5th European Joint
Theoretical/Experimental
Meeting on Membranes

EJTEMM2017

6-8 December, 2017
Kraków, Poland

JAGIELLONIAN UNIVERSITY
IN KRAKÓW

Contents

Welcome from the Organisers

Dear Colleagues,

It is with great pleasure that we welcome you to Kraków for the 5th European Joint Theoretical/Experimental Meeting on Membranes – EJTEMM2017. We are happy that 78 membrane scientists from 14 countries are meeting in Kraków to share their knowledge, learn, discuss, and present the results of their research.

The Meeting provides an active forum for exchanging expertise and to create personal contacts between experimentalists, computer modellers and theoreticians with the expectation that a better understanding of methodological approaches and limitations will trigger closer collaboration among researchers in solving problems related to membrane studies.

We believe that the diverse range of oral and poster presentations will be inspiring for all participants. We thank the speakers, those who have contributed posters and attendees for coming to the Meeting, and especially the keynote and invited speakers for accepting our invitation despite financial restrictions.

We wish you all an enjoyable time in Kraków and a fruitful and stimulating conference.

Marta Pasenkiewicz-Gierula

Organisers

Local Organising Committee

Marta Pasenkiewicz-Gierula – chair of the committee
Department of Computational Biophysics and Bioinformatics
Faculty of Biochemistry, Biophysics and Biotechnology
Jagiellonian University

e-mail: marta.pasenkiewicz-gierula@uj.edu.pl
tel.: +48 12 664 65 18

Bożena Milanović – secretariat and financial matters
Magdalena Tworzydło – coordinator, layout
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Acknowledgements/Sponsors

The Meeting is organised under the auspices of the Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, who have generously subsidised EJTEMM2017 from KNOW funds.

We gratefully acknowledge the support of the Polish Academy of Sciences (PAN) and the Polish Biochemical Society (PTBioch).

Special thanks are due to Professor Zygmunt Wasylewski Foundation (FZW) for funding the award for the Students' Oral Presentation and Poster Competition.

We thank our two great Sponsors – NanoTemper Technologies Sp. z o.o. and Selvita S.A. – for their support that enabled us to organize the Meeting.

General information

Date and location
The 5th European Joint Theoretical/Experimental Meeting on Membranes – EJTEMM2017 will take place from 6th to 8th December 2017 at the Jagiellonian University Przegorzały Conference Center/Guest House, Jodłowa Street 13, Kraków, Poland.

Language
The official language of EJTEMM2017 is English.

Registration desk
The registration desk will be situated just inside the Guest House entrance hall (see map nr 1). The desk will be open between 11.00 and 18.30 on Wednesday 6th December. Those participants who arrive after 18.30 will be able to check in on Thursday 7th December from 8.00 to 10.30 am.

Hotel accommodation
Payment for accommodation in the Przegorzały Guest House will be handled by the hotel during check-in (or check-out). The hotel accepts cash, credit and debit cards (VISA, MasterCard).

Badges
We kindly request all EJTEMM2017 participants to wear their identification badges throughout the meeting. Members of the organising committee can be recognised by green badges.

Posters
The poster session will take place on Wednesday 6th December between 19.45 and 22.30. Posters taking part in the Students' Poster Competition will be exhibited in Room F; all the other posters will presented in the Aula entrance hall (see floor plan nr 2).

Posters should be mounted on Wednesday no later than 18.30 (we encourage participants to mount their posters immediately after registration) and taken down after the EJTEMM2017 closing ceremony on Friday.

The numbers found in the overview of the poster presentations (see pages 19-23) correspond to the numbers on the poster boards.

Oral presentations
All plenary lectures will take place in Aula (see Floor plan nr 2).

- The time allocated for each keynote presentation is limited to 30 minutes, plus 10 minutes of discussion (40 min in total).
- The time allocated for each PhD student presentation participating in the student competition is limited to 20 minutes, plus 5 minutes of discussion (25 min in total).
- The time allocated for presentations by other speakers is limited to 25 minutes, plus 5 minutes of discussion (30 min in total).

Coffee breaks
Coffee, tea and snacks will be served in the exhibition area near Aula.

Dinner and lunch
All lunches and dinner on Wednesday will be served in hotel's two-story restaurant.

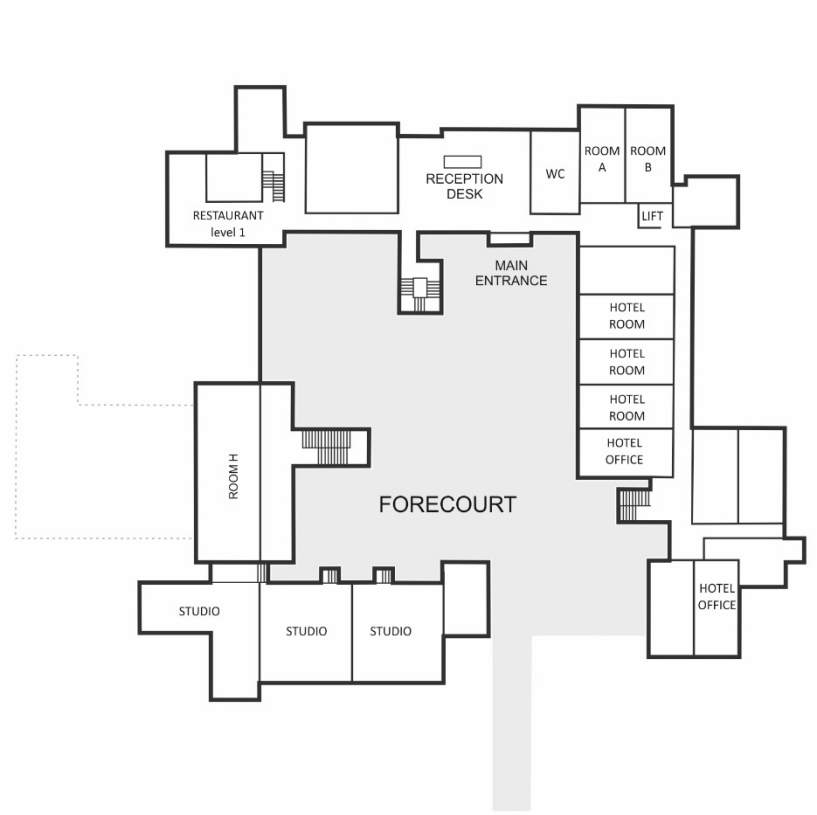
Banquet
The conference dinner will be held at the Szara Gęś Restaurant located on the picturesque Market Square (*Rynek Główny* 17) on Thursday evening (7th December).

The organisers will provide shuttle transport (4 minibuses) from Przegorzały Conference Centre to the Market Square and back. Participants are kindly requested to gather in the Przegorzały Guest House forecourt at 19.30 and occupy the seats in the buses allocated by the organisers (see Banquet invitation).

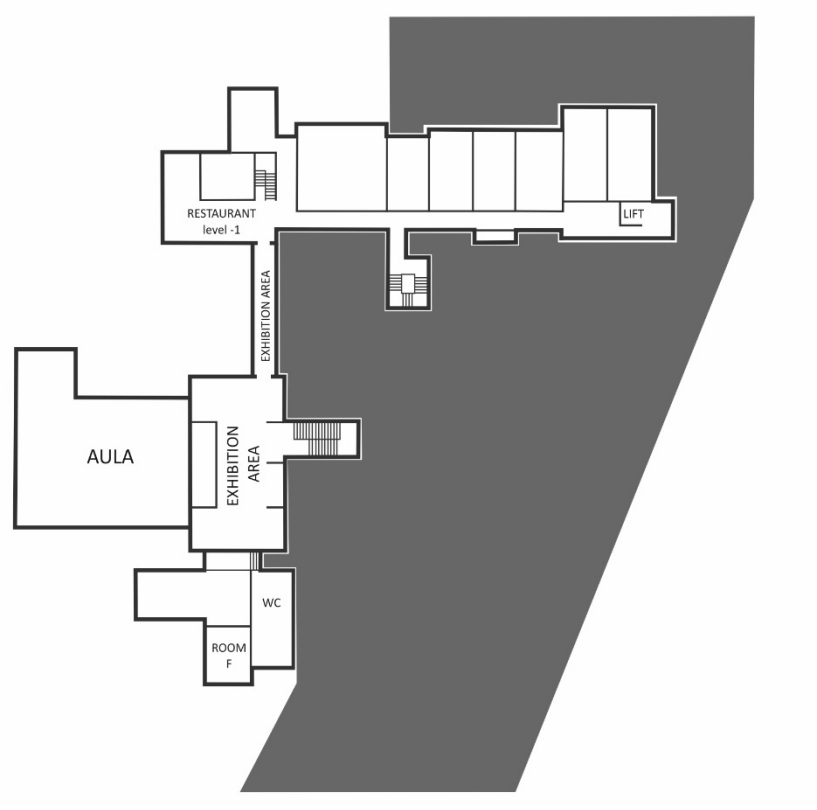
Announcements
Announcements will be displayed in Aula between sessions.

Liability
The organisers cannot be held responsible for damage, loss or theft during the conference.

Floor plans



Floor plan nr 1.
Ground floor of the Przegorzały Conference Center/Guest House



Floor plan nr 2.
Underground floor of the Przegorzały Conference Center/Guest House

Programme

Programme at a glance

Wednesday, 6 December	**Thursday, 7 December**	**Friday, 8 December**
11.00-18.30 REGISTRATION	07.30-09.00 BREAKFAST	07.30-09.00 BREAKFAST
13.00-14.00 LUNCH	09.00-10.45 **SESSION 2** M. Hof G. Pabst S.S. Iyer	09.00-10.10 **SESSION 6** I. Vattulainen G. Kneller
14.15-14.30 **EJTEMM OPENING**		10.10-10.25 COFFEE BREAK
14.30-15.50 **SESSION 1** O.G. Mouritsen W.K. Subczyński	10.45-11.00 PHOTOGRAPHY	10.25-11.20 **SESSION 5 cont.** W. Gruszecki I. Ermilova
15.50-16.20 COFFEE BREAK	11.00-11.15 COFFEE BREAK	
16.20-17.55 **SESSION 1 cont.** A. Kusumi H. Khandelia D. Przystupski	11.15-12.15 **SESSION 3** A. Wiśniewska-Becker B. Milanović	11.20-12.20 **SESSION 7** K. Riske F. Di Meo
17.55-18.20 **SPONSOR SESSION**	12.15-13.15 **SESSION 4** W. Kulig A. Olżyńska	12.20-12.35 COFFEE BREAK
18.30-19.45 DINNER	13.15-14.45 LUNCH	12.35-13:55 **SESSION 8** S. Knippenberg B. Chantemargue M.T. Abdelwahab
19.45-22.30 **POSTER SESSION**	14.45-16.15 **SESSION 4 cont.** M. Tarek Ł. Ćwiklik P. Jurkiewicz	13:55-14:35 **EJTEMM CLOSING**
	16.15-16.35 COFFEE BREAK	14:35-16:00 LUNCH
	16.35-18.05 **SESSION 5** N. Kučerka D. Uhríková H. Martinez-Seara	
	18.05-18.30 **CONFERENCE SPECIAL** O.G. Mouritsen	
	19:30 DEPARTURE TO MARKET SQUARE	
	20.00-22.30 BANQUET	
	22.30-23.00 RETURN FROM MARKET SQUARE	

WEDNESDAY, 6. DECEMBER

11.00-18.30	REGISTRATION (Hotel entrance hall)
13.00-14.00	LUNCH (Hotel restaurant)
14.15-14.30	**CONFERENCE OPENING** (Aula)
	SESSION 1: Membranes, membrane components and membrane functions (Aula) Chair – **Marta Pasenkiewicz-Gierula**, Jagiellonian University, Poland
14.30-15.10	Higher sterols – a prerequisite for higher life? Keynote speaker – **Ole G. Mouritsen**, University of Copenhagen, Denmark
15.10-15.50	Biological evolution selected membranes with a particular cholesterol content to perform certain functions in the cells of eukaryotic organisms Keynote speaker – **W. Karol Subczyński**, Medical College of Wisconsin, USA
15.50-16.20	COFFEE BREAK (Aula entrance hall)
	SESSION 1 cont.: Membranes, membrane components and membrane functions (Aula) Chair – **Ole G. Mouritsen**, University of Copenhagen, Denmark
16.20-17.00	Signal transduction by transient molecular complexes: findings by single-molecule tracking Keynote speaker – **Akihiro Kusumi**, Okinawa Institute of Science and Technology, Japan
17.00-17.30	About simulations of the Zika virus, and membrane bending by electrostatic potentials (flexo-electricity) Invited speaker – **Himanshu Khandelia**, University of Southern Denmark, Denmark
17.30-17.55	Membranes protection by freezing medium in cells exposed to variable temperature, pressure, overload and radiation in the stratosphere Students competition – **Dawid Przystupski**, Wroclaw Medical University, Poland
	SPONSOR SESSION (Aula) Chair – **Anna Wiśniewska-Becker**, Jagiellonian University, Poland
17.55-18.20	When protein matters **Jakub Nowak**, NanoTemper Technologies
18.30-19.45	DINNER (Hotel restaurant)
	POSTER SESSION (Aula hall/room F)
19.45-21.00	Students'/PhD students' poster competition (room F)
19.45-22.30	Poster session with beer and wine (Aula hall)
	WORKING MEETING (Aula)
20.15-21.00	EJTEMM Advisory Board meeting

THURSDAY, 7. DECEMBER

07.30-09.00	BREAKFAST (Hotel restaurant)
	SESSION 2: Membrane organisation (Aula) Chair – **Norbert Kučerka**, Comenius University, Slovakia
09.00-09.40	Lipid driven nanodomains are fluid Keynote speaker – **Martin Hof**, Czech Academy of Sciences, Czech Republic
09.40-10.20	Asymmetric membranes: The difference it makes Keynote speaker – **Georg Pabst**, University of Graz, Austria
10.20-10.45	Identifying liquid-liquid phase coexistence: Insights from local non-affine deformation and topological rearrangements Students competition – **Sahithya S. Iyer**, Indian Institute of Science, India
10.45-11.00	CONFERENCE PHOTOGRAPHY (Aula)
11.00-11.15	COFFEE BREAK (Aula entrance hall)
	SESSION 3: Lipid nanostructures (Aula) Chair – **Alex Bunker**, University of Helsinki, Finland
11.15-11.45	Nanodiscs vs liposomes – which model of biological membranes is more relevant? Invited speaker – **Anna Wiśniewska-Becker**, Jagiellonian University, Poland
11.45-12.15	Structural studies of nanodiscs from molecular dynamics and spectral simulations Invited speaker – **Bożena Milanović**, Jagiellonian University, Poland
	SESSION 4 – Lipid oxidised forms (Aula) Chair – **Piotr Jurkiewicz**, **Łukasz Ćwiklik**, Czech Academy of Sciences, Czech Republic
12.15-12.45	Presence of oxysterols affects permeability of lipid bilayers Invited speaker – **Waldemar Kulig**, University of Helsinki, Finland
12.45-13:15	How does the presence of oxysterol change biophysical properties of model membranes – experimental studies Invited speaker – **Agnieszka Olżyńska**, Czech Academy of Sciences, Czech Republic
13.15-14.45	LUNCH (Hotel restaurant)
	SESSION 4 cont. – Lipid oxidised forms (Aula) Chair – **Piotr Jurkiewicz**, **Łukasz Ćwiklik**, Czech Academy of Sciences, Czech Republic
14.45-15.15	Reactive oxygen species action on cell membranes: Unraveling a potential mechanism of electroporation in the biological context using Molecular Simulations **Mounir Tarek**, University of Lorraine, France

15.15-15.45	Oxidized phospholipid membranes as seen via molecular simulations Invited speaker – **Łukasz Ćwiklik**, Czech Academy of Sciences, Czech Republic
15.45-16.15	Oxidized phospholipid – fluorescence spectroscopy Invited speaker – **Piotr Jurkiewicz**, Czech Academy of Sciences, Czech Republic
16.15-16.35	COFFEE BREAK (Aula entrance hall)
	SESSION 5: Membrane modifiers (Aula) Chair – **Burkhard Bechinger**, University of Strasbourg, France
16.35-17.05	Interactions in the pre-AD mimicking model membranes Invited speaker – **Norbert Kučerka**, Comenius University, Slovakia
17.05-17.35	Divalent metal cations as a mediator of DNA-phospholipid bilayer binding Invited speaker – **Daniela Uhríková**, Comenius University, Slovakia
17.35-18.05	Determinants of sodium and calcium adsorption onto neutral lipid bilayers **Hector Martinez-Seara**, Czech Academy of Sciences, Czech Republic
	CONFERENCE SPECIAL (Aula) Chair – **Karin Riske**, Universidade Federal de São Paulo, Brazil
18.05-18.30	The science of deliciousness **Ole G. Mouritsen**, University of Copenhagen, Denmark
19.30	DEPARTURE FROM PRZEGORZAŁY TO MARKET SQUARE (Przegorzały hotel forecourt)
20.00-22.30	BANQUET (Szara Gęś restaurant, Market Square 17)
22.30-23.00	RETURN FROM MARKET SQUARE TO PRZEGORZAŁY

FRIDAY, 8. DECEMBER

07.30-09.00	BREAKFAST (Hotel restaurant)
	SESSION 6: Diffusion of membrane components (Aula) Chair – **Martin Hof**, Czech Academy of Sciences, Czech Republic
09.00-09.30	Diffusion of lipids and transmembrane proteins in membranes – from dilute conditions to crowding Invited speaker – **Ilpo Vattulainen**, University of Helsinki, Finland
09.30-10.10	Anomalous lateral diffusion of lipid molecules in lipid bilayers Keynote speaker – **Gerald Kneller**, University of Orleans/CNRS, France
10.10-10.25	COFFEE BREAK (Aula entrance hall)
	SESSION 5 cont.: Membrane modifiers (Aula) Chair – **W. Karol Subczyński**, Medical College of Wisconsin, United States

10.25-10.55	Xanthophylls in membranes Invited speaker – **Wiesław Gruszecki**, Maria Curie-Sklodowska University, Poland
10.55-11.20	Studying the behaviour of cholesterol in different phospholipid bilayers: combining classical molecular dynamics with 2-D metadynamics simulations on a microseconds' time-scale Students competition – **Inna Ermilova**, Stockholm University, Sweden
	SESSION 7 – Membrane transport (Aula) Chair – **Daniela Uhríková**, Comenius University, Slovakia
11.20-11.50	Edge tension of anionic membranes **Karin Riske**, Universidade Federal de São Paulo, Brazil
11.50-12.20	Drug membrane permeation: Towards semi-quantitative structure permeation relationship **Florent Di Meo**, University of Limoges, France
12.20-12.35	COFFEE BREAK (Aula entrance hall)
	SESSION 8 – Methods (Aula) Chair – **Patrick Trouillas**, University of Limoges, France
12.35-13.05	Atomistic pictures of fluorescent probes in lipid bilayer membranes enhance lipid phase recognition through combined (non-) linear optical and fluorescence analyses Invited speaker – **Stefan Knippenberg**, Royal Institute of Technology, Sweden
13.05-13.30	Exploration of landscapes of ABC membrane exporter Students competition – **Benjamin Chantemargue**, University of Limoges, France
13.30-13.55	Chemical compositions of ordered and disordered domains in phase-separated lipid membranes via CARS microscopy Students competition – **M. Tarek Abdelwahab**, Max Planck Institute for Polymer Research, Germany
13:55-14:25	**CLOSING CEREMONY** (Aula)
14:25-14:35	**ANNOUNCEMENT OF THE WINNERS OF THE STUDENTS' ORAL PRESENTATION AND POSTER COMPETITION** (Aula)
14:35-16:00	LUNCH (Hotel restaurant)

Posters overview

A1 Christopher **Aisenbrey**
Packing of peptides on the surface of lipid membranes
CNRS UMR 7177, Membrane Biophysics and NMR, Faculté de Chimie, Université de Strasbourg, France

A2 Borislav **Angelov**
Organization of TrkB neutrophin receptors in membranes revealed by STED microscopy
Institute of Physics, ELI Beamlines, Academy of Sciences of the Czech Republic, Prague, Czech Republic

A3 Neha **Awasthi**
Geometric shape of lipids versus molecular interactions in membrane pore formation
Department of Structural Molecular Biology, Georg August University, Goettingen, Germany

A4 Karel **Berka**
What drives drug-membrane interactions?
Faculty of Science, Palacky University Olomouc, Czech Republic

A5 Alex **Bunker**
Substrate differentiation between isoforms of Catechol-O-methyltransferase
Faculty of Pharmacy, University of Helsinki, Finland

A6 Kamila **Butowska**
Assessing caffeine impact on cell membrane integrity
Intercollegiate Faculty of Biotechnology UG-MUG, Gdańsk, Poland

A7 Alessia **Centi**
Free energy calculations to understand lipid domain reorganisation by small molecules
Max Planck Institute for Polymer Research, Mainz, Germany

A8 Paweł **Chodnicki**
Unveiling the driving forces of lipid raft formation by means of molecular dynamics
Department of Physical Chemistry, Gdansk University of Technology, Poland

A9 Anna **Choromańska**
Modulation of glucose transporter type 1 (GLUT1) by WZB117 and electroporation in melanoma cells to increase membrane permeability for oat β-glucan
Department of Medical Biochemistry, Wroclaw Medical University, Poland

A10 Aleksander **Czogalla**
How different species of phosphatidic acid behave in model lipid monolayers and bilayers
Faculty of Biotechnology, Uniwersity of Wroclaw, Poland

A11 Pauline **Delcroix**
Towards realistic models of lung surfactant – MD simulations with improved water and ion force fields
J. Heyrovsky Institute of Physical Chemistry, Prague, Czech Republic

A12 Joanna **Juhaniewicz-Dębińska**
On the influence of antimicrobial peptides on model biological membranes – Langmuir monolayer, QCM-D and AFM studies
Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Poland

A13 Mariusz **Kępczyński**
Polycation-lipid membrane interaction – experimental and MD simulations studies
Faculty of Chemistry, Jagiellonian University, Kraków, Poland

A14 Julita **Kulbacka**
Cell membrane permeabilization in glioma cells to support enhanced drug delivery
Department of Medical Biochemistry, Wroclaw Medical University, Poland

A15 Urszula **Kwolek**
Effect of phosphatidic acid on zwitterionic lipid membrane: experimental and molecular dynamics simulations study
Faculty of Chemistry, Jagiellonian University, Kraków, Poland

A16 Jadwiga **Maniewska**
The interaction of new oxicams derivatives with lipid bilayers as measured by calorimetry and fluorescence spectroscopy
Department of Chemistry of Drugs, Wroclaw Medical University, Poland

A17 Roberto **Menichetti**
Computational high-throughput screening of drug-membrane thermodynamics
Max Planck Institute for Polymer Research, Mainz, Germany

A18 Łukasz **Nierzwicki**
How γ-secretase bind substrates: conformational dynamics of the enzyme active site and substrate binding pathways for the amyloid precursor protein
Department of Physical Chemistry, Gdansk University of Technology, Poland

A19 Lukáš **Opálka**
The effects of omega-*O*-acylceramides on microstructure and permeability of model skin lipid membranes
Faculty of Pharmacy, Charles University, Hradec Kralove, Czech Republic

A20 Silvio **Osella**
Triggering on/off states of photoswitchable probes in biological environments
Centre of New Technologies, University of Warsaw, Poland

A21 Markéta **Paloncýová**
Effect of lipid composition on membrane immersion of cytochrome P450 3A4
Regional Centre of Advanced Technologies and Materials, Faculty of Science, Palacky University in Olomouc, Czech Republic

A22 Łukasz **Peplowski**
Interactions of carbon nanotubes with a model cell membrane – steered molecular dynamics studies
Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University, Toruń, Poland

A23 Katia **Perez**
Interaction of the antimicrobial peptide ocellatin pt7 with biomemetical membranes
Department of Biophysics, Federal University of São Paulo, Sao Paulo, Brazil

A24 Kamila **Riedlová**
Structure of pore-forming colicins in POPC membrane
J. Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences, Prague, Czech Republic
Faculty of Science, Charles University, Prague, Czech Republic

A25 Tomasz **Róg**
Can one use DPH to probe the behavior of itraconazole in lipid membrane systems?
Department of Physics, University of Helsinki, Finland
Laboratory of Physics, Tampere University of Technology, Finland

A26 Elżbieta **Rudolphi-Skórska**
Antioxidant protection of cell membranes from ozone-induced stress
Biophysics and Biotechnology Institute of Biology, Pedagogical University of Cracow, Poland

A27 Radek **Šachl**
Oligomerization of the protein FGF2 at the lipid membrane leads not always to pore formation
J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic

A28 Jakub **Suchodolski**
New application of Laurdan and di-4-ANEPPS in a study of *Candida albicans*' plasma membrane biophysics
Faculty of Biotechnology, University of Wroclaw, Poland

A29 Robert **Vácha**
Peptide translocation across phospholipid membranes
CEITEC, Masaryk University, Brno, Czech Republic
Faculty of Science, Masaryk University, Brno, Czech Republic

Students/PhD students Poster Competition

B1 Agnieszka **Borowik**
C60 fullerene impact on cell membranes integrity
Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Poland

B2 Anna **Chmielińska**
Myelin model for studying membrane active compounds
Faculty of Biochemistry Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

B3 James **Goodchild**
Substrate support for supported lipid bilayers affects domain mobility and phase behaviour
Department of Physics and Astronomy, University of Leeds, United Kingdom

B4 Ewa **Grela**
Study of interaction of an antifungal antibiotic amphotericin B with lipid membranes based on fluorescence anisotropy
Institute of Physics, Maria Curie-Sklodowska University, Lublin, Poland

B5 Ivo **Kabelka**
Optimizing peptide properties for translocation across lipid membranes
CEITEC and Faculty of Science, Masaryk University, Brno, Czech Republic
Faculty of Science, Masaryk University, Brno, Czech Republic

B6 Dorota **Konarzewska**
Interactions of short synthetic lipopeptide with model membranes containing phospatidylcholine and phosphatidyl-serine
Faculty of Chemistry, University of Warsaw, Poland

B7 Fabio **Lolicato**

A computational study on how cholesterol and PI(4,5)P_2 trigger oligomerization of FGF2 on the membrane surface

Department of Physics, University of Helsinki, Finland
Department of Physics, Tampere University of Technology, Finland

B8 Krzysztof **Makuch**

Molecular dynamics studies of lutein and zeaxanthin molecules in the phospholipid bilayer

Faculty of Biochemistry Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

B9 Olga **Michel**

Enhancing the effectiveness of electrochemotherapy – in vitro study with green tea catechin on sensitive and resistant pancreatic cancer

Department of Medical Biochemistry, Wroclaw Medical University, Poland

B10 Veronika **Navrátilová**

The role of distal mutation that alters rat CYP1A1 activity towards persistent organic pollutants

Regional Centre of Advanced Technologies and Materials, Faculty of Science, Palacký University Olomouc, Czech Republic

B11 Tomasz **Pieńko**

The role of BtuB dynamics in the transport of vitamin B_{12} through *E. coli* outer membrane

Centre of New Technologies, University of Warsaw, Poland
Faculty of Pharmacy with the Laboratory Medicine Division, Medical University of Warsaw, Poland

B12 Martin **Šrejber**

Cytochrome P450 reductase Simulations: Conformation changes and cytochrome P450 complex

Regional Center of Advanced Technologies and Materials, Faculty of Science, Olomouc, Czech Republic

B13 Pablo Andrés **Zambrano**

An in vitro study of the effects of labetalol on human erythrocytes and molecular models of cells membranes

Faculty of Chemical Sciences, University of Concepción, Chile
Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland

Oral presentation abstracts

The abstracts for oral presentations are presented in the same order as in the conference programme.

Higher sterols – a prerequisite for higher life?

O.G. Mouritsen

Department of Food Science, Faculty of Science, University of Copenhagen, Denmark

Higher sterols are universally present in large amounts (20-30%) in the plasma membranes of all eukaryotes whereas they are universally absent in prokaryotes. It is remarkable that each kingdom of the eukaryotes has chosen, during the course of evolution, its preferred sterol: cholesterol in animals, ergosterol in fungi and yeast, phytosterols in higher plants, and e.g., fucosterol and desmosterol in algae. The question arises as to which specific properties do sterols impart to membranes and to which extent do these properties differ among the different sterols. Using a range of biophysical techniques, we have found that fucosterol and desmosterol, found in red and brown macroalgae (seaweeds), similar to cholesterol support liquid-ordered membrane phases and induce coexistence between liquid-ordered and liquid-disordered domains in lipid bilayer. Fucosterol and desmosterol induce acyl-chain order in liquid membranes, but less effectively than cholesterol and ergosterol in the order: cholesterol > ergosterol > desmosterol > fucosterol, possibly reflecting the different molecular structure of the sterols at the hydrocarbon tail.

REFERENCES:

[1] Life – As a Matter of Fat: Lipids in a Biophysics Perspective (O. G. Mouritsen and L. A. Bagatolli) *Springer*, New York (2016).

[2] Small-scale structure in fluid cholesterol-lipid bilayers (M. C. Rheinstädter and O. G. Mouritsen) Curr. Opin. *Colloid Int. Sci*. 18, 440-447 (2013).

[3] A new outlook on organization of lipids in membranes: searching for a realistic connection with the organization of biological membranes (L. A. Bagatolli, J. H. Ipsen, A. C. Simonsen, and O. G. Mouritsen) *Prog. Lip. Res*. 4, 378-389 (2010).

[4] Spatial distribution and activity of Na/K-ATPase in lipid bilayer membranes with phase boundaries (T. Bhatia, F. Cornelius, J. Brewer, L. A. Bagatolli, A. C. Simonsen, J. H. Ipsen, and O. G. Mouritsen) *Biochim*. *Biophys. Acta* 1858, 1390-1399 (2016).

[5] Effects of seaweed sterols fucosterol and demosterol on lipid membranes (O. G. Mouritsen, L. A. Bagatolli, L. Duelund, O. Garvik, J. H. Ipsen, and A. C. Simonsen) *Chem. Phys. Lipids* 205, 1-10 (2017).

Biological evolution selected membranes with a particular cholesterol content to perform certain functions in the cells of eukaryotic organisms

W.K. Subczyński[1], M. Pasenkiewicz-Gierula[2]

[1]Department of Biophysics, Medical College of Wisconsin, Milwaukee, USA
[2]Department of Computational Biophysics and Bioinformatics, Jagiellonian University, Kraków, Poland

We assert that lipid composition diversity determines the membrane properties that allow the membranes to perform certain cellular functions. Cholesterol (Chol) plays a major role in this determination. Thus, we will concentrate mainly on the role of Chol in modifying the main properties (such as fluidity, permeability, and hydrophobicity [dielectric properties]) of the lipid bilayer of biological membranes, and also on Chol's role in the formation of separated phases and domains in the bilayer. The lateral organization of membranes is crucial in regulating a variety of cellular functions. We hypothesize that, during biological evolution, membranes with a particular Chol content were selected to perform certain functions in the cells of eukaryotic organisms. This hypothesis is in agreement with the long-standing opinion that the Chol content in the eukaryotic cell membranes increases along the secretory pathway, being very low in the endoplasmic reticulum, higher in the Golgi apparatus, and highest in the plasma membrane. The effects of Chol on the phospholipid bilayer will be illustrated with bilayers made of dimyristoylphosphatidylcholine and Chol, which form one of the simplest paradigms for the study of formation, coexistence, and separation of phases and domains. Our EPR spin-labeling studies of lipid and biological membranes, supported in some places by our molecular modeling studies, resulted in the major findings discussed in this presentation.

ACKNOWLEDGEMENTS:
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Signal transduction by transient molecular complexes: findings by single-molecule tracking

A. Kusumi

Membrane Cooperatively Unit, Okinawa Institute of Science and Technology (OIST), Onnason, Okinawa, Japan

Single-molecule imaging and tracking of molecules in living cells provide researchers with the unprecedented ability to directly observe molecular dynamics and interactions in/on the cell membrane (for a review, see, for example, [1]). My group is primarily responsible for advancing single-molecule observations in living cells and high-speed single-molecule imaging at time resolutions up to 20 μs.

The knowledge gained by single-molecule technologies is now revolutionizing our understanding of the molecular dynamics, structure, and signal transduction mechanisms that occur in the cell membrane. This is because these technologies allow us to "directly see" how individual molecules in the plasma membrane jostle around, collide with, bind to, and dissociate from each other, and how they become assembled, organized, and disengaged, right in front of our eyes, as if we are watching a group of ballet dancers in the theatre.

We have recently found that signal transduction in the plasma membrane is often enabled by very transient molecular interactions, rather than by stable molecular complexes and the solid-state-like circuits they produce, at variance with the prevalent views shown in many cell-biology and biochemistry textbooks and reviews. I plan to talk about these recent results in my presentation.

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About simulations of the Zika virus, and Membrane bending by electrostatic potentials (flexo-electricity)

D. Bruhn[1], C. Wewer[1] and H. Khandelia[1]

[1]MEMPHYS: Center for Biomembrane Physics and the Department of Physics Chemistry and Pharmacy, University of Southern Denmark, Odense, Denmark

I will talk about first: large-scale molecular dynamics (MD) simulations of the entire Zika virus particle aiming to determine why the virus is more stable and can survive in bodily fluids for a much longer time after initial infection. We will compare the footprints of the Dengue and Zika viruses in the viral lipidome. The second part of the talk will revisit flexo-electricity: the coupling between membrane potential and curvature in lipid bilayers. Using coarse-grained simulations, we show that application of electrical potentials across a POPC lipid bilayer causes the membrane to bend, and this can have appreciable consequences in several cellular and model lipid systems [2]. We also show how the flexoelectric effect is manifest in the tubulation of giant unilamellar vesicles induced by calcium ions [1].

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Membranes protection by freezing medium in human cells exposed to variable temperature, pressure, overload and radiation in the stratosphere

D. Przystupski[1], B. Ziętek[2], A. Choromańska[1], O. Michel1, J. Kulbacka[1]

[1]Department of Medical Biochemistry, Wroclaw Medical University, Wrocław, Poland
[2]Faculty of Mechanical Engineering, Wroclaw University of Science and Technology, Wrocław, Poland

The aim of the study was the verification how the preservation of biological membranes can be affected by the external environment. The experiment was carried out to send a stratospheric balloon to a height of 35 km above the surface of the Earth with human gingival fibroblasts, SKOV-3 and CHO cells. In this way, the research material got into the stratosphere, where the low temperature, pressure and UV radiation levels are similar to these currently presenting on the surface of Mars. This enabled the determination of the effect of subcosmic conditions on the functioning of human cells, in particular on the continuity of biological membranes under extreme conditions. There was examined whether the type of freezing medium (DMSO+FBS; Bambanker®; sucrose solution) in which the cells were suspended effects on the cell membrane properties and whether preincubation with various antioxidants protect membranes from damage and disintegration in the stratosphere. The results were compared with the data obtained from laboratory-simulated low temperature effects on cell membranes in vitro, which revealed that cells from different tissues respond differently to subcosmic conditions. Significant differences in the results after laboratory simulation and after the balloon flight indicate that various parameters of the extra-terrestrial environment – the pressure, radiation and the overload associated with the balloon flight have a significant effect on the cell membranes.

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Lipid driven nanodomains are fluid

R. Šachl[1], M, Amaro[1], A. Koukalová[1], L.Veľas[1], G. Gröbner[2], P.T.F. Williamson[3], M. Hof[1]

[1]J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[2]Department of Chemistry, University of Umeå, Sweden
[3]Centre for Biological Sciences/Institute for Life Sciences, University of Southampton, United Kingdom

It is a fundamental question whether sphingomyelin (SM)- and cholesterol (Chol)- driven nanodomains exist in cells and in model membranes. Studies on model membranes revealed SM and Chol driven micrometer-sized liquid-ordered domains. Although the existence of such microdomains has not been proven for the plasma membrane, such lipid mixtures have been used as a model system for 'rafts'. On the other hand, super resolution results indicate that the plasma membrane might organize into nanocompartments. However, due to the limited resolution of those techniques their unambiguous characterization is still missing. In this lecture, a combination of Förster resonance energy transfer and Monte Carlo simulations (MC-FRET) [1] identifies directly 10 nm large nanodomains in liquid-disordered model membranes composed of lipid mixtures containing SM and Chol [2]. Our MC-FRET approach can determine the sizes and concentrations of nanodomains down to 2 nm and enables studying the nanodomain inter-leaflet coupling. Combining MC-FRET with solid-state wide-line and high resolution magic angle spinning NMR as well as with fluorescence correlation spectroscopy [3] we demonstrate that these nanodomains containing hundreds of lipid molecules are fluid [2]. Addition of GM1 ganglioside, a molecule which forms already fluid 6 nm sized clusters in fluid phosphatidylcholine bilayers [4,5], leads to growth of those nanodomains while preserving the fluidity [5]. Similarly, addition of oxidized phospholipids increases the size of the nanodomains. Our MC-FRET approach indicates that the latter nanodomains are registered in between the leaflets.

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Asymmetric membranes: The difference it makes

G. Pabst[1,2]

[1]Institute of Molecular Biosciences, Biophysics Division, NAWI Graz, University of Graz, Austria
[2]BioTechMed-Graz, Graz, Austria

Biological membranes are asymmetric including the distribution of membrane lipids. Of recent, asymmetric large unilamellar lipid vesicles (aLUVs) have emerged as new platforms to study fundamental membrane biophysical issues by the application of a broad variety of experimental techniques [1]. We have focused primarily on neutron and X-ray scattering techniques, solution NMR or differential scanning calorimetry to interrogate leaflet specific structural properties of aLUVs on the sub-nanometer scale [2,3]. In particular, this allowed us to address transbilayer coupling and passive lipid flip/flop. Regarding the latter issue we found slow lipid flip/flop in the fluid phase and none in the gel phase. However, membrane defects associated e.g. with the lipid's melting transition caused an increase of lipid translocation. Regarding transbilayer coupling, we observed effects so far only for coexisting gel and fluid phases, but not for all-fluid bilayers. For DPPC/POPC aLUVs, for example, DPPC-enriched gel domains in the outer leaflet were significantly disordered by a coexisting fluid inner leaflet enriched in POPC. In the case of POPE/POPC aLUVs, we found transbilayer coupling when POPE was enriched on the inner leaflet, but not for the reversed system. Hence, transbilayer coupling depends strongly on lipid composition and in the case of DPPC/POPC most likely on partial hydrocarbon chain interdigitation, whereas intrinsic lipid curvature apparently dominates the coupling of leaflets in POPE/POPC aLUVs.

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Identifying liquid-liquid phase coexistence: Insights from local non-affine deformation and topological rearrangements

S.S. Iyer, M. Tripathy, A. Srivastava

Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India

We quantify the degree of topological rearrangements in heterogeneous lipid membranes to identify liquid-liquid phase co-existence. By simply measuring the local non-affine deformation around every lipid, we are able to characterize liquid-ordered (Lo) and liquid-disordered (Ld) phases in model lipid bilayers without any knowledge of chemical identity of the lipids. We use the technique on all atom (AA) [1,2] and coarse-grained (CG) [3] lipid bilayer trajectories to validate our method. Using this method, we define the instantaneous Lo-Ld domain boundaries in complex multicomponent model bilayer systems. In this framework, we study the effect of cholesterol and line active molecules on phase boundaries and domain mixing in biological membranes.[4]. This method provides a framework to explore the molecular origin of spatial and dynamical heterogeneity in biomembranes systems.

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Nanodiscs vs liposomes – which model of biological membranes is more relevant?

P. Stępień[1,2], A. Polit[2], A. Wiśniewska-Becker[1]

[1]Department of Biophysics, Jagiellonian University, Kraków, Poland
[2]Department of Physical Biochemistry, Jagiellonian University, Kraków, Poland

Biological membranes which consist of two main components, lipids and proteins, serve many important functions in cells. To better understand interactions between basic membrane components on a molecular level, simplified artificial models of biological membranes have been developed. One of recently developed membrane models used for protein studies are nanodiscs which are self-assembling systems constituted by the lipid bilayer stabilized by homodimers of modified apolipoprotein called membrane scaffold proteins (MSPs) [1]. Using the EPR spectroscopy technique and different PC-based spin labels we investigated the structure and dynamics of DMPC and POPC bilayer in nanodiscs in comparison with liposomes, which are most commonly used membrane models. We showed that the effect of MSP on lipid bilayer is similar to the effect of cholesterol [2]. We also showed that proximity of lipids and proteins influences lipids mobility. By fitting of experimental spectra with microscopic-order macroscopic-disorder (MOMD) model we distinguished and described two fractions of lipids – boundary lipids bound to the protein and bulk lipids. To study the effect of cholesterol on lipid dynamics in nanodiscs, cholesteryl hemisuccinate (CHS) has been used in POPC/POPG membrane. We showed that cholesterol and CHS caused a similar organizing effect in the hydrocarbon region of the lipid bilayer of both liposomes and nanodiscs, while CHS, in contrast to cholesterol, did not increase solvent permeability in the polar headgroup region in both membrane systems. Although nanodiscs with addition of CHS are quite good approximation of plasma membrane with high concentration of proteins and cholesterol, CHS seems to be buried deeper than cholesterol in the membrane. Therefore, one has to be cautious when replacing cholesterol with CHS in model membranes.

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Structural studies of nanodiscs from molecular dynamics and spectral simulations

B. Milanović[1], P. Stępień[2], C. Poojari[3], W. Gałan[1], A. Polit[4], A. Wiśniewska-Becker[2], I. Vattulainen[3], T. Róg[3]

[1]Department of Computational Biophysics and Bioinformatics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
[2]Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
[3]Department of Physics, Faculty of Science, University of Helsinki, Finland
[4]Department of Physical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

Membrane proteins possess diverse structure and function which are important in carrying out various biological functions and are also major drug targets. The challenge in the field has always been to obtain membrane protein structures which are biologically relevant. Over the recent years, the use of discoidal shaped nanodiscs (ND) has emerged as a novel tool in studying membrane proteins. The ND are composed of phospholipid bilayer which are stabilized by the homodimers of membrane scaffold proteins (MSP). The use of ND is not limited by the size of protein as they can accommodate protein of various sizes and the major advantage of ND is that they provide control over the system components. Previous studies have suggested that the lipids in the ND and planar lipid bilayers have different molecular properties in spite of both the models comprising phospholipid bilayer structure. The difference is likely linked to the presence of MSP in ND, which has the ability to alter lipid property. To that end, combining molecular dynamics (MD) simulations and electron paramagnetic resonance (EPR) spectroscopy, we aim to rationalize the difference in lipid properties in ND and planar bilayer membrane models composed of either POPC/DMPC lipids. The results put forward the effect of MSP on lipid conformation and the data clearly indicates that the ND systems are not homogeneous as the planar lipid bilayer systems.

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Presence of oxysterols affects permeability of lipid bilayers

W. Kulig[1,2], H. Mikkolainen[1], A. Olżyńska[3], P. Jurkiewicz[3], Ł. Ćwiklik[3,4], T. Róg[1,2], M. Hof[3], I. Vattulainen[1,2,5], P. Jungwirth[4,1]

[1]Department of Physics, Tampere University of Technology, Finland
[2]Department of Physics, University of Helsinki, Finland
[3]J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[4]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[5]MEMPHYS-Center for Biomembrane Physics, University of Southern Denmark, Odense, Denmark

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.

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How does the presence of oxysterol change biophysical properties of model membranes – experimental studies

A. Olżyńska, P. Jurkiewicz, M. Hof

Department of Biophysical Chemistry, J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Oxysterols, which are oxidized derivatives of cholesterol with one or more additional oxygen-containing functional groups, are present in healthy mammalian tissues at low concentrations. However, their number was found to be elevated under pathological conditions. The influence of oxysterols, both ring- (usually products of non-enzymatic reactions) and tail-oxidized (typically produced in enzymatic process), on biophysical properties of phospholipid membrane was studied. Dynamic light scattering (DLS) measurements demonstrated no influence of any oxysterol on the morphology of large unilamellar vesicles. However, using time dependent fluorescence shift method (TDFS), we learned that ring-oxidized sterols significantly increased the lipid mobility at the glycerol level (comparing with cholesterol), while tail-oxidized sterols did not show this effect [1]. The recent fluorescence steady-state quenching studies revealed significantly altered membrane properties in presence of the tail-oxidized sterol (27-OH) that can be rationalized by increased membrane permeability and/or flip-flop (transversal lipid diffusion between bilayer leaflets). This effect is likely the result of a complex dynamics of 27-OH along the bilayer normal as indicated by MD simulations. Fluorescence quenching confocal microscopy measurements on POPC/27-OH giant unilamellar vesicles indicated that observed quenching mechanism was via permeation of dithionite trough the membrane rather than flip-flop. Moreover, recent developments of calcium permeation assay are discussed.

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Reactive oxygen species action on cell membranes: Unraveling a potential mechanism of electroporation in the biological context using Molecular Simulations

P. Campomanes, M. Tarek

CNRS, UMR 7565 Université de Lorraine, Nancy, France

In this contribution we harness the capabilities of computational resources and the predictive power of advanced atomistic and quantum level molecular dynamics techniques to decipher key steps in several chemical and biophysical processes occurring during and following Electric field stimulations of cell membranes. We show that under low-voltage conditions, and predict that under nanosecond pulse electroporation conditions, peroxidation of model cell membranes by potent reactive oxygen species (OH• and OOH•) is significantly enhanced. We quantify then the permeability of the peroxidized membranes to a host of species including ions and mall molecules, to demonstrate that electrically mediated chemical effects may play a significant role in several processes experimentally evidenced following exposure of cells to high electric fields.

Oxidized phospholipid membranes as seen via molecular simulations

Ł. Ćwiklik

J. Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences, Prague, Czech Republic

Oxidative stress occurring in biosystems due to imbalance between the damage caused by reactive oxygen species and the combined action of antioxidants and repairing mechanisms leads to formation of oxidized lipids. In particular, unsaturated phospholipids, major components of cellular membranes, are prone to oxidative damage. This results in alterations of essential membrane properties, such as water permeability. In extreme cases, membrane integrity may be lost leading to membrane breakdown. Molecular dynamics (MD) simulations are well suited to investigate in detail the changes of phospholipid membrane properties resulting from oxidation of its lipid constituents. As witnessed and explained at the molecular level employing MD, phospholipid oxidation in membranes leads to enhanced water penetration, lipid reorientation, modulation of lipid mobility [1-4]. Furthermore, MD simulations were instrumental in explaining of such complex phenomena as membrane poration and disintegration due to oxidation on the one hand [5], and oxidized membrane repairing by cholesterol on the other hand [6]. While studying membrane oxidation, MD simulations can be directly combined with advanced experimental techniques thus providing a comprehensive description of the changes experienced by phospholipid membranes under oxidative stress [7].

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Oxidized phospholipids – fluorescence spectroscopy

P. Jurkiewicz[1], S. Cyboran-Mikołajczyk[2], M. Hof[1]

[1]Department of Biophysical Chemistry, J. Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences, Prague, Czech Republic
[2]Department of Physics and Biophysics, Wroclaw University of Environmental and Life Sciences, Poland

Products of lipid oxidation are commonly, although in limited quantities, present in our bodies both under physiological and pathological conditions. While their recognition by proteins triggers many signalling pathways, their very presence can also have severe effects on the physical properties of lipid membranes [1]. Herein we would like to summarize our experimental research on the effect of well-defined oxidized lipid on model lipid membranes. We focus on the methodological advantages and drawbacks of chosen fluorescence techniques: fluorescence correlation spectroscopy, time-dependent fluorescence shift method, diphenylhexatriene anisotropy, nitrobenzoxadiazol-dithionite quenching, calcium permeation measurements and a few other spectroscopic methods. The changes in lipid membranes induced by the presence of truncated oxidized lipids include complex alterations in membrane hydration profile, membrane destabilization manifested in increased lipid mobility and membrane permeability, loss of bilayer asymmetry, and enhanced calcium adsorption [2-3]. The role of cholesterol as well as lipid oxidation leftovers, like hydroxynonenal will be also discussed [4-5]. Our experience shows that fluorescence spectroscopy pairs well with computer simulations. The two approaches are complementary and gain from each other [6]. Simulations can remarkably well visualize and explain lipid interactions on the molecular level being a great help in interpreting experimental results.

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Interactions in the pre-AD mimicking model membranes

T. Kondela[1,2], B. Demé[3], N. Kučerka[1,2]

[1]Faculty of Pharmacy, Comenius University in Bratislava, Slovakia
[2]Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research in Dubna, Russia
[3]Institute Laue-Langevine in Grenoble, France

Neutron diffraction has over the years proven to be a useful technique in structural biology, biophysics and materials science. Our recent experimental data revealed several intriguing structural properties of biomimetic membranes. Amongst other, membrane structure depends strongly on the chemical composition of its constituents. For example, cholesterol is known to increase the order of lipid hydrocarbon chains while increasing the stiffness of membrane. In the contrary, our previous experiments revealed the fluidizing effect of melatonin in neat lipid membranes [1]. We have extended our investigations recently by including transmembrane amyloid-beta (Ab) peptide in these model membranes to shed a light on the melatonin's potential role in preventing the development of Alzheimer's disease (AD). AD is a devastating neuro-degenerative disease caused by the formation of senile plaques, primarily consisting of Ab. The crucial role in this process is imparted by peptide-membrane interactions, changing the structural properties of membrane. These changes are known to be modulated also by membrane composition, the presence of cholesterol and melatonin in particular. Small angle neutron diffraction (SAND) measured at four different contrast conditions was utilized for an unambiguous determination of structure in transversal direction. The obtained structures reflected the elevated amounts of cholesterol by the increase in the lipid phosphate-phosphate distance, while the effect was decreasing in the case of melatonin loaded membranes. However, the resulting effects on the overall thickness of membrane in the case of both cholesterol and/or melatonin seem to be suppressed in the presence of peptide. This corroborates the crucial role of Ab in the onset of AD [2].

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Divalent metal cations as a mediator of DNA-phospholipid bilayer binding

D. Uhríková[1], N. Kučerka[1,2], J. Teixeira[3], S.S. Funari[4]

[1]Faculty of Pharmacy, Comenius University in Bratislava, Bratislava, Slovakia
[2]Frank Laboratory of Neutron Physics, JINR, Dubna, Russia
[3]Laboratoire Léon Brillouin, CEA-CNRS, CEA Saclay, France
[4]Hasylab at DESY, Hamburg, Germany

Divalent metal cations are actively involved in cell's physiology and biochemistry. In addition to calcium and magnesium, essential and ubiquitous to all known living organisms, there are traces elements like zinc playing fundamental role in several critical cellular functions such as protein metabolism, gene expression, structural and functional integrity of biomembranes, and in metabolic processes. In phospholipid membranes, the binding site of cations^{2+} is close to the phosphate group of P^{-}-N^{+} dipole of the phospholipid headgroup. Our small angle neutron diffraction (SAND) and scattering (SANS) experiments have shown that calcium and zinc affect differentially the structure of membrane from dipalmitoylphosphatidylcholine (DPPC) [1,2]. Divalent metal cations mediate DNA interaction with neutral phospholipid bilayer forming supramolecular aggregates able to transfer DNA into a cell. The microstructure of formed aggregates was studied using small-angle X-ray diffraction (SAXD). Structural polymorphism induced by cations, ionic strength, and temperature generates a large variety of liquid-crystalline phases: condensed lamellar phases with or without DNA regular packing, coexistence of two lamellar phases, and also volume phases separation were identified in DNA-phopsholipid-cations mixtures.

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Determinants of sodium and calcium adsorption onto neutral lipid bilayers

M. Javanainen[1], A. Melcrová[2], A. Magarkar[3], P. Jurkiewicz[2], M. Hof[2], P. Jungwirth[3], H. Martinez-Seara[3,1]

[1]Tampere University of Technology, Finland
[2]J. Heyrovský Institute of Physical Chemistry, Prague, Czech Republic
[3]Institute of Organic chemistry and Biochemistry, Prague, Czech Republic

Metal cations adsorption to cellular membranes change a number of key functions, such as interaction with charged moieties, cell volume, membrane fusion or cell membrane potential. However, it is unclear how or whether cells regulate this adsorption and hence the related functions through adjusting the local lipid composition of their membranes. We have employed both fluorescence techniques and computer simulations to study how the presence of cholesterol – a key molecule in inducing membrane heterogeneity – and temperature can affect the adsorption of sodium and calcium on neutral phosphatidylcholine (PC) bilayers. We find that whereas transient sodium binding is dependent on the sole number of exposed PC head groups, the strong adsorption of calcium is determined by the available surface area of the membrane. Notably, cholesterol plays an indirect role in enlarging the total membrane area, therefore, increasing calcium adsorption, while having no effect on the adsorption of sodium [1].

These findings improve our understanding of how lateral lipid heterogeneity, that regulates surface charge density, regulates numerous ion-induced processes including adsorption of peripheral molecules such as the usually charged components of the glycocalyx [2].

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Diffusion of lipids and transmembrane proteins in membranes – from dilute conditions to crowding

I. Vattulainen[1,2]

[1]Department of Physics, University of Helsinki, Finland
[2]Laboratory of Physics, Tampere University of Technology, Finland

Cell membranes are dynamic structures that act as hosts for membrane-associated proteins to carry out their functions. Meanwhile, lipids as key components of cell membranes are also functional as highlighted by their ability to modulate protein function either allosterically or through membrane-mediated interactions. In both cases, the process driving the formation of functional lipid-protein complexes is lateral diffusion where lipids and proteins migrate in the membrane plane largely in a stochastic fashion. Here we discuss how lipids and proteins move in membranes [1-4], how their motion is not just random but arises from dynamical correlations [5-6], and how crowding with proteins found in native membranes affects the diffusion [6,7]. Biological consequences of the mechanisms observed to govern the diffusion processes are discussed.

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Anomalous lateral diffusion of lipid molecules in lipid bilayers

G. Kneller

Centre de Biophysique Moléculaire, CNRS/University of Orléans, France

Anomalous, i.e. non-Fickian diffusion is a phenomenon which is frequently observed in “crowded systems”. Lateral diffusion of single lipid molecules in lipid bilayers is an example where this phenomenon has been observed experimentally and by molecular dynamics simulation. The talk gives an overview about the theoretical background of anomalous diffusion from the point of view of liquid state theory, establishing in particular rigorous conditions for its observation on the basis of microscopic dynamical variables as well as a generalised Kubo relation for the fractional diffusion constant. Using molecular dynamics simulations of lipid bilayers with all-atom and coarse-grained force fields, it is shown how anomalous diffusion manifests itself in different physical observables which are also accessible by experiments. In this context, the problem of statistical accuracy is discussed by estimating the fractional exponent and diffusion constant of lipid molecules in a POPC membrane with the Bayesian inference method and fractional Brownian motion as model for their diffusive motion.

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Xanthophylls in membranes

W.I. Gruszecki

Department of Biophysics, Institute of Physics, Maria Curie-Sklodowska University, Lublin, Poland

Carotenes and xanthophylls (polar carotenoids) are ubiquitous in nature and play numerous important physiological functions, such as protection against oxidative damage. In living organisms, carotenoids appear in the form of pigment-protein complexes but also are constituents of biomembranes, present directly in their lipid phase. Functioning of carotenoids in biomembranes is tightly related to their molecular organization, localization and orientation in lipid bilayers. In most cases, orientation of carotenoid pigments in membranes has been analysed in lipid multibilayer systems and relatively high pigment concentration with respect to lipid, in order to assure sufficiently high signal-to-noise ratio. Recently, we developed an experimental approach which enables analysis of organization and orientation of fluorescing molecules in a single lipid bilayer [1] and we have applied this system to reanalyse xanthophylls in lipid membranes [2]. The results show very similar transmembrane localization and roughly vertical orientation of both zeaxanthin and lutein. Such results prompt us to revise molecular mechanisms associated with physiological activity of xanthophylls in the retina of the human eye.

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Studying the behaviour of cholesterol in different phospholipid bilayers: combining classical molecular dynamics with 2-D metadynamics simulations on a microseconds' time-scale

I. Ermilova, A. Lyubartsev

Department of Materials and Environmental Chemistry, Stockholm University, Sweden

Cholesterol and lipids are important components of biological systems such as human cells. Certain amount of cholesterol and lipids present or absent in different tissues of the human body can be related to diseases such as Alzheimer's disease, cancer, schizophrenia and many others. [1,2] This fact makes it interesting to study how the behaviour of cholesterol differs depending on saturation of lipids in lipid bilayers and their phases. In this work we have chosen two approaches in order to learn more about this phenomena: classical molecular dynamics simulations and molecular dynamics simulations with metadynamics. All simulations have been performed on a microsecond time-scale for the bilayers 12:0-12:0 PC, 14:0-14:0 PC, 18:0-18:0 PC, 18:2-18:2 PC, 18:0-18:2 PC, 20:0-20:0 PC, 20:4-20:4 PC, 22:0-22:0 PC, 22:6-22:6 PC. Metadynamics simulations for a single cholesterol molecule give an idea on preferable locations and positions of cholesterol inside the membrane depending on what phospholipids are building bilayers while classical molecular dynamics simulations for higher concentrations of cholesterol show how it can build clusters inside biomembranes.

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Edge tension of anionic membranes

R. B. Lira[1,2], B. R. Casadei[1], R. Dimova[2], K. A. Riske[1]

[1]Biophysics Department, Universidade Federal de Sao Paulo, Brazil
[2]Department of Theory and Bio-systems, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

Preservation of cellular membrane integrity is fundamental to sustaining life. Transient pores across the membrane can be induced by electric pulses and mechanical/osmotic stress, for instance. Once the stimulus is removed, membranes usually reseal quickly because of their high edge tension, a parameter that reflects the energy penalty per unit length for having frustrated lipids at the pore rims. Pore resealing can be observed with optical microscopy for giant unilamellar vesicles (GUVs) composed of the ubiquitous lipid phosphatidylcholine (PC) when subjected to strong electric pulses. Macropores open in response to the application of a single pulse, and reseal within ~50 ms. Quite differently, electric pulses can cause bursting of GUVs containing anionic lipids, as phosphatidylglycerol (PG): The macropores open continuously and the vesicle collapses within ~100 ms [1]. In cases in which the macropores resealed, a large fraction of the charged GUVs exhibited long-lasting (minutes) permeable states, allowing the passage of dyes of different sizes. The probability of the bursting event/permeable state increases with the fraction of PG in the bilayer. To explain the observed effects, the membrane edge tension of GUVs composed of PC:PG, the lipid extract of erythrocyte membranes and other biomimetic compositions was measured from the dynamics of macropore closure [2]. The presence of ~20 mol% anionic lipids caused an up to threefold decrease in the edge tension, showing that the presence of surface charges critically impacts the stability of membranes.

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Drug membrane permeation: Towards semi-quantitative structure permeation relationship

F. Di Meo[1], M Paloncýová[2], T. Ossman[1], L. Monticelli[3], M. Otyepka[2], C. Rossi[4], P. Trouillas[1,2]

[1]INSERM UMR850, University of Limoges, France
[2]Regional Centre for Advanced Technologies and Materials, Department of Physical Chemistry, Faculty of Science, Palacky University, Olomouc, Czech Republic
[3]Laboratory of Molecular Microbiology and Structural Biochemistry, CNRS UMR 5086, IBCP, Lyon, France
[4]Sorbonne Universités; CNRS Enzyme and Cell Engineering Laboratory, Université de Technologie de Compiègne, France

Drug-membrane crossing is a crucial pharmacological event that affects drug action as well as toxicity. Drug can either passively cross lipid bilayers or through membrane protein transporters. Over the past decade, molecular dynamics (MD) have become a powerful tool to better understand these processes at the atomic-scale, supporting experimental evidences. We here investigated passive permeation events by means of MD simulations, focusing on real-life drugs.

The elucidation of passive membrane crossing events for a given drug is a challenging task that requires a thorough analysis of the driving forces that ease both insertion into and crossing through biological membranes. Drug permeation through membranes might be calculated by unbiased MD simulations, however it would require very large timescale sampling (i.e., up to a few ms for small drugs) that is virtually unaffordable by current computational power. Joining biased MD and the inhomogeneous solubility diffusion model is a relevant strategy to predict permeability coefficients of series of drugs.

Gibbs energy and diffusion profiles were computed trough all slices/windows along z-axis of the bilayer (normal to membrane surface). This allows converting the atomic description of all crossing events into permeability coefficients for a series of drugs (e.g., ganciclovir, ciclosporin, mycophenolic acid). Structure property relationships were established, which highlighted the key role of H-bonding interactions, pKa, hydration as well as solvent surface accessible surface area. A (semi-quantitative) assessment of passive permeation timescales has been reached for various drugs used in organ transplantation. Such theoretical predictions may support (already existing and further) experimental and clinical data.

Atomistic pictures of fluorescent probes in lipid bilayer membranes enhance lipid phase recognition through combined (non-) linear optical and fluorescence analyses

S. Knippenberg[1], S. Osella[1,2], G. Fabre[3], N. A. Murugan[1], F. Di Meo[4], M. Ameloot[5], P. Trouillas[4,6]

[1]Division of Theoretical Chemistry and Biology, KTH Royal Institute of Technology, Stockholm, Sweden
[2]Centre of New Technologies, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland.
[3]LCSN – EA1069, Faculté de Pharmacie, Université de Limoges, France
[4]UMR 850 INSERM, Faculté de Pharmacie, Université de Limoges, France
[5]Biomedical Research Institute, Hasselt University, Belgium
[6]Regional Centre of Advanced Technologies and Materials, Palacký University Olomouc, Czech Republic

The interest in phase recognition of lipid membranes has led to the use of different probes and techniques in order to differentiate between healthy and ill biological environments. The focus is put upon the DiI-C18 probe, known to fluoresce in the red spectral region, as well as upon a newly developed blue Bodipy-derivative. Their positions and orientations in the liquid disordered (Ld) phase of DOPC are investigated, as well as the ones in the gel (So) and Ld phases of DPPC, and those in the liquid ordered (Lo) phase of a 2/1 mixture of Sphingomyelin/Cholesterol. Although in confocal microscopy it was seen that both probes are found in the same membrane phases [1], they behave differently. Since no unambiguous answer was available about the nature of the preferred phase, Gibbs free energy profiles are calculated and sound answers can be obtained [2].

It is shown that (non-)linear optical analyses such as One Photo Absorption (OPA), Two Photon Absorption (TPA) and Second Harmonic Generation (SHG) as well as the study of the fluorescence decay time lead to an enhanced screening of membrane phases (Ld, Lo and So) for the fluorescent DiI probe [3]. By means of electrostatic embedding QM/MM calculations, it was found that DiI can screen between the three natural occurring membrane phases when the aforementioned analyses are combined. In particular, the joint TPA and SHG (considering both the Hyper-Rayleigh scattering and EFISH analysis) and fluorescent analysis prove the efficiency of DiI as a versatile probe for phase recognition.

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Exploration of landscapes of ABC membrane exporters

B. Chantemargue[1,2], F. Di Meo[1], M. Paloncýová[2], M. Otyepka[2], P. Trouillas[1,2]

[1]U850 INSERM, Fac. Pharmacy, Univ Limoges, France
[2]RCPTM, Dpt. Physical Chemistry, Fac. Sciences, Palacký University, Olomouc, Czech Republic

Molecular dynamics simulations enable the study of landscape of membrane transporters, picturing energetics and conformational changes related to drug transport. Human ATP binding cassette (ABC) transporters excrete drugs from inner to outer cellular compartments. This process is catalyzed by: i) ATP hydrolysis in active sites; ii) substrate binding; iii) surrounding lipid bilayer, which makes that a proper lipid membrane environment should be systematically included in MD simulations.

Drug transport through ABC exporters requires large conformational changes from Inward-Facing (IF) to Outward-Facing (OF) conformers. This closing-opening process is far beyond the reach of classical MD simulations and to be explored, it requires the use of biased MD simulations. Here we propose a metadynamics study to describe the landscape of this process, following four collective variables. Several intermediate conformers were thus identified, which allowed thorough understanding of drug efflux process, especially in the IF-occluded region where all domains are in close contact, triggering domain swapping.

Chemical compositions of ordered and disordered domains in phase-separated lipid membranes via CARS microscopy

M. Tarek Abdelwahab, M. Bonn, S.H. Parekh

Department of Molecular Spectroscopy, Max Planck Institute for Polymer Research, Mainz, Germany

Ternary mixtures of lipids have evolved into suitable models to study cell membrane biophysics, in large part because of their predictable ability to form liquid domains (i.e. phase separate) [1]. Phase separation has been proposed as the underlying mechanism of the "lipid raft" hypothesis for plasma membrane organization [2] and also for regulating membrane protein function. Different techniques have been used to quantify lipid domains both in cells and reconstituted ternary lipid mixtures [3] in order to understand their physical origins. Fluorescence microscopy was, and is still, the primary tool for lipid domain visualization and has led to many breakthroughs in our understanding of lipid membranes. Indeed, the conclusions from fluorescence experiments have been pivotal in our understanding of membrane biophysics. However, these studies have also played a huge role in arousing debates between different research groups for years, e.g. on whether or not the anaesthetic concentrations of n-alcohols alter the physical and compositional properties of lipid membranes, due to the inability of fluorescence to report on lipid composition directly [4, 5]. Here, we exploit a chemical microscopy technique, coherent anti-Stokes Raman scattering (CARS), for its extreme sensitivity to the C-H bonds [6, 7] (the lipids' main component) and diffraction-limited optical resolution, to extract the chemical fingerprint of lipid domains under different experimental conditions (e.g. temperature and lipid compositions). This method will be used in future studies to explore how lipid phase transitions and chemistry are modified by interaction with small molecule anaesthetics.

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Poster presentation abstracts

Abstracts for poster presentations are divided into two sections.
The majority of posters are presented in section A.
Section B is dedicated to posters enrolled
in the Students'/PhD Students' Poster Competition.
In both sections abstracts are arranged in alphabetical order
by the surname of the participant.

Packing of peptides on the surface of lipid membranes

C. Aisenbrey, B. Bechinger

CNRS UMR 7177, Membrane Biophysics and NMR, Faculté de Chimie, Université de Strasbourg, France

When polypeptides bind to the membrane surface they become confined to a restricted quasi two-dimensional space where peptide-peptide interactions become highly relevant and the concept of a crowded medium is appropriate. Within this crowded environment interesting effects like clustering, separation of phases, cooperative alignment and common movements occur. Here we investigated such effects by measuring distances between fluorophore-labeled peptides in the range ≤ 1 nm by fluorescence self-quenching. For helical peptides with dimensions of approximately 1 x 3 nm such a small 'ruler' is sensitive to the packing of the labeled peptides and thereby to their molecular arrangement. A novel approach to characterize peptide-peptide interactions within membranes is presented using the designer peptide LAH4. This sequence changes membrane topology in a controlled manner being transmembrane at neutral conditions but oriented parallel to the surface at low pH. Experimental measurements of the fluorescence self-quenching of close-by chromophores, and the changes that occur upon dilution with unlabeled peptides are used to analyze the peptide distribution within the membrane surface. The data shows a strong effect of electrostatic interactions and under some experimental conditions clustering of the peptides. Furthermore the results suggest that at pH 4 the peptides arrange along the membrane surface in an ordered mesophase-like arrangement.

Mag2 and PGLa are antibiotic peptides discovered in the skin of the African clawed frog. The mechanism of the synergism of the two peptides is still under discussion. We present the results of fluorescence self-quenching experiments, which give new insights into the interaction of both peptides.

Organization of TrkB neutrophin receptors in membranes revealed by STED microscopy

B. Angelov[1], A. Angelova[2]

[1]Institute of Physics, ELI Beamlines, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[2]Institut Galien Paris-Sud, CNRS UMR 8612, Univ. Paris-Sud, Université Paris-Saclay, LabEx LERMIT, Châtenay-Malabry, France

The organization on the nanoscale of the tropomyosin-related kinase receptor type B (TrkB) is examined by stimulated emission depletion (STED) microscopy. TrKB is a promising therapeutic target for neurodegenerative and psychiatric disorders. The performed fluorescence subdiffraction imaging of the membrane receptor localization reveals that clusters of oligomeric TrkB states and randomly organized nanodomains are formed in the membranes of differentiated human neuroblastoma SH-SY5Y cells. These studies serve as an in vitro model of neurodegeneration. Despite that the isolated monomeric states of TrKB cannot be distinguished from its dimeric forms in such nanoscopy images, TrkB receptor dimers are visualized as single pixels in the STED images. The clusters of higher-order TrkB oligomers are of dynamic nature rather than of a fixed stoichiometry. The membrane protein clustering as well as the dissociation of the TrkB receptors nanodomains can be modulated by neurotherapeutic formulations containing ω-3 polyunsaturated docosahexaenoic acid (DHA). Nanomolar amount of DHA easily changes the receptor topology and leads to destruction of the clusters. In this way the mobility, activity and the dynamic distribution of the TrKB receptors can be controlled in the cell membranes.

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Geometric shape of lipids versus molecular interactions in membrane pore formation

N. Awasthi, J.S. Hub

Department of Structural Molecular Biology, Georg August University, Goettingen, Germany

Phosphoethanolamine (PE) lipids are considered crucial lipids for intracellular transport, viral entry via membrane fusion, antimicrobial peptide activity, and translocation of ions and cell-penetrating peptides. Transient membrane defects and pores appear across all these processes, and it is widely believed that PE lipids favorably influence pore nucleation via their inverted cone molecular shape and intrinsic curvature.

Using a new reaction coordinate [3], we demonstrate that the molecular shape and the intrinsic curvature of lipids do not determine the free energy of nucleating a membrane defect or pore [1,2]. In fact, interactions between lipid head groups, and head-groups and solvent determine the energetics of pore nucleation, and define whether pore nucleation is favored by enthalpy or entropy [1]. Hence, contrary to belief, nucleating transmembrane pores in a bilayer with PE lipids is energetically expensive due to extensive hydrogen bonding network of PE lipids. In addition, we find qualitative differences between PE and Phosphocholine (PC) lipids in their roles as zwitterionic components of model bacterial membranes. Finally, we use potential of mean force (PMFs) to quantify the action of membrane active polyarginines as model cell penetrating peptides [1].

Our work highlights the critical nature of lipid composition and arising interactions, and the limitations of shape based arguments, derived from geometric models in explaining the thermodynamics of membrane pore nucleation.

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What drives drug-membrane interactions?

M. Paloncýová[1], V. Navratilova[1], V. Bazgier[1], G. Fabre[2], J. Juracka[2], F. Di Meo[2], P. Trouillas[1,2], M. Otyepka[1], K. Berka[1]

[1]Department of Physical Chemistry, RCPTM, Faculty of Science, Palacky University Olomouc, Czech Republic
[2]U850 INSERM, Faculty of Pharmacy, Universite de Limoges, France

Drugs interact with membranes at different levels stemming from the accumulation on the membrane surface, partitioning into the membrane at various depths, or permeation through the membrane [1]. The major tool for evaluation of drug-membrane interactions is drug's free energy profile along a membrane normal, which shows the affinity as well as penetration resistance for any given drug-membrane pair. Since we have gathered hundreds of calculated free energy profiles based on our benchmarking study [2] within our newly built MolMeDB database together with available experimental information, we can start to distil general trends from the drug-membrane interaction data. We will discuss, i) where drugs are typically located on the membrane [3], ii) how interactions can be altered by different membranes [4], iii) how the interactions can be altered by the drug metabolism [5], and finally what the effect of individual functional groups is. Observed trends might be further used for *in silico* pharmacology predictions during drug design in the future.

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Substrate differentiation between isoforms of Catechol-O-methyltransferase

A. Bunker[1], A. Magarkar[1,2], P. Parkkila[1], T. Viitala[1], T. Lajunen[1], E. Mobarak[3,4], G. Licari[5], O. Cramariuc[3], E. Vauthey[5], T. Róg[3,4]

[1]Drug research programme, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Finland
[2]Institute of Organic Chemistry and Biochemistry, Academy of the Sciences of the Czech Republic, Prague, Czech Republic
[3]Department of Physics, Tampere University of Technology, Finland
[4]Department of Physics, University of Helsinki, Finland
[5]Department of Physical Chemistry, University of Geneva, Switzerland

There are two isoforms of the enzyme Catechol-O-Methyltransferase (COMT): 1) water soluble (S-COMT) and 2) membrane associated, bitopic (MB-COMT), a drug target in relation to the treatment of Parkinson's disease[1]. Substrate profiles of S-COMT and MB-COMT differ[2], however, until now the mechanism for this has been unknown; this insight holds the key to selective targeting of MB-COMT. Using a combination of computational and experimental protocols, we have determined this. Our Molecular dynamics (MD) simulation results, verified by a combination of Quartz Crystal Microbalance (QCM), Isothermal Titration Calorimetry (ITC), Surface Second Harmonic Generation (SSHG) and Surface Plasmon Resonance (SPR) studies demonstrates: 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, a mechanism that differentiates its catalytic activity from S-COMT. Data mining of the UniProt and Drugbank databases reveals evidence of this mechanism in other proteins, including several existing drug targets.

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Assessing caffeine impact on cell membrane integrity

A. Woziwodzka, A. Borowik, K. Butowska, Jacek Piosik

Laboratory of Biophysics, Intercollegiate Faculty of Biotechnology UG-MUG, Gdańsk, Poland

In the era of multidrug resistant microorganisms, finding a new way for effective treatment of infectious diseases appears as a major challenge for modern medicine. One of the promising approaches to improve antibiotic treatment efficacy is the combination therapy, in which antibiotic and an additional substance improving overall antimicrobial effects is used. Caffeine, due to its stimulant activity, is the most commonly consumed alkaloid worldwide. It is recognized as safe up to 500 mg per day, therefore is a good candidate for combination antibacterial regimen. Indeed, caffeine has been shown previously to exhibit antimicrobial properties. However, the mechanism of antibacterial action of caffeine remains unclear.

In this work we investigated the impact of caffeine on the cell membrane integrity. We will present the data for caffeine influence on artificial model bacterial membranes using large unilammelar vesicles and carboxyfluorescein leakage assay. Moreover, the impact of caffeine on Staphylococcus aureus cell membrane will be demonstrated with laurdan fluorescence anisotropy measurements. The data on bioavailability of two commonly used antibiotics (tetracycline, ciprofloxacin) in the presence of caffeine will be provided with the in vitro model of passive, transcellular permeation – PAMPA assay.

Presented study will contribute to better understanding of the mechanism of action of caffeine as a modulating compound in antimicrobial treatment.

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Free energy calculations to understand lipid domain reorganisation by small molecules

A. Centi, K. Kremer, T. Bereau

Max Planck Institute for Polymer Research, Mainz, Germany

Hydrophobic and amphiphilic compounds, including many alcohols and anaesthetics, are known to affect cell membrane organisation by preferentially partitioning between lipid domains. By doing so they do not simply alter membrane properties (e.g. bilayer thickness, interfacial tension, miscibility transition temperature), but they also influence the functionality of the associated proteins. Because of its biological relevance, the process of domain stabilisation/destabilisation has been widely investigated, both experimentally [1] and computationally [2]; however, its exact mechanism as well as the physical and/or chemical properties responsible for it remain largely unknown. A more detailed knowledge of the interactions governing lipid membrane reorganisation would allow us to achieve a better understanding of the mechanism by which hydrophobic and amphiphilic compounds carry out their functions, hence opening the way for the design of novel drugs, which could act by targeting lipids domains rather than proteins.

In this work, the processes underlying domain reorganisation triggered by small molecules have been investigated by means of free energy calculations in combination with the MARTINI [3] coarse-grained model. This approach allowed us to obtain values of membrane partitioning free energies for a variety of systems (i.e. different lipid membranes, solutes and concentrations) at a reduced computational cost in comparison to atomistic simulation and, therefore, explore the effect of parameters such as lipid saturation/unsaturation or the relative ratio of the different membrane components for the process under evaluation.

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Unveiling the driving forces of lipid raft formation by means of molecular dynamics

P. Chodnicki, Ł. Nierzwicki, J. Czub

Department of Physical Chemistry, Gdansk University of Technology, Poland

Lipid rafts are ordered microdomains in cell membranes enriched in cholesterol and sphingolipids. These microdomains are the suitable environment for the proper functioning of certain transmembrane proteins. The presence of cholesterol contributes to an increase in the membrane lipid order, also increasing its thickness. Moreover, proteins found in the lipid raft may adopt different conformations than in non-raft environment, what leads to their altered activity or metabolism.

Huge fraction of cholesterol (25% of total cholesterol) is located in the brain and nerve cells. Furthemore, it is known, that lipid rafts are involved in pathogenesis of many diseases, such as Alzheimer's disease or Huntington's chorea [1]. Understanding the molecular underpinnings of raft formation may be crucial in comprehensing the organisation of a lipid bilayer around the raft-residing proteins.

In this work, we are using all-atom molecular dynamics simulations to reveal the molecular driving forces governing the parition of cholesterol to ordered microdomains and thereby to characterize the mechanism behind the formation of lipid rafts Also we focus on how the sterol concentration affects the physical properties of the membrane.

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Modulation of glucose transporter type 1 (GLUT1) by WZB117 and electroporation in melanoma cells to increase membrane permeability for oat β-glucan

A. Choromańska[1], J. Kulbacka[1], A. Szewczyk[2], J. Harasym[3], J. Saczko[1]

[1]Department of Medical Biochemistry, Wroclaw Medical University, Poland
[2]Department of Animal Developmental Biology, Institute of Experimental Biology, University of Wroclaw, Poland
[3]Department of Food Biotechnology, Wroclaw University of Economics, Poland

The internal composition of the cell is maintained because the plasma membrane is selectively permeable to small molecules. There are still not specified mechanisms how beta-glucan molecules are transported into cells. Supposing, beta-glucan toxicity against tumor cells may be related to the overexpression of the transporter responsible for the transport of glucose molecules in the cells.

Malignant cells require high energy levels via glycolytic generation of ATP to proliferate and survive. In cancer-induced starvation, GLUT1 overexpression governs mechanisms that favor tumor growth at the expense of host tissues. In this case, glucans – polymers composed of glucose units are much more up-taken by tumor than normal cells. Increased GLUT1 (Glucose Transporter Type 1) expression has been demonstrated earlier in malignant melanomas [1]. GLUT1 expression promotes glucose uptake and cell growth in that cells [2]. Also in human melanoma tissues a significant correlation between GLUT1 expression and mitotic activity was found [1]. Overexpression of GLUT1 protein confers poor prognosis in a wide range of solid tumors [3]. The aim of our study was to examine the effect of oat β-glucan (OβG) and OβG delivered with electroporation (EP) on viability and the level of GLUT-1 expression in human primary and metastatic melanoma cell lines (MeWo and Me45). The viability evaluation was performed by MTT assay. Immunofluorescent method was applied for GLUT-1 determination in melanoma cells. Moreover, the effect of OβG and OβG-EP was assessed after GLUT-1 blocking by WZB 117.

The obtained results bring us to elucidate the mechanism of transport of the oat β-glucan into the cells.

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How different species of phosphatidic acid behave in model lipid monolayers and bilayers

A. Czogalla, K. Penkal, M. Szymanowski, A. F. Sikorski

Labolatory of Cytobiochemistry, Faculty of Biotechnology, Uniwersity of Wroclaw, Poland

Phopsphatidic acid is one of the simplest glycerophospholipids that occurs in tiny amounts in biological membranes. However, its unique role stems from its function as an intermediate in multiple lipid synthesis pathways and as one of the key signaling lipids in cells. Remarkably, the involvement of phosphatidic acid in multiple cellular events raises the question how all these processes could be fine-tuned. Our data show that within lipid mono- and bilayers cholesterol may interact with phosphatidic acid, as revealed by lipid monolayer approach combined with fluorescence microscopy as well as membrane packing and morphology observations of lipid vesicles. Our protein-membrane binding studies show that the presence of cholesterol in lipid bilayers exerts also a huge impact on the recognition of phosphatidic acid by various peripheral proteins. Moreover, these effects are not the same for different molecular species of phosphatidic acid and clearly correlate with acyl chain structure of the lipid. Our data reveal new modulatory mechanisms of the biological activity of phosphatidic acid and help to clarify how different species of signaling lipids could be spatially and functionally distinguished within cells.

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

P. Delcroix, A. Olżyńska, Ł. Ćwiklik

J. Heyrovsky Institute of Physical Chemistry, Prague, Czech Republic

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

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

Finally, the role of cholesterol and the so-called “Cholesterol Mystery” will be investigated. The effect of cholesterol removal on the properties and behavior of the monolayer will be analyzed. The results will be compared to those obtained with cholesterol.

On the influence of antimicrobial peptides on model biological membranes – Langmuir monolayer, QCM-D and AFM studies

J. Juhaniewicz-Dębińska, S. Sęk

Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Poland

The discovery of antibiotics was a milestone in the human fight against infectious diseases and has led to the substantial increase in quality of human's life. However, rapidly increasing number of multi-drug resistance pathogens resulted in a continuous need for new active compounds with strong activity. One of the most promising groups of compounds includes antimicrobial peptides (AMPs) [1,2]. They display a broad range of activity on bacteria, fungi and parasites as well as on several cancer cell lines. Therefore, they have enormous potential as novel therapeutic agents.

Here we present the results of our studies on interactions of two natural antimicrobial peptides: cecropin B (from the hemolymph the giant silk-moth *Hyalophora cecropia*) and melittin (from the venom of the honey bee *Apis mellifera*) with model lipid membranes. [3,4] The biomimetic lipid films were composed of: a) phosphatidylcholine and cholesterol, b) phosphatidylethanolamine and phosphatidylglycerol, reflecting the eukaryotic and bacterial cell membrane, respectively. Firstly, the influence of peptides on the behavior of lipid membranes was examined by means of surface pressure and Brewster Angle Microscopy. To better mimic the natural membrane, we prepared lipid bilayers by transferring the lipids on solid support using the combination of Langmuir-Blodgett and Langmuir-Schaefer techniques. The action of antimicrobial peptides was investigated by means of Quartz Crystal Microbalance with dissipation and Atomic Force Microscopy. We found that the peptide mode of action strongly depends on a lipid composition of the membrane.

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Polycation-lipid membrane interaction – experimental and MD simulations studies

U. Kwolek[1], N. Wilkosz[1], M. Zatorska[1], D. Jamróz[1], K. Nakai[2], S. Yusa[2], D. Wnuk[3], J. Bednar, M. Michalik[3], M. Kępczyński[1]

[1]Faculty of Chemistry, Jagiellonian University, Kraków, Poland
[2]Department of Applied Chemistry, University of Hyogo, Japan
[3]Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

Interactions between polycations, both natural and synthetic, and phospholipid membranes are of fundamental importance for various biophysical and biomedical applications of these polymers, such as gene delivery, antibacterial usage, or the preparation of stabilized liposomes by covering with polymer films. In this work we applied complementary experimental and computational approaches to examine the effect of linear synthetic polycations, both strong and weak, on zwitterionic and negatively charged lipid membranes used as models of cellular membranes. Atomic-scale MD simulations allowed us to gain insight into the polycation – lipid interactions at the molecular level. We focused mainly on four aspects: polycation adsorption on the membrane surface, the effect of such adsorption on the stabilization/aggregation of the vesicles, the possibility of polycation penetration into the lipid bilayers, and the formation of hydrophilic pores or the disruption of the membranes. Liposomes were prepared from POPC, DPPC, or POPC/DOPA (a negatively charged lipid). The vesicles were treated with aqueous solutions of the polycations and several experimental techniques were used to study the polycation−membrane interactions. Dynamic light scattering (DLS) and zeta potential measurements were used to confirm the polycation adsorption on the liposome surface. Microdifferential scanning calorimetry (microDSC) was used to study the effect of polycations on the thermotropic behavior of membranes. The permeability of the polycation-treated membranes was monitored using calcein-leakage experiments. Additionally, intermolecular interactions between lipid molecules and the polycations were studied using Langmuir monolayer measurements. Finally, the polycation cytotoxicity were estimated using in vitro experiments.

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Cell membrane permeabilization in glioma cells to support enhanced drug delivery

N. Niedzielska[1], E. Krzywiecka[1], M. Kotulska[1], S. Kraszewski[1], A. Chwiłkowska[2], J. Saczko[2], A. Choromańska[2], O. Michel[2], A. Szewczyk[3], N. Rembiałkowska[2], J. Kulbacka[2]

[1]Faculty of Fundamental Problems of Technology, Department of Biomedical Engineering, Wroclaw University of Science and Technology, Poland
[2]Department of Medical Biochemistry, Wroclaw Medical University, Poland
[3]Department of General Zoology, University of Wroclaw, Poland

Cell membranes isolate interior of the cells from the external environment and separate intracellular structures. The main problem of currently applied therapeutic protocols in an increasing secondary drug resistance is not sufficiently efficient drug biodistribution. The present study indicates possible methods to modulate glioma cell membranes and enhanced calcium ions uptake, which show anti-cancer activity. We used the electroporation (EP) method involving the application of the pulsed electric field (PEF) of sufficiently high energy, which induces the formation of short-lived nanopores in cell membranes [1, 2]. The research was performed on human glioma cells (SNB19). Glioma cells were exposed to microsecond PEF which affect external cell membrane and nanosecond PEF which affect all cell membranes. In case of the microsecond PEF the following parameters were used: 8 pulses of 100 µs, 1 Hz interval; 800-1600 V/cm. For the nanosecond PEF: 200 pulses of 10 ns, 10 Hz interval; 12.5-50 kV/cm. The experiments were performed with and without calcium ions in a buffer of low electric conductivity. There was determined cell viability after 24 and 72 h. Cell membrane integrity and lipid oxidation were visualized by the CLSM method. We also investigated membrane structure changes at molecular level using Molecular Dynamic (MD) simulations.

The obtained results indicate significant morphological changes in cell membranes and increased lipid oxidation induced by electroporation. Moreover, an increased cytotoxic effect was observed after microsecond PEF in combination with calcium ions. In case of the nanosecond PEF the modulative effect of Ca^{2+} was observed, dependent on the ions concentration. Our results show a great potential of EP to improve the intracellular drug delivery in anti-cancer therapy of glioma cells and intracellular effects of PEF. Simultaneously, we proved anti-cancer effect of calcium ions at appropriate concentration delivered into cells using PEF.

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Effect of phosphatidic acid on zwitterionic lipid membrane: experimental and molecular dynamics simulations study

U. Kwolek[1], P. Wydro[1], W. Kulig[2], M. Nowakowska[1], T. Róg[2], M. Kępczyński[1]

[1]Faculty of Chemistry, Jagiellonian University, Kraków, Poland
[2]Department of Physics, Tampere University of Technology, Finland

Phosphatidic acid (PA, 1,2-diacyl-sn-glycero-3-phosphate) is the simplest diacyl-glycerophospholipid, negatively charged at physiological pH. PA occurs in small amounts in biomembranes, but plays a very significant role in cells. In particular, PA is a precursor in biosynthesis of other phosphorlipids and participates in the regulation of cell proliferation [1]. Thus, study of the effect of PA on a model zwitterionic lipid membrane seems to be an interesting issue with relevance to biological systems.

We have investigated the interactions between lipids in model membranes prepared from a mixture of phosphatidylcholines (PCs) and PAs. Lipids with different combinations of saturated and unsaturated acyl chains have been studied. The PC - PA interactions were explored both experimentally and computationally. Firstly, measurements using the Langmuir monolayer technique were performed at physiological pH. Brewster angle microscopy (BAM) was employed to study the morphology of the lipid films. Finally, atomistic-scale molecular dynamics (MD) simulations were used to gain insight into mechanism of interaction between lipids in the PC - PA mixed bilayers.

In conclusion, using the comprehensive approach applying experimental and computational techniques, we have provided detailed information on the effect of PA on zwitterionic membranes, the role of saturation/unsaturation of acyl chains in the PC - PA interactions and their molecular mechanisms.

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The interaction of new oxicams derivatives with lipid bilayers as measured by calorimetry and fluorescence spectroscopy

J. Maniewska[1], J. Gąsiorowska[2], B. Szczęśniak-Sięga[1], K. Środa-Pomianek[2], K. Michalak[2]

[1]Department of Chemistry of Drugs, Wroclaw Medical University, Poland
[2]Department of Biophysics, Wroclaw Medical University, Poland

The group of the oxicams (e.g. piroxicam, meloxicam), which are known as a non-steroidal anti-inflammatory drugs (NSAIDs), is mainly used in the treatment of chronic rheumatic diseases. Most solid tumours express the cyclooxygenase-2 (COX-2) protein, which is the target of NSAIDs, and that is why those drugs are also evaluated as cancer preventive. Chemopreventive properties of NSAIDs may be, at least partially, related to their ability to interact with lipid phase of biological membranes, because in order to achieve their main target -membrane associated enzyme COX- NSAIDs have first to pass through the membranes.

The purpose of the present work, was to assess the ability of new oxicams analogues, to interact with the lipid bilayers. The results of calorimetric and fluorescence spectroscopic experiments of a few newly synthesized analogues of oxicams, on the phase behavior of phospholipid bilayers and fluorescence quenching of fluorescent probes (Laurdan and Prodan), which molecular location within membranes is known with certainty.

All studied oxicams analogues abolished DPPC pretransition. They also affected the main phase transition of DPPC by decreasing the phase transition temperature and the enthalpy, as well as by broadening of the transition peaks. The presented results revealed that, depending on the details of chemical structure, the studied compounds penetrated the phospholipid bilayers.

Computational high-throughput screening of drug-membrane thermodynamics

R. Menichetti, K. H. Kanekal, K. Kremer, T. Bereau

Max Planck Institute for Polymer Research, Mainz, Germany

The partitioning of small molecules in cell membranes, a fundamental parameter for pharmaceutical applications, typically relies on experimentally-available bulk partitioning coefficients.

Computer simulations provide structural resolution over the permeation thermodynamics through the potential of mean force. However, the extensive computational resources required by atomistic molecular dynamics simulations, together with the overwhelming size of chemical space, seriously hamper the possibility of employing the potential of mean force in computational drug-screening.

Coarse-grained models represent an efficient alternative to address both issues. On one hand, they significantly mitigate the computational expense while still capturing the relevant physical properties of the system. Moreover, coarse-grained models significantly reduce the size of chemical space, as similar chemical fragments can be mapped onto the same coarse-grained interaction site.

In this work[1], we introduce a high-throughput screening of a small subset of chemical space via coarse-grained molecular dynamics simulations of the Martini[2] model. This screening allows us to identify simple linear relationships between bulk quantities and key features of the potential of mean force. More specifically, the study allows for a semi-quantitative reconstruction of the main transfer free-energy barriers of a compound across a membrane environment only given its partition coefficients and Martini[2] representation. The potential of mean force hereby becomes an easily accessible quantity—already recognized for its high predictability of certain properties, e.g., passive permeation.

By connecting the analyzed coarse-grained compounds to atomistic ones, we show that these results are representative of the transmembrane behavior of a set of more than 400000 small molecules.

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How γ-secretase bind substrates: conformational dynamics of the enzyme active site and substrate binding pathways for the amyloid precursor protein

Ł. Nierzwicki[1], M. Olewniczak[1], I. Grochowina[2], J. Czub[1]

[1]Department of Physical Chemistry, Gdansk University of Technology, Poland
[2]Laboratory of Evolutionary Biochemistry, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Poland

γ-secretase is a multimeric membrane-embedded aspartyl protease that mediates regulated intramembrane proteolysis of type I transmembrane proteins. The enzyme complex consists of nicastrin, anterior pharynx defective 1, presenilin enhancer 2 and presenilin, with the latter bearing the catalytic aspartates. Aberrant activity of this enzyme against amyloid precursor protein results in overproduction of β-amyloid polypeptides that eventually leads to neurodegeneration and Alzheimer's Disease, while abnormal Notch processing is linked to several types of cancer.

In this work, we use molecular dynamics simulations to investigate the substrate binding mechanism of γ-secretase, using as an example the βCTF protein, which is the direct precursor of β-amyloid polypeptides. We characterize the conformational changes of γ-secretase that have been proposed to govern the access to the binding site,and show that actual binding site is located between the helices 6 and 9 of presenilin. The presented results also suggest that substrates bind to the active site of γ-secretase according to the induced fit rather than conformational selection model. We also show that initial substrate binding to γ-secretase is at least to some extent promoted by hydrophobic mismatch.

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The effects of omega-*O*-acylceramides on microstructure and permeability of model skin lipid membranes

L. Opálka[1], A. Kovacik[1], J. Maixner[2], K. Vávrová[1]

[1]Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy in Hradec Kralove, Charles University, Hradec Kralove, Czech Republic
[2]University of Chemistry and Technology Prague, Prague, Czech Republic

Omega-*O*-acylceramides (acylCer) are a subclass of ceramides with an ultralong *N*-acyl chain esterified in ω-position with linoleic acid. AcylCer are crucial components of mammalian skin permeability barrier where they are responsible for the formation of so-called long periodicity lamellar phase (LPP), which is essential for preventing water loss from the body. Decreased levels of acylCer in stratum corneum and disruptions in LPP accompany several skin diseases, such as atopic dermatitis.

We studied how the concentration and structure of acylCer influence the organization (X-ray powder diffraction) and permeability barrier properties (flux of model drugs and transepidermal water loss) of model lipid membranes. Two models with different complexity were constructed. For the simple model membranes, the LPP formed at 10% of acylCer EOS and the short periodicity phase disappeared at 30%. Surprisingly, membranes with the observed LPP had higher permeabilities compared to control membrane without acylCer.

In the complex models, acylCer decreased the membrane permeability to model permeants at physiological concentration (10%) with the strongest effect for acylCer EOP and EOdS compared to control without acylCer. However, the formation of LPP in the complex model was observed only in the most complex model with the mixture of acylCer. The individual acylCer at 10% concentration did not form the LPP.

The relationships between acylCer structure, LPP formation and permeability barrier function seem to be more complicated. The lipid heterogeneity is essential, because only the most complex model with nine Cer subclasses mimicked both the organization and permeability of stratum corneum lipid membranes.

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Triggering on/off states of photoswitchable probes in biological environments

S. Osella[1], S. Knippenberg[2]

[1]Centre of New Technologies, University of Warsaw, Poland
[2]Division of Theoretical Chemistry and Biology, School of Biotechnology, Royal Institute of Technology, Stockholm, Sweden

The use of hybrid systems for which the change on properties of one component triggers the change in properties of the other is of outmost importance when 'on/off' states are needed. For such a reason, azobenzene compounds are one of the most used probes due to their high photoswitching efficiency. In this study, we consider a new derivative of azobenzene interacting with different lipid membrane phases as a versatile fluorescent probe for phase recognition. By means of a multiscale approach, we found that the cis and trans conformers have different positions and orientations in the different lipid membranes (DOPC for the liquid disordered phase and DPPC for the gel phase), and these have a profound effect on the optical properties of the system, for both one and two photon absorption. In fact, we found that the cis state is the 'on' state when the probe is inserted into the DOPC membrane, while it is in the 'off' state in the DPPC membrane. This behaviour enhances the selectivity of this probe for phase recognition, since the different environments will generate different response on the same conformer of the probe. The same effect is found for the fluorescence anisotropy analysis, for which the trans (cis) isomer in DOPC (DPPC) presents a fast decay time. Due to the 'on/off' effect it is possible to screen the different membrane phases via fluorescence decay time analysis, making this new probe versatile for phase detection.

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Effect of lipid composition on membrane immersion of cytochrome P450 3A4

V. Navrátilová, M. Paloncýová, K. Berka, M. Otyepka

Regional Centre of Advanced Technologies and Materials, Department of Physical Chemistry, Faculty of Science, Palacky University in Olomouc, Czech Republic

Microsomal cytochrome P450 enzymes (CYPs) are membrane attached enzymes that play indispensable roles in biotransformations of numerous endogenous and exogenous compounds. Although recent progress in experiments and simulations has allowed many important features of CYP-membrane interactions to be deciphered, many other aspects remain underexplored. Using 50ns+ molecular dynamics simulations, we analyzed interaction of CYP3A4 with phosphatidylcholine membranes with differing amount of cholesterol [1]. We observed significant interactions between CYP3A4 and cholesterol and changes in the interior structure of access and egress channels. We also observed slow motions of the enzyme visible only on a very long simulations. Further, we embedded CYP3A4 into bilayers composed of lipids differing in their polar head groups, i.e., phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylglycerol [2]. In the negatively charged lipids, CYP3A4 was immersed deeper and more inclined toward the membrane because of favorable electrostatic and hydrogen bonding interactions between the CYP catalytic domain and lipid polar head groups. The identified significant role of electrostatics in CYP-membrane interactions may explain the experimentally observed preferences of individual CYP isoforms to distribute in (dis)-ordered membrane microdomains.

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Interactions of carbon nanotubes with a model cell membrane – steered molecular dynamics studies

Ł. Peplowski, W. Nowak

Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University, Toruń, Poland

Drug delivery to selected tissues or specific cells is nowadays one of the hottest items in science [1]. One of the plausible mechanisms assumes using carbon nanotubes (CNT) as a medium delivering drugs directly to target (for example cancer) cells. Experiments show it is possible to pierce a living cell by CNT without destroying bacteria [2,3]. In this presentation we will show results of a theoretical version of such an experiment. We simulated piercing of POPC model phospholipid bilayer by CNT having cisplatin molecule inside the nanotube. Results of our investigations show all details: how piercing changes the structure of the membrane, what is the effect of pulling out of some phospholipids from of the membrane and how it is possible to transport cisplatin through the membrane using the open CNT. Our simulations show that a force needed to pierce membrane bilayer is about 1200 pN, this value depend on piercing rate. Calculations show that piercing the first layer of the membrane is easier than piercing the second layer and/or dragging out the carbon nanotube out of it. We observed that some phospholipids were pulled out in this process, as well. After piercing the membrane rebuilds its structure.

To model this phenomenon molecular dynamics (MD) simulations have been performed. External force has been applied through Steered MD. The NAMD Code [4] with Charmm 27 force field [5] have been used. Our data present a possibility of using CNT to transport cisplatin through a cell membrane.

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Interaction of the antimicrobial peptide ocellatin pt7 with biomimetical membranes

E. A. Carvalho[1], M. A. Rodrigues[2], J. R. S. A. Leite[3], M. P. Bemquerer[2], K. A. Riske[1], K. R. Perez[1]

[1]Department of Biophysics, Federal University of São Paulo, Sao Paulo, Brazil
[2]Embrapa- Genetic Resources and Biotechnology, Brasilia, Brazil
[3]Biotec, Federal University of Piaui, Brazil

Antimicrobial peptides (AMPs) are a promising alternative to antibiotics since some bacteria have shown resistance to conventional antibiotics. The main mode of action of AMPs described in the literature involves the destabilization of the cell membrane. Ocellatin PT7 (GVFDIIKGAGKQLIAHAMGKIAEKVGLNKDGN), isolated from the skin secretion of the Brazilian frog *Leptodactylus pustulatus*, has a potential to be used as an AMP. In this work we studied the interaction of Ocellatin PT7 with large (LUVs) and giant (GUVs) unilamellar vesicles as membrane models. Vesicles were composed of POPC, POPG and cholesterol in different molar ratios to mimic bacterial and mammal membranes. The peptide interaction with membranes was investigated employing fluorescence measurements of 5(6)-carboxyfluorescein leakage from LUVs, isothermal titration calorimetry (ITC), circular dichroism spectroscopy (CD) and optical microscopy of GUVs. Ocellatin PT7 increased permeability of pure POPC, pure POPG and mixed POPC:POPG membranes, but it was not able to permeabilize membranes of POPC:cholesterol (6:4 mol:mol). ITC studies showed that the binding of Ocellatin PT7 to LUVs is exothermic for all membrane compositions. CD spectra showed that the peptide acquires alfa helical structure in the presence of membranes of pure POPG, but seems to acquire an amyloid assembly in membranes of POPC:POPG (1:1 mol). All results together indicate that the peptide interacts with both neutral and negatively charged membranes causing changes in membrane permeability but not with neutral membranes with cholesterol. Furthermore, the variety of peptide structures suggests that the mode of action of Ocellatin PT7 is dependent on membrane composition.

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Structure of pore-forming colicins in POPC membrane

K. Riedlová[1,2], R. Fišer[2], T. Dolejšová[2], Ł. Ćwiklik[1]

[1]Department of Theoretical Chemistry, J. Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences, Prague, Czech Republic
[2]Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague, Czech Republic

Colicins are toxins produced by *Escherichia coli* which at the same time are toxic to some strains *E. coli.* These proteins exhibit their cytotoxic effect while embedded in cytoplasmic membrane [1]. In this work, we study individual helices from C-terminal pore-forming domain (CTD) of colicin U [2] embedded in POPC lipid bilayer at the atomistic level. For this purpose, the all-atom molecular dynamics (MD) simulation method is used. The simulations include also longer segments of CTD in colicin U as well as other colicins (A, S4, B, N, E1, Ia). We focus on an embedding mechanism and stability of the protein fragments in the lipid bilayer. Furthermore, pore-forming properties and modulation of bilayer permeability are investigated in detail. MD simulations are supported by experimental permeability measurements employing Black Lipid Membranes with embedded helical peptides.

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Can one use DPH to probe the behavior of itraconazole in lipid membrane systems?

C. Poojari[1], N. Wilkosz[2], P. Jurkiewicz[3], M. Dzieciuch-Rojek[2], I. Vattulainen[1,4], M. Kępczyński[2], T. Róg[1,4]

[1]Department of Physics, University of Helsinki, Finland
[2]Faculty of Chemistry, Jagiellonian University, Kraków, Poland
[3]J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[4]Laboratory of Physics, Tampere University of Technology, Finland

1,6-diphenyl-1,3,5-hexatriene (DPH) is a fluorescent probe that is frequently used to study the ordering and the mobility of lipids in model and biological membranes. Additionally, DPH can also be employed for investigations of drug-membrane interactions. In this work, we combined atomistic molecular dynamics (MD) simulations and fluorescence anisotropy measurements to study the behavior of DPH in lipid membranes that host an antifungal drug known as itraconazole (ITZ). In particular, we explored how DPH and ITZ affect each other in model membranes. ITZ is a long, rigid, amphipathic molecule locating in lipid membranes below the membrane-water interface and orienting itself parallel to the membrane surface [1]. MD studies showed that, overall, DPH does not affect the membrane properties to a significant degree, but ITZ increases the ordering of hydrocarbon chains close to the glycerol backbone and slightly decreases the order towards the acyl chain end. The findings based on MD simulations are supported by steady state fluorescence anisotropy measurements, which indicated a large disordering effect by ITZ. In membranes containing both DPH and ITZ, MD simulations suggest that ITZ mainly influences the behavior of DPH rather than the ordering of lipid chains, as is assumed in the traditional interpretation. Our results suggest that there limitations in the use of DPH in lipid membranes containing drug molecules that should be accounted for, as DPH may report the behavior of drugs, such as ITZ, instead of providing information of the behavior of lipid hydrocarbon chains.

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Antioxidant protection of cell membranes from ozone-induced stress

E. Rudolphi-Skórska[1], B. Dyba[1], B. Kreczmer[1], M. Filek[1,2]

[1]Department of Biochemistry, Biophysics and Biotechnology Institute of Biology, Pedagogical University of Cracow, Poland
[2]Institute of Plant Physiology, Polish Academy of Sciences, Kraków, Poland

Cellular membranes representing the first place of contact with environment are responsible for defending the cells against stressogenic action of various pollutants. The degree of membrane damage caused by lipid oxidation depends on both: lipid nature and the presence/formation of substances which are capable of reducing the effects of oxidative stress and restoring redox equilibrium. Increased level of ozone in polluted atmosphere is responsible for appearance of ROS - an oxidative stress inducer.

The work compares the impact of water- (green tea extracts and polyphenolic compounds) and oil-soluble (a-tocopherol) antioxidants on the ozone-induced oxidation of Langmuir monolayers formed from phosphatidylcholines of various structure of hydrophobic part.

The protective ability of antioxidants was quantified on the basis of the level of lipid monolayer destruction. Layer degradation was quantified by the ratio of areas per molecule corresponding 1 mN/m increase of surface pressure (lift-off values) of not- to oxidized layers, occurring to be a sigmoidal-shape function of ozone concentration.

The results shows that:

1. in the degree of ozone-induced degradation of lipid layers a decisive role plays composition and structure of hydrophobic part of lipid, in particular – a content of unsymmetrical lipid with one saturated hydrocarbon chain,
2. water-soluble polyphenols, by diminishing ROS level in bulk reaction, shift the threshold of ozone concentration (causing noticeable layer damage) to higher level,
3. oil-soluble antioxidant practically does not influence the threshold ozone level but its oxidation products, staying within lipid layer helps to maintain its integrity.

Oligomerization of the protein FGF2 at the lipid membrane leads not always to pore formation

R. Šachl[1], S. Čujová[1], M. Hof[1], J. Steringer[2], W.Nickel[2]

[1]J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[2]Heidelberg University Biochemistry Center, Germany

Fibroblast Growth Factor 2 (FGF2) is a protein that has many biological functions from wound healing, cell differentiation to functioning as a signaling molecule in tumor-induced angiogenesis. To execute its extracellular functions, FGF2 is secreted from cells by an unconventional, ER/Golgi-independent pathway. Based on mainly biochemical assays, it has been suggested that FGF2 oligomerizes into pores at cellular plasma membranes from which it is released into the extracellular space. To study the process of oligomerization in detail we have developed a fluorescence approach which combines determination of protein oligomeric state by the analysis of brightness of individually diffusing oligomers and a leakage assay performed on giant unilamellar vesicles (GUVs) into a single method. This enables to monitor FGF2 oligomerization selectively on leaky vs. non-leaky GUVs, i.e. on such GUVs that are containing or missing membrane pores. Moreover, the oligomerization state of FGF2 can be monitored on the same GUV over time, which allows for correlating the formation of pores to the oligomerization of FGF2. Details on the clustering behaviour of FGF2 will be given in this contribution.

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New application of Laurdan and di-4-ANEPPS in a study of *Candida albicans*' plasma membrane biophysics

J. Suchodolski, A. Krasowska

Department of Biotransformation, Faculty of Biotechnology, University of Wroclaw, Poland

Candida albicans is a pathogenic fungus that causes mucosal and systemic infections in immunocompromised patients with a mortality rate up to 50% [1]. Ergosterol in the *C. albicans*' plasma membrane is the primary target for antifungal agents (such as azoles and polyenes).

The changes in plasma membrane ergosterol concentrations modulate the activity of ATP-binding cassette (ABC) transporters, which play the main role in multidrug resistance (MDR) among *C. albicans'* clinical isolates [2]. Ergosterol could be a modulator of plasma membrane fluidity and transmembrane potential; therefore, adaptation and implementation of new biophysical methods for visualising the *C. albicans'* membrane seem to be important in candidemia treatment.

We used the fluorescent dyes Laurdan and di-4-ANEPPS and adopted previously used methods for measuring membrane fluidity in human cells [3] in order to evaluate *C. albicans'* plasma membrane fluidity and transmembrane electrochemical potential. Lauran and di-4-ANEPP bound to *C. albicans'* membranes and showed specific changes in membrane fluidity or electrochemical potential. According to our knowledge, this is the first report on the use of both fluorescent dyes in *C. albicans* cells *in vivo*.

We applied both dyes in a *C. albicans'* mutant deficient in lanosterol 14α-demethylase (*erg11Δ/erg11Δ*) and in cells after treatment with azoles, which are inhibitors of ergosterol biosynthesis pathway. We conclude that both *ERG11* gene deletion and azole treatment depolarize and rigidify *C. albicans'* plasma membrane.

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Peptide translocation across phospholipid membranes

I. Kabelka[1,2], R. Božek[1,2], R. Vácha[1,2]

[1]CEITEC, Masaryk University, Brno, Czech Republic
[2]Faculty of Science, Masaryk University, Brno, Czech Republic

Amphiphilic peptides with right properties can translocate inside the cells by passing through its semi-permeable protective membrane. The translocation is particularly important for antimicrobial and cell-penetrating peptides, which can carry drugs and kill cells by interacting with internal molecules. However, the properties and conditions that enable peptides to spontaneously translocate across the membrane are not well understood. We have developed and tested new collective variable for peptide translocation and calculated the translocation free energy profile for amphiphilic helical peptides using MARTINI coarse-grained model. The determined advantageous properties of peptides and lipids for translocation may be useful for the rational design of peptides that are more efficient and specific against given target cells or bacteria.

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C_{60} fullerene impact on cell membranes integrity

A. Borowik, A. Woziwodzka, J. Piosik

Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Poland

C_{60} fullerene (FC_{60}) is reported to directly interact with numerous groups of drugs and penetrate into cells. Therefore, it is extensively studied as a drug carrier in novel therapies. However, some reports suggest FC_{60} toxicity, which might be connected with its hydrophobicity, ability to interact with fatty acids and to affect cellular membranes integrity. From application point of view, it seems important to characterize FC_{60} interactions with diverse types of membranes.

Model Gram+ and Gram- bacteria were chosen to study changes in cell membranes parameters caused by presence of FC_{60}. Using bacterial mutagenicity assay (Ames test) FC_{60} biological activity was determined. Atomic Force Microscopy was employed to visualize FC_{60} adhesion to bacterial cell walls. Subsequently, combined Live/Dead staining was used to define FC_{60} uptake and its impact on the cell wall continuity and cells viability. FC_{60} influence on cell growth rate was assessed by spectro-photometrical measurements. Finally, Parallel Artificial Membrane Permeability Assay (PAMPA) was developed to define interactions of FC_{60} with membranes composed of lipids in diverse proportions. As a result, differences in membranes continuity caused by FC_{60} are visualized.

The obtained findings shed light on FC_{60} impact on various membranes integrity. Precise understanding of FC_{60} behavior during movement inward cells is crucial for its future application as a drug transporter in nanomedicine.

Myelin model for studying membrane active compounds

A. Chmielińska[1], A. Polit[1], M. Dziedzicka-Wasylewska[1,2]

[1]Department of Physical Biochemistry, Faculty of Biochemistry Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
[2]Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

Lipid vesicles are the most commonly utilized models for studying interactions of small molecules with the lipid membrane. In case of externally supplied compounds, if the mechanisms of transmembrane transport are absent, only the most outer membrane layer will be affected. In order to analyze the interaction quantitatively, the amount of available lipid is essential. This, in turn, can be evaluated only for unilammelar vesicles of known diameter and small polydispersity. Studying myelin membrane active compounds is challenging. The isolated myelin is a suspension of lipo-protein multilammelar vesicles. The lammelae are kept tightly together by peripheral and transmembrane proteins [1]. This suspension is extremely heterogeneous in size, shape and the number of lammelae [2]. The estimation of available lipid for myelin is impossible. Moreover the scattering from the suspension interferes with most of spectroscopic measurements.

Here we present the protocol for obtaining rat myelin vesicles of small size, low polydispersity and highly reduced lammelarity. We have found that disrupting protein scaffold was necessary for producing highly homogenous unilammelar vesicles. The peripheral proteins were removed from membrane surface with sodium hydroxide wash; the remaining protein bounds were diminished by proteinase K digestion. Then the standard method were employed: cycles of freeze and thaw combined with the extrusion through polycarbonate filters. The reduction of diameter and polydispersity was observed using dynamic light scattering (DLS). The vesicles shape and number of lammelae was evaluated using transmission electron microscopy imaging.

Myelin vesicles obtained according to the presented protocol are convenient models for studying membrane active compounds.

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Substrate support for supported lipid bilayers affects domain mobility and phase behaviour

J. A. Goodchild, D. Walsh, H. Laurent, S. D. Connell

Department of Physics and Astronomy, University of Leeds, United Kingdom

A wide range of biophysical techniques have enabled a detailed structure of the phospholipid bilayer to be established. In particular, Fluorescence Microscopy, AFM, NMR and XRD have been used to elucidate the lateral heterogeneity of phase separating membranes. Different techniques can use different model membranes such as GUVs, Black Lipid Membranes and Supported Lipid Bilayers (SLBs). Heterogeneity in SLBs can be investigated using many surface sensitive techniques, but different substrates are often used. AFM uses Mica, Fluorescence Microscopy/FRAP/FCS generally use glass, and QCM-D uses Silicon Oxide. Another substrate of recent interest for SLBs is polydimethylsiloxane (PDMS). This cheap and relatively inert polymer is simple to mould into different patterns and can be used to design a wide-range of biotechnological devices. These include drug-screening chips and microfluidic devices.

We are interested in how substrates affect the phase behaviour of SLBs. We have investigated how different substrates affect 1) the hydrophobic and thermodynamic drive for bilayers to form at the substrate, 2) the diffusion of individual lipids, domain formation and hydrodynamic motion of domains and 3) the lipid ordering and melting transition on different substrates. We find that whilst molecular diffusion is hardly affected by changes in substrate, domain mobility is significantly hindered on glass and PDMS compared to mica, likely due to a combination of increased surface roughness and increased hydrophobic recovery. We also show that nanoscale domains on PDMS can only be observed using 'Partial Penetration AFM', where imaging force is controlled to selectively break through different phases.

Study of interaction of an antifungal antibiotic amphotericin B with lipid membranes based on fluorescence anisotropy

E. Grela, R. Luchowski, W. Grudziński, W.I. Gruszecki

Department of Biophysics, Institute of Physics, Maria Curie-Sklodowska University, Lublin, Poland

Amphotericin B (AmB) is a polyene antibiotic, which is synthetized by the bacteria *Streptomyces nodosus*. AmB is a gold standard in treatment of systemic mycotic infections, due to its high effectiveness, a broad spectrum of action and rare pathogens resistant to this antibiotic. Although, the exact mechanism of action of AmB is still unknown, it is used as a lifesaving drug. Understanding the mechanism of action of AmB would enable to minimize its toxic side effects, and possibly increase its therapeutic effects.

The molecular organization of different spectral forms of AmB and their incorporation to the lipid membranes was studied with application of fluorescence anisotropy. The results show that in the presence of sterols, AmB binds into lipid bilayers, while lack of sterols causes AmB to be located on the surface of lipid membranes. The effect strongly depends also on a lenght of lipid acyl chains. Based on confocal fluorescence microscopy the orientation of molecules of AmB was determined with respect to a single lipid bilayer. Measurements in model systems were performed using Giant Unilamellar Vesicles (GUV) formed with lipids and AmB. Analysis of fluorescence lifetime of AmB incorporated to liposomes revealed appearance of the drug in the form of different supramolecular structures.

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Optimizing peptide properties for translocation across lipid membranes

I. Kabelka[1,2], R. Vácha[1,2]

[1]CEITEC and Faculty of Science, Masaryk University, Brno, Czech Republic
[2]Faculty of Science, Masaryk University, Brno, Czech Republic

Antimicrobial peptides belong to a diverse group of membrane-active peptides that can be lethal to a large variety of microorganisms. Some of these peptides act via spontaneous translocation across lipid membranes. Using Metropolis Monte Carlo simulations and coarse-grained models, we have calculated the free energies associated with the process and its dependence on various peptide properties. All peptides were found to adsorb at membrane parallel with its plane, to tilt when inserting deeper into the hydrophobic core, and to be perpendicular or tilted to membrane plane in the inserted/transmembrane state. Both peptide hydrophobic content and its distribution affect the depth of adsorption as well as the free energy barrier for translocation. The optimal parameters of a peptide (length, hydrophobic content and its distribution) could help the rational design of membrane-active peptides and contribute towards better understanding of the translocation process.

Interactions of short synthetic lipopeptide with model membranes containing phospatidylcholine and phosphatidylserine

D. Konarzewska, S. Sęk

Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Poland

The use of currently available medicines or drugs to treat cancer is often related with serious cardiovascular toxicity. Because of it, development of new and less harmful substances with anticancer activity is very important. Among them, lipopeptides can be considered as promising class of drugs which primary target is the cancer cell membrane. Lipopeptides are amphiphilic molecules, which contain one or more lipid chains attached to a peptide headgroup [1]. They are produced by some bacteria and fungi however, could be synthesized in laboratory as well. Modulation of chirality the amino acids at peptide chains or the length of the hydrophobic part, can cause increase or minimize the activity of lipopeptides. In this work, the Langmuir technique and atomic force microscopy were used to investigate the interactions of novel lipopeptide with lipid films mimicking cancer cells membrane. Lipopeptide was composed of Trp-Lys-D-Leu-Lys amino acids and palmitoyl chain linked to N-terminus of peptide [2]. Lipid films contained: 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphoserine (DMPS) at molar ratio 7:3. Britton-Robinson buffers of pH 5,5 and 7,4 were used as subphases. Also, the stability of lipid monolayers with lipopeptide was examined and we have determined the maximum insertion pressure (MIP) – parameter which allows evaluation of lipid-lipopeptide interactions.

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A computational study on how cholesterol and PI(4,5)P_2 trigger oligomerization of FGF2 on the membrane surface

F. Lolicato[1,2], C. Poojari[1], J. K. Kuisma[1], I. Vattulainen[1,2,3]

[1]Department of Physics, University of Helsinki, Finland
[2]Department of Physics, Tampere University of Technology, Finland
[3]MEMPHYS – Center for Biomembrane Physics, University of Southern Denmark, Odense, Denmark

Fibroblast Growth Factor II (FGF2) is secreted from cells by an unconventional secretory pathway. This process depends on sequential interactions of FGF2 with the phosphoinositide PI(4,5)P_2 at the inner leaflet and heparin sulfate proteoglycans at the outer leaflet of the plasma membrane. Here we show how the recruitment by PI(4,5)P_2 triggers oligomerization of FGF2 on the membrane surface.

Atomistic molecular dynamics simulations were used to shed light on the initial steps of PI(4,5)P2-driven FGF2 oligomerization and to predict the most likely interfaces for FGF2-FGF2 interactions [1]. The simulations were complemented by biochemical experiments [1].

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Molecular dynamics studies of lutein and zeaxanthin molecules in the phospholipid bilayer

K. Makuch, M. Markiewicz, M. Pasenkiewicz-Gierula

Department of Computational Biophysics and Bioinformatics, Faculty of Biochemistry Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

Carotenoids are the second largest group of plant pigments. They are part of the photosynthetic complex, responsible for light harvesting and energy dissipation. They also play a role as membrane photoprotectors. Carotenoids are synthesised only by plants and bacteria, while animals have to receive them from food. Animals need carotenoids as light filters and membrane protectors. Decreased consumption of lutein is associated with increased risk of eye diseases, especially age related macular degeneration.

Lutein and zeaxanthin are two main carotenoids of retina. Although they have similar structures, their localisation within the retina is different – concentration of zeaxanthin is higher in the middle and that of lutein is higher in the outer regions of the retina. Moreover, model studies indicate that their orientation in the lipid bilayer is different. To verify this, computational methods were used to analyse the orientation of lutein and zeaxanthin in the phosphatidylcholine bilayer. For lutein, both transmembrane and horizontal orientations were observed. In contrast, for zeaxanthin only transmembrane orientation was observed. Orientation of the carotenoid molecules may affect the fluidity of the membrane as well as their interactions with potential partners.

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Enhancing the effectiveness of electrochemotherapy – in vitro study with green tea catechin on sensitive and resistant pancreatic cancer

O. Michel[1], J. Mączyńska[1], A. Szewczyk[2], J. Rossowska[3], J. Kulbacka[1], J. Saczko[1]

[1]Department of Medical Biochemistry, Wroclaw Medical University, Poland
[2]Department of Animal Developmental Biology, Institute of Experimental Biology, University of Wroclaw, Poland
[3]Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

The acquisition of the resistance mechanisms makes the pancreatic ductal adenocarcinoma (PDA) one of the most lethal cancers [1]. Therefore, many studies aim to limit the expression of proteins present in the cell membrane, such as ABCB1 – a P-glycoprotein efflux pump. It has been shown that green tea polyphenols can inhibit the photolabeling of P-gp by 75% in multidrug-resistant cells [2]. Electrochemotherapy (ECT) is an experimental method based on the cell exposition of external electric pulses to induce permeabilization of the cell membrane. Combining the increased drug delivery with the modulation of drug resistance could potentially maximize the cytotoxicity. The aim of our study was to investigate the efficiency of cisplatin delivered through the membrane via electropulses and to examine the possibility of enhancing that effect using catechin – green tea bioflavonoid. The research material consisted of three cell lines of PDA: EPP85-181P (sensitive to daunorubicin), EPP85-181RDB (resistant to daunorubicin) and EPP85-181RNOV (multidrug-resistant). Firstly, we examined the influence of electrochemotherapy with cisplatin on cells viability using MTT assay. Further, we determined the toxicity of catechin and preincubated cells for 24 h or 2 h with two non-toxic catechin concentrations (10 and 50μM, respectively). Subsequently we performed ECT with cisplatin and investigated the influence of preincubation on drug uptake via flow cytometry and on the expression of ABCB1 using confocal microscopy and immunocytochemical staining ABC. Obtained results indicate that catechin preincubation in combination with electroporation can sensitize membranes of resistant PDA cells to cisplatin and hereby increase the therapeutic effect.

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The role of distal mutation that alters rat CYP1A1 activity towards persistent organic pollutants

V. Navrátilová[1], M. Paloncýová[1], K. Berka[1], S. Mise[2], Y. Haga[3], C. Matsumura[3], T. Sakaki[4], H. Inui[2,5], M. Otyepka[1]

[1]Regional Centre of Advanced Technologies and Materials, Department of Physical Chemistry, Faculty of Science, Palacký University Olomouc, Czech Republic
[2]Graduate School of Agricultural Science, Kobe University, Japan
[3]Hyogo Prefectural Institute of Environmental Sciences, Kobe, Japan
[4]Biotechnology Research Center, Faculty of Engineering, Toyama Prefectural University, Imizu, Japan
[5]Biosignal Research Center, Kobe University, Japan

Cytochromes P450 (CYP) enzymes are important in the metabolism of many xenobiotics including highly toxic and poorly degradable environmental pollutants such as persistent organic pollutants (POPs) which are mainly catalyzed by cytochromes P450 1A1 isoforms. For this reason we rationalize the biodegradation of two different POPs - 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 3,3',4,4'-tetrachlorobiphenyl (PCB77) by rat cytochrome P450 1A1 (CYP1A1). Using molecular dynamics simulations and experiments, we showed that the enhanced activity/binding affinity of the rat CYP1A1 mutant towards TCDD was due to more efficient binding of the substrate in the active site even though the mutated site was over 2.5 nm away from the catalytic center. Moreover, this mutation decreased activity towards PCB77. These results show that not only active site mutations but also distal mutations may change the enzyme activity and can be possible targets for rational enzyme design.

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The role of BtuB dynamics in the transport of vitamin B_{12} through *E. coli* outer membrane

T. Pieńko[1, 2], J. Trylska[1]

[1]Centre of New Technologies, University of Warsaw, Poland
[2]Department of Drug Chemistry, Faculty of Pharmacy with the Laboratory Medicine Division, Medical University of Warsaw, Poland

BtuB is a transmembrane beta-barrel protein that belongs to the family of TonB-dependent receptors engaged in the transport of vitamin B_{12} and iron siderophores in many bacteria. In *E. coli*, it was postulated that vitamin B_{12} transport through BtuB is feasible due to unfolding of the luminal domain of BtuB resulting from the interactions with an inner membrane protein TonB [1]. However, detailed molecular description of this process still remains unclear.

We have investigated the mechanism of passage of vitamin B_{12} through BtuB embedded in an asymmetric heterogeneous outer membrane of *K-12 E. coli* strain using molecular dynamics (MD) simulations. The BtuB barrel is occluded by its N-terminal luminal domain that has to either unfold or otherwise make space for vitamin B_{12} passage to the periplasm. Vitamin B_{12} binds at the top of luminal domain being partially covered by the BtuB extracellular loops.

Recent EPR studies on BtuB [2] showed that dynamics of BtuB external loops is crucial for vitamin B_{12} transport and that there is some coupling between the N-terminus of luminal domain and extracellular loops. Thus we combined steered MD to unfold the luminal domain with local heating of the loops via imposing additional random forces. The simulations were performed with NAMD 2.12 [3] and AMBER force field parameters [4,5].

We found that the BtuB loops demonstrate diverse conformational dynamics suggesting that they may play different roles in BtuB activity. The BtuB loops' dynamics is also affected by vitamin B_{12} loading and the luminal domain folding/unfolding state.

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Cytochrome P450 reductase simulations: Conformation changes and cytochrome P450 complex

M. Šrejber, V. Navrátilová, M. Paloncýová, K.Berka, M. Otyepka

Regional Center of Advanced Technologies and Materials, Department of Physical Chemistry, Faculty of Science, Olomouc, Czech Republic

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

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

An in vitro study of the effects of labetalol on human erythrocytes and molecular models of cells membranes

P. A. Zambrano[1,2], M. Suwalsky[1], M. Jemioła-Rzemińska[2,3], K. Strzałka[2,3]

[1]Faculty of Chemical Sciences, University of Concepción, Chile
[2]Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland
[3]Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

Labetalol is a drug used to treat high blood pressure. With the aim to better understand the molecular mechanisms of the toxic effect of labetalol it was assayed on human erythrocytes and in bilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidyl-ethanolamine (DMPE), classes of phospholipid located in the outer and inner monolayers of the human erythrocyte membrane, respectively. The capacity of labetalol to interact with the bilayer structures of DMPC and DMPE was determined by X-ray diffraction and differential scanning calorimetry (DSC) whereas intact human erythrocytes were observed by scanning electron microscopy. X ray diffraction results of DMPC bilayers shows that after exposure to labetalol there was a gradual weakening of the lipid reflection intensities. From these results it can be concluded that labetalol produced a structural perturbation of both the polar and the acyl hydrophobic regions of DMPC bilayers. In addition, experimental findings demonstrated that labetalol in vitro interacted with erythrocytes inducing morphological changes.

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