UNIVERSITY of DEBRECEN

EJTEMM

European Joint Theory/Experiment Meeting on Membranes

June 12-14, 2024, Debrecen, Hungary

PROGRAM

The Organizing Comittee of EJTEMM 2024 are grateful for the support of its

Sponsors and exhibitors

GOLD SPONSOR:

UNICAM

Exhibitor:

SARTORIUS

Supported by:

Debrecen University Symposium, 2024

UNIVERSITY of DEBRECEN

EBSA
Biophysics in Europe

BIO-SCIENCE

European Joint Theory/Experiment Meeting on Membranes (EJTEMM)

June 12-14, 2024
Debrecen

Dear Colleagues,

It is our pleasure to invite you to the next **European Joint Theory/Experiment Meeting on Membranes (EJTEMM)** to be organized in the nice Hungarian city of Debrecen between June 12-14, 2024.

Membrane research has always been an interdisciplinary subject inviting and requiring biologists, physicists and chemists to establish the fundamental principles of membrane structure and function. This tenet is ever more relevant with the significant advancement of structural biological and computational tools, in which even a single experiment may require the cooperation between scientists representing different disciplines. It is our intention to organize the meeting with the aforementioned principle in mind, and to provide a stimulating environment for fostering exchange of knowledge and for establishing future collaborations by putting together a program that covers a wide range of subjects of theoretical and experimental membrane research. While several large meetings feature topics about membranes, a regular, topical conference covering membrane-related problems from the standpoint of structural, computational and cell biological research is required to fill a void in the professional life of membrane researchers.

We would like to promote the participation of PhD students and early career researchers by sending out a special invitation to them to present their findings as poster and oral contributions.

We hope that many of you will join us for this three-day meeting that will not only provide ample opportunities for formal and less formal interactions and networking but will also be memorable for the enjoyable social programs and for the hospitality of the host institution.

Peter Nagy and György Vámosi
University of Debrecen

Scientific organizers:

Péter Nagy and György Vámosi
Department of Biophysics and Cell Biology,
Faculty of Medicine University of Debrecen, Hungary

Program committee:

György Vámosi, Peter Nagy (University of Debrecen, Hungary)
Győző Garab (Biological Research Center, Hungary)
Judith Peters (Grenoble Alpes University, France)
Edit Buzás (Semmelweis University, Hungary)
Veronika Iglič (University of Ljubljana, Slovenia)
István Mándity (Semmelweis University, Hungary)
Ines Neundorf (University of Köln, Germany)
Norbert Kučerka (Comenius University, Slovakia)
Daniela Uhrikova (Comenius University, Slovakia)
John Seddon (Imperial College, UK)
Sandro Keller (University of Graz, Austria)
Hector Martinez-Seara (Czech Academy of Sciences, Czech Republic)
Michal Otyepka (Palacký University, Czech Republic)

Venue:

University of Debrecen
LifeScience Building
1. Egyetem Square, 4032 Debrecen

Organizing Agency:

Remedicon Kft.
Galagonya str. 7. 1036
Budapest, Hungary
info@remedicon.hu
Tel: +36-1-225-0188
www.remedicon.hu

Registration fee:

Category	EARLY BIRD until 15/04/2024	REGULAR from 16/04 until 8/06/2024
Senior	300 EUR	350 EUR
PhD students	150 EUR	200 EUR

The registration fee includes:

- Admission to the Conference
- Book of Abstracts
- Conference materials
- 2 Lunches
- Coffee breaks
- 2 Dinners
- Social events

Registration Desk at the venue:
LifeScience Building, ground floor
Opening hours:
12th JUNE 10:00-17:00
13th JUNE 08:30-17:00
14th JUNE 08:30-13:00

Oral presentations:
For the lectures MS Office/PowerPoint presentation facilities will be provided. Lectures are kindly asked to give their presentations on USB stick to the technician before the morning or the afternoon session. Any special needs (e.g. the use of own laptop) should be discussed in time with the technician.

Poster presentations:
The poster sessions will take place in the Life Science Building Aula. Posters are expected to be mounted on the opening day, before the poster sessions and can remain displayed for 3 days, until the end of the Conference.
Recommended size of posters: AO, 90 x 120 cm, portrait format, and numbered in advance. (Poster stands are 100 cm X 200 cm)
Tools will be provided at the site to help install the posters.

Social events:

June 12, Wednesday

19:00- Dinner

Venue: University of Debrecen,

Address:„Nagyerdei Restaurant" next to the Life Science Building

June 13, Thursday

19:00-20:00 Concert of the Debrecen Dixiland Jazz Band

20:00- Gala dinner with music

Venue: Hotel Divinus, Ignis conference room

Address: Nagyerdei krt.1, 4032 Debrecen (1 km far from the venue)

www.hoteldivinus.hu

Exhibition:

Exhibitor's stands will be placed on the ground floor and will be on display throughout the Conference next to the conference room.

Meals:

During the coffee breaks coffee, tea, refreshments, and snacks will be served. Lunch for 12th, 13th and 14th of June while dinner for 12th and 13th of June will be served. The cost of the meals is included in the registration fee. Tickets for lunch and dinners will be provided in the name badge.

Please make sure to wear your badge at all times during the conference.

Free wifi:

Free wifi is available at the Conference venue.

Network: ejtemm2024

Password: Unideb2024

PROGRAM

June 12, Wednesday

10:00	*Registration*	
10:45- 11:00	*Opening*	
11:00-12:55	**SESSION 1: MEMBRANE PROTEINS AND CELLULAR ASPECTS** **Chairs: Peter Nagy,** *University of Debrecen* **György Vámosi,** *University of Debrecen*	
11:00-11:25	**Mario Brameshuber** MONTE CARLO SIMULATIONS FOR THE EVALUATION OF QUANTITATIVE SINGLE MOLECULE FLUORESCENCE MICROSCOPY	1
11:25-11:50	**Aleš Iglič** ON THE ROLE OF ACTIN FORCES AND PROTEIN INTRINSIC CURVATURES IN PHAGOCYTOSIS AND MIGRATION OF CELLS ON CURVED SURFACES	2
11:50-12:15	**Jerker Widengren** (on-line) FLUOROPHORE BLINKING – A SOMEWHAT OVERLOOKED SOURCE OF INFORMATION FOR BIOMOLECULAR AND CELLULAR STUDIES	3
12:15-12:30	**Hector Martinez-Seara** ENHANCING ELECTROSTATIC INTERACTION MODELS THROUGH ELECTRONIC POLARIZATION:INSIGHTS FROM THE PROSECCO75 BIOMOLECULAR FORCE FIELD	4
12:30-12:55	**Zsolt Török** MILD HYPERTHERMIA INDUCED INTRACELLULAR THERMOGENESIS DEFINES STRESS RESPONSE MECHANISMS	5
12:55-14:00	*Lunch*	

14:00-15:50	**SESSION 2: EXTRACELLULAR VESICLES** **Chairs: Edit Buzás,** *Semmelweis University* **Veronika Iglič,** *University of Ljubljana*	
14:00-14:25	**Veronika Kralj-Iglic** ASSESSMENT OF NUMBER DENSITY AND SIZE OF EXTRACELLULAR PARTICLES IN DIFFERENT BIOLOGICAL FLUIDS WITH INTERFEROMETRIC LIGHT MICROSCOPY	6
14:25-14:50	**Tamás Beke-Somfai** RED BLOOD CELL DERIVED VESICLES AS INTERACTING PARTNERS OF AMPHIPHILIC ANTIMICROBIAL PEPTIDES	7
14:50-15:10	**Tamás Visnovitz** RELEASE OF EN BLOC SECRETED AMPHIECTOSOME-DERIVED SMALL EXTRACELLULAR VESICLES	8
15:10-15:30	**Bartosz Różycki** MEMBRANE CURVATURE SENSING BY MODEL BIOMOLECULAR CONDENSATES	9
15:30-15:50	**Emese Sinkó,** *Bio-Science Ltd* SHIFT PERSPECTIVE, ACHIEVE MORE: INTRODUCING THE CYTOFLEX NANO FLOW CYTOMETER	10
15:50- 16:30	*Coffee break*	
16:30-17:15	**Pleanary lecture I.** Chair: Stefan Knippenberg **John Seddon,** *Imperial College, UK* INVERSE BICONTINUOUS AND DISCONTINUOUS PHASES OF LIPIDS	11
17:15-19:00	POSTER SESSION I.	
19:00-21:00	*Dinner*	

June 13, Thursday

08:45-09:30	**Plenary lecture II.** Chair: Aleš Iglič **Douglas Kell** *University of Liverpool, UK* A PROTET-BASED MODEL FOR OXIDATIVE AND PHOTOSYNTHETIC PHOSPHORYLATION	12
09:30-11:10	**SESSION 3: LIPID PROTEIN INTERACTIONS** **Chairs: John Seddon,** *Imperial College, UK* **Sandro Keller,** *University of Graz*	
09:30-09:55	**Ana Nicoleta Bondar** PROTONATION-COUPLED WATER-MEDIATED HYDROGEN BOND NETWORKS FOR SIGNALING ACROSS CELLULAR MEMBRANES	13
09:55-10:20	**Alessandra Luchini** UNDERSTANDING PROTEIN-LIPID INTERACTIONS ASSOCIATED WITH THE MALARIA INFECTION	14
10:20-10:40	**Tamás Földes** COARSE-GRAINED MOLECULAR DYNAMICS MODELLING OF HUMAN LOW-DENSITY LIPOPROTEIN	15
10:40-10:55	**Nicolas Carvalho** DECIPHERING INTERDOMAIN ALLOSTERIC EFFECTS ON THE pH-DEPENDENT MEMBRANE INSERTION PROCESS OF THE DIPHTHERIA TOXIN	16
10:55-11:10	**Marketa Paloncyova** INTERACTIONS OF CARBON NANOMATERIALS WITH LIPID MEMBRANES	17
11:10-11:40	*Coffee break*	

11:40-13:20	**SESSION 4: MEMBRANE STRUCTURE, CURVATURE AND DYNAMICS** **Chairs: Hector Martinez-Seara,** *Czech Academy of Sciences* **Marketa Paloncyova,** *Palacký University*	
11:40-12:00	**Alex Bunker** THREE VERY DIFFERENT EXAMPLES OF MEMBRANE SIMULATIONS IN PHARMACEUTICAL RESEARCH	18
12:00-12:20	**Dominique J. Bicout** THE DYNAMICAL MATRYOSHKA MODEL OF LIPID BILAYERS	19
12:20-12:40	**Marion Alix** AOC3 ACTIVE SITE ACCESSIBILITY AND HELICES DIMERIZATION REVEALED BY MEMBRANE MOLECULAR DYNAMIC	20
12:40-13:00	**Denys Biriukov** ULTRA-LARGE-SCALE ALL-ATOM MOLECULAR DYNAMICS SIMULATIONS OF CELLULAR MEMBRANES AND EXTRACELLULAR INTERACTIONS	21
13:00-13:20	**Silvio Osella** PHOTOPHYSICS IN BIOMEMBRANES: COMPUTATIONAL INSIGHT INTO THE INTERACTION BETWEEN LIPID BILAYERS AND CHROMOPHORES	22
13:20-14:30	*Lunch*	
14:30-16:25	**SESSION 5: NON-BILAYER MEMBRANE STRUCTURES** **Chairs: Győző Garab,** *Biological Research Center* **Judith Peters,** *Grenoble Alpes University*	
14:30-14:55	**Edward Gasanoff** AN UPGRADE ON THE PROTON CIRCUIT IN THE INNER MITOCHONDRIAL MEMBRANE THAT INVOLVES FORMATION OF NON-BILAYER INVERTED MICELLES	23
14:55-15:20	**Bence Fehér** MOLECULAR DYNAMIC SIMULATION OF LIPID ASSEMBLIES MIMICKING THYLAKOID MEMBRANES	24

15:20-15:40	**Ondrej Dlouhy** LIPID POLYMORPHISM OF PLANT THYLAKOID MEMBRANES – INSIGHT INTO THE INVERTED HEXAGONAL PHASE	25
15:40-16:00	**Judith Peters** NEUTRON AND X-RAY SCATTERING STUDIES OF NON-BILAYER MEMBRANE STRUCTURES	26
16:00-16:15	**Kinga Böde** LIPID POLYMORPHISM IN PHOTOSYNTHETIC MEMBRANES. THE FUSION OF PHOTOSYSTEM-II ENRICHED MEMBRANE PAIRS, ASSISTED BY ISOTROPIC LIPID PHASE	27
16:15-16:25	**Győző Garab** THE WHYS AND WHEREFORES OF NON-BILAYER LIPID PHASES IN ENERGY-CONVERTING BIOLOGICAL MEMBRANES – CO-CHAIR'S CONCLUDING COMMENTS AND HYPOTHESES	28
16:25-18:25	POSTER SESSION II.	
19:00-19:30	*Concert of the Debrecen Dixieland Jazz Band at Hotel Divinus*	
19:30-	*Gala dinner at Hotel Divinus*	

June 14, Friday

08:45-09:30	**Plenary lecture III.** Chair: Dominique J. Bicout **Amitabha Chattopadhyay** *Center for Cellular and Molecular Biology, India* G PROTEIN-COUPLED RECEPTORS AND CHOLESTEROL SENSITIVITY: EXCITEMENTS AND CHALLENGES	29
09:30-11:30	**SESSION 6: MEMBRANE MIMETICS** **Chairs: Norbert Kučerka,** *Comenius University* **Daniela Uhrikova,** *Comenius University*	
09:30-09:55	**Petra Pullmanova** LIPID BIOPHYSICS - THE INDISPENSABLE SKIN LIPID BARRIER	30
09:55-10:20	**Hanne Antila** INTRODUCING THE NMRLIPIDS DATABANK: A GATEWAY TO LARGE-SCALE ANALYSIS OF MEMBRANE SIMULATIONS	31
10:20-10:35	**Adam Tywoniak** INVESTIGATION OF DRUG-MEMBRANE INTERACTIONS BY NEW LIPOSOMAL CO-PERMEATION ASSAY	32
10:35-10:50	**Daniela Uhríková** EXOGENOUS LUNG SURFACTANT INTERACTIONS WITH LIPOPOLYSACCHARIDE AND POLYMYXIN B AS SEEN THROUGH NEUTRON DIFFRACTION	33
10:50-11:05	**Petra Čechová** MECHANISTIC INSIGHTS INTO INTERACTIONS BETWEEN IONIZABLE LIPID NANODROPLETS AND BIOMEMBRANES	34
11:05-11:35	*Coffee break*	

11:35-13:35	**SESSION 7: MEMBRANE ACTIVE PEPTIDES, CELL PENETRATING PEPTIDES** **Chairs: István Mándity,** *Semmelweis University* **Ines Neundorf,** *University of Cologne*	
11:35-12:00	**Monika Wojciechowska** IMPROVING THE PROPERTIES OF MEMBRANE-ACTIVE PEPTIDES BY STABILIZING THEIR SECONDARY STRUCTURES	35
12:00-12:25	**Evgeniya Save-Trofimenko** CELL-PENETRATING PEPTIDE UPTAKE AND TRAFFICKING IN CELLS	36
12:25-12:40	**Christopher Aisenbrey** DISTRIBUTION OF TWO SYNERGISTIC ANTIMICROBIAL PEPTIDES ON THE LIPID MEMBRANE SURFACE AND SIDECHAIN DYNAMICS:A FLUORESCENCE STUDY	37
12:40-12:55	**Tamás Kovács** INVESTIGATION OF THE EFFECTS OF SH-42, A POTENTIAL STATIN SUBSTITUTE, ON MEMBRANE BIOPHYSICAL PARAMETERS AND PENETRATIN UPTAKE	38
12:55-13:10	**Olivér Pavela** EXAMINING THE MEMBRANE PERTURBING EFFECT OF THE ANTIFUNGAL PEPTIDE NFAP2 WITH MOLECULAR DYNAMICS SIMULATIONS	39
13:10-13:35	**Wojciech Bal** SDS MODEL FOR ENHANCED CU(II) DELIVERY TO CELLS BY AMPHIPHILIC PEPTIDES	40
13:35	Closing	
13:45-14:45	*Lunch*	

LECTURE ABSTRACTS

1 MONTE CARLO SIMULATIONS FOR THE EVALUATION OF QUANTITATIVE SINGLE MOLECULE FLUORESCENCE MICROSCOPY

C. Bodner, D. Kiesenhofer, G. J. Schütz, M. Brameshuber

Signaling processes at the cell surface and inside the cell, as well as the cellular function itself depend on protein-protein interactions. For example, T cell antigen recognition requires T cell antigen receptors (TCRs) engaging MHC-embedded antigenic peptides (pMHCs) within the contact region of a T cell with its conjugated antigen-presenting cell. Oligomerization of TCR to higher order complexes has been postulated to maintain high antigen sensitivity and trigger signaling. However, quantifying such oligomerization events in a live cell context challenges currently available microscopy techniques1.

In the past, we presented an approach to single out interactions of fluorescently labeled membrane proteins by combining photobleaching and single-molecule microscopy. With this approach, termed "Thinning Out Clusters while Conserving the Stoichiometry of Labeling" (TOCCSL)[2], oligomerization can be detected even at physiologically high surface densities of fluorescently labeled proteins. In TOCCSL, an aperture-restricted region of the plasma membrane is irreversibly photobleached by applying a high-intensity laser pulse. During a recovery time, in which illumination is turned off, non-photobleached molecules from the non-illuminated area of the plasma membrane re-populate the aperture-restricted region. At the onset of this recovery process, these molecules can be detected as well-separated, diffraction-limited signals and their oligomerization state can be quantified.

To date, TOCCSL revealed the oligomerization state of various membrane proteins. For the TCR we found that monomeric TCR-CD3 complexes drive the recognition of antigenic pMHCs[3], which underscores the exceptional capacity of single TCR–CD3 complexes to elicit robust intracellular signaling.

To provide a theoretical framework for the quantitative interpretation of such TOCCSL measurements, we now used extensive Monte Carlo simulations. We determined the influence of experimental parameters and intrinsic characteristics of the investigated system on the outcome of a TOCCSL experiment. We identified the diffraction-affected laser intensity profile and the diffusion of molecules at the aperture edges during photobleaching as major sources of generating partially photobleached oligomers. They are falsely detected as lower order oligomers and, hence, higher order oligomers might be prevented from detection. Such *in silico* TOCCSL experiments allow for optimizing experimental parameters and oligomerization data analysis to increase the accuracy in quantifying protein oligomerization.

1 Brameshuber, M., Klotzsch, E., Ponjavic, A. & Sezgin, E. Understanding immune signaling using advanced imaging techniques. Biochem Soc Trans 50, 853-866 (2022). https://doi.org:10.1042/BST20210479

2 Moertelmaier, M., Brameshuber, M., Linimeier, M., Schütz, G. J. & Stockinger, H. Thinning out clusters while conserving stoichiometry of labeling. Applied Physics Letters 87 (2005). https://doi.org:10.1063/1.2158031

3 Brameshuber, M. et al. Monomeric TCRs drive T cell antigen recognition. Nat Immunol 19, 487-496 (2018). https://doi.org:10.1038/s41590-018-0092-4

2

ON THE ROLE OF ACTIN FORCES AND PROTEIN INTRINSIC CURVATURES IN PHAGOCYTOSIS AND MIGRATION OF CELLS ON CURVED SURFACES

Raj Kumar Sadhu[1], Samo Penič[2], Luka Mesarec[2], Veronika Kralj-Iglič[3], Nir Gov[4], Aleš Iglič[2]

1 Institut Curie, PSL Research University, CNRS, France

2 Laboratory of Physics, Fac. of Electrical Eng., University of Ljubljana, Ljubljana, Slovenia

3 Lab. of Clinical Biophysics, Fac. of Health Sciences, Univ. of Ljubljana, Ljubljana, Slovenia

4 Department of Chem. and Biol.I Physics, Weizmann Institute of Science, Rehovot, Israel

We present a theoretical study of the cell movement on the surface of different geometry as well as the mechanism of efficient phagocytosis and the mechanism of coiling of cellular protrusions around fibers. Our theoretical model describes the membrane leading-edge that are produced by curved membrane proteins that recruit the protrusive forces of actin polymerization, and identifies the role of bending and adhesion energies. Among other our model recovers the observed cell migration on the sinusoidal substrate, where cells move along the grooves (minima), while avoiding motion along the ridges. Further we predicted in accordance with experimental results that the cell's leading-edge may coil on fibers with circular cross-section (above some critical radius), but the coiling ceases for flattened fibers of highly elliptical cross-section. We also considered the phagocytosis of spherical and non-spherical particles and found that non-spherical particles are more difficult to engulf in comparison to the spherical particles of the same surface area. For non-spherical particles, the engulfment time crucially depends on the initial orientation of the particles with respect to the vesicle. Our model also offers a mechanism for the spontaneous self-organization of the actin cytoskeleton at the phagocytic cup, in good agreement with high-resolution experimental observations.

References:

1.R.K. Sadhu, M. Luciano, W. Xi, C. Martinez-Torres, M. Schröder, C. Blum, M. Tarantola, S. Villa, S. Penič, A. Iglič, C. Beta, O. Steinbock, E. Bodenschatz, B. Ladoux, S. Gabriele, Nir S. Gov: A minimal physical model for curvotaxis driven by curved protein complexes at the cell's leading edge. PNAS 121 (12): e2306818121, 2024.

2. R.K. Sadhu, C. Hernandez-Padilla, Y.E. Eisenbach, S. Penič, L. Zhang, H.D. Vishwasrao, B. Behkam, K. Konstantopoulos, H. Shroff, A. Iglič, E. Peles, A. S. Nain, N. S. Gov: Experimental and theoretical model for the origin of coiling of cellular protrusions around fibers. Nature Communications 14: 5612, 2023.

3. R.K. Sadhu, S. R. Barger, S. Penič, A. Iglič, M. Krendel, N.C. Gauthiere, N.S. Gov: A theoretical model of efficient phagocytosis driven by curved membrane proteins and active cytoskeleton forces. Soft Matter 19, 31-43, 2023.

4. Mesarec, W. Góźdź, V. Kralj-Iglič, S. Kralj, A. Iglič: Coupling of nematic in-plane orientational ordering and equilibrium shapes of closed flexible nematic shells. Scientific Reports 13:10663, 2023.

3

FLUOROPHORE BLINKING – A SOMEWHAT OVERLOOKED SOURCE OF INFORMATION FOR BIOMOLECULAR AND CELLULAR STUDIES

Jerker Widengren[1]

1 Department of Applied Physics, Royal Inst of Technology (KTH), Stockholm, Sweden (jwideng@kth.se)

Reversible dark state transitions of fluorophores are central for all forms of fluorescence-based, single-molecule and super-resolution microscopy and spectroscopy, as limiting factors in single molecule studies, at the same time prerequisites for super-resolution imaging techniques. Here, an additional aspect of such transitions is highlighted, namely that such transitions can be used to sense a manifold of biomolecular environments, dynamics and interactions. To make monitoring of dark state transitions widely applicable for studies on biological samples, we have developed so called transient state (TRAST) imaging.[1] In TRAST, fluorophore dark state transitions are monitored via the time-averaged fluorescence intensity, and from how it varies with the modulation of the excitation light.

Here, the concept of TRAST is described, and how it can be experimentally realized within different microscope modalities. Examples of biological applications are given, demonstrating how biologically relevant environmental and molecular interaction parameters can be monitored in solutions, live cells, and tissue,[2,3] which are difficult, if possible at all, to follow via regular fluorescence readout parameters. Finally, recent work is presented, on the characterization of near-IR fluorophores and their dark state transitions,[4] and how they can offer both prerequisites and restrictions in super-resolution imaging measurements.

Acknowledgements: This work was supported by grants from the Swedish Research Council (VR), the Swedish Foundation for Strategic Research (SSF), and the European Commission (H2020, NanoVIB).

References:

[1] Widengren, J. in Fluorescence Spectroscopy and Microscopy in Biology, Springer 2023 (Sachl, R., Amaro, M.M. Eds),

[2] Kitamura, A. et al, Nucl. Acids. Res. 2023, 51(5),

[3] Sandberg, E. et al., Sci. Rep. 2023, 13, 1, 16829

[4] Sandberg, E. et al., J. Phys. Chem. B. 2023, 127, 14, 3208.

4

ENHANCING ELECTROSTATIC INTERACTION MODELS THROUGH ELECTRONIC POLARIZATION: INSIGHTS FROM THE PROSECCO75 BIOMOLECULAR FORCE FIELD

Ricky Nencini[1,2], Carmelo Tempra[1], Denys Biriukov[1,3,4], Miguel Riopedre-Fernandez[1], Victor Cruces Chamorro[1], Jakub Polák[5], Philip E. Mason[1], Daniel Ondo[5], Jan Heyda[5], O. H. Samuli Ollila[2,6], Pavel Jungwirth[1], Matti Javanainen[1,2], Hector Martinez-Seara[1]

1 Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic

2 Institute of Biotechnology, University of Helsinki, Helsinki, Finland

3 CEITEC – Central European Institute of Technology, Masaryk University, Brno, Czech Republic

4 National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic

5 Department of Physical Chemistry, University of Chemistry and Technology, Prague, Czech Republic

6 VTT Technical Research Centre of Finland, Espoo, Finland

The prosECCo75 force field introduces an innovative approach to improving the precision of molecular dynamics (MD) simulations by incorporating electronic polarization via charge scaling. This method addresses the critical challenge faced by traditional nonpolarizable force fields: their inability to accurately capture the complex electrostatic interactions among water, ions, proteins, lipids, and polysaccharides, which are vital for biological functions. By refining partial charges within the CHARMM36 framework, prosECCo75 effectively reduces overbinding issues and aligns more closely with experimental observations across various biomolecular systems, without altering the detailed molecular structures defined by the original force field. Through rigorous testing against experimental benchmarks involving lipid membranes, amino acids/proteins, and sugars, prosECCo75 demonstrates substantial improvements in simulating ion binding to membranes and the interactions among charged biomolecules. As a result, prosECCo75 stands out as a computationally efficient option, offering enhanced accuracy and wider relevance for studying the complex dynamics of biomolecular interactions, essential for advancing our comprehension of biological mechanisms.

5

MILD HYPERTHERMIA INDUCED INTRACELLULAR THERMOGENESIS DEFINES STRESS RESPONSE MECHANISMS

Barbara Dukic[1], Imre Gombos[1], Zsófia Ruppert[1], Miklós Erdélyi[2], Mária Péter[1], Ákos Hunya[1], Gábor Balogh[1], László Vígh[1] and Zsolt Török[1]

1 HUN-REN, Biological Research Centre, Institute of Biochemistry, Laboratory of Molecular Stress Biology

2 Department of Optics and Quantum Electronics, Institute of Physics, University of Szeged

Cells adjust their membrane composition in response to a temperature change so that membrane fluidity, and therefore function, is conserved. Such compensatory changes in membrane composition are considered ‘‘homeoviscous adaptation’’ develops relatively slowly. Much less is known how cells maintain membrane homeostasis during stress.

We investigated the membrane events which contribute to the development of stress tolerance upon a fever-like hyperthermia (40 °C) without heat shock protein expression in mammalian cells. Our results indicate that mild heat triggers a distinct, dose-dependent remodeling of the cellular lipidome followed by the expression of heat shock proteins only at higher heat dosages. RNAseq experiments, superresolution microscopy and lipidomic analysis revealed that mild heat initiates ER stress-related signaling cascades resulting in a rapid lipid rearrangement and ultimately in an elevated resistance against membrane fluidization by benzyl alcohol. By using ER thermo yellow, a temperature-sensitive organelle-specific fluorescence probe, we observed a cell dependent intracellular thermogenesis reaching as high as approximately 47 °C in the ER as a consequence of an uncoupled SERCA Ca pump activity.

The distinct layers of stress response elicited by different heat dosages highlight the capability of cells to utilize multiple tools to gain resistance against or to survive lethal stress conditions.

6

ASSESSMENT OF NUMBER DENSITY AND SIZE OF EXTRACELLULAR VESICLES IN DIFFERENT BIOLOGICAL FLUIDS WITH INTERFEROMETRIC LIGHT MICROSCOPE

Veronika Kralj-Iglič[1], Matevž Arko[1], Maxence Berry[2], David Drobne[3], Aleš Iglič[4,5], Boštjan Korenjak[1], Anna Romolo[1], Gitta Schlosser[6], Armando Tratenšek[7], Tomaž Vovk[7]

1 University of Ljubljana, Faculty of Health Sciences, Laboratory of Clinical Biophysics, Ljubljana, Slovenia
2 University of Poitiers, Poitiers, France
3 University of Ljubljana, Faculty of Electrical Engineering, Laboratory of Physics, Ljubljana, Slovenia
4 University Medical Centre Ljubljana, Department of Gastroenterology and Hepatology, Ljubljana, Slovenia
5 University of Ljubljana, Faculty of Medicine, Laboratory of Clinical Biophysics, Ljubljana, Slovenia
6 Eotvos Lorand University, Institute of Chemistry, Budapest, Hungary
7 University of Ljubljana, Faculty of Pharmacy, Chair of Pharmaceutical Technology, Ljubljana, Slovenia

Processing and characterization of the samples with extracellular vesicles (EVs) presents a challenge due to EV small size (within nano range) and transient identity. There are various techniques used for estimation of the size and number density of EVs (e.g. dynamic light scattering (DLS), nanotracking analysis, resistive pulse sensing, flow cytometry (FCM), scanning and transmission electron microscopy (SEM and TEM, respectively)), however the golden standard has not yet been claimed. Interferometric light microscopy (ILM) is a relatively new method (issued in 2017) based on interference pattern of incident and scattered LED light directed at the sample. The particles are identified and counted and their positions are tracked by recording a movie. Analysis of the movement yields the hydrodynamic diameter of the particles. The advantage of the method is that moderate presence of larger particles does not present an obstacle for the measurement. We report on the ILM measurements of different samples containing EVs: blood, blood plasma, isolate from blood plasma, isolate from aged erythrocytes, nanoalgosomes in conditioned media and in isolates, fractions of isolation of EVs from spruce homogenate, liposomes and spruce hybridosomes. We have compared the results of ILM with the results of DLS and obtained agreement within the error of the methods. We report on the population studies on diluted canine, equine and human plasma, diluted human blood and conditioned media of different microalgae, by using ILM. Our results indicate that ILM is a high throughput method for determination of number density and hydrodynamic diameter of biological samples. Pilot study of EVs assessed directly in diluted blood is reported for the first time.

8

RELEASE OF EN BLOC SECRETED AMPHIECTOSOME-DERIVED SMALL EXTRACELLULAR VESICLES

Tamás Visnovitz[1,2], Dorina Lenzinger[1], Anna Koncz[1,3], Tünde Bárkai[1], Krisztina V Vukman[1], Alicia Galinsoga[1], Krisztina Németh[1,3], Kelsey Fletcher[1], Péter Lőrincz[4], Gábor Valcz[1,5], Edit I Buzás[1,3,6]

1 Institute of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary

2 Department of Plant Physiology and Molecular Plant Biology, Eötvös Loránd University, Budapest, Hungary

3 HUN-REN-SU Translational Extracellular Vesicle Research Group, Budapest, Hungary

4 Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary

5 Department of Image Analysis, 3DHISTECH Ltd, Budapest, Hungary

6 HCEMM-SU Extracellular Vesicle Research Group

Recent studies suggest an unexpected complexity of extracellular vesicle (EV) biogenesis pathways. Based on our previous findings, human colorectal cancer cells in vivo release large EVs with small intraluminal vesicles. Here we focus on these multivesicular large EVs (MV lEVs). We characterized them with different protein markers and investigated whether this EV subtype was unique for colon cancer.

We used confocal microscopy, transmission electron microscopy, super-resolution live-cell imaging and Western blot analysis. The detection of the MV-lEVs was made possible by our novel in situ fixation method. Our marker studies focused on classical (CD63, CD81, ALIX, TSG101) and non-conventional (TSPAN4, LC3) EV molecules. We have successfully modulated the level of EV production by inhibition of cytoskeleton, endo-lysosomal system and autophagy.

All tested cell types (cell lines and tissue environment) released MV lEVs. We described that upon spontaneous rupture of the limiting membrane of the MV-lEVs, their intraluminal vesicles escaped to the extracellular environment by a “torn bag mechanism”. MV lEVs were distinct from migrasomes by morphology and protein markers and we proposed that they were of amphisome origin. Taking into consideration their intracellular origin and release mechanism, we designated them “amphiectosomes”.

Our model suggests that during amphisome formation, the inner LC3 positive membrane of autophagosomes fragments and forms vesicles. These intraluminal vesicles are secreted by the “torn bag” mechanism into the extracellular space. Our data reveal a novel small EV (40-200 nm) secretion pathway, distinct from the previously widely accepted exocytosis-based small EV release.

9

MEMBRANE CURVATURE SENSING BY MODEL BIOMOLECULAR CONDENSATES

Midhun Mohan Anila[1], Rikhia Ghosh[2] and Bartosz Różycki[1]

1 Institute of Physics, Polish Academy of Sciences, Aleja Lotników 32/46, 02-668 Warsaw, Poland

2 Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Pl, New York, NY 10029, USA

Biomolecular condensates (BCs) are fluid droplets that form in biological cells by liquid-liquid phase separation. Their major components are intrinsically disordered proteins. Vast attention has been given in recent years to BCs inside the cytosol and nucleus. BCs at the cell membrane have not been studied to the same extent so far. However, recent studies provide increasingly more examples of interfaces between BCs and membranes which function as platforms for diverse biomolecular processes. Galectin-3, for example, is known to mediate clathrin-independent endocytosis and has been recently shown to undergo liquid-liquid phase separation, but the function of BCs of galectin-3 in endocytic pit formation is unknown. Here, we use dissipative particle dynamics simulations to study a generic coarse-grained model for BCs interacting with lipid membranes. In analogy to galectin-3, we consider polymers comprising two segments – one of them mediates multivalent attractive interactions between the polymers, and the other one has affinity for association with specific lipid head groups. When these polymers are brought into contact with a multi-component membrane, they spontaneously assemble into droplets and, simultaneously, induce lateral separation of lipids within the membrane. Interestingly, we find that if the membrane is bent, the polymer droplets localize at membrane regions curved inward. Although the polymers have no particular shape or intrinsic curvature, they appear to sense membrane curvature when clustered at the membrane. Our results indicate toward a generic mechanism of membrane curvature sensing by BCs involved in such processes as endocytosis.

Reference: M. M. Anila, R. Ghosh, B. Różycki. Soft Matter 19, 3723-3732 (2023).

10 SHIFT PERSPECTIVE, ACHIEVE MORE: INTRODUCING THE CYTOFLEX NANO FLOW CYTOMETER

Emese Sinkó PhD, product manager
Bio-Science Ltd, Budapest, Hungary

The CytoFLEX nano Flow Cytometer (Beckman Coulter Life Sciences) is the first nanoscale flow cytometer that enables you to simultaneously detect particles as small as 40 nm (based polystyrene particles when triggering on violet side scatter) and characterize particles with precision thanks to its 6 channels of fluorescent measurement and ability to count via volumetric delivery. It is the newest member of the well-known CytoFLEX Platform. With a workflow-focused software and complementary suite of reagents, the CytoFLEX nano flow cytometer has been created with user accessibility and data quality in mind, so you can focus on creating meaningful results.

The CytoFLEX nano flow cytometer features:
- sensitivity to detect and characterize EVs
- consistency in instrument performance and data quality to trust results
- flexibility to design EV experiments that suit you best
- built on the same principles as the CytoFLEX Platform, with similar footprint and user-friendly software interface.

The platform also includes the CytoFLEX, CytoFLEX S, and the CytoFLEX LX flow cytometers, along with the CytoFLEX SRT cell sorter for research, and the DxFLEX flow cytometer for diagnostic use.

11

INVERSE BICONTINUOUS AND DISCONTINUOUS PHASES OF LIPIDS

John M Seddon[1]

1 Chemistry Department, MSRH, Imperial College London, London W12 0BZ, UK.

Lyotropic liquid crystals of 1-, 2-, or 3-dimensional periodicity spontaneously assemble when lipids are mixed with aqueous solvent under various conditions of temperature, pressure and hydration. The most relevant non-lamellar phases from a biological perspective are the inverse hexagonal HII phase, and the inverse cubic phases. There are two quite distinct types of inverse cubic phase: *bicontinuous* ones based on underlying periodic minimal surfaces, and *discontinuous* ones based on simple or more complex packings of discreet inverse micelles. In this lecture I will briefly review lipid self-assembly, interfacial curvature and phase diagrams. I will then go on to describe how the phase behaviour can be controlled, and the structure of lyotropic phases can be tuned, by various parameters such as temperature, hydrostatic pressure, or the addition of amphiphilic molecules such as fatty acids, diacylglycerols, and cholesterol. For potential medical applications, bulk lipid phases can be dispersed into lipid nanoparticles of the order of 100 – 200 nm in diameter: hexosomes when formed from the HII phase, cubosomes from inverse bicontinuous cubic phases, and micellosomes when based on discontinuous cubic phases.

By incorporation of charged phospholipids, we can swell inverse bicontinuous cubic phases to lattice parameters of approx. 500 Å, with water channels of approx. 220 Å diameter, potentially expanding the range of usefulness of such phases for applications such as drug delivery or encapsulation of enzymes.

We have previously shown that addition of weakly-polar amphiphiles such as diacylglycerols to phospholipids can lead to the formation of a discontinuous cubic phase of spacegroup Fd3m. We have recently demonstrated that Fd3m micellosomes can be formed in buffer at pH 7.4 by mixtures of monoolein and oleyl alcohol, containing a small amount of an ionizable lipid. By lowering the pH to below pH 6, the zwitterionic lipid becomes cationic, triggering a phase transition within the lipid nanoparticle from an internally-confined Fd3m structure (micellosome), to a porous inverse hexagonal H_{II} phase (hexosome), favouring release of any encapsulated contents.

We have recently developed a microfluidic hydrodynamic focussing technology for the production of cubosomes and hexosomes, whose size is relatively monodisperse and can be controlled by varying the flow rate ratio between the buffer and ethanolic streams.

12

A PROTET-BASED MODEL FOR OXIDATIVE AND PHOTOSYNTHETIC PHOSPHORYLATION

Douglas B. Kell[1,2]

1 Department of Biochemistry, Cell & Systems Biology, University of Liverpool, UK

2 Novo Nordisk Foundation Centre for Biosustainability, Danish Technical University, Lyngby, DK, dbk@liv.ac.uk http://dbkgroup.org/publilcations/ @ dbkell

Two-stage (e.g. light-dark) phosphorylation experiments showed that there is a 'high-energy' intermediate linking electron transport and phosphorylation. Large, artificial electrochemical proton gradients can also drive phosphorylation, a fact seen as strongly supportive of the chemiosmotic coupling hypothesis that a pmf **is** the 'high-energy' intermediate. However, in such experiments there is a threshold (pmf >170 mV, equivalent to ΔpH ~2.8) below which **no** phosphorylation is observed, and 220 mV are required to recreate in vivo rates. This leads to the **correct** question, which is then **whether those values of the pmf generated by electron transport are large enough.** Even the lower ones are below the threshold [1, 2], whether measured directly or via the use of membrane-permeant ions and/or acids/bases (which are always transporter substrates [3], so all such measurements are artefactual). The single case that seemed large enough (220 mV) is now admitted to be a diffusion potential artefact [4]. Many other observables (inadequate bulk H^+ in 'O_2-pulse'-type experiments, dual-inhibitor titrations, uncoupler-binding proteins, etc.) are consistent with the view that values of the pmf, and especially of $\Delta\psi$, are very low. I review this widespread, voluminous and self-consistent evidence in detail. A protet-based charge separation model [2], a protonic version analogous to how energy may be stored in devices called electrets, provides a high-energy intermediate that can explain the entire relevant literature, including the very striking demonstration [5] that close proximity is required between electron transport and ATP synthase complexes for phosphorylation to occur.

[1] D.B. Kell, On the functional proton current pathway of electron transport phosphorylation: an electrodic view, Biochim. Biophys. Acta, 549 (1979) 55-99.

[2] D.B. Kell, A protet-based, protonic charge transfer model of energy coupling in oxidative and photosynthetic phosphorylation, Adv Micr Physiol, 78 (2021) 1-177; also OSF preprint http://osf.io/172xsz178.

[3] D.B. Kell, The transporter-mediated cellular uptake and efflux of pharmaceutical drugs and biotechnology products: how and why phospholipid bilayer transport is negligible in real biomembranes., Molecules, 26 (2021) 5629.

[4] D.G. Nicholls, S.J. Ferguson, Bioenergetics 4, Academic Press, Amsterdam, 2013.

[5] J. Sjöholm, J. Bergstrand, T. Nilsson, R. Sachl, C. von Ballmoos, J. Widengren, P. Brzezinski, The lateral distance between a proton pump and ATP synthase determines the ATP-synthesis rate, Sci Rep, 7 (2017) 2926.

13

PROTONATION-COUPLED WATER-MEDIATED HYDROGEN BOND NETWORKS FOR SIGNALING ACROSS CELLULAR MEMBRANES

Eva Bertalan[1], Matthew Joseph Rodrigues[2], Gebhard FX Schertler[2], Ana-Nicoleta Bondar[3,4]

1 Physikzentrum, RWTH Aachen University, Aachen, Germany;
2 Laboratory of Biomolecular Research, Paul Scherrer Institut, Villigen, Switzerland;
3 University of Bucharest, Faculty of Physics, Bucharest-Magurele, Romania;
4 Forshungszentrum Jülich, Institute for Computational Biomedicine (IAS-5/INM-9), Jülich, Germany

G Protein Coupled Receptors (GPCRs) mediate communication across eukaryotic cell membranes. The binding of an activating (agonist) ligand at the extracellular side of the receptor triggers structural rearrangements and activation of the receptor, which binds and activates G protein partners at the cytoplasmic side. Water-mediated Hydrogen(H)-bond networks are central to the mechanism of receptor activation, yet mechanisms by which GPCRs use such networks for long-distance couplings across the membrane remain open questions. The talk will present the development and applications of graph-based algorithms to analyze dynamic water-mediated H-bond networks in biomolecules, and the usefulness of these algorithms to dissect the protonation-coupled dynamics of water-mediated H-bond networks of GPCRs. The picture that emerges for the systematic atomic-level simulations and graph-based analyses is that the inactive receptors host water-mediated H-bond networks that can rapidly respond to changes in protonation and/or binding of the ligand, and which can recruit additional H-bonds -resulting in extensive water-mediated H-bond networks that couple key functional regions of the receptor.

References

Bertalan E, Rodrgues MJ, Schertler GFX, Bondar A-N. Graph-based algorithms to dissect long-distance H-bond networks for conformational couplings in GPCRs. British Journal of Pharmacology, 10.1111/bph.16387

Bertalan E, Lesca E, Schertler GFX, Bondar A-N. C-Graphs tool with graphical user interface to dissect conserved hydrogen-bond networks: applications to visual rhodopsins. Journal of Chemical Information and Modeling, 61, 5692-5707,10.1021/acs.jcim.1c00827 (2021)

Siemers M, Lazaratos M, Karathanou K, Brown LS, Bondar A-N. Bridge: A graph-based algorithm to analyze dynamic H-bond networks in membrane proteins. Journal of Chemical Theory and Computation 15, 6781-6798, 10.1021/acs.jctc.9b00697 (2019)

14

UNDERSTANDING PROTEIN-LIPID INTERACTIONS ASSOCIATED WITH THE MALARIA INFECTION

Alessandra Luchini[1], Sai S.R. Raghavan[2], Louise Turner[2], Thomas Lavstsen[2]

1 Department of Physics and Geology, University of Perugia, Perugia, Italy

2 Centre for Medical Parasitology, Department of Immunology and Microbiology, University of Copenhagen, and Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark

The malaria infection is widely spread in low-income countries with more than 250 000 cases reported in World Malaria Report 2023[1]. Plasmodium falciparum is the parasite responsible for the most severe cases of malaria. The parasite enters the human body during a mosquito bite from the female. During the "blood stage" of the infection the parasite infects red blood cells (RBCs) and induces the production of transmembrane proteins named PfEMP1 [2]. These proteins are responsible for the binding to human protein receptors which results in the adhesion of the infected RBCs to blood vessel walls, which triggers strong inflammatory responses and in narrow blood vessel can cause vessel occlusion and ischemia (celebral malaria) [3].

In this project we are combining different biophysical methods, including Quartz crystal microbalance with dissipation monitoring (QCM-D) and Neutron reflectometry (NR), to investigate the protein-lipid interaction occurring between PfEMP1 proteins and the surrounding RBC membrane and their potential role during binding with epithelial protein C receptor (EPCR). The collected data indicates that PS lipids, which are abundant on the infected RBC outer leaflet, impacts the conformation of PfEMP1 at the membrane surface. This interaction does not interfere with the PfEMP1 complex with EPCR.

References

[1] https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023

[2] Introini et al. Frontiers in Cellular and Infection Microbiology 2022

[3] Raghavan et al. Structure 2023

15

COARSE-GRAINED MOLECULAR DYNAMICS MODELLING OF HUMAN LOW-DENSITY LIPOPROTEIN

Tamás Földes[1], Judith Peters[2,3], Ambroise Desfosses[4], Karin Kormüller[5]

1 Institut de Biologie Structurale, UGA, Grenoble, France

2 Université Grenoble Alpes, CNRS, LiPhy, Institut Laue-Langevin, Grenoble, France

3 Institut Universitaire de France, Paris, France

4 Institut de Biologie Structurale, UGA, CEA, CNRS, Grenoble, France

5 Gottfried Schatz Research Center for Cell Signaling, Metabolism and Aging, Medical Physics and Biophysics Division, Medical University of Graz, Graz, Austria

Cardiovascular disease is accounted for 32% of death worldwide, and is considered to be the leading cause of premature mortality. For its risk assessment and treatment, modern guidelines indicate serum low-density lipoprotein (LDL) cholesterol level as the primary target. These LDLs (~17-28 nm) [1] are complex amphiphilic nanoparticles that are instrumental in the metabolism and homeostasis of cholesterol and lipid transportation.

Recent developments in cryo-electron microscopy (cryoEM), sample homogeneity, and artificial intelligence-based algorithms opened new avenues towards solving the structure. In this current study, we aimed to apply molecular dynamics simulations based on mid-resolution cryoEM data, AlphaFold2 predictive software, and computational data refinement. We utilized GROMACS.2024 and the Martini3 force field [2] for coarse-grained simulations. Our modelling approach first disassembled the LDL particle to three main parts: **a)** the Apolipoprotein B100 (apo-B100, ~550kDa, with 17 N-glycans), **b)** the phospholipid membrane surface (phosphatidylcholine lipids, cholesterol), **c)** the lipid core (cholesterol, cholesterol esters, triglycerides). Structure predictions indicate the amphiphilic nature of apo-B100 originates from the well-tuned sequence of the 3-4 nm-wide β-belt motif with over 2000 residues that surrounds the LDL lipid core. The core forms three double-layers at lower temperatures, and is stabilized by the lipophilic side of the β-belt. With clustering analysis, we were able to discover surface domains with specific lipid compositions that suggests the formation of cholesterol-rich reservoirs in the LDL surface membrane. Finally, our simulation shows the dynamics of relatively flexible protein chains appearing as surface protrusions. These findings allow us to further expand our understanding of this particle and pave out the road towards further investigations.

[1] a, Prassl, R. and P. Laggner, Eur Biophys J, **2009,** 38(2), 145-58 b, Kuklenyik, Z., et al., PLoS One, **2018,** 13(4), e0194797 c, Cisse, A., et al., Int J Biol Macromol, **2023,** 252, 126345.

[2] Souza, P.C.T., Alessandri, R., Barnoud, J. et al. Nat Methods **2021,** 18, 382–388.

16

DECIPHERING INTERDOMAIN ALLOSTERIC EFFECTS ON THE PH-DEPENDENT MEMBRANE INSERTION PROCESS OF THE DIPHTHERIA TOXIN.

Nicolas Carvalho[1], Gaia Scilironi[1], Corentin Léger[1], Dorothée Raoux-Barbot[1], Mathilde Briday[2], Sébastien Brier[2], Phillipe Minard[3], Marielle Valerio-Lepiniec[3], Agathe Urvoas-Cissé[3], Iñaki Guijarro[2], Daniel Ladant[1], Alexandre Chenal[1]

1 Department of structural biology and chemistry, Biochemistry of Macromolecular Interactions Unit, Institut Pasteur, Université de Paris Cité, CNRS UMR3528, Paris, France

2 Department of structural biology and chemistry, Biological NMR and HDX-MS Technological Platform, Institut Pasteur, Université de Paris Cité, CNRS UMR3528, Paris, France

3 Protein Engineering and Modeling Unit, Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CNRS UMR9198, Gif-sur-Yvette, France

Most of the studies on the pH-dependent membrane insertion process of the diphtheria toxin (DT) focus on the translocation (T) domain, isolated or linked to its catalytic (C) domain. However, the contribution of the receptor-binding (R) domain, as well as the crosstalk between the three domains, on the pH-dependent membrane insertion of the full-length toxin has been poorly investigated until yet. Here, we investigate the pH-dependent membrane insertion process of DT to characterize its conformational and dynamic changes occurring on the entire toxin. A combination of biophysical approaches including fluorescence spectroscopy, NMR, quartz crystal microbalance with dissipation, vesicle permeabilization assays, small-angle X-ray scattering, specular neutron reflectometry and hydrogen-deuterium exchange mass spectrometry allows us to identify the respective contributions of pH, lipid composition, and the DT interdomain allosteric effects to the toxin membrane insertion process. Moreover, using a R domain binder (RDB) selected by phage display, we show that the interaction of R with RDB induces dramatic allosteric effects on the C and T domains favoring membrane interaction. We propose that this effect mimics DT interaction with its cell receptor, HB-EGF, and may significantly contribute to the DT membrane insertion process. Finally, we propose a model of the membrane insertion process considering the interplay between DT and its environment, i.e., the lipid composition, the pH acidification, DT interaction with its cell receptor, and allosteric effects arising from DT interdomain interactions.

17

INTERACTIONS OF CARBON NANOMATERIALS WITH LIPID MEMBRANES

Markéta Paloncýová[1], Andrea Nedělníková[1,2], Martin Šrejber[1], Jindřich Lněnička[1,2], Michal Otyepka[1,3]

1 Czech Advanced Technology and Research Institute (CATRIN), Palacký University Olomouc, Olomouc, Czech Republic

2 Department of Physical Chemistry, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic

3 IT4Innovations, VŠB – Technical University of Ostrava, Ostrava, Czech Republic

Carbon nanomaterials (CNMs) have gathered significant attention for their optoelectronic properties and promising biocompatibility, making them compelling candidates for diverse medical applications. From electrodes for brain implants to exhibiting antimicrobial activity, CNMs offer a broad spectrum of potential uses. However, designing effective applications demands a detailed understanding of their atomistic interactions. In this study, we employ molecular dynamics simulations to explore various models of CNMs, derived from graphene, characterized by differences in size, type, and functionalization. By evaluating their interactions with lipid membrane models designed to mimic specific environments — such as neuronal, microglial, and myelin membranes reflecting brain tissue, and Escherichia Coli mimicking bacterial membranes — we uncover insights crucial for tailored design. We investigated the unique properties of these cell models, notably influenced by cholesterol content (in brain membranes) or the presence of lipopolysaccharide layers (in bacterial membranes). Further, we placed the varying CNMs models on the membrane models and by both all-atom and coarse-grained simulations, we investigated the behavior of CNMs on the membranes and the mutual membrane-CNM effect. Apart from the dynamics and localization of CNMs in the lipid membranes, we identified that CNMs do not have to enter the membrane to affect its internal organization.

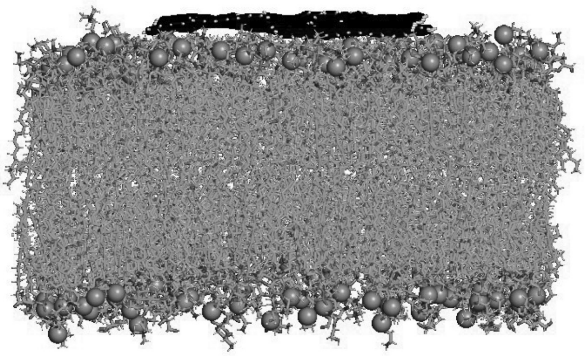


Figure 1: Model of hydrophobic unfunctionalized CNM on lipid membrane model, interacting with the membrane surface

18

THREE VERY DIFFERENT EXAMPLES OF MEMBRANE SIMULATIONS IN PHARMACEUTICAL RESEARCH

Alex Bunker[1], Suvi Heinonen[1], Ruifeng Wang[1], Saara Iautala[1], Mohammad Habibi[1], Tapani Viitala[1], Riccardo Provenzani[1], Ilari Tarvainen[1], Hanan Sa'd[1], Artturi Koivuniemi[1], Henri Xhaard[1], Raimo Tuominen[1], Virpi Talman[1], Jari Yli-Kauhaluoma[1], Katia Sirna[1], S. Tuuli Karhu[1], Antti K. Lehtinen[1], Evgeni Grazhdankin[1], Elina Vuorimaa-Laukkanen[2], Chunzhu Li[3], Matthew Davies[4], Mikko Karttunen[4], Camilla Foged[5]

1 Faculty of Pharmacy, University of Helsinki, Helsinki, Finland

2 Faculty of Engineering and Natural Sciences, Tampere University, Tampere, Finland

3 College of Food Science and Technology, Huazhong Agricultural University, Wuhan, China

4 Department of Chemistry, Western University, London, Canada

5 Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark

I discuss three pieces of research that involve the use of molecular dynamics simulation of lipid membranes in the context of pharmaceutical research: 1) orientation in the membrane of diacyl glycerol (DAG) mimics 2) polyphenol effect on lipid raft formation and 3) propensity for interdigitation of synthetic monomycoloyl glycerol (MMG) analogues. The very different nature of the phenomena that these three studies have focused on will hopefully highlight the broad range of pharmaceutically relevant insight that molecular dynamics simulations of lipid membranes can provide. The first study[1] involved the development of drug candidate molecules designed to mimic diacyl glycerol (DAG) that binds to the C1 domain of protein kinase C (PKC), activating it; moderate activation of PKC is of therapeutic benefit. We developed MD simulation of the drug candidate molecules interacting with the membrane as a screen for DAG mimics that not only bind to the C1 domain of PKC but also orient in the membrane in the same fashion as DAG. The second study involves the effect of polyphenols on lipid raft formation as a possible mechanism for their role in preventing obesity. It is hypothesized that they interfere with the signaling activity of raft domains thus we have studied three different polyphenols interacting with models of raft (Lo) and nonraft (Ld) domains. Dimeric polyphenols were found to bind strongly to both domains and we found some evidence that the binding is stronger to the Lo domain, thus indicating the effect of inhibiting lateral diffusion is amplified in the raft domains, which could, in turn, lead to disruption of signaling activities. In the third work discussed, we studied two MMG analogs representing native (MMG-6) and non-native (MMG-1) stereochemistry. MMG-1 showed a greater extent of tail ordering and reduced polar interactions; we hypothesized that this explains its greater propensity for interdigitation. MMG analogs are used as vesicular vaccine adjuvants and elucidating their properties will assist in their design.

[1] S. Lautala *et al. J. Med. Chem.* 66 (7) pp. 4588 – 4602 (2023)

19

THE DYNAMICAL MATRYOSHKA MODEL OF LIPID BILAYERS

Dominique J. Bicout[1,2], Aline Cisse[1], Tatsuhito Matsuo[1,3], Judith Peters[1,4]
1 Institut Laue-Langevin, 38042 Grenoble, France
2 Univ. Grenoble Alpes, CNRS, Grenoble INP, VetAgro Sup, TIMC, 38000 Grenoble, France
3 Institute for Quantum Life Science, QST, Tokai, Ibaraki 319-1106, Japan
4 Univ. Grenoble Alpes, CNRS, LiPhy, Grenoble, France

Biological membranes constitute fundamental interfaces and selective permeable barriers that define cells and organisms. Fluid lipid bilayers are the building blocks of biological membranes and lipids are the most abundant constituents of membranes. Dynamics of lipids in membrane layers is very crucial to ensure membrane functions. Due to their complex structure and dynamics, lipid bilayers are characterized by a hierarchy and heterogeneity of motions over a wide range of time and space. These dynamics include local lipid movements (e.g., rotational, in-and-out of the plane, in-plane diffusion, …) and collective lipid movements (e.g., density fluctuations, flip-flop (transmembrane lipid translocation), undulation and bending modes). The dynamical Matryoshka model describes the local dynamics of lipid molecules in membrane layers as a nested hierarchical convolution of three motional processes: (i) individual motions described by the vibrational motions of H-atoms, (ii) internal motions including movements of the lipid backbone, head groups and tails, and (iii) molecule movements of the lipid molecule as a whole.

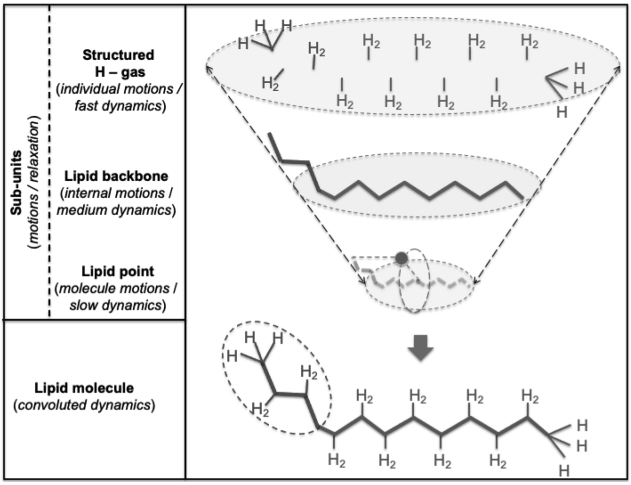


Figure 1: The dynamical Matryoshka model for the dynamics of the lipid molecule represented as a funnel of three - level convoluted dynamic processes [1].

Reference:

[1] - D. J. Bicout, A. Cisse, T. Matsuo and J. Peters. The dynamical Matryoshka model: 1. Incoherent neutron scattering functions for lipid dynamics in bilayers. *BBA - Biomembranes* **1864** (2022) 183944. (https://doi.org/10.1016/j.bbamem.2022.183944)

20

AOC3 ACTIVE SITE ACCESSIBILITY AND HELICES DIMERIZATION REVEALED BY MEMBRANE MOLECULAR DYNAMIC.

Marion Alix[1], Saara Lautala[2, 3], Alex Bunker[3], Tiina Salminen[1]

1 Department of Biochemistry, Science and Engineering, Åbo Akademi University, Turku, Finland

2 Frankfurt Institute for Advanced Studies, Max Planck Institute of Biophysics, Frankfurt, Germany

3 Department of Pharmacy, University of Helsinki, Helsinki, Finland

Human primary amine oxidase (AOC3) plays a crucial role in various inflammation-related diseases, as it is upregulated on endothelium during inflammation for extravasation of leukocytes into inflammatory site. Therefore, investigating the dynamics of this protein provides valuable insights for designing inhibitors. AOC3 exists in two forms: as a transmembrane protein and as soluble protein resulting from proteolytic cleavage of its membrane bound form. AOC3 structure exhibits a heart-shaped envelop composed of two identical chains, two active sites often including crystal waters, and two transmembrane helices.

Previously we focused on the dynamic of the soluble form of AOC3 protein, revealing greater accessibility to the active site. However, in this project, we investigated the membrane-bound form of AOC3 embedded in both phosphatidylcholine (POPC) membrane and a mixture of POPC and cholesterol (CHL) membranes.

Molecular dynamics simulations were performed for 1.5 to 2microseconds using Gromacs software, considering two helix conformations and membrane compositions. Analysis is still on going, however molecular dynamics revealed the heart shape structure could bend towards the membrane enhance access to one active site. Helixes exhibited a preference for crossed conformation, either in dimeric form or not. Analysis of the simulation revealed minimal effects on the active site size and water environment surrounding catalytic residues, consistent to crystal structures.

Our finding shed light on the behavior of AOC3 when embedded in the membrane, including its bending capability, active site accessibility, active site size providing valuable insight for future in silico experiments.

21

ULTRA-LARGE-SCALE ALL-ATOM MOLECULAR DYNAMICS SIMULATIONS OF CELLULAR MEMBRANES AND EXTRACELLULAR INTERACTIONS

Denys Biriukov[1,2,3], Hector Martinez-Seara[3]

1 National Centre for Biomolecular Research, Faculty of Science, Masaryk University, CZ-62500 Brno, Czech Republic

2 CEITEC – Central European Institute of Technology, Masaryk University, CZ-62500 Brno, Czech Republic

3 Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, CZ-16610 Prague 6, Czech Republic

Cellular interactions and processes near the plasma membrane are highly complex and dynamic. For decades, theoreticians and experimentalists have been trying to understand the self-organization of cell membranes and their interactions with extracellular biomolecules. However, the complexity and functional length scales have, until now, made it unfeasible to model a significant portion of the membrane with the extracellular interaction network on top of it. The primary obstacles included the limited computational power, a lack of precise and consistent simulation models, and uncertainty regarding which molecular entities should be modeled.

In this work, we harness the latest technological advancements to construct a comprehensive model of the extracellular matrix at an unparalleled scale. Our simulations encompass various biomolecules—including lipids, proteins, sugars, ions, and water—each modeled at full atomic resolution. Our model but realistic systems, ranging from lipid membranes with thoroughly detailed lipid composition to huge lipid membranes/glycocalyx complexes containing a wide variety of biologically relevant molecules, account for millions of atoms simulated at the microsecond timescale. The preliminary results of our work uncover complex structural and interaction patterns within these systems, offering novel insights into the molecular architecture and communicative processes of biomacromolecules under realistic conditions. Furthermore, our pioneering large-scale molecular dynamics simulations provide an unprecedented in-silico view of numerous biological phenomena at the atomic level. Our research enhances our understanding of the extracellular matrix and establishes a new benchmark for studying biological processes at the membrane.

22

PHOTOPHYSICS IN BIOMEMBRANES: COMPUTATIONAL INSIGHT INTO THE INTERACTION BETWEEN LIPID BILAYERS AND CHROMOPHORES

S. Osella[1], S. Knippenberg[2,3]

1 Chemical and Biological Systems Simulation Lab, Centre of New Technologies, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland.

2 Hasselt University, Theory Lab, Agoralaan Building D, 3590 Diepenbeek, Belgium.

3 Université Libre de Bruxelles, Spectroscopy, Quantum Chemistry and Atmospheric Remote Sensing (SQUARES), 50 Avenue F. Roosevelt, C.P. 160/09, B-1050 Brussels, Belgium.

Email addresses: s.osella@cent.uw.edu.pl

Light is a powerful tool to probe the structure and dynamics of biomolecules and biological. In most cases, this cannot be done directly with visible light because of the absence of absorption by those biomolecules. This problem can be overcome by incorporating organic molecules (chromophores) that show an optical response in the vicinity of those biomolecules. Since those optical properties are strongly dependent on the chromophore's environment, time-resolved spectroscopic studies can provide a wealth of information on biosystems at the molecular scale in a non-destructive way. In this work, we give an overview on the multiscale computational strategy developed by us in the last 8 years and prove that theoretical studies and simulations are needed to explain, guide and predict observations in fluorescence experiments. As we challenge the accepted views on existing probes, we discover unexplored abilities that can discriminate surrounding lipid bilayers and their temperature- as well as solvent-dependent properties. We focus on two archetypal chromophores: Laurdan and azobenzene. Thanks to its pronounced first excited state dipole moment, Laurdan has long been known as a solvatochromic probe. Since this molecule has however two conformers, we prove that they exhibit different properties in different lipid membrane phases. We see that the two conformers are only blocked in one phase but not in another. Supported by fluorescence anisotropy decay simulations, Laurdan can therefore be regarded as a molecular rotor. Finally, the conformational versatility of azobenzene in saturated Ld lipid bilayers is simulated along with its photoisomerization pathways. By means of non-adiabatic QM/MM surface hopping analyses (QM/MM-SH), a dual mechanism is found with a torsional mechanism and a slow conversion for transto-cis. For cis-to-trans, simulations show a much higher quantum yield and a so-called 'pedal-like' mechanism. The differences are related to the different potential energy surfaces as well as the interactions with the surrounding alkyl chains. When tails of increased length are attached to this probe, cis is pushed towards the polar surface, while trans is pulled towards the centre of the membrane.

23

AN UPGRADE ON THE PROTON CIRCUIT IN THE INNER MITOCHONDRIAL MEMBRANE THAT INVOLVES FORMATION OF NON-BILAYER INVERTED MICELLES

Edward Gasanoff[1,2], Zeng Zhuoyan[1], Wei Mingsi[1], Tao Yuxuan[1]

1 STEM Research Center, Chaoyang KaiWen Academy, Beijing, China

2 Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia

The chemiosmotic theory of cell bioenergetics developed by Peter Mitchell postulates that energy releases from the transport of electrons through the protein complexes of electron transport chain (ETC) is consumed to generate the concentration gradient of protons in the bulk solutions across both sides of the IMM. This generates proton motif force (PMF) to support movement of protons from the matric to the intermembrane space through the Fo subunit of the ATP synthase needed for ATP synthesis. However, existence of free protons in bulk solution is not possible as a free proton would be immediately solvated to form hydroxonium ion H_3O^+ via creation of coordinate covalent bond with water molecule which would disrupt structural integrity of IMM due to the release of a huge amount of energy from solvation of free protons. Also, hypothetical accumulation of free protons in the solution of intermembrane space, which is the vastly extended external volume, would greatly dilute concentration of protons and the entropic component of the PMF would be lost. In addition, a concentration gradient of protons on both sides of the IMM would create unphysiological pH in the intermembrane space and the matrix which can be detrimental to the structure and functions of biological molecules. An alternative hypothesis supported by experimental evidence suggests the movement of protons from the matrix to the inter-membrane space via the H^+ pumps in IMM and then on the intermembrane side protons do not disperse into solution but move on the surface of IMM from the H^+ pumps to the Fo subunit of ATP synthase where protons move upward via a proton wire to Glu 58 residue of the c subunit of the F_o rotor. It is proposed that while F_o rotates, protons detach from the Glu 58 and move through the hydrophobic space in the center of the IMM and return to the protein complexes of ETC. We raise a question on the movement of protons through the hydrophobic center of IMM which is a low dielectric environment not compatible with the charged entity as a proton. We propose based on our experimental and in silico data that H^+ ions moving though the F_o subunit increase the conical shape of cardiolipin mole-cules and trigger formation of inverted micelles that carry H^+ ions in the inner volume of micelles through the hydrophobic center of IMM to the nearest protein complexes of ETC.

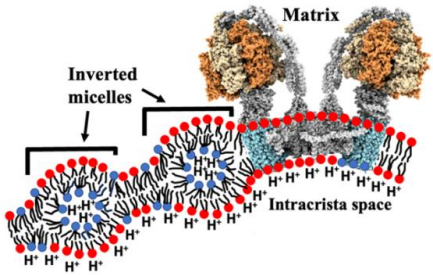


Figure 1. The inverted micelles predominantly made of cardiolipin serve as nanocarrier of protons for transporting protons from the F_o subunit of ATP synthase to the respiratory complexes in the IMM. The concentration of protons at the F_o subunit of ATP synthase increases the conical shape of cardiolipin molecules and triggers formation of inverted micelles carrying H^+ ions through the hydrophobic center of IMM to the nearest protein complex of ETC.

24

MOLECULAR DYNAMIC SIMULATION OF LIPID ASSEMBLIES MIMICKING THYLAKOID MEMBRANES

Bence Fehér[1,2,3], Győző Garab[4,5], Gergely Nagy[6]

1 HUN-REN-SU Nanobiophysics Research Group, Budapest, Hungary
2 Institute of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary
3 HUN-REN-SU Biophysical Virology Research Group, Budapest, Hungary
4 HUN-REN Biological Research Centre, Szeged, Hungary
5 Faculty of Science, University of Ostrava, Ostrava, Czech Republic
6 Neutron Scattering Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

Thylakoid membranes (TMs) represent pivotal energy-converting membranes characterized by a distinctive lipid composition. While the light reactions of photosynthesis are performed by protein (super-)complexes, the molecular organization of TMs and the operation of the photosynthetic apparatus depend largely on their lipid composition. Particularly intriguing is the structural and functional roles of the main lipid species of TMs, the neutral galactolipid, monogalactosyldiacylglycerol (MGDG), because of its high propensity to form non-bilayer structures.

Experimental characterization of these diverse structures in protein-free environments poses significant challenges. A promising approach to explore the structural landscape of TMs involves molecular dynamics (MD) simulations on their bulk lipid assemblies. Through MD simulations conducted across a broad range of temperatures, we elucidated the physical parameters of TM lipid membranes pertinent to photosynthetic functions. Our investigations revealed a pronounced temperature-dependent impact on membrane permeability, attributed to increased membrane compressibility and curvature at elevated temperatures. Furthermore, our study delved into the structural dynamics of TM lipid bilayers, comparing configurations with and without MGDG across different hydration levels and temperatures. Our simulations underscore the pivotal role of MGDG in modulating membrane properties critical for the self-assembly, structural dynamics, permeability and functional activity of TMs. We also unveiled that the presence of MGDG, coupled with low hydration levels, is indispensable for the formation of hexagonal structures. These findings shed light on the intricate interplay between lipid composition, hydration, and temperature in shaping the structural and functional landscape of TMs, with strong implications for better understanding plant photosynthesis.

Reference: Fehér et al. (2023) Photosynthetica 61(4):441-450

25

LIPID POLYMORPHISM OF PLANT THYLAKOID MEMBRANES – INSIGHT INTO THE INVERTED HEXAGONAL PHASE

Ondřej DLOUHÝ[1], Kinga BÖDE[1,2,3], Uroš JAVORNIK[4], Irena KURASOVÁ[1], Pavel ROUDNICKÝ[5], Zbyněk ZDRÁHAL[5], Primož ŠKET[4], Janez PLAVEC[4], Vladimír ŠPUNDA[1], Győző GARAB[1,2]

1 Department of Physics, Faculty of Science, University of Ostrava, Czech Republic
2 HUN-REN Biological Research Centre, Szeged, Hungary
3 Doctoral School of Biology, University of Szeged, Szeged, Hungary
4 Slovenian NMR Center, National Institute of Chemistry, Ljubljana, Slovenia
5 Mendel Centre for Plant Genomics and Proteomics, Central European Institute of Technology, Masaryk University, Brno, Czech Republic

Thylakoid membranes (TMs) represent pivotal energy-converting membranes characterized by a distinctive lipid composition. While the light reactions of photosynthesis are performed by protein (super-)complexes, the molecular organization of TMs and the operation of the photosynthetic apparatus depend largely on their lipid composition. Particularly intriguing is the structural and functional roles of the main lipid species of TMs, the neutral galactolipid, monogalactosyldiacylglycerol (MGDG), because of its high propensity to form non-bilayer structures.

Experimental characterization of these diverse structures in protein-free environments poses significant challenges. A promising approach to explore the structural landscape of TMs involves molecular dynamics (MD) simulations on their bulk lipid assemblies. Through MD simulations conducted across a broad range of temperatures, we elucidated the physical parameters of TM lipid membranes pertinent to photosynthetic functions. Our investigations revealed a pronounced temperature-dependent impact on membrane permeability, attributed to increased membrane compressibility and curvature at elevated temperatures. Furthermore, our study delved into the structural dynamics of TM lipid bilayers, comparing configurations with and without MGDG across different hydration levels and temperatures. Our simulations underscore the pivotal role of MGDG in modulating membrane properties critical for the self-assembly, structural dynamics, permeability and functional activity of TMs. We also unveiled that the presence of MGDG, coupled with low hydration levels, is indispensable for the formation of hexagonal structures. These findings shed light on the intricate interplay between lipid composition, hydration, and temperature in shaping the structural and functional landscape of TMs, with strong implications for better understanding plant photosynthesis.

Reference: Fehér et al. (2023) Photosynthetica 61(4):441-450

26

NEUTRON AND X-RAY SCATTERING STUDIES OF NON-BILAYER MEMBRANE STRUCTURES

Judith Peters[1,2,3]
1 Univ. Grenoble Alpes, CNRS, LiPhy, 38400 St. Martin d'Hères, France
2 Institut Laue Langevin, 38000 Grenoble, France
3 Institut Universitaire de France, 75005 Paris, France

Lipids in biological membranes are organized in bilayers, a two-dimensional matrix consisting of amphipathic lipid molecules with a polar head group and a hydrophobic tail; this matrix gives fluidity and elasticity to the membrane. Bilayers separate the inner and outer aqueous phases of membrane vesicles. They possess low permeability to water and most water-soluble molecules and to ions, and protons, in particular – and thus allow the buildup and utilization of the proton motive force for ATP synthesis, an essential feature of energy converting biological membranes. In an aqueous environment, the so-called bilayer lipids spontaneously form bilayer structures. However, all biological membranes contain lipid molecules that, by their intrinsic structure (non-conically shaped geometry) prefer not to form a bilayer in the presence of water; instead, they are assembled into different non-lamellar or non-bilayer lipid phases, such as the inverted hexagonal (HII) and cubic phases (Figure 1).

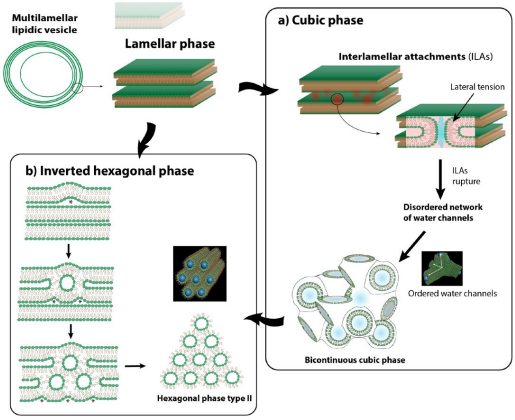


Figure 1: Lipids self-assembly into non-lamellar phases.

Here we will present recent results obtained by neutron and X-ray scattering on bilayer and non-bilayer membrane structures and how they change under temperature and pressure [1, 2]. In addition, first results from a very recent neutron experiment on a lipid mixture of PC:PE mimicking thylakoid membranes will be presented.

E-mail for corresponding author: jpeters@ill.fr

1. Salvador-Castell, M., et al., *Characterisation of a synthetic Archeal membrane reveals a possible new adaptation route to extreme conditions.* Commun Biol, 2021. **4**(1): p. 653.
2. Salvador-Castell, M., et al., *Induction of non-lamellar phases in archaeal lipids at high temperature and high hydrostatic pressure by apolar polyisoprenoids.* Biochim Biophys Acta Biomembr, 2020. **1862**(2): p. 183130.

27

LIPID POLYMORPHISM IN PHOTOSYNTHETIC MEMBRANES. THE FUSION OF PHOTOSYSTEM-II ENRICHED MEMBRANE PAIRS, ASSISTED BY ISOTROPIC LIPID PHASE

Kinga BÖDE[1,2,3], Ondřej DLOUHÝ[3], Tibor PÁLI[1], Krisztina SEBŐK-NAGY[1], Ildikó DOMONKOS[1], Gábor STEINBACH[1], Petar H. LAMBREV[1], Vladimír ŠPUNDA[3] and Győző GARAB[1,3]

1 HUN-REN Biological Research Centre, Szeged, Hungary
2 Doctoral School of Biology, University of Szeged, Szeged, Hungary
3 Faculty of Science, University of Ostrava, Ostrava, Czech Republic

Plant thylakoid membranes (TMs) are largely impermeable to protons and most hydrophilic molecules, thus warrants the build-up and utilization of the proton motive force according to the chemiosmotic theory. This is ensured by the bilayer organization of their bulk lipid molecules. Yet, the major lipids of TMs, monogalactosyldiacylglycerol, being a non-bilayer lipid, lend the lipid mixture a strong non-bilayer propensity. Intensive research over the past decade, using 31P-NMR spectroscopy, discovered that the bulk lipids in functional TMs, in addition to the bilayer (lamellar, L) phase, are found in at least two isotropic (I) lipid phases and an inverted hexagonal (HII) phase [1]. Studies on the effects of different proteases and lipases have revealed that these non-bilayer lipid phases are located outside the protein-dense regions containing the photosynthetic supercomplexes [2]. However, the structural and functional roles of these non-bilayer phases in the complex vesicular TM network remained elusive. Using isolated Photosystem-II (PSII) enriched stacked membrane pairs, BBY particles, we recently identified the role of I phase(s) in the fusion of TMs [3]. BBY membranes are extended sheets which are formed via lateral fusion of grana membrane patches. Using 31P-NMR spectroscopy, we identified L and I phases, similar to TMs but no HII phase. As in TMs, wheat germ lipase (WGL) selectively eliminated the I phase of BBY without compromising the structural and functional integrity of PSII. The elimination of I phase(s) led to the disassembly of the extended membrane sheets into their constituent units, as demonstrated by sucrose density gradient, magnetic linear dichroism spectroscopy, and scanning electron microscopy [3]. Fluorescence lifetime analysis of DPH-stained BBY confirmed the heterogeneity of the physicochemical environment of the bulk lipid molecules and revealed that WGL selectively suppressed the fastest decay component of this lipophilic fluorescent probe. The presently available data strongly suggest the crucial role of non-bilayer, isotropic lipid phases in the self-assembly and integrity of the highly organized TM network of plant chloroplasts.

[1] G. Garab et al. **(2022)** Progress in Lipid Research **86**, 101163.
[2] O. Dlouhý et al. **(2022)** Cells **11**, 2681.
[3] K. Böde et al. **(2024)** Photosynthesis Research (in press).

28 THE WHYS AND WHEREFORES OF NON-BILAYER LIPID PHASES IN ENERGY-CONVERTING BIOLOGICAL MEMBRANES – CO-CHAIR'S CONCLUDING COMMENTS AND HYPOTHESES

Győző Garab[1,2]
1 HUN-REN Biological Research Centre, Szeged, Hungary
2 Faculty of Science, University of Ostrava, Ostrava, Czech Republic

The bulk lipid molecules in biological membranes are organized in lamellar (bilayer) structures, which embed and anchor intrinsic and membrane-associated proteins and also contain lipophilic compounds and provide the basis for their 2D-restricted diffusions. Lipid bilayers are largely impermeable to water and most water-soluble ions, and for protons, in particular – warranting the efficient separation of the inner and outer aqueous compartments of cells and cellular organelles. This impermeability ensures the build-up of the electrochemical potential gradient for protons ($\Delta\mu H^+$) (the proton motive force, pmf) and its utilization for ATP synthesis in all energy-converting biological membranes. However, in these membranes the most abundant lipid species belong to the family of non-bilayer lipids.

Our session has paid special attention to enigmatic questions of the structural and functional roles of non-bilayer lipid assemblies in energy-converting biological membranes. Based on the submitted abstracts and recent publications, it can now be concluded that functional plant thylakoid membranes (TMs) and inner mitochondrial membranes (IMMs) contain substantial amounts of non-bilayer lipid phases. Non-bilayer lipids contribute to the structural dynamics of these membranes and have been shown to play key roles in membrane fusions as well as in the formation of structurally and/or functionally important lipid:protein assemblies in the aqueous phases. The presently available data allow us to hypothesize that (i) non-bilayer lipids facilitate the sub-compartmentalization of TMs and IMMs, as opposed to the equipotential inner and outer aqueous phases over the entire organelle; (ii) there is a compromise between the structural flexibility of the membranes and their enhanced permeability due to the presence of non-bilayer lipids in the bilayer; (iii) the lipid polymorphism of TMs and IMMs is modulated by variations in the hydration of the membranes; and (iv) the strong non-bilayer propensity of TM and IMM lipids, via their segregation capability, warrant the homeostatic high protein density of these membranes, which permits the formation and stability of protein mega-complexes and/or extended protein networks – which thus are capable of accommodating protons in their membrane-sequestered domains and utilizing them according to refined chemisosmotic mechanism(s).

29

G PROTEIN-COUPLED RECEPTORS AND CHOLESTEROL SENSITIVITY: EXCITEMENTS AND CHALLENGES

Prof. Amitabha Chattopadhyay
CSIR Bhatnagar Fellow
Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad, India
amit@ccmb.res.in
URL: http://e-portal.ccmb.res.in/e-space/amit/Pages/Index.htm

G protein-coupled receptors (GPCRs) are the largest class of molecules involved in signal transduction across membranes, and represent major drug targets. Serotonin1A receptors are important neurotransmitter receptors of the GPCR superfamily and are implicated in generation and modulation of cognitive, behavioral and developmental functions. We previously demonstrated that membrane cholesterol is necessary for ligand binding, G-protein coupling and signaling of serotonin1A receptors. In our recent work, we explored the molecular basis of cholesterol sensitivity exhibited by the serotonin1A receptor by site-specific mutations and MD simulations. We show that a lysine residue (K101) in one of the transmembrane helices is crucial for sensing altered membrane cholesterol levels (Kumar *et al.* (2021) *Science Advances* 7: eabh2922 (recommended in Faculty Opinions (F1000Prime)). Our results constitute one of the first reports comprehensively demonstrating that cholesterol sensitivity could be knocked out by a single point mutation at a cholesterol binding site. I will end my talk by presenting our recent observations on the role of cholesterol in GPCR endocytosis and trafficking.

30

LIPID BIOPHYSICS - THE INDISPENSABLE SKIN LIPID BARRIER

Petra Pullmannová[1], Norbert Kučerka[2], Robert Georgii[3], Bruno Demé[4], Jaroslav Maixner[5], Kateřina Vávrová[1]

1 Skin barrier research group, Charles University, Faculty of Pharmacy, Hradec Králové, Czech Republic

2 Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, Russia

3 Heinz Maier-Leibnitz Zentrum, Technische Universität München, Garching, Germany

4 Institut Laue-Langevin, Grenoble, Cedex 9, France

5 University of Chemistry and Technology Prague, Faculty of Chemical Technology, Prague, Czechia

The mammalian skin barrier is located in the outermost skin layer – the stratum corneum (SC). It was evolved to enable the terrestrial life of mammals, including humans. The indispensable barrier function of the skin includes protection against excessive water loss and the entrance of harmful substances from the environment[1]. The SC fulfills its role due to its unique architecture. The flattened cornified cells – corneocytes – are coated with a monolayer of covalently bound ceramide-dominated lipids. The unbound free lipids fill up the extracellular space of SC and are arranged into the oligolamellar lipid matrix. 50 weight % of SC lipids are ceramides (Cer), approximately 25 weight % is cholesterol and the rest are free fatty acids and minor components[2]. Ceramides are a structurally heterogeneous group of sphingolipids derived from a long-chain sphingoid base by attaching a fatty acid by an amide bond to an amino group in position 2. Skin lipid matrix does not show lyotropic mesomorphism and has limited interactions with water. The molecular arrangement of the extracellular SC lipid lamellae has not been satisfyingly resolved. The competing skin lipid molecular models are based on the various conformations of the ceramide molecule (either the hairpin or the splayed-chain conformation) and various extents of the microsegregation of hydrophobic chains. Our research group in collaboration with foreign laboratories and large research facilities brought experimental evidence on the molecular arrangement of the skin lipid model. The results from Fourier-transformed infrared spectroscopy, X-ray and neutron diffraction, and ^{2}H solid-state nuclear magnetic resonance point to the microsegregation of hydrophobic chains and fully spayed-chain Cer conformation.

(1) Matsui, T.; Amagai, M. Dissecting the Formation, Structure and Barrier Function of the Stratum Corneum. *Int Immunol* **2015,** 27 (6), 269–280.

(2) Elias, P. M.; Friend, D. S. The Permeability Barrier in Mammalian Epidermis. *Journal of Cell Biology* **1975,** *65* (1), 180–191.

31 INTRODUCING THE NMRLIPIDS DATABANK: A GATEWAY TO LARGE-SCALE ANALYSIS OF MEMBRANE SIMULATIONS

Hanne Antila
University of Bergen, Norway

We present the NMRlipids Databank—a community-driven, open-for-all database of molecular dynamics (MD) simulation data of lipid membranes, which have been quality-evaluated against experimental data. Using the overlay databank format, it collects data scattered in various locations and formats, and makes them accessible both through a GUI (Graphical User Interface) and an API (Application Programming Interface). We envision that the programmatic access through the API will enable flexible implementation of data-driven and machine learning applications, and unlock possibilities beyond current MD simulation and experimental studies to understand cellular membranes. We have already demonstrated such application: the analysis of water diffusion through membranes which, as a rare event, is beyond the sampling capabilities of regular simulation volumes, but highly relevant for applications such as understanding diffusion tensor magnetic resonance imaging and drug penetration through membranes. For the practitioners of MD, the embedded quality evaluation metrics will provide a tool for choosing the best lipid force field (MD model) for the system being modelled, for detecting typical errors in the existing models, and eventually provide training data for using machine learning and other data-driven algorithms to improve the force fields. Furthermore, we hope that by creating this resource, we will encourage data upcycling and open sharing of simulation data.

32

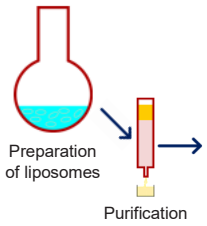
INVESTIGATION OF DRUG-MEMBRANE INTERACTIONS BY NEW LIPOSOMAL CO-PERMEATION ASSAY

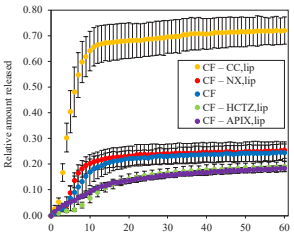
Adam Tywoniak[1]; Klára Odehnalová[1]; Martin Balouch[1]; Kateřina Storchmannová[2]; Aleš Zadražil[1]; Karel Berka[2]; František Štěpánek[1]

1 Department of Chemical Engineering, University of Chemistry and Technology Prague, CZ

2 Department of Physical Chemistry, Faculty of Science, Palacký University Olomouc, CZ

Phospholipid bilayers present an energy barrier to permeating molecules. For a given membrane composition, permeability is considered a property only of the permeating substance. We present experimental results calling for a more complex explanation. In co-permeation experiments, liposomes were loaded with a selected pharmaceutical together with carboxyfluorescein (CF; 7.5 mg/ml), and a concentration gradient was set up by gel chromatography. Release of CF was monitored by measuring fluorescence (490 / 522 nm) at 40 °C. As carboxyfluorescein self-quenches at higher concentrations, an increase in fluorescence corresponds to its release from liposomes (the slope proportional to its permeability coefficient). Then, liposomes were disrupted by a surfactant, releasing dye molecules from their membrane sheets as well. From this additional increase, modified partition coefficients of CF were determined. For a set of commonly prescribed drugs (both hydrophilic & lipophilic), we show that the presence of a co-permeant can either enhance or suppress the permeation rate of a probe molecule, as well as modulate its partitioning, up to five-fold, independently of the solubility or permeability of the drug itself. We suspect that such interactions during drug-membrane permeation might explain some knowledge gaps in pharmacokinetics, incl. certain poorly understood drug-drug interactions. Currently, we are expanding the selection of drug molecules, as well as investigating concentration-dependent properties of this permeation enhancement or retardation, and the possible additivity of effects in multi-drug mixtures.





Fluorescence measurement

Drug (abbreviation)	Effect on permeability of CF	Effect on partitioning of CF
Ascorbic acid *	↑	→
Hydrochloro-thiazide (HCTZ)	↑	↑
Kanamycin *	→	↑
Norfloxacin (NX)	↑	↑
Candesartan cilexetil (CC)	↑	↓
Apixaban (APIX)	↓	↑

** presented in a separate plot (see preprint)*

Reference:

Odehnalová, K.; Balouch, M.; Storchmannová, K.; Zadražil, A.; Berka, K.; Štěpánek, F. *Liposomal Co-Permeation Assay Reveals Unexpected Membrane Interactions of Commonly Prescribed Drugs;* ChemRxiv preprint, 2023. https://doi.org/10.26434/chemrxiv-2023-gsjtk

33

EXOGENOUS LUNG SURFACTANT INTERACTIONS WITH LIPOPOLYSACCHARIDE AND POLYMYXIN B AS SEEN THROUGH NEUTRON DIFFRACTION

Daniela Uhríková[1], Norbert Kučerka[1], Nina Královič[1], Bruno Demé[2]

1 Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius University Bratislava, Bratislava, Slovakia

2 Institut Laue – Langevin, Grenoble, France

Pulmonary surfactant (PS) is a mixture of lipids (~90 %) and 8-10 % specific surfactant associated proteins. PS lines the interior of the lung alveoli and acts to lower interfacial tension (Fig. 1). The absence of PS due to prematurity, or its damage, is treated with exogenous (EPS) in neonatal medicine and also for ventilated patients with Covid-19 sickness in experimental treatment. In pulmonary infection, the bacterial endotoxin, lipopolysaccharide (LPS), interferes with PS and alters its structure and function [1]. Polymyxin B (PxB) is an antimicrobial peptide primarily used in clinical practice to treat infections by resistant Gram-negative bacteria. Furthermore, PxB improves the surface properties of EPS and combined therapy (PxB – EPS) was found to be beneficial in the treatment of animal models [2]. We investigated structural changes of the EPS model lipid system free of proteins (mPS) in the presence of LPS and PxB using small- angle neutron diffraction (SAND). Aligned mPS bilayers deposited on a silicon wafer and hydrated in vapors (at four different RH %) of 8% D2O allowed us to unravel the effect of both, LPS and PxB, on the thickness of the mPS bilayer in this complex system. We found that LPS increases the thickness of the mPS bilayer, and PxB compensates for the effect up to a certain threshold. The findings correspond to the effect observed in vitro and in vivo in studies [1,2].

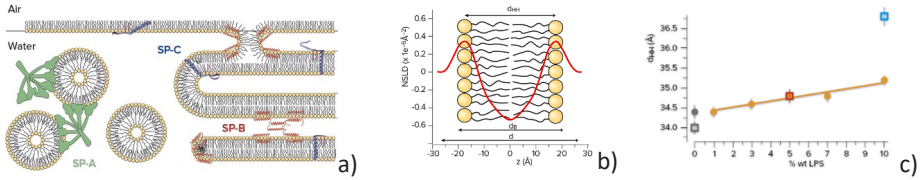


Fig. 1. a) Sketch of lung surfactant; b) Neutron scattering length density profile of mPS (99 % RH); c) the thickness of the bilayer dHH as a function of the LPS content (solid points) and the effect of PxB (open squares).

Acknowledgment. SAND experiments were performed on the D16 spectrometer of ILL, Grenoble. The experiments were supported by the projects APVV-17-0250 and VEGA 1/0305/24.

References

[1] M. Kolomaznik et al., Int. J. Mol. Sci. 19 1964 (2018)

[2] A. Calkovska et al., Sci Report 11, 22 (2021)

34

MECHANISTIC INSIGHTS INTO INTERACTIONS BETWEEN IONIZABLE LIPID NANODROPLETS AND BIOMEMBRANES

Petra Čechová[1], Markéta Paloncýová[1], Martin Šrejber[1], Michal Otyepka [1,2]

1 Czech Advanced Technology and Research Institute (CATRIN), Palacký University Olomouc, Olomouc, Czech Republic

2 IT4Innovations, VŠB – Technical University of Ostrava, Ostrava, Czech Republic

Lipid-based drug delivery systems, such as lipid nanoparticles (LNPs), are a promising branch of current medical research, as demonstrated by the LNP-mRNA COVID-19 vaccines [1].

There are several proposed mechanisms of LNP's cargo endosomal release, however, the exact sequence of this process is challenging to study experimentally. In contrast, molecular dynamics simulations can provide an insight into complex systems with atomic and femtosecond resolution. In this project, biased and unbiased molecular dynamics is used to visualize and describe the interactions between the ionizable and membrane lipids, to help understand the fine details of a LNP entering the cell on a molecular level [2].

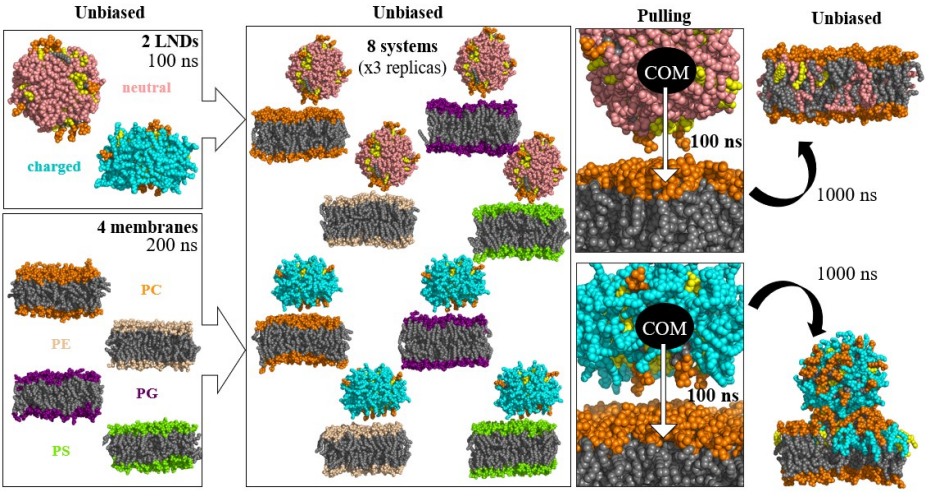


Figure 1: Scheme of the simulation protocol.

References:

1. M. Paloncýová, M. Šrejber, P. Čechová, P. Kührová, F. Zaoral, M. Otyepka, J.Phys.Chem B (2023), DOI: 10.1021/acs.jpcb.2c07671
2. P. Čechová, M. Paloncýová, M. Šrejber, M. Otyepka, J.Biomol.Struct.Dyn (2024), DOI: 10.1080/07391102.2024.2329307

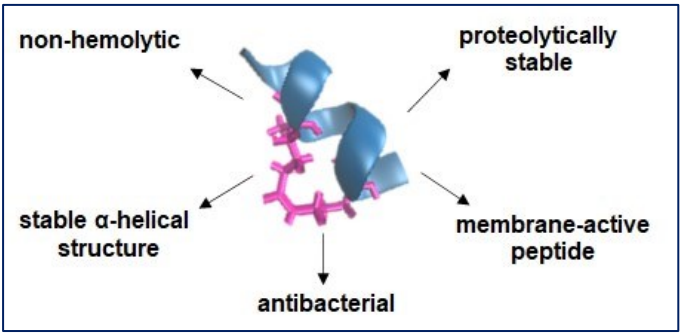
35

IMPROVING THE PROPERTIES OF MEMBRANE-ACTIVE PEPTIDES BY STABILIZING THEIR SECONDARY STRUCTURES

Monika Wojciechowska[1], Julia Macyszyn[1], Małgorzata Lobka[1], Michał Burmistrz[1], Joanna Miszkiewicz[1,2], Joanna Trylska[1]

1 Centre of New Technologies, University of Warsaw, Warsaw, Poland

2 College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Warsaw, Poland



The stabilization of the active, secondary structures of membrane-active peptides allowed us to induce or improve their antibacterial properties. We introduced two unnatural amino acids ((S)-2-(4'-pentenyl)-alanines) into the peptide sequences and covalently linked their side chains to form "staples" [1], [2]. For example, we showed that such modification of anoplin, a weak antimicrobial peptide, significantly improves its proteolytic stability and antimicrobial activity [1]. Similarly, the introduction of “staples” into (KFF)3K, a cell-penetrating peptide, endowed it with antimicrobial activity [2]. Furthermore, we designed new amphipathic peptides, rich in lysine and leucine, which became antibacterial after being stapled. The stapled peptides are active against gram-negative and gram-positive bacteria already at concentrations of 2-4 μM. Importantly, we confirmed that the introduction of "staples" to the peptides does not necessarily induce their hemolytic activity. As a result, our research has led to the development of new antibacterial peptides.

Acknowledgements: This work was supported by National Science Centre Poland (2019/35/D/NZ1/01957 and 2020/37/B/NZ1/02904)

References:

[1] M. Wojciechowska *et al.*, “Stapled Anoplin as an Antibacterial Agent,” *Front Microbiol,* vol. 12, Dec. 2021, doi: 10.3389/fmicb.2021.772038.

[2] J. Macyszyn *et al.,* “Structural dynamics influences the antibacterial activity of a cell-penetrating peptide (KFF)3K,” *Sci Rep*, vol. 13, no. 1, Dec. 2023, doi: 10.1038/s41598-023-38745-y.

36

CELL-PENETRATING PEPTIDE UPTAKE AND TRAFFICKING IN CELLS

Evgeniya Trofimenko[1], **Christian Widmann**[1]
1 Department of Biomedical Sciences, University of Lausanne, Lausanne, Switzerland

Cell-penetrating peptides (CPPs) are short and typically cationic peptides that facilitate the intracellular delivery of various cargos for research and therapeutic purposes. Their uptake mechanisms involve both direct translocation across the plasma membrane and endocytosis, each governed by intricate cellular processes.

Direct translocation of CPPs occurs through the formation of ~2nm-wide water pores, a phenomenon triggered by the hyperpolarization of the plasma membrane to approximately -150 mV. This hyperpolarization is orchestrated by the synergistic action of potassium channels and the accumulation of positive charges at the cell surface, allowing CPPs to traverse the membrane barrier efficiently.

On the other hand, CPP endocytosis follows a distinct pathway, characterized by the involvement of small GTPase Rab proteins. While conventional endocytic pathways typically converge at Rab5-positive early endosomes, our investigations reveal a novel route utilized by CPPs. This pathway bypasses Rab5 and Rab7, instead relying on Rab14 for intracellular trafficking, ultimately directing cargos towards non-acidic Lamp1-positive late endosomes.

Despite the established significance of endocytosis in CPP uptake, our research highlights its limitations in achieving cytosolic delivery. Specifically, endosomal escape proves to be inefficient, potentially hindering the therapeutic efficacy of CPP-mediated cargo delivery. To address this challenge, we conducted a systematic screening of small molecule compounds, identifying candidates capable of enhancing CPP endosomal escape.

In summary, our findings elucidate the complex mechanisms underlying CPP uptake into cells, offering valuable insights for the development of enhanced drug delivery strategies. By comprehensively understanding and manipulating these processes, we can harness the full potential of CPPs in biomedical applications, advancing both research and therapeutic interventions.

37

DISTRIBUTION OF TWO SYNERGISTIC ANTIMICROBIAL PEPTIDES ON THE LIPID MEMBRANE SURFACE AND SIDECHAIN DYNAMICS: A FLUORESCENCE STUDY

Jasmin Schlauch[1], Christopher Asenbrey[1], Burkhard Bechinger[1,2]

1 CNRS / University of Strasbourg, UMR7177, Chemistry Institute, Strasbourg, France

2 Institut Universitaire de France, 75005 Paris, France

Antimicrobial peptides represent an effective and promising alternative to conventional antibiotics as they attack bacterial cells via different pathways which are less prone for resistance development. A big class of antibiotic peptides are characterized by an in-planar topology of amphipatic helices in their membrane bound form. Aspects of the way of action of this class is best described by the carpet[1] and the smart model[2]. For both models the distribution of peptides on the membrane surface plays an essential role. Here we focus on two members of this family, PGLa and magainin 2, which interact with biological membranes in a synergistic manner.

Fluorescence quenching is used to determine the peptides' packing on membrane surfaces. PGLa is labelled at different positions (1, 10, 16, 21) by introducing the artificial amino acid diaminopropionic acid (Dap) with NBD (nitrobenzoxadiazole) attached to the sidechain amide. The labeling scheme allows to refine the supramolecular structures of PGLa on lipid membrane interface previously detected by N-terminal labeled PGLa[3].

Time resolved fluorescence anisotropy measurements allows the characterization of the sidechain dynamics in the ns time range. The orientation of the absorption transition dipole of NBD in combination with the short sidechain of diaminopropionic acid as a linker brings the dynamics into the sensitive range. The side chain dynamics can be an indicator of the local environment. In addition, the dynamics in the ns timescale might be easily correlated with molecular dynamics simulations.

1 Pouny, Y., Rapaport, D., Mor, A., Nicolas, P. & Shai, Y. Interaction of Antimicrobial Dermaseptin and Its Fluorescently Labeled Analogs with Phospholipid-Membranes. Biochemistry 31, 12416-12423, doi:DOI 10.1021/bi00164a017 (1992).

2 Bechinger, B. The SMART model: Soft Membranes Adapt and Respond, also Transiently, in the presence of antimicrobial peptides. Journal of Peptide Science 21, 346-355 (2014).

3 Aisenbrey, C., Amaro, M., Pospisil, P., Hof, M. & Bechinger, B. Highly synergistic antimicrobial activity of magainin 2 and PGLa peptides is rooted in the formation of supramolecular complexes with lipids. Sci Rep 10, 11652, doi:10.1038/s41598-020-68416-1 (2020).

38

INVESTIGATION OF THE EFFECTS OF SH-42, A POTENTIAL STATIN SUBSTITUTE, ON MEMBRANE BIOPHYSICAL PARAMETERS AND PENETRATIN UPTAKE

Florina Zakany[1], Barbara Vigh[1], Rosemary Kothalawala[1], Mate Szabo[1], Zoltan Varga[1], Gyorgy Panyi[1], Istvan Mandity[2], Peter Nagy[1], Tamas Kovacs[1]

1 Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary

2 Department of Organic Chemistry, Semmelweis University, Budapest, Hungary

Various human diseases including hypercholesterolemia and certain tumors (e.g. breast cancer), are characterized by a pathogenically relevant accumulation of cholesterol both in serum and the cell membrane. The latter is associated with alterations in membrane biophysical properties (fluidity, hydration, dipole potential), which may play a role in disease development and progression. In these conditions, cholesterol-lowering compounds have beneficial therapeutic effects. However, applicability of the most commonly used statins is limited by serious adverse effects. SH-42, a recently described sterol analogue that inhibits a key enzyme in cholesterol biosynthesis, 24-dehydrocholesterol reductase, can represent an alternative with a more favorable action profile. Given that reduction of membrane cholesterol levels and consequent lowering of dipole potential facilitate the uptake of cell-penetrating peptides, SH-42 might also enhance the cellular entry of penetratin, a commonly applied cationic cell-penetrating peptide.

We investigated the effects of SH-42 and, as a positive control, atorvastatin on membrane biophysical properties in different breast tumor cell lines. Using environment-sensitive fluorophores and fluorescence-based approaches in living cells we demonstrated that both SH-42 and atorvastatin altered these parameters in a manner consistent with the reduction in membrane cholesterol levels, i.e. significantly increased membrane fluidity and hydration and decreased membrane dipole potential. At the in vivo relevant nanomolar concentrations, SH-42 showed superior efficiency compared to atorvastatin. Currently, we are examining the effects of SH-42 and atorvastatin on the cellular entry and endo-lysosomal escape of fluorophore-conjugated penetratins using a flow cytometer-based time-dependent experimental approach.

Our results suggest that SH-42 has beneficial effects on the biophysical properties of the cell membrane, and therefore, given its more favorable action profile compared to statins, it may be an effective therapeutic alternative for cholesterol lowering from a membrane biophysical point of view.

Support: OTKA FK146740 and UNKP-23-4-II-DE-169 (F.Z.); UNKP-23-4-II-DE-169 (M.S.); OTKA FK143400 and UNKP-23-5-DE-488 (T.K.)

39 EXAMINING THE MEMBRANE PERTURBING EFFECT OF THE ANTIFUNGAL PEPTIDE NFAP2 WITH MOLECULAR DYNAMICS SIMULATIONS

Pavela Olivér, Tünde Juhász, László Galgóczy, Tamás Beke-Somfai
HUN-REN TTK, Budapest

Antimicrobial resistance (AMR) is a major problem in the healthcare sector. Over the past five years, an estimated 5 million human deaths have been associated with AMR almost every year." Antimicrobial peptides are a promising class of molecules, that can destroy various microorganisms, primarily by targeting their biomembranes. These peptides can be found in living organisms, but they can also be synthesized. An antifungal peptide from filamentous ascomycetes, the so-called Neosartorya fischeri antifungal protein 2 (NFAP2), is able to inhibit the growth of fluconazole-resistant Candida albicans. We used computational methods in order to reveal more about the mechanisms of fungal killing by NFAP2. We used molecular dynamics (MD) simulations to investigate the membrane affinity and membrane binding of the protein. We also investigated whether NFAP2 forms oligomers and whether oligomers play an active role in membrane disruption. In this poster we're presenting our latest findings on this topic.

40 SDS MODEL FOR ENHANCED CU(II) DELIVERY TO CELLS BY AMPHIPHILIC PEPTIDES

Dobromiła Sudzik, Maciej Plichta, Iwona Ufnalska, Wojciech Bal
Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

The mechanisms of trafficking of metal ions between cells and extracellular fluids have not been fully elucidated, despite their importance in human health and disease. The controlled cellular import and export of physiologic metal ions is executed by dedicated membrane channels/ transporters of various specificity. Alternatively, metal ions can cross the cellular membrane when assisted by ionofores, organic molecules which can complex metal ions while maintaining hydrophobicity necessary for passive transfer across the phospholipid bilayer. Such molecules are used for experimental enhancement or depletion of metal ion contents of cells, and are also developed towards novel drugs. A prominent example was recently provided by elesclomol, a selenium-based experimental drug, which introduces Cu(II) ions specifically to cells, eliciting the apoptotic process (dubbed cuproptosis) [1]. Human body contains a number of amphiphilic peptides serving regulatory/hormonal roles, e.g. the tachykinin family [2]. Some of them bear metal binding sites, e.g. neurokinin B (NKB) which contain N-terminal ATCUN motifs specific for Cu(II) ions [3]. NKB was proposed to act as natural ionophore, introducing Cu(II) ions directly to cells [3, 4]. This is a troublesome proposal, because intracellular Cu(II) ions may be a source of oxidative stress. Because of this, the Ctr1 receptor, which collects the extracellular Cu(II) ions, assists their reduction to Cu(I) prior to the transmembrane transfer [5]. We used the SDS micellar model [6], to explore the effect of membrane environment on Cu(II) binding of NKB [7]. We found that the Cu(II) binding was weakened nearly 1000-fold, and proposed that this is a feasible mechanism for enhanced Cu(II) delivery to cells, by facilitating the Cu(II) uptake by Ctr1. The validation and extension of this model will be discussed.

References

[1] Tsvetkov P et al. Science 2022, 375, 1254.
[2] Campo A et al. Front. Endocrinol. 2022, 13, 1056939.
[3] Russino D et al. ACS Chem. Neurosci. 2013, 4, 1371.
[4] Shahzad R et al. J. Inorg. Biochem. 2016, 162, 319.
[5] Eisses JF and Kaplan JH. J. Biol. Chem. 2005, 280, 37159.
[6] Bal W. et al. J. Inorg. Biochem. 1994, 55, 41.
[7] Stokowa-Sołtys K. et al. Dalton Trans. 2022, 51, 14267.

POSTER ABSTRACTS

P01

EFFECT OF PROTEIN CORONA ON THE INTERACTION OF SMALL EXTRACELLULAR VESICLES WITH MODEL MEMBRANES

Csilla Adorján[1], András I. Försönits[2], Ádám Zolcsák[3], Bence Fehér[1,3,4]

1 HUN-REN-SU Nanobiophysics Research Group, Budapest, Hungary

2 Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary

3 Institute of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary

4 HUN-REN-SU Biophysical Virology Research Group, Budapest, Hungary

Small extracellular vesicles (sEV) are nanoscale membranous particles and one of the most important species when it comes to intercellular communication. They are diverse and have various biological functions such as delivering small molecules (proteins, nucleic acids, lipids), contribution to developing T cells, maintaining homeostasis, and they are present in almost all body fluids. However, to utilize them as drug delivery systems further studies have to be carried out in order to understand the interaction of sEVs with various particles, e.g. proteins and DNA.

Furthermore, it is important to understand the interactions between sEVs and cell membrane mimicking model bilayers. Some studies already demonstrated the capability of sEVs to fuse with solid supported phospholipid bilayers [1] However, it is known that serum albumin can be absorbed on the surface of the sEVs. [2] The effect of the adsorbed protein layer on the fusion mechanism remains to be unknown.

In our work we produced small extracellular vesicles from monocytes and studied the adsorption of BSA in the function of physicochemical parameters. Furthermore, by means of atomic force microscopy we characterized the fusion properties with dipalmitoylphosphatidylcholine (DOPC) model membranes.

References

[1] F. Perissinotto et al., Nanoscale, 2021, 13, 5224

[2] E.I. Buzas, Nature Cell Biology, 2022, 24, 1322

P02

THE EFFECT OF TARANTULA VENOM DERIVED PEPTIDES ON HUMAN HV1 ION CHANNELS

Ali Rahpeymaei[1,3], Sepideh Mehrabi[1,3] , Samuel Cardoso-Arenas[2], Herlinda Clement[2], Iván Arenas[2], Gyorgy Panyi[1], Zoltan Varga[1], Gerardo Corzo[2], Ferenc Papp[1]

1 Department of Biophysics and molecular biology, University of Debrecen, Debrecen, Hungary

2 Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Avenida Universidad, 2001, Apartado Postal 510-3, Cuernavaca Mor., 62210, México.

3 Department of Pharmacy, University of Tor vergata, Rome, Italy

Spider venoms contain various peptide toxins that modify ion channel currents, mainly of excitable insect cells. Consequently, scientific research on spider venoms has revealed a broad range of peptide toxins with different pharmacological properties, even for mammalian species.

Hv1 channels facilitate reactive oxygen species (ROS) production during the respiratory burst in immune cells, contributing to inflammation. Additionally, Hv1 channels contribute to carcinogenesis by maintaining an acidic tumor microenvironment that supports tumor growth, invasion, and resistance to apoptosis.

In this study, thirty animal venoms underwent screening against hHv1. The complete venom extracted from the spider Grammostola rosea resulted in the modulation of hHv1 expressed in CHO cells during whole-cell patch clamp measurements.

To identify the active component, HPLC reverse-phase fractionation was performed on the G. rosea venom, and the active peptides, named fraction #7 and #9, were isolated, offering promising prospects for therapeutic interventions targeting Hv1 channel inhibition.

Through the application of patch clamp techniques in our laboratory, coupled with subsequent data analysis, we verified that two fractions extracted from spider venom are accountable for suppressing the ion channel. While both fractions act as inhibitors of Hv1, slight variations in the strength of inhibition were observed based on our measurements at different concentrations (Kd values are in the micromolar range). The inhibition is membrane potential dependent, and the threshold voltage for Hv1 activation is also influenced by these peptides, shifting it towards a more positive membrane potential.

Based on current knowledge, this study marks the first examination of the fractions extracted from the spider G. rosea in hHv1. It reveals their capability to alter and suppress the ion current, suggesting a potential lead compound for drug development purposes.

P03

INTRACRINE SIGNALING OF THE EGF RECEPTOR

Noémi Bilakovics[1], Julianna Volkó[1], István Csomós[1], Chinzorig Jeppesen[1], Andrea Bodnár[1], Katalin Tóth[1], György Vereb[1], Péter Nagy[1], György Vámosi[1]

1 Department of Biophysics & Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

The epidermal growth factor receptor (EGFR) is a transmembrane protein. Its activation starts with binding its ligand, the epidermal growth factor (EGF). The mature EGF ligand is derived from a membrane-anchored transmembrane precursor (preEGF).

EGFR is involved in cancer development by gene amplification or by mutations occurring in the EGFR gene. Hence, the EGFR is an important target in anticancer therapies. Monoclonal antibodies are used as inhibitors of EGFR function. These mAbs target the extracellular ligand binding domain of membrane-localized EGFR.

Previously, we defined the intracrine (intracellular autocrine) signaling mechanism of the IL-2 receptor in ATL lymphoma cells expressing both IL-2R and its ligand, IL-2. In this mechanism the receptor and the ligand could form a complex and start signaling already in the Golgi. We were interested whether a similar intracrine signaling mechanism can also take place in cells expressing both EGFR and the soluble EGF or the preEGF ligand.

We studied the steps of the signaling process with various methods. We used CHO cells co-transfected with the EGF or preEGF ligand and EGFR. We applied an anti-phospho-EGFR antibody to detect receptor phosphorylation in the Golgi visualized by a TagBFP-labeled giantin, a Golgi-resident marker. To test our hypothesis that the different forms of the EGF ligand can bind to the EGFR in the Golgi we used FLIM-FRET measurement. Energy transfer was measured between different donor-acceptor pairs.

We obtained zero FRET efficiency between the N-terminally tagged EGFP-EGFR and mScarlet3-preEGF, and low positive FRET (2-4%) between C-terminally tagged protein pairs (EGFR-EGFP and mScarlet3-preEGF), suggesting that preEGF is unlikely to bind to the EGFR. Weak EGFR phosphorylation was detected only from the plasma membrane, not from the Golgi. In contrast, our FRET results show that the mature EGF ligand binds to EGFR and causes its oligomerization and phosphorylation in the Golgi, while EGFR is practically not present in the plasma membrane. If such intracrine signaling occurs before the receptor reaches the plasma membrane, antibody therapies targeting the extracellular ligand binding domain of the EGFR are inefficient in cancer cells expressing both EGFR and its mature EGF ligand. In such cases therapies against intracellular targets are necessary.

P04

TEMPERATURE DEPENDENT EFFECT OF VERAPAMIL ON THE ACTIVITY OF P-GLYCOPROTEIN

Carmila Owusu-Ansah[1], Kevin William Tinkamanyire Rweyemamu[1], Szabolcs Tarapcsák[1], Gábor Szabó[1] and Katalin Goda[1]

1 Department of Biophysics and Cell Biology, University of Debrecen, Hungary

Multidrug resistance (MDR) is responsible for more than 50% of ineffective cancer chemotherapies. One of the major causes of MDR is the overexpression of certain members of the ATP-Binding Cassette (ABC) superfamily, such as P-glycoprotein (Pgp). Pgp is expressed in the plasma membrane and it can export a broad range of substrates out of cells including many anti-neoplastic drugs. During its transport cycle, Pgp is believed to alternate between conformations facing the cytoplasm (inward-facing (IF) conformer) and the extracellular side (outward-facing (OF) conformer). UIC2 is a conformation sensitive antibody selectively recognising the IF Pgps. Previous research has suggested that Pgp is present both in raft and non-raft microdomains. However, little is known about the functional significance of its dual localization. In confocal microscopy-based co-localization studies carried out with raft and non-raft markers, it was found that Pgps spontaneously taking up the UIC2-reactive IF conformation are predominantly raft-localized (pool I), while those that take up this conformation in the presence of Pgp inhibitors, such as cyclosporine A (CsA), reside in the non-raft microdomains (pool II). Raft and non-raft membrane microdomains may each have a different melting temperature (Tm), which may affect the function of raft and non-raft resident Pgps differently. Therefore, we investigated how different temperatures (22, 30 & 37°C) affect the activity of Pgp in the absence and presence of a known substrate/activator, verapamil (Vp).

The function dependent IF to OF conformation change of Pgp was studied by following the kinetics of UIC2 dissociation. CsA completely inhibited the dissociation of UIC2, while in the absence of Pgp ligands we observed comparable UIC2 dissociation from pool I at 30 and 37°C supporting that this temperature decrease is not sufficient to inhibit Pgp activity. Interestingly, 1 μM Vp had noticeable stimulatory effect on UIC2 dissociation at 37°C, while at 30°C we have not observed any stimulation by Vp. We assume that the temperature decrease reduces membrane fluidity and increases membrane packing, which affects the interaction of Pgp with Vp.

The two pools of Pgp were studied in sequential labelling experiments: pool I was labelled with red fluorescent UIC2 mAb followed by labelling of pool II in the presence of 2 μM CsA with green fluorescent UIC2. At 37°C, pool I and pool II Pgps showed comparably slow UIC2 dissociation kinetics, which was accelerated about 4 to 6-fold by Vp in case of both pools. However, at 22°C and 30°C in the absence of Vp, we did not observe significant UIC2 dissociation suggesting the lack of Pgp activity. However, addition of 2 μM Vp noticeably stimulated the activity of both Pgp pools, although the effect on pool II was stronger in accordance with its non-raft localisation. Collectively, the distinct activity of the two Pgp pools further supports their different membrane localization.

P05

STUDIES ON MODEL MYELIN MEMBRANES DEMONSTRATE THAT MYELIN SHEATH MAY SERVE AS A STRONG PROTON CAPACITOR

Jiang Chenchang[1], Zhang Jian Xuan[1], Xie Boyu[1], Ren Jiazhen[1], Edward Gasanoff [1,2]

1 STEM Research Center, Chaoyang KaiWen Academy, Beijing, China

2 Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia

It has been suggested that myelin serves as electrical insulation on nerve fibers. However, nerve impulses are not transmitted through axons the way electrons flow through a metal wire, and the myelin sheath must be far more than an insulator. In this work we prepared model myelin membranes made of phospholipids found in myelin and an acidic protein isolated from bee venom which has the same molecular mass and isoelectric point as the myelin protein (**Figures 1 and 2**). The concentrations of H^+ ions absorbed by acidic protein in H_2O, by lipid membranes or by myelin, was calculated as the difference between initial concentration of H^+ ions in pure H_2O and final concentration of H^+ ions when lipid membrane or myelin are added to H_2O. **Figure 3** compares values of negative logarithm of concentration of H^+ ions absorbed by lipid membranes with that of myelin of various lipids + acidic protein (VP). The lower the negative logariphm value the higher the concentration H^+ ions absorbed. One can see that the concentration of H^+ ions absorbed by myelin membrane is always higher than that of membrane made of only lipids. This finding suggests that the physiological role of myelin could be in serving as a strong proton capacitor.

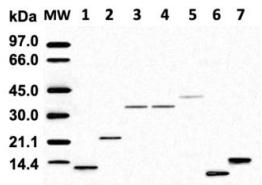


Figure 1

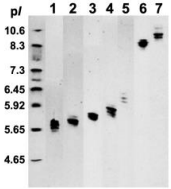


Figure 2

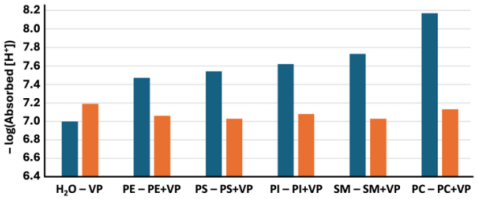


Figure 3

Figure 1. SDS-polyacrylamide 12% gel electrophoresis of the bee venom fractions 1 to 7. Fraction 2 is an acidic protein selected for a model myelin membrane.

Figure 2. Isoelectic focusing of the bee venom fractions 1 to 7 with ampholytes making pH gradient from 3.0 to 10.5. Fraction 2 is acidic protein selected for model myelin membrane.

Figure 3. Negative logarithm of [H^+] absorbed by an acidic protein (VP) in H_2O and by membrane of various lipids or myelin (various lipids + VP). Emerald bars: H_2O; PE –phosphatidylethanolamine; PS – phosphatidylserine; PI – phosphatidylinositol; SM – sphingomyelin; PC – phosphatidylcholine. Orange bars: VP – acidic protein in H_2O; the rest of bars represent myelin which are membranes made of various lipids + VP.

P06

SIGNIFICANT CHANGES IN THE ELECTROPHYSIOLOGICAL PROPERTIES OF WHITE BLOOD CELLS IN MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME DURING HYPEROSMOTIC STRESS

Clarke K.S.P.[1], Kingdon C.C[2], Lacerda E.M[2], Kruchek E.J[1], Griffiths O[1], Hoque R[1], Lewis R[3], Hughes M.P[1,4], and Labeed F.H[1,5].

1 Centre for Biomedical Engineering, University of Surrey, Guildford, United Kingdom

2 Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom

3 Department of Comparative Biomedical Sciences, School of Veterinary Medicine, University of Surrey, Guildford, Surrey, GU2 7XH, United Kingdom

4 Biomedical Engineering Department. Healthcare Engineering Innovation Centre, Khalifa University, Abu Dhabi, UAE.

5 Department of Biology, United Arab Emirates University, Al Ain, UAE

No reliable and validated laboratory tests currently exist to quantifiably diagnose myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS); a condition characterised by profound fatigue which is not relieved by rest and is exacerbated by mental or physical exercise. Recent evidence suggests that the electrophysiological properties of blood samples may pave the way towards a diagnostic tool. Esfandyarpour et al. (2019) identified a significant change in the electrical impedance pattern of ME/CFS samples consisting of peripheral blood mononuclear cells (PBMCs) incubated in their own plasma supplemented with NaCl (to impart hyperosmotic stress) compared with healthy controls. To understand these electrophysiological differences in more detail and their diagnostic potential, we used dielectrophoresis to characterise changes in the electrophysiological properties of PBMC membranes during hyperosmotic stress, and in disease controls.

PBMCs from a total of 12 donors across four donor cohorts (severe ME/CFS, mild/moderate ME/CFS, healthy controls, and multiple sclerosis (MS)) were incubated in physiological and hyperosmotic cell culture media supplemented with NaCl. After 1.5 hours the electrophysiological properties of PBMCs were characterised using the 3DEP (DEPtech). Normalised arbitrary values of measured electrophysiological parameters were found to significantly differentiate severe ME/CFS and mild/moderate ME/CFS donors from both healthy controls and MS donors.

These results demonstrate differential electrophysiological responses to hyperosmotic stress in ME/CFS intrinsic to PBMCs. Furthermore, the electrophysiological response is significantly different from MS disease controls. As a low cost, high-throughput technique using the commercially available 3DEP device, these results show promise towards the development of an urgently needed quantitative and specific diagnostic biomarker for ME/CFS using dielectrophoresis.

P07

EFFECTS OF PROTEIN CORONA ON THE ELASTIC PROPERTIES OF SMALL EXTRACELLULAR VESICLES

Kevin Csordás[1], András I. Försönits[2], Ádám Zolcsák[3], Bence Fehér[1,3,4]

1 HUN-REN-SU Nanobiophysics Research Group, Budapest, Hungary

2 Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary

3 Institute of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary

4 HUN-REN-SU Biophysical Virology Research Group, Budapest, Hungary

Small extracellular vesicles (sEV) are nanoparticles with outmost importance in intercellular communication. They are related to various biological functions and have great diversity. They are present in almost all bodily fluids and have important roles in delivering small molecules between cells and also in maintaining the body's homeostasis.

However, to understand how we could utilize them as drug delivery systems, further studies need to be carried out, to understand how they can interact with other particles such as proteins and how the interaction changes their properties and biodistribution.

Furthermore, it is important to understand how protein absorption can change the mechanical properties of vesicles, which are believed to be important in their biodistribution. Some studies suggest that changes in membrane proteins can influence their elastic properties [1]. However, the exact effect of protein absorption is still unknown.

In our work we produced small extracellular vesicles from monocytes and studied the effect of bovine serum albumin (BSA) protein adsorption on the vesicles mechanical parameters. By the means of atomic force microscopy, we compared the mechanical properties of vesicles without having protein corona and ones with BSA adsorbed on their surface.

References

[1] Stridfeldt, Fredrik, et al. "Surface adhesion and membrane fluctuations influence the elastic modulus of extracellular vesicles." bioRxiv (2024): 2024-02.

P08

COMPUTATIONAL RESOURCES FOR ANALYZING TRANSMEMBRANE PROTEIN STRUCTURES, AND TOPOLOGY

Dobson László; Tusnády Gábor
Research Centre for Natural Sciences, Budapest, Hungary

In 2021, AlphaFold2 (AF2) opened new frontiers for almost all fields of structural biology and provided 3D structures for almost all known protein sequences. In the Transmembrane AlphaFold database (TmAlphaFold database, https://tmalphafold.ttk.hu/) we use a simple geometry-based method to visualize the likeliest position of the membrane plane for AF2 predicted structures of transmembrane proteins. In addition, we calculate several parameters to evaluate the predicted structure

We also overhauled several other popular resources and combined them in the The UNIfied database of TransMembrane Proteins (UniTmp, https://www.unitmp.org/). UniTmp is a comprehensive and freely accessible resource of transmembrane protein structural information at different levels, from localization of protein segments, through the topology of the protein to the membrane-embedded 3D structure. We not only annotated tens of thousands of new structures and experiments, but we also developed a new system that can serve these resources in parallel. UniTmp is a unified platform that merges TOPDB (Topology Data Bank of Transmembrane Proteins), TOPDOM (database of conservatively located domains and motifs in proteins), PDBTM (Protein Data Bank of Transmembrane Proteins), and HTP (Human Transmembrane Proteome) databases and provides interoperability between them. These resources are very useful a starting point for downstream bioinformatics studies, as well as for experiments in the wet lab.

In the near future we plan to integrate more databases and web servers into the framework of UniTmp - with a special emphasis on the TmAlphaFold database. We critically show how different information from AlphaFold can be incorporated, including lipid binding, redox sensitivity and more.

P09

CHARACTERIZING SMALL MOLECULE MODULATORS OF THE HV1 PROTON CHANNEL.

Geraldo Domingos[1], Katinka Gyuris[1], Adam Feher[1], Eva Korpos [1,2], Tibor G. Szanto[1], Martina Piga[3], Tihomir Tomasic[3], Nace Zidar[3], Adrienn Gyongyosi[4], Ferenc Papp[1], Zoltan Varga[1]

1 Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Hungary,

2 MTA-DE Cell Biology and Signalling Research Group, Faculty of Medicine, University of Debrecen, Hungary,

3 Department of Pharmaceutical Chemistry, University of Ljubljana, Slovenia

The human voltage-gated proton channel (hHV1) plays an important role in immune and cancer cells being involved in variety of patho-physiology of the cells. HV1, contrary to the other voltage-gated ion-channels does not have a conventional ion-conducting pore, the conduction occurs through the voltage-sensing domain. This difference may be the reason for the lack of selective hHV1 inhibitors. Our project focuses on verifying the presence of the Hv1 proton channel in Human Aortic Vascular Smooth Muscle Cells (HAoVSMCs) and understanding its functional role using synthesized small molecules as inhibitors. Currently, 5-chloro-2-guanidinobenzimidazole (ClGBI) is the most widely used inhibitor of HV1 (IC50 $\approx$ 26 μM), however, it presents a low selectivity for the channel. This could lead to misinterpretation of functional assays addressing the role of HV1. Thus, our aim was first to find potent and more selective inhibitors for hHV1, which could be useful research tools and serve as lead molecules for the development of drug molecules targeting HV1 for therapeutic goals. We used manual patch-clamp whole cell configuration and measured in transfected CHO model cells, using small molecules inhibitors synthesized from our pharmaceutical chemist collaborators in Slovenia. Our results showed several molecules that effectively blocked the channel. NMP85, NMP132-33, NZ66-2, GHK21, NGS40 and NGS42, showed lower IC50 comparable to that of ClGBI. From those, NGS42 showed the highest affinity with an IC50 = 11 μM. Our next task will be to screen for selectivity in other related voltage gated ion channels, specifically hKv1.3, hK11.1, hNav1.4 and hNav1.5.

P10

ANALYSIS OF UNILATERAL WALKER A AND A-LOOP MUTANTS INDICATE THAT A SINGLE ACTIVE CATALYTIC SITE IS SUFFICIENT TO PROMOTE TRANSPORT ACTIVITY IN ABCB1

Nimrah Ghaffar[1,2], Zsuzsanna Ritter[1,2], Szabolcs Tarapcsák[1], Orsolya Bársony[1], Thomas Stockner[3], Gergely Szakács[4], Katalin Goda[1]

1 Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Hungary

2 Doctoral School of Molecular Cell and Immune Biology, University of Debrecen, Hungary

3 Medical University of Vienna, Centre for Physiology and Pharmacology, Institute of Pharmacology, Austria

4 Institute of Cancer Research, Medical University of Vienna, Austria

Human ABCB1 (P-glycoprotein, Pgp) is an exporter type ABC protein that can expel numerous chemically unrelated xeno- and endobiotics from cells using the energy of ATP hydrolysis. When expressed in tumor cells, ABCB1 may cause multidrug resistance contributing to the failure of chemotherapy. ABCB1 contains two transmembrane domains (TMDs) that collectively form a substrate binding pocket and two nucleotide binding domains (NBDs) that build up two composite nucleotide binding sites (NBSs). The conserved tyrosine in the A-loop of NBSs aligns the adenine ring of the bound ATP, while the Walker A lysine interacts with the α and β phosphate of ATP. The integrity of both NBSs is generally believed to be needed for transport and it is also supposed that the NBSs hydrolyse ATP in a strictly alternating order. Here we demonstrate that ABCB1 variants carrying bilateral mutations in the above residues are completely inactive, while unilateral exchange of these residues is compatible with both ATP hydrolytic activity and transport function. Characterization of the single mutants revealed an about 10-fold reduction of the apparent ATP binding affinity compared to wild-type (WT). Stabilization of the post-hydrolytic complex by phosphate mimicking anions, such as vanadate also occurred at higher ATP concentrations in the mutant variants. Although the basal catalytic activity was strongly reduced in accordance with the decreased ATP binding affinity of the single mutants, the degree of ATPase stimulation by verapamil was almost identical to that of the WT, showing that substrate-stimulation of the ATPase activity is preserved in the single mutants. Location of the mutation in the N or C terminal NBD did not affect the extent of ATPase stimulation. Taken together, in contrast to prevailing views, unilateral NBD mutants retain a significant uphill transport activity, suggesting that the WT catalytic site can hydrolyse ATP in repeated cycles without hydrolysis at the other NBS.

Funding: The work was supported by Hungarian National Science and Research Foundation (OTKA) grant K124815 and by the University of Debrecen Scientific Research Bridging Fund (DETKA).

P11

THE DIELECTRIC STUDY OF ANIMAL CHONDROGENESIS

Oreoluwa Griffiths[1], Srdjan Cirovic[1], Csaba Matta[2], Rebecca Lewis[3], Michael Hughes[4], Fatima Labeed[5]

1 University of Surrey, Centre for Biomedical Engineering, Guildford, UK
2 University of Debrecen, Department of Anatomy, Debrecen, Hungary
3 University of Surrey, School of Veterinary Sciences, Guildford, UK
4 Khalifa University, Department of Biomedical Engineering, Abu Dhabi, UAE
5 United Arab Emirates University, Department of Biology, Al Ain, UAE

Bioelectricity is a mechanism through which cells and tissues communicate and has an important role in developmental biology, wound repair, infection, and cancer development.

The study of embryogenesis in vitro provides insight into the developmental processes through which foetal development occurs. In vitro study has offered the opportunity to scrutinise development, understand crucial stages, cell-cell communication, and the impact of disruption. Ultimately to advance regenerative medicine and pioneer stem cell therapies for applications such as organ and limb replacement.

Chondrogenesis is the process through which cartilaginous tissue is formed, a crucial step in skeletal development that follows differentiation from chondrogenic mesenchymal stem cells to mature/hypertrophic chondrocytes.

Dielectrophoresis (DEP) can be used to characterise developmental stages, through a non-invasive, high throughput and label-free method. The DEPtech 3DEP reader utilises the principle of DEP to characterise and derive four electrophysiological parameters: cytoplasmic conductivity (σcyto), membrane conductance (Geff) and membrane capacitance (Ceff) and membrane potential (Vm). Together these parameters produce an electrical fingerprint that can be used to identify developmental stages and differentiate between cancerous and healthy cell types.

The research presented here studied the electrophysiological changes during in vitro chondrogenesis in the chicken micro-mass model: understanding these changes may allow for non-invasive determination of developmental stages, crucial in stem cell therapies.

The results have highlighted significant changes in three electrophysiological properties: σcyto, Geff, Ceff and Vm. These were characterised by the DEPtech 3DEP Reader platform. Primary chicken chondrogenic mesenchymal stem cells were isolated and cultured for a period of 15 days. 3DEP analysis was conducted on days 0, 1, 2, 3, 6, 10 and 15 to capture differentiation from stem cells to chondrocytes and further the progression to mature and potentially hypertrophic chondrocytes.

A dynamic pattern of electrophysiological changes during chondrogenesis was found. All four electrophysiological parameters remained high in days 0-3 of chondrogenesis before dropping by day 6 - this may indicate maturation to mature chondrocytes - before again rising towards day 15 indicating cellular establishment and possible hypertrophy. This may pave the way for a non-invasive method for the preliminary identification of developmental stages.

P12

INVESTIGATION OF THE TIME-DEPENDENCE OF IMMUNOLOGICAL SYNAPSE FORMATION INDUCED BY IL-15 TRANS-PRESENTATION

Éva Hegedüs[1], Tamás Fekete[2], Lóránd Kelemen[2], György Vámosi[1]

1 Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary

2 Biological Research Center, Szeged, Hungary

The effect of cytokines regulating immune processes is based on their release by the signaling cell into the extracellular environment, followed by their binding to the cell surface receptors of the receiving cell, which leads to the activation of downstream intracellular signaling cascades. However, the major mode of interleukin-15 (IL-15) signaling is a unique method referred to as trans-presentation. IL-15 binds the α subunit of its trimeric receptor with high affinity (Kd=10 pM) on the cell surface of antigen-presenting cells, which can interact with the β and γc subunits of the receptor expressed by a target cells (e.g. memory CD8+ T cell, NK cells), so IL-15 is a membrane-associated molecule that induces signaling at the immunological synapse (IS) between two cells.

We examined the time-dependence of the formation of stable ISs induced by IL-15 treatment. Our model system consists of Raji B cells stably transduced with EGFP-IL-15Rα and Jurkat T cells expressing IL-15Rβ and γc subunits. A microstructure was designed and prepared from Ormocomp photopolymer to allow trapping and manipulation of cells by optical tweezers. Raji-EGFP-IL-15Rα cells were treated with IL-15, and then mixed with Jurkat-IL-15Rβγc cells and microstructures. Cells were trapped and brought into contact with the other type of cell for various time intervals (5, 10, 30 and 60 sec), and then pulled away by optical tweezers. We examined how long it took until a stable contact was formed between them. The two cell types were identified by their fluorescence, Raji cells via the EGFP tag, whereas Jurkat-IL-15Rβγc cells via an A546-conjugated anti-MHC-I antibody.

The IS formed between two cells is a plastic and dynamic structure in which the cytoskeleton plays an active role. We were curious how the absence of actin filaments affects the time-dependence of IS formation, so we treated Jurkat-IL-15Rβγc cells with Latrunculin A, which binds actin monomers and inhibits the polymerization of actin filaments.

We found that the vast majority of the cells formed a stable IS as a result of cytokine treatment. In the absence of cytokine treatment, the cells could not or could only weakly stick to each other, and they could mostly be separated. Latrunculin A treatment significantly decreased the probability and slowed the kinetics of IS formation.

P13

INVESTIGATION OF THE ROLE OF THE HHV1 PROTON CHANNEL BY MOLECULAR BIOLOGY METHODS

Katinka Gyuris[1], Geraldo Domingos[1], Éva Korpos[2], Beáta Arnódi-Mészáros[1], Martina Piga[3], Nace Zidar[3] and Zoltán Varga[1]

1 University of Debrecen, Faculty of Medicine, Department of Biophysics and Cell Biology

2 University of Debrecen, Faculty of Medicine, Department of Biophysics and Cell Biology, MTA-DE Cell Biology and Signaling Research Group

3 University of Ljubjana Faculty of Pharmacy Department of Pharmaceutical Chemistry, Ljubjana

The hHv1 voltage-dependent proton channel is a passive transporter that selectively transfers protons across the membrane, and thus plays an important role in pH regulation of many cell types. Vascular smooth muscle cells (VSMCs) found in the arterial wall have a resting or contractile phenotype under physiological conditions. When the vessel wall is damaged, the cells switch to a synthetic, migratory and proliferative phenotype, which allows for tissue regeneration. Failure of migrating/proliferating cells to switch back to a contractile phenotype induces pathogenic vascular remodelling leading to medial clacification of the vessel wall.

In our work, we aim to demonstrate the presence of hHv1 on vascular smooth muscle cells and to elucidate the role of the channel in normal and pathological cellular activities through the regulation of intracellular pH. We investigate the role of hHv1 in VSMC survival, differentiation and matrix production.

If hHv1 plays a key role in the pathological activity of VSMCs during atherosclerosis but not in normal function, the channel may become an important pharmacological target to inhibit the pathological activity of VSMCs in calcification.

To confirm this hypothesis, we performed viability measurements using MTT assay, PCR and Western blot experiments to detect hHv1, alizarin red staining to visualize the effect on calcified extracellular matrix production, and microscopic studies.

Our results support the hypothesis that hHv1 is expressed in VSMCs and plays a role in their ability to produce calcified extracellular matrix and influences their viability. This conclusion may provide a basis for further experiments to quantify the difference in hHv1 expression between contractile and differentiated proliferative cells and to identify related functional differences.

Abbreviations:

hHv1 – human voltage-gated proton channel
VSMC – vascular smooth muscle cell
MTT – (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
PCR – Polymerase chain reaction

P14

ARRANGEMENTS OF PRE-AD MEMBRANE MIMETICS DUE TO THE PRESENCE OF AB(25-35) PEPTIDE

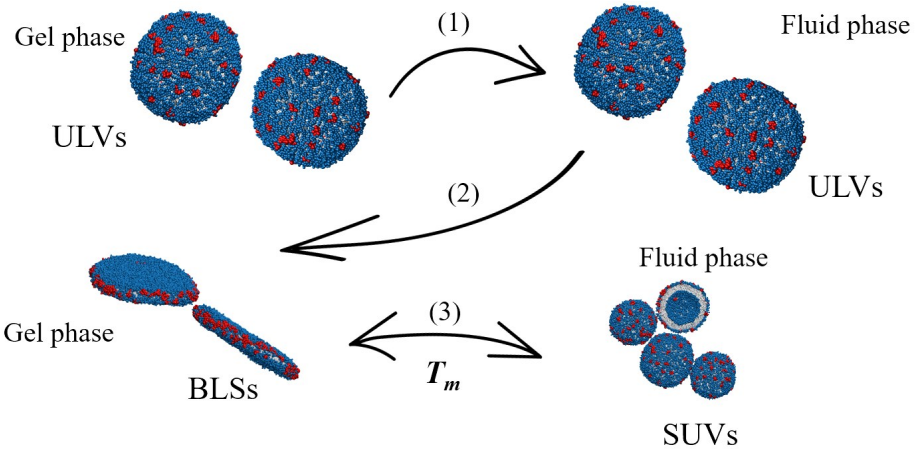
Norbert Kučerka[1,2], Sergei Kurakina[3], Dina Badreeva[1], Ermuhammad Dushanov[1], Tatiana Murugova[1], Oleksandr Ivankov[1], Elena Ermakova[1], Aleksander Kuklin[1], Kahramon Mamatkulov[1], Grigory Arzumanyan[1]

1 Joint Institute for Nuclear Research, Dubna - Moscow Region

2 Faculty of Pharmacy, Comenius University Bratislava, Bratislava, Slovakia

3 Kazan Federal University, Kazan, Russia

The complementary experimental data and molecular dynamics (MD) simulations results reveal the structure of previously observed lipid bicelle-like structures (BLSs) formed in the presence of amyloid-beta peptide Aβ(25-35) below the main phase transition temperature (Tm) of saturated phosphatidylcholine lipids and small unilamellar vesicles (SUVs) above this temperature. First, we show by using solid-state 31P nuclear magnetic resonance (NMR) spectroscopy that our BLSs being in the lipid gel phase demonstrate magnetic alignment along the magnetic field of NMR spectrometer and undergo a transition to SUVs in the lipid fluid phase when heated through the Tm. Secondly, thanks to the BLS alignment we present their lipid structure. Lipids are found located not only in the flat bilayered part but also around its perimeter, which is corroborated by the results of coarse-grained (CG) MD simulations. Finally, peptides appear to mix randomly with lipids in SUVs while assuming predominantly unordered secondary structures revealed by circular dichroism (CD), Raman spectroscopy, and all-atom MD simulations. Importantly, the former is changing little when the system undergoes morphological transitions between BLSs and SUVs. Our structural results then offer a platform for studying and understanding mechanisms of morphological transformations caused by the disruptive effect of amyloid-beta peptides on the lipid bilayer.



P15

INVESTIGATION OF THE INTERACTION BETWEEN SPIKE PROTEINS OF DIFFERENT SARS-COV-2 VARIANTS AND THE ACE2 RECEPTOR

Kitti Kurtan[1], Muhammad Umair Naseem[1], Kashmala Shakeel[1], Zoltan Varga[1], Peter Nagy[1], Gyorgy Panyi[1], Tamas Kovacs[1], Florina Zakany[1]

1 Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary

Initial membrane-coupled events of SARS-CoV-2 infection involve interactions between viral spike proteins and lipid raft resident ACE2 and TMPRSS2 proteins of the host cell. We found that remdesivir formulations containing cyclodextrins (CDs) inhibit ACE2 binding and internalization of spike proteins due to membrane cholesterol depletion and raft disruption independent of remdesivir, raising the therapeutic applicability of CDs in COVID-19.

We hypothesized that SARS-CoV-2 variants with different ACE2 affinities are characterized by different cholesterol and thus CD sensitivity. Our aim was to compare ACE2 binding affinities of spike receptor-binding domains (RBDs) of clinically relevant variants (WT, Alpha, Beta, Delta, Omicron Ba1 and Ba2), and examine the relationship between their binding affinity, cholesterol sensitivity and lipid raft preference. We are also investigating the effects of cholesterol extraction on the uptake of spike trimer variants.

In HEK293 cells stably expressing ACE2 and TMPRSS2, using flow cytometry we found that spike RBD variants exhibited different binding efficiency to ACE2, and a negative correlation was observed between binding efficiency and the extent of hydroxypropyl-β-cyclodextrin (HPβCD)-mediated inhibition of ACE2 binding. We also examined the correlation between receptor binding and cellular lipid raft content quantified using either fluorescently labeled GM1 ganglioside-binding choleratoxin B subunit or an environment-sensitive fluorophore (F66). We found that variants with lower ACE2 affinity (WT or Omicron Ba1) showed stronger preference for cells with higher lipid raft abundance than those with higher affinity (Delta). Using confocal microscopy and quantitative 3D image analysis, we are currently investigating if these changes are mirrored in an altered efficiency and HPβCD sensitivity of cellular uptake of different trimer variants.

SARS-CoV-2 spike protein variants are characterized by distinctive ACE2 binding efficiency and cholesterol sensitivity, which could originate from their altered preference to raft-resident ACE2 over receptors found in non-raft membrane regions. Consequently, receptor binding and internalization of the high affinity variant Delta exhibits lower cholesterol sensitivity compared to that of the lower affinity variant Omicron Ba1.

Support: UNKP-23-2-I-DE-197, NTP-HHTDK-23-0023 (K.K.); OTKA FK143400 and UNKP-23-5-DE-488 (T.K.); OTKA FK146740 and UNKP-23-4-II-DE-169 (F.Z.)

P16

LACTADHERIN-BASED MEMBRANE LABELLING IN FLUORESCENT MICROSCOPY

Dorina Lenzinger[1], Anna Koncz[1,2], Krisztina Németh[1,2], Tünde Bárkai[1], Krisztina V. Vukman[1], Edit I. Buzás[1,2,3], Tamás Visnovitz[1,4]

1 Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary

2 HUN-REN-SU Translational Extracellular Vesicle Research Group, Budapest, Hungary

3 HCEMM-SU Extracellular Vesicle Research Group, Budapest, Hungary

4 Department of Plant Physiology and Molecular Plant Biology, Eötvös Loránd University, Budapest, Hungary

Extracellular vesicles (EVs) are particles enclosed by a phospholipid bilayer in the extracellular space, without the ability self-reproduction. The defining component of EVs is the phospholipid membrane, and thus, its visualization during microscopic examination is crucial. The location of plasma membrane of the EV-releasing or the EV-recipient cells can be also essential. In addition, it may be helpful to determine whether the detected EV-specific markers are inside or outside of the cell during immunocytochemistry. Although there is no ideal membrane labeling, we developed an optimal approach for tracking of medium and large-sized EVs during confocal microscopy.

Lactadherin, a protein that binds to phosphatidylserine, offers advantages over annexinV due to its calcium-independent binding. Therefore, lactadherin-based labeling is more stable during immunocytochemistry. By conjugating lactadherin with fluorophores, we evaluated its efficacy in labeling membranes and EVs across various fixed cell lines (e.g., HEK293, HEK293T-palmGFP, HepG2, HT29, H9c2, HeLa) using a Leica SP8 Lightning confocal microscope.

Our findings demonstrate that lactadherin specifically labels the external leaflet of the plasma membrane in fixed, non-permeabilized cells. Moreover, as phosphatidylserine is enriched in the EV membrane, the fluorescence signal detected in the case of EVs is even stronger than that of the plasma membrane. We highly recommend the adoption of the lactadherin-based labeling system for fluorescence microscopic EV studies.

P17

ABC TRANSPORTERS WITH A WIDE-RANGE DRUG SPECTRUM INHIBIT THE TRANSMIGRATION OF HUMAN CYTOTOXIC T-LYMPHOCYTES ACROSS HUVEC CELL MONOLAYERS

Algirmaa Lkhamkhuu[1,2,4], Biwott Kipchumba[1,2], and Zsolt Bacso[1,2,3]

1 Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Hungary

2 Doctoral School of Molecular Cellular and Immune Biology, Faculty of Medicine, University of Debrecen, Hungary

3 Department of Biophysics and Cell Biology, Faculty of Pharmacy, University of Debrecen, Hungary

4 Department of Biomedicine and Pharmacy, Darkhan-Uul Medical School, Mongolian National University of Medical Sciences, Mongolia

Previous studies have highlighted ABCB1 blockade's role in hindering dendritic cell transmigration through human embryonic endothelial layers or emigration from skin explants, models for lymphatic vessel diapedesis. Extending this understanding to cytotoxic lymphocytes remains underexplored, especially regarding ABCB1 or other transporter mechanisms in diapedesis across various endothelial cells. Deciphering MDR transporters' contribution in T cell-endothelial interactions, notably in effector and immune-initiator contexts, bears significant implications for immunology and therapy.

We established a model system to probe MDR transporters in human CD8+ lymphocytes and their effect on transmigration across HUVEC monolayers, mimicking blood, and lymphatic vessel diapedesis. Preliminary assessment of Pgp expression in endothelial cells modeling vessel walls revealed relatively low levels in primary HUVEC cells compared to human Pgp-transfected NIH-3T3-MDR1 mouse cells.

Utilizing a Boyden chamber-based transwell system, we examined primary cytotoxic T lymphocyte (CTL) transmigration across HUVEC monolayers. CTLs were induced into the upper chamber, and chemotaxis was facilitated by CCL19 in the lower chamber, allowing transmigration for 4 hours. The evaluation involved quantifying nucleated cells on the lower surface of inserts via trypsinization and flow cytometry.

The HUVEC monolayer alone constrained CTL transmigration induced by CCL19 chemoattraction. While anti-Pgp antibodies UIC2 and 15D3 had no effect, specific inhibitors like Zosuquidar, Ko143, MK571, and Cyclosporine A inhibited CTL transmigration in both orientations. Zosuquidar demonstrated superior efficacy over Cyclosporine A, suggesting Pgp specificity. No disparity was observed between direct and reverse orientation experiments, indicating broad-spectrum ABC transporters' involvement in CTL transmigration across HUVEC monolayers during chemoattraction.

P18

INTERACTION OF ANTIMICROBIAL PEPTIDE CATH-1 WITH EXOGENOUS PULMONARY SURFACTANT: STRUCTURE AND THERMODYNAMICS

Rastislav Korfanta[1], Ali Asi Shirazi[1], Juan Carlos Martínez[2], Daniela Uhríková[1]

1 Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius University Bratislava, Bratislava, Slovakia

2 ALBA Synchrotron, Cerdanyola del Vallés, Barcelona, Spain

Pulmonary surfactant (PS) is a complex of lipids and proteins that are produced and secreted by the respiratory epithelium of the lungs, and it serves to minimize surface tension at the air-liquid interface of the alveolar space, which reduces the work of breathing. PS consists mainly of lipids (90 wt%), and dipalmitoylphosphatidylcholine (DPPC) is the most abundant lipid. Specific proteins make up the remaining 10% of its composition. PS is responsible for gas exchange in the alveolar space, and its deficiency can cause respiratory distress syndrome in newborns (NRDS). The condition is treated with the administration of exogenous pulmonary surfactant (EPS), such as Curosurf® (poractant alfa).

Cathelicidins are antimicrobial peptides (AMP) that belong to the innate immune system of various organisms, including humans. The cumulative positive charge of cathelicidins facilitates their interaction with negatively charged bacterial membranes. The utilization of AMPs has been complicated due to the difficulty in distributing AMPs to lung tissue. Recent studies have proposed the use of EPS as a drug carrier for other drugs, including antimicrobial agents.

We studied the interaction and impact of chicken cathelicidin CATH-1 on the structure of exogenous PS (Curosurf®). Bacterial lipopolysaccharide (LPS) was used to mimic the pathological condition. LPS is known as an inactivating agent for PS. The effect of CATH-1 on the temperature of the gel- to -fluid phase transition was determined using differential scanning calorimetry (DSC). The structural changes induced by CATH-1 in the multilamellar ordering of Curosurf® and LPS/Curosurf® were examined using small- and wide-angle X-ray scattering (SAXS/WAXS).

Acknowledgments: SAXD/WAXD experiments were performed at the NCD-BL11 beamline, ALBA Synchrotron, Barcelona, Spain, with the collaboration of Alba staff. The research was supported by the VEGA project 1/0305/24.

P19

EFFECTS OF HV1 VOLTAGE-GATED PROTON CHANNEL INHIBITION ON THE VIABILITY OF POLARIZED MACROPHAGES

Rosemary Kothalawala[1], Bence Cs. Szabo[1], Mate Szabo[1], Peter Nagy[1], Zoltan Varga[1], Gyorgy Panyi[1], Tamas Kovacs[1], Florina Zakany[1]

1 Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary

The human voltage-gated proton channel (HV1) is a proton-selective ion channel gated by membrane depolarization and pH. The proton efflux into the extracellular space following channel activation plays an important role in the regulation of membrane potential and pH in immune cells and the production of cytokines and free radicals in macrophages. Although HV1 inhibition has been shown to reduce the viability of lymphocytes, such effects have not been investigated in macrophages polarized along different pathways.

Our aim was to establish a simplified model system of polarized macrophages, to investigate the effects of HV1 inhibition on the viability of these cells, and to examine the channel specificity and the molecular mechanism of action of the effects. We differentiated THP-1 monocytes into M0 macrophages using PMA and subsequently polarized them along the classical M1 (LPS and IFNγ) and alternative M2 (IL-4 and IL-13) pathways. The success of polarization was confirmed by flow cytometric analysis of cell surface CD markers (CD64, CD71, CD80, CD86, CD206). We then examined effects of the small-molecule HV1inhibitor, 5-chloro-2-guanidinobenzimidazole (ClGBI), and other ion channel blocking agents (Zn2+, Vm24, tetraethylammonium and tetradotoxin) on the viability of polarized macrophages by labeling necrotic and apoptotic cells with Sytox Green and annexin V, respectively, and determining the relative fraction of double negative live cells by flow cytometry.

Among the treatments tested, only the HV1-inhibiting ClGBI and Zn^{2+} reduced cell viabilities in a dose-dependent manner and different macrophages showed altered sensitivities (M1 > M0 > M2). The ClGBI-induced cell death was ceramide-dependent, as our flow cytometry measurements revealed significant increases in membrane ceramide levels, whereas pretreatment with ceramide production inhibitors provided partial protection against the decreases in viability.

Our results suggest that HV1 inhibition reduces viability of THP-1-derived macrophages in a polarization- and ceramide-dependent manner. Since we hypothesize that perturbation of pH regulation due to channel inhibition may play a role in enhanced ceramide production, we are investigating the effects of ClGBI on cytoplasmic, lysosomal and phagosomal pH.

Support: UNKP-23-4-II-DE-169 (M.S.); OTKA FK143400 and UNKP-23-5-DE-488 (T.K.); OTKA FK146740 and UNKP-23-4-II-DE-169 (F.Z.)

P20

Graphene-based materials in biomedicine: a molecular approach

Martin Šrejber[1], Markéta Paloncýová[1], Andrea Nedělníková[1,2] and Michal Otyepka[1,3]

1Czech Advanced Technology and Research Institute (CATRIN), Palacký University Olomouc, Olomouc, Czech Republic

2 Department of Physical Chemistry, Faculty of Science, Palacký University Olomouc, Czech Republic

3 IT4Innovations, VŠB – Technical University of Ostrava, Ostrava, Czech Republic

In recent years, there has been a significant increase in the utilization of graphene and graphene-based materials in various biomedical fields, such as biomedical sensing of small molecules, drug delivery, imaging techniques or photothermal therapy applications. This is attributed to the exceptional properties exhibited by graphene, such as its high surface area, thermal and electrical conductivity, and remarkable mechanical strength. These properties arise from its single-layered, hexagonal "honeycomb" lattice nanostructure consisting of sp2 hybridized carbon atoms that can be covalently and non-covalently functionalized. This exceptional combination of characteristics makes graphene a promising candidate for various biomedical research applications. The understanding of the effects of graphene and graphene-based materials on biological membranes is critical to comprehending their potential for use in various biomedical disciplines since biological membranes are typically the initial barriers encountered by foreign substances upon administration.

Here we foccused on the investigation of the interaction between graphene (and its derivatives) and biological membranes models using molecular dynamics simulations (MD) at both atomistic and coarse-grained resolutions. Atomistic molecular dynamics (MD) simulations were employed to investigate the interaction mechanism between graphene-based materials and models of nerve cell membranes, which mimic real membranes such as neuronal or microglial membranes. Understanding the impact of these materials on such cell membranes holds significant potential for their application in neural implants. Alternatively, we used coarse grained simulations to depict the larger-scale dynamics of interaction of those kind of materials with complex bacterial models of Eschrichia coli membranes generally used as model organism in bacterial resitence.

P21

HYDRATION-TEMPERATURE DEPENDENT FLUORESCENCE SPECTRA OF LAURDAN CONFORMERS IN A DPPC MEMBRANE

S. Knippenberg[1,2], K. De[3], C. Aisenbrey[3], B. Bechinger[3,4], S. Osella[5]

1 Hasselt University, Theory Lab, Agoralaan Building D, 3590 Diepenbeek, Belgium; email: stefan.knippenberg@uhasselt.be

2 Université Libre de Bruxelles, Spectroscopy, Quantum Chemistry and Atmospheric Remote Sensing (SQUARES), 50 Avenue F. Roosevelt, C.P. 160/09, B-1050 Brussels, Belgium.

3 University of Strasbourg/CNRS, UMR7177, Institut de Chimie de Strasbourg, Strasbourg, France.

4 Institut Universitaire de France (IUF), Paris, France.

5 Chemical and Biological Systems Simulation Lab, Centre of New Technologies, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland.

The widely used Laurdan probe has two conformers, resulting in different optical properties when embedded in a lipid bilayer membrane as demonstrated by simulations . Up to now, the two conformers' optical responses have however not been investigated when the temperature and the phase of the membrane changes. Since Laurdan is known to be both a molecular rotor and a solvatochromic probe, it is subject to a profound interaction with both neighboring lipids and water molecules. In the current study, molecular dynamics simulations and hybrid Quantum Mechanics/ Molecular Mechanics calculations are performed for a DPPC membrane at eight temperatures between 270 K and 320 K, while the position, orientation, fluorescence lifetime and fluorescence anisotropy of the embedded probes are monitored. The importance of both conformers is proven through a stringent comparison with experiment, which corroborates the theoretical findings. It is seen that for Conf-I the excited state lifetime is longer than the relaxation of the environment, while for Conf-II the surroundings are not yet adapted when the probe returns to the ground state. Throughout the temperature range, the lifetime and anisotropy decay curves can be used to identify the different membrane phases. The current work might therefore be of importance for biomedical studies on diseases which are associated with cell membrane transformations.

P22

THE INFLUENCE OF CHOLESTEROL ON PHOSPHOLIPID CONFORMATIONS IN MIXED SATURATED LIPID MEMBRANES

Matthew Davies[1,2], Suvi Heinonen[3], Alex Bunker[3], Mikko Karttunen[1,2]

1 Department of Physics and Astronomy, The University of Western Ontario, London, Ontario, Canada

2 Department of Chemistry, The University of Western Ontario, London, Ontario, Canada

3 Drug Research Program, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland

Cholesterol affects the permeability of phospholipid membranes concentration-dependently [1,2]. Low cholesterol concentrations can induce points of leakage in rippled membranes, whereas high concentrations suppress the ripple phase completely [2,3]. Our study utilizes molecular dynamics (MD) simulations and machine learning to quantify lipid conformation types in ternary membranes with varying cholesterol concentrations. A similar approach was recently applied to identify four distinct lipid conformations in rippled DPPC membranes [4].

MD simulations of mixed CHOL/DMPG/DMPC membranes were performed using the GROMACS-2020.5 simulation package with the CHARMM36 force field and TIP3P water model. Five cholesterol concentrations (0, 4, 11, 20, and 33 mol%) were simulated for 1.5 to 2.0 μs at 297 K. The lipid hydrocarbon chain conformations were analyzed using a previously developed mixed radial-angular three-body correlation function method combined with unsupervised machine learning [4].

The membranes with a low cholesterol concentration contained all four of the lipid conformations found also in the DPPC ripple phase [4]. Increasing the cholesterol concentration was observed to suppress the number of splayed and disordered lipids and induced the emergence of a fifth lipid type with constrained, yet more fluid, hydrocarbon tails. This work exemplifies how MD simulation can be combined with machine learning methods to aid the rational design of liposomal formulations containing cholesterol.

[1] S. Raffy and J. Teissié Biophys. J. 1999 76, 2072-2080.
[2] E. Corvera et al. Biochim Biophys Acta 1992 1107, 261-70.
[3] A. Hicks et al. Biochim. Biophys. Acta Biomembr. 1987 903, 177-185.
[4] M. Davies et al. Biophys. J. 2023 122, 442-450.

P23

THE EFFECT OF DIPOLE POTENTIAL ON THE SIGNALLING EFFICIENCY OF THE INTERLEUKIN-2 RECEPTOR

Levente Szolyka, Tamás Kovács, Florina Zákány, Péter Nagy, György Vámosi

Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary

The electric potentials of the cell membrane are classified as the transmembrane potential, the dipole potential and the surface potential. The dipole potential is an electrostatic potential that results from the dipole moment of the membrane molecules and their associated water molecules. This electrical property may strongly influence the conformation of membrane proteins. It can change with the composition of the membrane. Metabolic diseases such as Gaucher's disease and SLO syndrome can disturb the lipid homeostasis of the body, leading to changes in the composition of the plasma membrane and causing immunological symptoms too. Interleukin-2 (IL-2) and its receptor (IL-2R) play a key role in regulating the immune system. They are essential for the proliferation and differentiation of T cells, and therefore play a significant role in the development of various diseases, including multiple sclerosis and T-cell leukemia. Previously we have shown that IL-2R signaling efficiency and mobility can change in response to changes (hyper- or depolarization) of the transmembrane potential. The aim of this research is to perform biophysical measurements to establish a correlation between changes in dipole potential and IL-2R signalling. This will provide a deeper understanding of the relationship between lipid metabolism and immune-related diseases.

P24

PEPTIDE-EXTRACELLULAR VESICLE INTERACTION: DISRUPTION, MEMBRANE TRESPASSING, AND PROTEIN CORONA CLEARANCE

Tasvilla Sonallya, Tünde Juhász,Imola Cs. Szigyártó, Priyanka Singh, Delaram Khamari ,Kinga Ilyés, Edit I. Buzás, Zoltán Varga and Tamás Beke-Somfai

Extracellular vesicles (EVs) play crucial roles in intercellular communication, with their surface interactions influencing cellular uptake and immune recognition. The external surface, often termed the "protein corona," comprises biomolecules adsorbed on the lipid surface of EVs. Despite their therapeutic potential, the functionality of EVs and their interactions with the host immune system remain incompletely understood. In this study, we investigated the potential of natural host defense peptides and synthetic antimicrobial peptides (AMPs) in modulating EV surface properties, aiming to inform future bioengineering strategies and targeted drug delivery approaches.

We conducted an in-depth biophysical characterization of 26 AMPs and membrane-active cell-penetrating peptides (CPPs) to assess their effects on red blood cell-derived vesicles (REVs). Peptides were categorized based on their interactions with REVs, focusing on protein corona elimination and membrane penetration mechanisms.

These results provide valuable insights into peptide-EV interactions, highlighting their potential for manipulating EV surface properties in bioengineering applications and targeted drug delivery. By elucidating the mechanisms underlying peptide-mediated effects on EVs, this study lays the foundation for further research into therapeutic peptide-EV formulations and delivery strategies. Further investigations into peptide-EV interactions are warranted to fully harness their therapeutic potential in clinical applications.

P25

EXAMINATIONS OF CELLULAR UPTAKE OF CELL PENETRATING PEPTIDES IN VITRO AND IN VIVO

Gabriella Tóth[1], Gyula Batta[1,2], Levente Kárpáti[3], Árpád Szöőr[1], István Mándity[3], Péter Nagy[1]

1 University of Debrecen, Faculty of Medicine, Department of Biophysics and Cell Biology

2 University of Debrecen, Faculty of Science and Technology, Institute of Biotechnology, Department of Genetics and Applied Microbiology

3 Semmelweis University, Faculty of Pharmacy, Institute of Organic Chemistry

Cell-penetrating peptides (CPPs) are peptides that enter cells by endocytosis and/or directly through the cell membrane. CPPs in general have been considered potential carriers of molecules that have difficulties entering cells. This is the feature that we would like to exploit and thereby establishing the opportunity for CPPs to have therapeutic applications in the long term. Our previously published results have shown that we can increase the cellular uptake and endosomal release of CPPs with statins. Our goal was to modify them and test if it is possible to make them enter the cells more efficiently. We also aimed to test the biodistribution of CPPs in mice after intravenous administration. We examined the cellular uptake and endosomal release by flow cytometry and confocal microscopy in SKBR-3 and MDA-MB-231 cell lines, while for the in vivo experiments a mouse model was applied. Fluorescently-labeled CPPs were used both in the in vivo and in vitro experiments. We compared the differences in the biophysical properties of the original and the modified CPPs, and we found that the cellular uptake of the modified version is more effective. There is a difference between the enhancement in the uptake of CPPs labeled by the pH-sensitive naphthofluorescein or Alexa Fluor 532. In the case of in vivo experiments, we found that peptides enter the mouse organs, including the liver, for which we have shown that CPPs is present in the intracellular space of hepatocytes. CPPs hold promise for increasing the efficiency and specificity of drug delivery to cells.

P26

INVESTIGATING THE ROLES OF MEMBRANE CERAMIDES IN THE PARKINSON'S DISEASE-ASSOCIATED PHOSPHORYLATION OF THE MICROGLIAL KV1.3 ION CHANNEL

Tamas Kovacs[1], Bence Cs. Szabo[1], Kitti Kurtan[1], Zoltan Varga[1], Peter Nagy[1], Gyorgy Panyi[1], Florina Zakany[1]

1 Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary

Parkinson's disease is characterized by the selective destruction of dopaminergic neurons, which is casually linked to the abnormal Kv1.3-dependent activation of microglia. Therefore, selective inhibition of the channel may represent an efficient novel therapeutic approach. Fyn kinase responsible for the phosphorylation of Kv1.3 is an important risk factor for Parkinson's disease that is also associated with increases in membrane ceramide (Cer) contents. In addition to cholesterol and glucosylceramide (GCer)-enriched lipid rafts, Cer platforms are unique microdomains that provide a specific signaling microenvironment in the cell membrane. However, the role of Cer platforms in Parkinson's disease-associated phosphorylation of Kv1.3 (pKv1.3: phosphorylated Kv1.3) has not been previously investigated.

We examined how treatments increasing the abundance of membrane Cer (exogenous loading, lipopolysaccharide (LPS) treatment) and GCer (loading) alter the levels and lateral microdomain distribution of Kv1.3 and pKv1.3 in a mouse microglial cell line.

Using quantitative confocal microscopy we found that while GCer loading increased Kv1.3-lipid raft colocalization, treatments leading to Cer accumulation (Cer loading, LPS) resulted in higher Kv1.3-Cer platform colocalization. Flow cytometry measurements demonstrated that Cer loading and LPS elevated pKv1.3 levels, which were also associated with increased pKv1.3-Cer platform colocalization, without any changes in pKv1.3-lipid raft colocalization. We are currently investigating whether the effects induced by LPS can be prevented by a simultaneous inhibition of Cer accumulation with blockers of enzymes involved in Cer production, such as serine palmitoyl transferase, acidic or neutral sphingomyelinases.

Our experiments demonstrated that in microglial cells increases in cell membrane Cer levels are accompanied by elevated Parkinson's disease-associated phosphorylation of Kv1.3 and pKv1.3 channels are enriched in Cer platforms, which suggest a direct link between Cer accumlation and increased pKv1.3 levels in Parkinson's disease.

Support: OTKA FK143400 and UNKP-23-5-DE-488 (T.K.); UNKP-23-2-I-DE-197 (K.K.); OTKA FK146740 and UNKP-23-4-II-DE-169 (F.Z.)

P27

STRUCTURAL CHANGES OF CATIONIC ANTIMICROBIAL PEPTIDES UPON INTERACTION WITH QUORUM SENSING MOLECULES

Kamilla Ujvári[1,2], Imola Csilla Szigyártó[2], Tamás Beke-Somfai[2]

1 Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Budapest, Hungary

2 Biomolecular Self-assembly Research Group, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary

Nowadays, large numbers of bacterial infections involves biofilm formations; moreover these recalcitrant infections often lead to substantial mortality. Antimicrobial peptides represent a promising approach to treat biofilm-related infections, as they have the ability to interfere with quorum sensing (QS) molecules. QS molecules regulate the communication between the cells in the biofilm and can control the release of outer membrane vesicles in Gram-negative bacteria enabling them to communicate and to suppress other bacteria.

We have investigated a set of natural and synthetic membrane active peptides, e.g. LL-37 (Zsila et al. 2021), FK-16, Dhvar4, IDR-1018 and 1037 in order to explore their binding affinity to compounds of bacterial cell-to-cell communication. QS molecules interact with peptides, inducing helical folding and forming co-assemblies. These interactions were characterized using circular dichroism and infrared spectroscopy, whereas the morphology of assemblies was investigated using dynamic light scattering and transmission electron microscopy. The promising combinations were further examined with model membrane systems of mammalian and bacterial cell membranes in order to better understand their action mechanism.

Reference

F. Zsila, M. Ricci, I.Cs. Szigyarto, P. Singh, T. Beke-Somfai: Quorum Sensing Pseudomonas Quinolone Signal Forms Chiral Supramolecular Assemblies With the Host Defense Peptide LL-37, Front. Mol. Biosci. 8:742023, 2021

Acknowledgement

This work was funded by the National Research, Development and Innovation Office, Hungary (TKP2021-EGA-31, 2020-1.1.2-PIACI-KFI-2020-00021 and KKP_22 Project n.o. 144180). Support from Eötvös Loránd Research Network, Grant Nos. SA-87/2021 and KEP-5/2021 are also acknowledged.

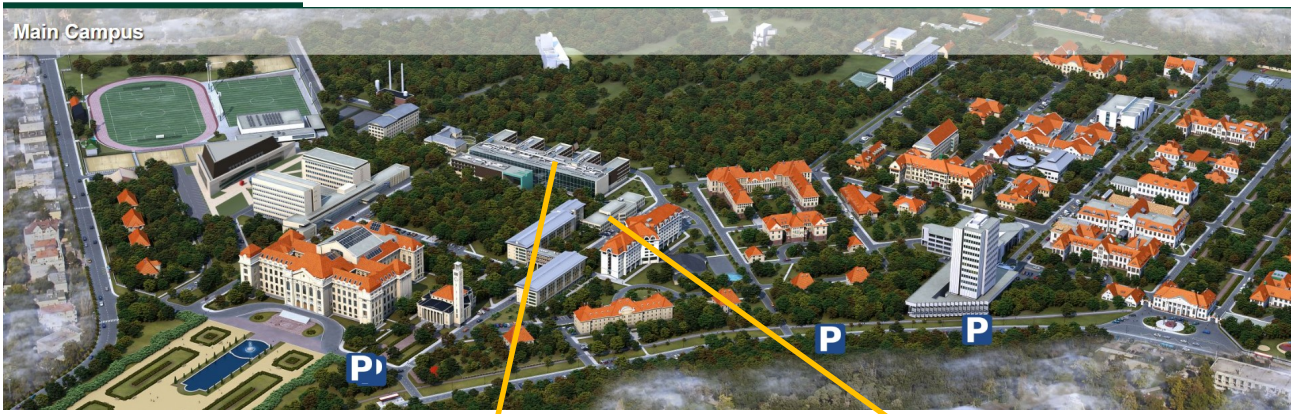
P28

PERILIPIN 3 PROTEIN RECOGNIZES DAG-CONTAINING MEMBRANES BY ITS CONSERVED PAT DOMAIN

Jiri Stribny, Roger Schneiter
Department of Biology, University of Fribourg, Switzerland

Perilipins (PLINs) constitute an evolutionarily conserved family of proteins that specifically associate with the surface of lipid droplets (LDs). These proteins function in LD biogenesis and lipolysis and help to stabilize the surface of LDs. PLINs are typically composed of three different protein domains. They share an N-terminal PAT domain of unknown structure and function, a central region containing 11-mer repeats that form amphipathic helices, and a C-terminal domain that adopts a 4-helix bundle structure. How exactly these three distinct domains contribute to PLIN function remains to be determined. Here, we show that the Nterminal PAT domain of PLIN3 binds diacylglycerol (DAG), the precursor to triacylglycerol, a major storage lipid of LDs. PLIN3 and its PAT domain alone bind liposomes with micromolar affinity and PLIN3 binds artificial LDs containing low concentrations of DAG with nanomolar affinity. The PAT domain of PLIN3 is predicted to adopt an amphipathic triangular shaped structure. In silico ligand docking indicates that DAG binds to one of the highly curved regions within this domain. A conserved aspartic acid residue in the PAT domain, E86, is predicted to interact with DAG, and we found that its substitution abrogates high affinity binding of DAG as well as DAG-stimulated association with liposome and artificial LDs. These results indicate that the PAT domain of PLINs harbor specific lipid-binding properties that are important for targeting these proteins to the surface of LDs and to ER membrane domains enriched in DAG to promote LD formation.

UNIVERSITY OF DEBRECEN



LifeScience Building,
Conference venue
EJTEMM 2024

Nagyerdei Restaurant
Lunch and dinner on 11 June

abberior INSTRUMENTS

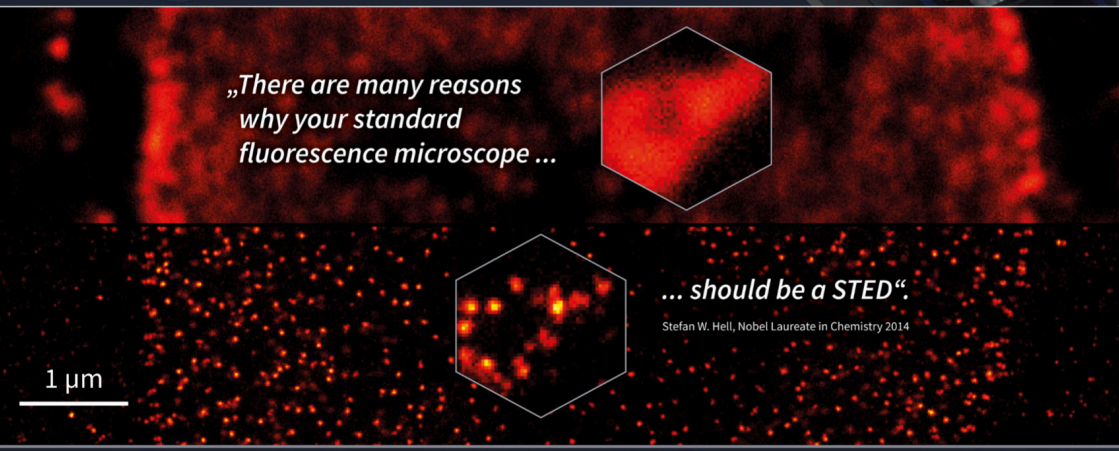
Superresolution STED
microscopy from its inventors

Facility Line

fastest time to result

Compact Line STEDYCON

expands any microscope to confocal & STED



UNICAM

1144 Budapest, Kőszeg utca 29.
Telefon: +36 1 221 5536
E-mail: unicam@unicam.hu
Web: www.unicam.hu