ligand interactions. Here we present a novel approach to measurements of fluorescence anisotropy and homoFRET and discuss its applications to studies of membrane proteins in living cells.