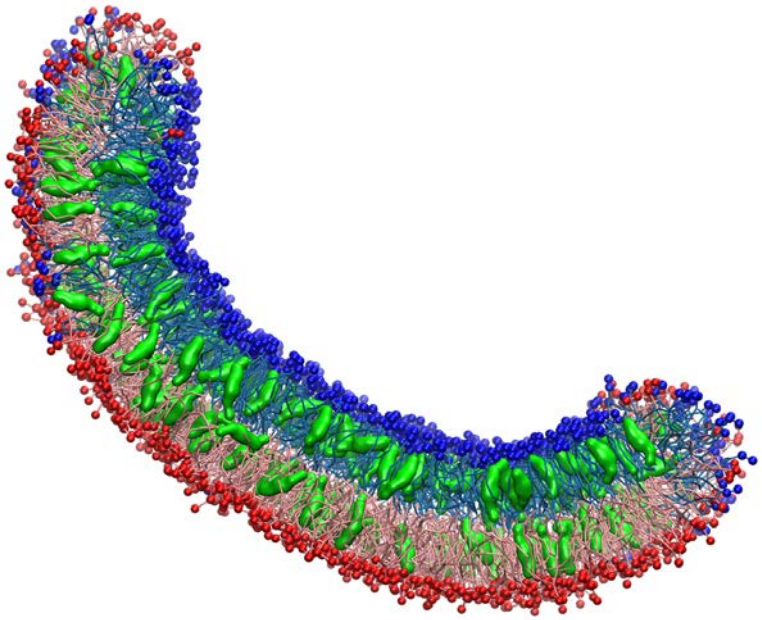
International workshop

Asymmetry of biological membranes: physical, biological and biomedical aspects



April 28-29 2016

Besançon, France

Université de Franche-Comté,
Laboratoire Chrono-Environnement CNRS - UMR 6249

Co-organizers:

- Department of Physics of Biological Systems, Institute of Physics of the National Academy of Sciences of Ukraine, Kyiv, Ukraine.
- Institute of Biomedical Engineering and Instrumentation,Wroclaw University of Technology, Wroclaw, Poland.

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Thursday, April 28

9:00-10:30	Arrival of participants
10:30-10:40	Welcome word from laboratory officials
10:40-11:00	*Christophe Ramseyer*, University of Franche-Comté, Besançon, France; *Semen Yesylevskyy*, Institute of Physics of NAS of Ukraine, Kiev, Ukraine **Introduction and goals of the meeting**
11:00-12:00	*Patrick Couvreur*, University of Paris-Sud, Paris, France **Nanotechnologies for the treatment of severe diseases**
12:00-13:00	Lunch
13:00-13:45	*Alexander Demchenko*, Institute of Biochemistry of NAS of Ukraine, Kiev, Ukraine **Fluorescence probing of biological membranes : new tools and ideas**
13:45-14:30	*Manuel Dauchez*, University of Champagne-Ardenne, Reims, France **To be announced**
14:30-15:00	*Semen Yesylevskyy*, Institute of Physics of NAS of Ukraine, Kiev, Ukraine **Simulations of curved and asymmetric membranes : where we are and what is ahead**
15:00-15:30	??
15:30-17:00	Round table discussion
17:00 ??	Welcome dinner

Friday, April 29

9:30-10:00	Reims group
10:00-10:30	Arvi Freiberg, University of Tartu, Estonia **Probing Membrane Interactions with Photosynthetic Chromoproteins**
10:30-11:00	Andrey Klymchenko, University of Strasbourg, Strasbourg, France **Fluorescent probes for lipid order and membrane asymmetry**
11:00-11:30	*Mikhail Bogdanov*, University of Texas-Houston, Houston, USA **Lipid and protein asymmetry in bacterial membranes : assessment, maintenance, dynamics and physiological significance**

11:30-12:00	*Sébastien Mongrand*, Université de Bordeaux, France **Plant plasma membrane lipids: role of sphingolipids in interdigitation of membrane leaflet and lipid asymmetry**
12:00-13:00	Lunch
13:00-13:30	*Sylvie Ricard-Blum*, Université Lyon, France **A 3D journey through the pericellular matrix and the glycocalyx**
13:30-14:00	Galina Dovbeshko, Institute of Physics of NAS of Ukraine, Kiev, Ukraine **Vibrational "signatures" of solid-supported asymmetric bilayers mimicking bacterial cellular membranes**
14:00-14:30	*Kirill Pyrshev*, Institute of Biochemistry of NAS of Ukraine, Kiev, Ukraine **The lipids distribution and their dynamics in plasma membranes of living and apoptotic cells**

Abstracts

Nanotechnologies for the treatment of severe diseases

Patrick COUVREUR, Member of the Académie des Sciences

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Even if new molecules are discovered to treat severe diseases, the clinical use and efficacy of conventional chemotherapeutics is hampered by the following limitations: (i) drug resistance at the tissue level due to physiological barriers (non cellular based mechanisms), (ii) drug resistance at the cellular level (cellular mechanisms), and (iii) non specific distribution, biotransformation and rapid clearance of the drugs in the body. It is therefore of importance to develop nanodevices able to overcome these limitations.

This will be illustrated by two nanomedicine platform developed in the laboratory: the design of biodegradable nanoparticles loaded with doxorubicin for the treatment of the resistant hepatocarcinoma (a nanomedicine currently in phase III clinical trials) (1) and the "squalenoylation" (2), a technology that takes advantage of squalene's dynamically folded conformation to link this natural and biocompatible lipid to anticancer (3), antimicrobial (4) or neuroprotective compounds (5) in order to achieve the spontaneous formation of nanoassemblies (100–300 nm) in water, without the aid of surfactants. The design of "multidrug" nanoparticles combining in the same nanodevice chemotherapy and imaging (ie., "nanotheranostics") or various drugs with complementary biological targets will be also discussed (6). Finally, it will be shown that the construction of nanodevices sensitive to endogenous (ie. pH, ionic strenght, enzymes etc.) or exogenous (ie., magnetic or electric field, light, ultrasounds etc.) stimuli may allow the spatio-temporal controlled delivery of drugs and overcome resistance to current treatments (7). The possibility to use other terpenes (natural or synthetic) than squalene to design nanoparticles for the treatment of resistant intracellular infections (8) or cancer will be discussed, too (9).

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Simulations of curved and asymmetric membranes: where we are and what is ahead

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Molecular dynamics (MD) simulations of the lipid membranes are now the method of choice for revealing precise atomic details of interactions in the membranes. They provide unique controllable environment for studying all components of the cell membranes with atomic resolution, which is not accessible in experimental techniques. MD is also an excellent complementary method for a wide range of experimental techniques which allows deciphering experimental results.

Although intrinsic asymmetry and curvature of the membranes is known to be critically important for a wide range of their functional properties, majority of MD simulations nowadays is still performed on flat model membranes with symmetric lipid distribution.

In this talk advances, challenges and perspectives of MD simulation of asymmetric and curved membranes are discussed. An emphasis is made on complexity of simulation setup and data analysis of asymmetric membranes simulations. Existing methods of analysis of the membrane curvature are discussed. Recent application to curvature-dependent cholesterol distribution in the membranes and the permeability of the membranes to anticancer drugs are shown.

Perspectives of simulations of other lipid supramolecular complexes, such as low density lipoprotein particles, are discussed.

Nanoparticles and nanocomposites for biomembrane studies

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In recent years a range of fluorescent reporters have been developed that scale from 5-10 nm (the size of average proteins to 100 nm and over (the size of viruses). Their advantages in optical studies are obvious: (1) They can be much brighter than organic fluorophores with the possibility of two-state ON-OFF switching in sensing. (2) Their excitation and emission spectra can be modulated in broad ranges, from near-UV to near-IR. (3) They can be up-converting, two-photonic, plasmonic, x-ray excited, etc. (4) Their surface can be engineered and functionalized up to the needs. In biomembrane studies they allow broad range of applications: (1) Visualizing cell receptors with their ligand-carrying forms. (2) Probing the membrane charge/potential by electrostatic binding. (3) Tracing their penetration into the cells and organelles on a small-ensemble and single-particle levels. A number of examples will be given to illustrate these new possibilities.

Fluorescent probes for lipid order and membrane asymmetry

Andrey S. Klymchenko

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Lipid organization in membranes of living cells controls a number of biological processes. In particular it concern formation of microdomains of ordered and disordered phases as well as asymmetric distribution of lipids in two leaflets of cell plasma membranes. In the recent years, we have developed a variety of small fluorescent molecules bearing specially designed anchor groups that partition into lipid membranes and monitor changes in their properties. Those probes are based on solvatochromic dyes, such as 3-hydroxyflavone, Nile Red and push-pull pyrene, which change their emission color in response to the local properties of membranes. They enable imaging lipid domains in model membranes and changes in the lipid order of cell plasma membranes on apoptosis, membrane blebbing, endocytosis, etc. Probes that stain specifically the outer leaflet with minimal flip-flop are particularly important to detect changes in the lipid asymmetry during apoptosis. Moreover, cell permeable probes can visualize differences in the lipid order between the plasma and intracellular membranes. The obtained toolkit of probes will help the advances in the biomembrane research.

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Lipid and protein asymmetry in bacterial membranes: assessment, maintenance, dynamics and physiological significance

Mikhail Bogdanov

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Whether bacterial membranes are asymmetric? How to assess a transmembrane (TM) phospholipid distribution in bacterial membranes? Can we create and maintain *in vivo* and in vitro a membrane with different and desired phospholipid asymmetry e.g. use a lipid engineering approach? If bacterial membranes are asymmetric whether phospholipid sidedness in bacteria is important?

In order to asses a bacterial phospholipid asymmetry *in vivo* and *in vitro* a novel radioactive and colorimetric non-radioactive phospholipid sidedness assays were developed and utilized to determine a of phosphatidylethanolamine (PE) or lysylphosphatidylglycerol (LPG) TM distribution across inner membrane of either naturally occurring or recombinant gram-negative *Escherichia coli* cells which imitate closely the cytoplasmic membrane of gram-positive *Staphylococcus aureus.* Since synthetic asymmetric liposomes are in a non-equilibrium thermodynamic state like real biological membranes the 'lifetime' of their asymmetry is quite limited. Thus a new "fliposome" technology was employed to determine a transient TM asymmetry of either net neutral PE or cationic LPG in proteoliposome system in which lipid composition can be systematically controlled before (liposomes) and after ("fliposomes") protein reconstitution.

According new paradigm a "flip-flopping" membrane proteins can flip back and forth in response to change of lipid composition, membrane depolarization events or even during function. The lipid-dependent bi-directional TM flipping is driven in the accordance with Charge Balance Rule as an extension of the Positive Inside Rule.

A novel assays and recombinant *Escherichia coli* model microorganisms allow to vary the efficient charge of either leaflet of cytoplasmic membrane and therefore can be used

- to monitor the effect of TM lipid asymmetry on orientation of either newly synthesized or reconstituted membrane protein
- to establish the mechanism which provides molecular basis for the membrane protein flipping dynamically driven by nascent protein-lipid interactions.
- to dissect the physical mechanisms that confer resistance of bacteria to cationic antibiotics.

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Plant plasma membrane lipids: role of sphingolipids in interdigitation of membrane leaflet and lipid asymmetry

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Unlike glycerophospholipids, sphingolipids and phytosterols in plant plasma membrane (PM) display striking differences by comparison with their animal's counterparts in term of chemistry and abundance. In this talk, I would like to reinvestigate the role for the major sphingolipid on earth, namely Glycosylinositol phosphoceramides, GIPC, in their roles in structuring membrane raft domains with sterols, in their asymmetrical distributions and structural diversity. GIPC are most likely only locate in the outer leaflet, they contain very long chain fatty acids up to 26 carbon atoms (often 2-hydroxylated) which can interdigitate the outer leaflet with the inner leaflet. GIPCs contain sugar with up to 14 residues, likely making a very bulky polar head. Broad methods are currently used to challenge PM structure from lipid purification, liposomes studies, environment-sensitive probes, MS-based lipidomics and solid-state NMR. The ins and outs of lipid asymmetry, raft formation, and interdigitation in plant membrane biology will be extensively discussed.

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A 3D journey through the pericellular matrix and the glycocalyx

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The extracellular matrix (ECM) is a scaffold providing the shape and mechanical properties of tissues. The pericellular matrix is the most dynamic part of the ECM, where the ECM meets the cells, interacts with receptors and controls cell behavior, cell-matrix interactions and ECM maturation, which are deregulated in pathological situations.

Many soluble molecules either released from the cells (e.g. cytokines, chimiokines) or from the ECM (matricryptins) travel within the pericellular space, where they participate in bidirectional signaling (from the ECM to the cell and from the cell to the ECM) and bidirectional trafficking (internalization of ECM proteins and release of intracellular proteins – moonlighting proteins - in the extracellular milieu).

The question arises of how these molecules travel within the pericellular matrix, which is a crowded environment, to reach their receptors. Indeed the pericellular matrix is comprised of, although not restricted to, glycocalyx made of an intricate, flexible, network of complex linear polysaccharides, called glycosaminoglycans (heparan and chondroitin sulfate, hyaluronan), proteoglycans and glycolipids. Are there pericellular highways with pericellular protein stores located on glycosaminoglycan chains? Are proteins crawling in the pericellular layer of glycosaminoglycans?

The cell membrane provides the floor of the pericellular matrix, whereas the supramolecular assemblies comprising the scaffold of the extracellular matrix provide the ceiling. Furthermore endostatin, an anti-angiogenic matricryptin released from collagen XVIII, binds to phosphatidylserine (Zhao et al., 2005 Biochemistry 44: 2857–2863) and associates with lipid rafts (Wickström et al., 2003 J Biol Chem 278:37895-901) and glycosaminoglycans influence lipid dynamics in supported phospholipid bilayers (Sahoo and Schwille, 2013, Soft Matter 9:3859). It is thus important to study the influence of membrane lipids on the organization of the glycocalyx and heparan sulfate proteoglycans (e.g. syndecans) and their dynamic interplay with glycosaminoglycans and pericellular proteins in order to better understand the molecular mechanisms regulating protein transport within the pericellular matrix

Probing Membrane Interactions with Photosynthetic Chromoproteins

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Integral membrane proteins are ubiquitous in many life-supporting functions, such as selective transmission of information and matter across the membrane, immune response, respiration, and photosynthesis. The biological machinery driving photosynthesis comprises an elaborate assemblage of membrane proteins that include various pigment chromophores as the main harvesters of sunlight. The purple non-sulfur bacteria are among the best-characterized photosynthetic species due to their relative simplicity, and genetic and physiological flexibility. Similarly, the light harvesting pigment–protein complexes from photosynthetic bacteria are among the most studied membrane chromoproteins. The relative spatial arrangement of the chromoprotein complexes in purple membranes has been previously determined by atomic force microscopy as well as by spectroscopic means. Herein, the spectroscopic and kinetic fingerprints will be introduced that distinguish excitons in individual detergent-solubilized chromoprotein complexes from them in the membrane-embedded complexes of purple photosynthetic bacteria. Significant changes in energetics and dynamics of the antenna excitons observed upon self-assembly of the proteins into intracytoplasmic membranes will be established and discussed [1-3].

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The lipids distribution and their dynamics in plasma membranes of living and apoptotic cells

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Local changes in the properties of plasma membrane lipid phases play an essential role in cell biology. Most of existing approaches to study them and, in particular, the presence and transformations of rafts in vitro are based on spectroscopic techniques. Being performed in non-physiological conditions, they provide only limited information on the plasma membrane surface without fine details. Novel fluorescence probing techniques should provide deeper insight into the memberane changes on apoptosis, which results in exposure of phosphatidylserine (PS) and influx of sphingomyelin from the outer to the inner leaflet. The latter undergoes hydrolysis to give ceramide, which produces dramatic effects not only on the membrane structure and properties, but on the whole signaling pathway (Tepper, Ruurs et al. 2000; Suzuki, Denning et al. 2013).

The aim of the current research was to study the properties and composition of the plasma membrane in connection with morphology and dynamics of intracellular membranous structures in native (non-fixed) conditions at 37ºC. The novel fluorescent probes NR12S, bNR10S, F2N12SM and PA (Kreder, Oncul et al. 2015; Kreder, Pyrshev et al. 2015; Niko, Didier et al. 2016) were used for lipid order detection. The results suggest the strong lateral heterogeneity of the outer plasma membrane leaflet organization and its significant difference in living and apoptotic cells. It was shown that, on the background of good correlation between caspase-3 activation, PS-exposure and general lipid redistribution, the PS becomes externalized on later steps of the changes in transmembrane lipid asymmetry. Further studies with recently developed FRET-based approaches (F2N12SM-NR12S; F2N12SM-bNR10S) allowed direct observation of separated lipid phases during all stages of programmed cell death. The application of PA allowed observation of subcellular membranous structures in parallel with plasma membrane. We show that decrease of the lipid order in plasma membrane is strongly coupled with its mixing with the lipids of less ordered interacellular membranes, which leads to formation of the large disordered phase' areas.

Vibrational “signatures” of solid-supported asymmetric bilayer mimicking bacterial cellular membranes

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Defining the roles individual lipids play in cell structure and function has lagged behind the progress made with other macromolecules due to the pleiotropic nature of lipid involvement in cellular processes and the diverse nature of their structures and properties. The lipid and protein components of the membrane are not held together by covalent interactions but are highly mobile individual molecules in dynamic equilibrium undergoing transient interactions organized into the three-dimensional supramolecular structure of biological membrane. Due to the variation in lipid hydrophobic acyl chains and hydrophilic polar headgroups, each of different lipid types is made up of a wide spectrum of structural variants, which as a whole determine membrane fluidity, lateral pressure, permeability and symmetric or asymmetric surface charge. Although much is known about physicochemical properties of individual lipid species a much less is known about behaviour of even simple mixtures of lipids in the test tube and it has been difficult to extrapolate this information to biological membranes containing proteins and complex lipid mixtures. Even less clear is an understanding of how these diverse physical and chemical properties translate into biological function.

Whether heterogeneous phospholipid head groups and/or aliphatic acyl chain are undergo a deformation and change conformational (orientational) order under supramolecular assembly? Whether conformational order of lipid head groups or acyl chains and their orientational parameters are interrelated and affected (e.g. conformationally restricted or not) by residing membrane proteins?

In order to answer these questions and investigate a vibrational signature of heterogeneous lipid mixtures qualitative and quantitative vibrational spectroscopies (FTIR, Raman SFG), as well as confocal microscopy and correlation spectroscopy) were utilized to establish indicative marker bands of either synthetic or natural phospholipid counterparts assembled into a supramolecular structure to mimic a real lipid compositions of intact bacterial cells with drastically different polar head group composition and net charge. According Charge Balance Rule a proper charge balance between interfacial topogenic signals of membrane protein required to achieve its proper transmembrane orientation (e.g. ability to flip or not to flip) is maintained by presence of lipids with net zero charge and appropriate charge density of the membrane which in turn determined by molar percent of anionic (polar) and net zero charged phospholipids (Bogdanov et al., *Biochim. Biophys. Acta*. 1843:1475 2014.

Since a SFG spectroscopy is intrinsically sensitive to the property of biological interfaces including interfacial regions of supramolecular lipid structures (Lis et al., *ChemPhysChem*, 2013, 14,1227) non-linear vibrational responses of both lipid acyl chains and head groups will be measured in future experiments with either “empty” solid-supported asymmetric bilayer mimicking bacterial cellular membranes or bilayers containing either non-flipping or flip-flopping membrane proteins.