The future of lipid oxidation

3RD STRASBOURG WORKSHOP ON MEMBRANE BIOPHYSICS

Institut Charles Sadron Strasbourg, France

TALKS

Probing photoinduced lipid hydroperoxidation in Langmuir monolayers

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Photodynamic therapy (PDT) efficiency depends on many factors including the incorporation of the photosensitizer (PS) in cell membranes and possible lipid hydroperoxidation. Herein, we show that hydroperoxidation may be photoinduced when eosin Y is incorporated into Langmuir monolayers that serve as cell membrane models. This occurs for Langmuir monolayers of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), which have unsaturation in their hydrophobic chains. In contrast, light irradiation had no effect on monolayers of saturated 1,2-dipalmitoyl-sn-glycero-3- phosphocholine (DPPC). Evidence of hydroperoxidation was obtained from the area increase in eosin-containing DOPC and POPC monolayers upon irradiation, which was accompanied by a decrease in monolayer thickness according to grazing incidence X-ray off-specular scattering (GIXOS) data. Furthermore, the changes in polarization-modulated infrared reflection absorption spectroscopy (PM-IRRAS) induced by irradiation were consistent with hydroperoxide migration toward the lipid hydrophilic heads. In summary, this combination of experimental methods allowed us to determine the effects of eosin Y interaction with cell membrane models under irradiation, which may be associated with the underlying mechanisms of eosin Y as photosensitizer in PDT.

Photochemistry of photoinduced membrane leakage

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Irreversible membrane damage, which lead to membrane leakage, is key to the efficiency of cell death caused either by photosensitizers (PS) used in Photodynamic Therapy (PDT) or by PS naturally present in the skin. Experiments based in absorption/emission and mass spectroscopic, as well as, theoretical simulations, showed that lipid aldehydes have a key role in the formation of transmembrane pores. Processes depending on direct contact between photosensitizers and lipids were revealed to be essential for the progress of peroxidation and for aldehyde formation, providing a molecular-level explanation of why membrane binding is so well correlated with the efficiency of photosensitizers. Consequences of this find will be showed and discussed in experimental models of PDT and of skin aging.

In situ characterization of lipid vesicle dynamics undergoing photooxidation

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Due to their macrocycle, tetrapyrrole molecules - porphyrins, chlorins, phthalocyanines ... - present very special photo-physical properties. Their light irradiation generates, through their triplet state, reactive species such as singlet oxygen or oxyradicals. These species cause, in the area both irradiated and marked by the tetrapyrrole (photosensitizer), molecular alterations. Specific targeting photosensitizers to one or the other cell compartments thus is the basis of their potential to modify and control the physiology of the cells. For example, the photo-chemical internalization (PCI) of macromolecules into cells is based on the photo-induced alteration of endosomal membranes - before their maturation in lysosomes - allowing the escape of the macromolecules, free to reach their targets within cell. More extensive photo-induced changes, in particular to the mitochondria, lead to cell death by necrosis or apoptosis. This photo-induced cell death is basis of an anticancer therapy so-called PDT.
To better apprehend such complex effects, we turned to model systems. In particular, we focussed on photo-oxidation of membranes lipids, that are important targets of the photodynamic effect, as well as for photodynamic therapy than for photochemical internalization. We extensively studied their modifications under photo-oxidation, involving major différentes modifications within the membranes which can be highly destabilized. Our purpose is to demonstrate that the photo-induced permeabilization of the membranes is correlated with a deep physical stress, which can be relaxed by various pathways, depending on its lipids composition, which is characteristic of the targeted cellular compartment.

Upconversion at lipid bilayers: sensitivity to dioxygen and ROS production

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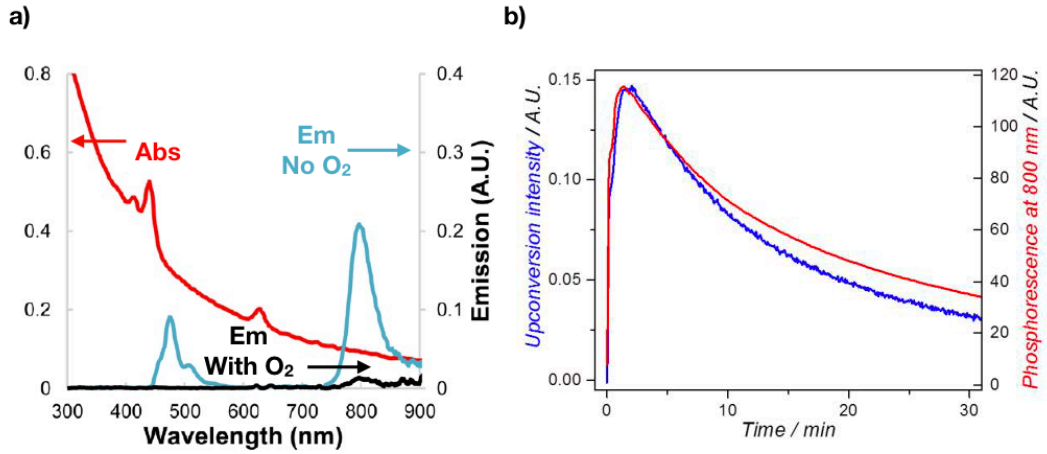
Photoactivated chemotherapy (PACT) and photodynamic therapy (PDT) consists in a range of techniques aimed at activating with light photosensitive medicinal compounds, either in oxygen-poor or oxygen-rich tumors, respectively. These techniques are regarded as a promising alternative to chemotherapy as it might limit side effects and increase treatment efficacy. However, many photosensitive compounds require UV or blue light to be activated, which penetrates sub-optimally in biological tissues. We have developed upconverting liposomes, polymersomes, and lipid-coated upconverting nanoparticles, that can produce blue light upon red or near infrared light irradiation, to activate blue-light sensitive ruthenium-based anticancer compounds. In upconverting vesicles, the triplet-based upconversion emission is quenched in presence of O_2 (Figure 1a). However, antioxidants simply added to the solution can stabilize for hours the upconversion intensity in air. By introducing antioxidants in the membrane composition of polymersomes, it is even possible to stabilize upconversion in air without a need for exogeneous antioxidants (Figure 1b). In lipid-coated upconverting nanoparticles, ruthenium PDT sensitizers added to the lipid formulation generate reactive oxygen species upon 980 nm light irradiation, but it is difficult to detect the primary nature of the light-generated ROS because we miss the appropriate probe. In this presentation I will compare the lipid-containing upconverting nanosystems developed in Leiden and their interactions with dioxygen and ROS.



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Electrostatic interactions in phospholipid membranes: influence of ions and hydroxyl radicals

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Electrostatic interactions in monolayers of acidicphospholipids are studied by thermodynamical and optical techniques inconjunction with model calculations. The focus is the LE/LC (liquid expanded/liquid condensed) phasetransition. The phase transition pressure depends on the composition of the aqueoussolution. For cardiolipins and monovalent cations (Na+, K+, Cs+) weak binding and anonmonotonic behavior of the phase transition surface pressure is found:Raising the monovalent salt concentration increases the transition surfacepressure by charging the TMCL monolayer until 0.1 mol/l, then screening effectsdominate and decrease the transition surface pressure by reducing electrostaticrepulsion between lipid head groups. Strong binding for divalent cations isfound, in the sequence Sr^{2+}< Mg^{2+} < Ca^{2+} ≈Zn^{2+} ≈ Mn^{2+}.
The reaction of DPPC monolayerswith hydroxyl radicals(HO˙fromFenton solutions) was investigated using infrared reflection absorption spectroscopy (IRRAS), grazing incidence X-ray diffraction, X-ray reflection and fluorescence microscopy. The DPPC monolayer is exposed to different HO˙concentrations.The decrease in the lateral pressure was used as a measure of the efficiency ofthe HO˙attack. With increasing HO˙concentration,the surface transition pressure decreased; eventually it disappeared. Fluorescence microscopy during the HO˙attack showed that new domains in the condensed phase nucleate immediately. In the LC phase a reduced tilt angle of the alkyl chains was found. IRRAS experiments indicated a partial cleavage of the head group leading to a reduced head group size, the reduced phase transition pressure is attributed to Fe^{2+} binding.

Molecular Simulations of Oxidised Phospholipids

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I will first provide a perspective of our prior work on oxidized lipids, which includes the first demonstration of reversal[1] and reorientation[2] of oxidized acyl chains in lipid bilayers, the pairing of cholesterol and fully oxidized lipid species in bilayers[3], and the unusual conformation of double-headed, 4-tailed Schiff base oxidized lipids[4]. In particular, I will focus on the protective property of cholesterol on oxidized membranes, and make the unusual claim that the protection arises not from the ordering effect of cholesterol, but from complementary shape factors of cholesterol and oxidized lipid species which drive them closer together, preventing membrane leakage by an excess of non-cylindrical surfactant-like polar lipids in the membrane. The simulations have consistently been complemented by biophysical experiments demonstrating a critical need for theoretical and experimental biophysicists to work together.

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Imaging lipid oxidation using molecular rotors

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Molecular rotors are small synthetic fluorophores, in which the speed of rotation about a sterically hindered bond is viscosity-dependent [1-2]. We have demonstrated that molecular rotors allow imaging of the oxidative stress in lipid membranes, live cells and in atmospheric aerosols, upon exposure to ozone, singlet molecular oxygen, or radicals. We find that in an environment where cross linking or oligomerisation are possible, viscosity increases significantly following oxidative stress, and can be quantitatively imaged using molecular rotors combined with either Fluorescence Lifetime Imaging (FLIM) or ratiometric detection. [3-8]

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Microdomain structure and mechanical properties of lipid monolayer mimicking red cell membranes: influence of oxidative stress

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Red blood cells (RBCs) have remarkable mechanical properties that are major determinants of blood flow. This is made possible by the unique structure of the erythrocytes. The bilayer lipid membrane accounts for the bending rigidity of RBCs whereas the membrane skeleton, built mainly of spectrin dimers in the form of a network sparsely anchored to the lipid bilayer, regulates the shear elasticity.[1] Deformation of RBC is a key property to ensure efficient passage through the microcirculation. A number of diseases such as diabetes may cause changes in the RBC's deformability and the physical properties of the erythrocyte plasma membrane due to oxidative and other chemical stresses.[2] To reduce the complexity of the system, model membranes and lipid monolayers have been used to determine membrane properties.[3]

Figure1. Langmuir monolayer compression isotherms, storage moduli from dilational rheology experiments and fluorescence micrographs at 25 mNm-1 for the RBC inner leaflet mixture with cholesterol (grey) or 7-ketocholesterol (black).We investigate the microdomain structure of synthetic lipid monolayers mimicking the composition of the inner or outer leaflet of the erythrocyte plasma membrane by means of the Langmuir trough technique coupled with fluorescence microscopy, gracing incidence X-ray diffraction (GIXD) and X-ray reflectivity (XRR) to study domain structures. Surface relaxation (dilational rheology) is used to quantify the dynamic interfacial rheological properties of the lipid film and Molecular Dynamics (MD) simulations reveal details in monolayer organization at molecular length scales. In particular, we investigate the effect of oxidation of key membrane constituents to clarify the role of oxidative stress on the plasma membrane organisation and physical properties.

We show that a replacement of cholesterol by 7-ketocholesterol as a main oxidation product leads to a significant stiffening of the monolayer at surface pressures above 20 mN/m (Figure1), in the range of the bilayer equivalence surface pressure ($\pi \approx 30$ mN/m). Fluorescence imaging was used to reveal crystallite-like microdomain structures of the monomolecular layers containing 7-ketocholesterol whereas the monolayer with non-oxidized cholesterol features more circular domain shapes. We also report differences in the monolayer organisation as detected by GIXD and XRR.

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Consequences of oxidation of plasma membrane lipids in cultured cells

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The lipid bilayer is a key site for oxidative damage; unsaturated lipids are particularly labile to oxidation. In vitro models have demonstrated that oxidation alters key properties of the lipid bilayer including permeability, morphology, and phase state. However, it remains unclear to what extent oxidation persists in the membranes of living cells, which incorporate mechanisms for preventing oxidative damage and regenerating plasma membrane lipids. Here, we expose cells in culture to a variety of oxidative environments, extract lipids from their plasma membranes, and use lipidomic techniques to quantify the extent of oxidation.

In our previous work, we have shown that at low degrees of oxidation (fewer that 3% of unsaturated lipids oxidized), bilayers demonstrate radically increased permeability to small molecules. This represents a potentially catestrophic compromising of the barrier properties of the plasma membrane. Oxidation is also capable of radically altering the morphology of lipid bilayers, altering the dimensions of lipids in a manner that leads to changes in membrane tension and subsequent pore formation. Similary, oxidation can lead to processes that inhibit the capacity of lipid bilayers to form high-curvature structures.

To better model modes of oxidative damage to the cellular plasma membrane, we have exposed cells in culture to both chemical oxidation and growth in a high oxygen partial pressure environment. Cell viability was analyzed and lipids were extracted from the cell to assess the mechanical properties and permeability of lipid bilayers constructed from them. Extracted lipids were also subject to lipidomic analysis to clarify the compositional changes that occur in plasma membranes in an oxidative environment.

Spatially Resolved Optical Experiments to Monitor the Singlet Oxygen Initiated Oxidation of Lipid Droplets in Oil-in-Water Emulsions

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We have established that singlet molecular oxygen, $O_2(a^1\Delta_g)$, can be selectively produced with high spatial localization in micro- and nano-heterogeneous samples using a variety of laser-based methods. For lipid droplets in oil-in-water emulsions, this means that we can produce $O_2(a^1\Delta_g)$ inside a droplet or in the aqueous medium right next to the oil-water interface. We then use fluorescent probes to monitor the resultant oxidation reactions in spatially resolved imaging experiments. By using a hydrophobic probe, we monitor oxidation inside the droplet. With an amphiphilic probe, we monitor oxidation at the oil-water interface. With this approach, we can monitor the spatial evolution of an oxidation reaction in/on a given droplet, and the evolution from one droplet to another droplet.

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C. Banerjee, M. Westberg, T. Breitenbach, M. Bregnhøj, and P. R. Ogilby, *Anal. Chem.*, **2017**, *89*, 6239-6247. Monitoring Interfacial Lipid Oxidation in Oil-in-Water Emulsions Using Spatially Resolved Optical Techniques.

Lipid oxidation in cellular delivery applications

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Delivery agents cross cellular membranes to penetrate cells. Membrane translocation may take place on the surface of the cell or, following endocytic uptake, within the endosomal pathway. Notably, lipid oxidation can modulate these various translocation processes. In particular, I will describe how membrane oxidation modulates the extent by which cell-penetrating peptides traverse the plasma membrane of live human cells. I will also describe how lipid oxidation can be exploited to mediate the selective permeation of endosomal membranes.

2D Time-resolved singlet oxygen luminescence scanning on microorganisms on surfaces

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The concept of monitoring the distribution of photosensitizers (PS) in biological systems is a well-known and common procedure but it is limited to obtaining information about its localization only. Even evaluating the PS fluorescence does not necessarily correlate one-to-one with singlet oxygen ($^1O_2$) generation efficiency.

It is well accepted that $^1O_2$ is the main mediator of the photodynamic effect.[1] For this reason, numerous efforts have been made to detect $^1O_2$ *in vitro* as well as *in vivo*. The detection methods of $^1O_2$ range from indirect methods using monitor molecules, other indirect methods that do not require additional drugs, to direct $^1O_2$ detection via its weak near-infrared (NIR) phosphorescence at around 1270 nm.[2-8] Beside using the photodynamic effect in photomedicine for the treatment of different diseases it is also important for the inactivation of microorganisms like bacteria, phototrophic organisms and fungi.

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Frontiers in Peroxidation of lipid membranes: Key molecular level aspects using computational chemistry

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Membrane oxidative stress is connected to the emergence of various diseases ranging from inflammation to cancer and ageing. While lipid oxidation is an active field of research, there is an ever-increasing need for understanding the microscopic details involved in its occurrence in cell membranes. Recently, we have been investigating membranes peroxidation in realistic environments using state of the art Quantum Mechanics/Molecular Mechanics modeling. In the spirit of this workshop, we will present our most recent efforts in understanding and characterizing several key steps of the phenomena in order to convey the exquisite predictive power or today's computational techniques and some aspects of the results that could greatly benefit from experimental validation.

Structural and dynamic changes in photo-oxidized membranes: insights from a coarse-grained model, and a kinetic puzzle

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A few years ago, the in-situ photo-oxidation of POPC and DOPC vesicles brought a proof of the stability of fully peroxidized lipid bilayers and provided an estimate of their relative increase in area per lipid [1,2]. These results were comforted by self-consistent mean-field Monte-Carlo simulations. A coarse-grained model was then introduced, which reproduces semi-quantitatively the experimental findings [3]. We will review the main predictions of this model in terms of membrane structure and mechanics.

When photo-oxidation is not restricted to peroxidation, irreversible damages to the bilayer occur. Different experiments point to a non trivial relationship between the rate of lipid alteration and the time of occurrence of the damages in DOPC bilayers, as inferred from the sudden increase in membrane porosity [4,5]. We argue that a lateral lipid separation mechanism could account for this non-trivial kinetics of the membrane degradation.

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How do the membranes sense the oxidation of cholesterol?

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Cholesterol is one of the vital components in regulating the physical properties of animal cell membranes. There are further numerous membrane proteins whose function is dependent on cholesterol. Given the importance of cholesterol in regulating cellular function, it is tempting to ask how its oxidation due to enzymes or oxygen radicals would be sensed in cells, and how these structural modifications would alter cholesterol function. In this talk, we consider these two topics on the basis of recent computer simulation studies. We discuss how oxysterols resulting from the oxidation of cholesterol affect membrane properties in a manner that is distinct from cholesterol, and how the oxidation of cholesterol alters the binding affinity for proteins that have allosteric cholesterol-specific binding sites.

Plasma-induced oxidation of the lipid bilayer:
Insights from molecular level modeling

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Near room temperature or cold atmospheric plasmas (CAPs) are gaining increasing interest for cancer treatment [1-3]. The underlying mechanisms, however, are still not fully understood, but they are important in terms of safe utilization. It is largely accepted that CAP-generated reactive oxygen and nitrogen species (RONS) play a key role [3-5]. Indeed, a noticeable rise of intracellular ROS has been observed in cancer cells compared to normal cells upon CAP treatment [6-9], which can eventually result in oxidative damage inside the cells. The CAP-generated species first interact with the cell membrane, thereby oxidizing its lipids, and it is important to study the behavior of the oxidized membrane and its effect on the penetration abilities of the RONS across the oxidized lipid bilayer.

This talk will give an overview of our recent simulation results on the interaction of ROS with the lipid bilayer which causes lipid oxidation, thereby leading to increased membrane fluidity. Moreover, insights on the permeation of various (hydrophilic and hydrophobic) RONS through both oxidized and non-oxidized membranes will be provided, and the synergistic effect of the electric field and lipid oxidation, both arising from CAP, on the permeability of cell membranes, will be explained, as well as the phosphatidylserine (PS) flip-flop induced by lipid oxidation, which plays a vital role in apoptosis signaling. Finally, the results on the different permeability of H_2O_2 across aquaporin transmembrane channel vs. the lipid bilayer will also be demonstrated.

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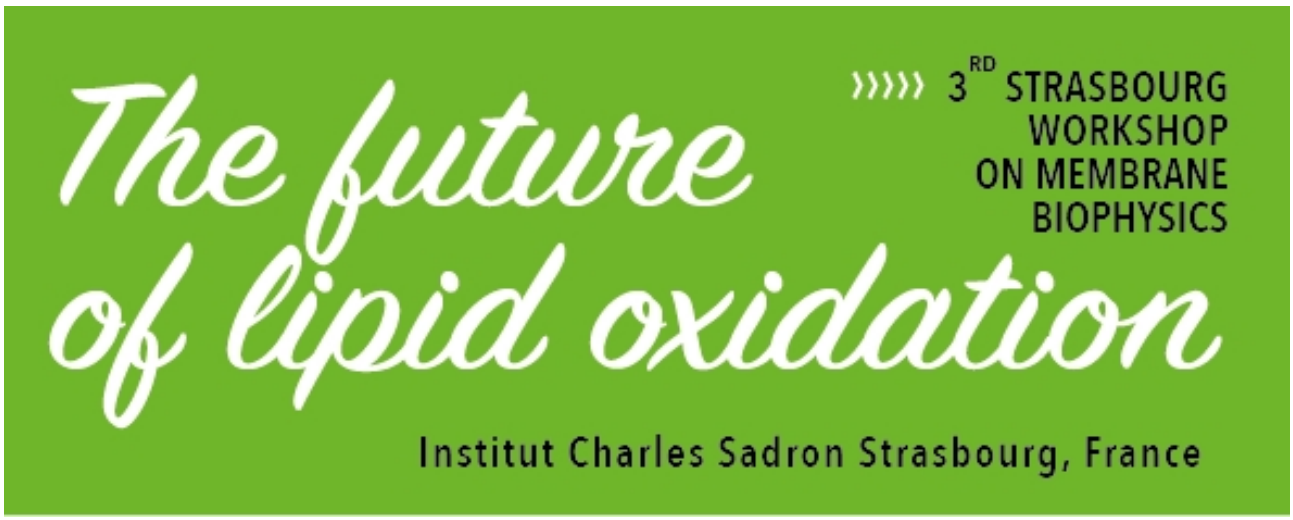
The future of lipid oxidation
3RD STRASBOURG WORKSHOP ON MEMBRANE BIOPHYSICS
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POSTERS

Permeation of RONS and glucose across native and oxidized membranes: answers from molecular dynamics simulations

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Cold atmospheric plasmas (CAPs) are being used in various medical applications, such as bacterial decontamination, wound healing [1, 2], blood coagulation [3], as well as cancer treatment [4-6]. CAP-generated reactive oxygen and nitrogen species (RONS) interact with and permeate through the cell membrane, inducing modifications in cellular components, thereby influencing signaling processes. In order to better understand the transfer of RONS through native and oxidized membranes (induced by CAP), we performed umbrella sampling (US) molecular dynamics [7].

The calculated free energy profiles show that hydrophobic species, like NO, NO_2, N_2O_4, O_2 and O_3, can significantly better penetrate across both native and oxidized membranes, compared to hydrophilic ROS, such as OH, HO_2 and H_2O_2, due to the considerably lower free energy barriers [7]. Oxidation of the membrane does not strongly affect the free energy barriers of NO, NO_2, N_2O_4, O_2 and O_3, whereas it reduces the barriers of OH, HO_2 and H_2O_2, thereby increasing their translocation probability across oxidized membranes [7].

Recent experiments have evidenced that CAP can also enhance the intracellular uptake of glucose molecules, important in diabetes therapy [8]. In this respect, it is essential to understand the underlying mechanisms of intracellular glucose uptake induced by CAP, which is still unclear. Hence, we elucidated the possible mechanism of glucose uptake by cells, performing again US simulations. Specifically, we studied the transport of glucose through native and oxidized membranes. Our simulation results show that the free energy barrier for the permeation of glucose molecules across the membrane decreases upon increasing degree of oxidized lipids in the membrane [9].

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A Versatile Molecular Toolbox for Membrane Investigations

A poster by Line BOUREL, Maria Vittoria SPANEDDA and Benoît FRISCH
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As chemists working at the interface of molecular biology and biophysics, we have gained a strong experience in the design, the synthesis and the characterization of a large set of original molecular tools. Over the years, unique lipid tools based either on fluorescence, photochemistry or nanophysics experiments, were specially developed to image, target and/or characterize *in vitro* or reconstituted cellular events. Examples of molecular contributions to membrane investigations - especially in the field of fusion peptide identification, viral entry simulation, molecular motors study and intracellular trafficking modeling - will be given.

Effect of reactive oxygen and nitrogen species on gold supported POPC and POPC:Coenzyme Q10 lipid bilayers

A poster by Mehdi Ravandeh/1, Heike Kahlert/1, Johanna Striesow/2, Jan-Wilm Lackmann/2, Kristian Wende/2
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Effect of reactive oxygen and nitrogen species on lipids have been implicated in several human diseases and exposures such as cancer, diabetes, and neurodegenerative disorders. Efforts have been devoted to understanding the mechanism of lipid peroxidation and preventing the deleterious effects of this process1. The current study reports antioxidant protection of lipid bilayer by coenzyme Q10 against reactive species generated by the kINPen cold atmospheric plasma jet2. Q10 is mostly known for its role in electron and proton transfer in aerobic cellular respiration, and its function as a powerful antioxidant3. POPC and POPC:Q10 liposomes were formed by sonication technique and then the structure and morphology of small unilamellar vesicles (SUV) were characterized with cryogenic transmission electron microscopy (Cryo-TEM). Generalized polarizations for POPC and POPC:Q10 were calculated based on Laurdan assay experiments which shows that the addition of Q10 to a lipid film leads to a more fluid bilayer. In the next step, a gold supported lipid bilayer was prepared by the potential assisted vesicle fusion method4. After formation of the lipid bilayer, a decrease in the oxidation and reduction peak currents of redox probe $[Fe(CN)6]^{4-}$ were observed. Electrochemistry and high-resolution mass spectroscopy were used to monitor effect of ROS and RNS generated by kINPen on lipid bilayer. According to the electrochemical results, the peak current of redox system increased during plasma treatments which indicates the degradation and hole formation in lipid bilayer. However, incorporation of Q10 into lipid bilayer was accompanied by a dramatic decrease in the electrochemical peak current. The results confirm the antioxidant activity of Q10 in protecting lipid bilayer against reactive species. The result of MS and tandem MS/MS spectroscopy in positive and negative modes showed that there were less modifications on head group (phosphocholine) but several modifications and fragmentations on alkyl chains during plasma treatments. For instance, a peak at 649.5 m/z indicates formation of Poxno (16:0-09:0) PC lipid after peroxidation of POPC lipid bilayer by reactive oxygen species such as singlet oxygen.

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Red Cell Mechanical Properties and Biochemical Signalling in Health and Disease

A poster by Beth McGill
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Red blood cells (RBCs) are highly specialised for transporting oxygen around the body. Their unique composite membrane (lipid bilayer and membrane skeleton) provides the elasticity required to maintain shape and withstand deformation by external forces throughout the microcirculation. A number of diseases, such as diabetes mellitus, may cause an alteration of the RBC deformability and membrane physical properties due to oxidative and other chemical stresses.

Here, we investigate the role of the RBC as an effective regulator of vascular tone, by its ability to release the vasodilator ATP as a result of mechanical deformation. We correlate the amount of released ATP with relevant mechanical parameters of the membrane, i.e. membrane bending and shear moduli. Further, we investigate how this control mechanism is compromised in disease, mimicking elevated oxidative stress with chemical treatments that selectively alter specific membrane components. RBCs from blood samples of healthy volunteers were analysed by thermal fluctuation spectroscopy to quantify the bending and shear moduli of individual cells. Whole blood preparations were then treated with an in vitro shearing device (producing physiologically-relevant shear stress) and subjected to testing via a chemiluminescence assay to determine the total ATP release induced by the shear deformation.

The chemical treatments increase the bending and/or shear moduli, stiffening the membrane, which in turn causes a reduction in the amount of released ATP after shearing. The results suggest that in disease, cells could show a reduced ability to release ATP and hence reduced control of blood flow. This could be a contributing factor to complications observed in a number of conditions characterised with increased levels of oxidative stress.

The impact of lectin valence and structure on the organization of the plasma membrane

A poster by Taras Sych1, 2, Thomas Schubert1, Ramin Omidvar1, Ludovic Richert2, Yves Mély2, Josef Madl1 and Winfried Römer 1
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The plasma membrane represents an outstanding example of self-organization in biology. Lipids and proteins assemble this semi-permeable barrier between the cytosol and extracellular fluid. The variety of membrane components is impressive, and their distribution in the lipid bilayer is not homogeneous. Highly dynamic, ordered plasma membrane nanodomains, so-called lipid rafts, are involved in a plethora of cellular processes, such as cell adhesion, signaling and endocytosis. The spatial organization of the plasma membrane is being adjusted constantly in order to maintain cell integrity, especially during the exchange of material with the extracellular space. For instance, the lipid environment responds to the interaction of host cell glycosphingolipids (GSL) with various carbohydrate binding proteins (lectins). The membrane reorganization induced by specific GSL-lectin interactions can be thoroughly studied by the reconstitution in synthetic membrane systems. In such systems, the microscopic “raft-like” (i.e. liquid-ordered – Lo) phase domains can be observed and characterized using conventional fluorescence microscopy techniques. In addition, rebuilding allows isolating single membrane components in order to elucidate their unique functions. We use Supported Lipid Bilayers (SLBs) that contain the GSL globotriaosylceramide (Gb3) to mimic the plasma membrane. Gb3 is known as host cell receptor for the lectins Shiga toxin (Stx) from S. dysenteriae and LecA from P. aeruginosa. Both lectins bind to the Gb3 carbohydrate moiety and cluster on the lipid bilayer surface. However, the subsequent behavior of the lectins differs. The homopentameric B-subunit of Shiga toxin (StxB) with in total 15 binding pockets binds to preformed Lo domains and also induces the formation of novel ones. On the contrary, the homotetrameric LecA with 4 binding sites decreases the size of preformed Lo domains until they completely disappear. Furthermore, huge membrane defects emerge and, as a result, the lipid bilayer is destroyed. Sharing the same receptor, both lectins induce different (maybe even opposite) events. This can be explained by variations in the number of binding pockets, their positions and orientations.

Red wine tannins protect membrane lipids from oxidation: a proton NMR study

A poster by Julie Géan
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Polyphenols supplied by foods have been reported to possess antioxidant properties that could have positive effects on human health by protecting cell membranes from lipid oxidation damages. In this study, we propose a new in situ and non-invasive method based on proton liquid-state nuclear magnetic resonance (NMR) to determine the antioxidant efficiency of red wine tannins on a twice-unsaturated phospholipid, 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLiPC), embedded in isotropic bicelles used as membrane model. Four tannins were studied: (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG). The kinetics of lipid degradation induced by a radical initiator, 2,2'-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) was determined by measuring the loss of the bis-allylic methylene moiety, an indicator of lipid oxidation. The antioxidant efficiency, i.e. the ability of tannins to slow down the lipid oxidation rate, was shown to be higher for galloylated tannins, ECG and EGCG. Furthermore, the mixture of four tannins was more efficient than the most effective tannin, EGCG, demonstrating a synergistic effect. To better understand the antioxidant action mechanism of polyphenols on lipid membranes, the tannin location was investigated by NMR and molecular dynamics. The location of tannins at the membrane interface (inserted at the glycerol backbone level) certainly plays a key role in their antioxidant action and their protective effect on membranes.

Probing phase separation on DOPC/DPPC/cholesterol ternary systems as outcome of hydroperoxidation

A poster by Maria Julia Bistaffa, Pedro Aoki
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Challenged by the recent progress on unraveling ternary phase diagrams of lipid mixtures, we have probed here the membrane phase behavior of DOPC/DPPC/cholesterol ternary system under oxidative stress induced by the photo-activated erythrosin. Excited states of erythrosin can transfer energy to the surrounding oxygen (O_2) towards single oxygen ($^1O_2$) generation, a very reactive specie capable of hydroperoxidize both DOPC and cholesterol. Indeed, membrane fluctuations followed by a surface area increase was observed as result of hydroperoxidation, independently of the evaluated composition. Besides, the liquid-ordered (Lo) and liquid-disordered (Ld) coexistence emerged from lipid compositions originally in a uniform liquid phase, suggesting a displacement at the boundary lines of Lo-Ld phases. This phase behavior was dictated by the hydroperoxidized species of DOPC rather than cholesterol, as revealed by the DOPC-OOH(2)/DPPC/cholesterol ternary diagram built. In summary, this combination of experimental methods allowed us to determine the effects of the photo-induced hydroperoxidation of ternary lipid mixtures, which may be associated with the underlying mechanisms of biological signaling.

Oxidation-responsive polymersomes based on amphiphilic diblock polypeptoids

A poster by Yangwei Deng/1, 2, Hui Chen/1, Xinfeng Tao/1, 2, Fangyi Cao/2, Sylvain Trepout/3, Jun Ling/2 and Min-Hui Li/1, 2

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Stimuli-responsive polymersomes formed in water by amphiphilic block copolymers have attracted much attention as nano- or micro-containers for drug delivery and nano/micro-reactors. We developed amphiphilic diblock copolypeptoids, poly(*N*-3-(methylthio)propyl glycine)-*block*-polysarcosine (PMeSPG-*b*-PSar) that self-assemble into unilamellar vesicles. These vesicles can be destabilized under the action of reactive oxygen species (ROS) either triggered by hydrogen peroxide or by light in the presence of a photosensitizer, with spatiotemporal control. We selected polysarcosine (also called poly *N*-methyl glycine) as the hydrophilic block, due to its resistance to protein adsorption and low toxicity, similar to poly(ethylene glycol) (PEG). As hydrophobic block, we synthesized a new polypeptoid, poly(*N*-3-(methylthio)propyl glycine), because of its hydrophobicity, its structure with the same backbone as PSar, and most importantly its *N*-substituted thioether side-chains that can be oxidized and transformed from hydrophobic moieties to hydrophilic sulfoxide ones. This new oxidation-responsive polymersome based on polypeptoids may find applications as nano-/micro-containers in drug delivery, biosensing, biodetection and nano-/micro-reactors.

Voltage-dependent formation of stable, ion conductive pores in suspended lipid bilayers from oxidized lipids.

A poster by André P. Schroder, Ekaterina Zaitseva, Carlos M. Marques and Jan C. Behrends

Oxidation of membrane lipids is a ubiquitous phenomenon in eukaryotic cells occurring as a consequence of the generation of reactive oxygen species by mitochondrial or peroxisomal activity and irradiation with photons or ions. Lipid hydroperoxidation, the primary step of the oxidative reactions, has been shown to induce molecular area increase, enhanced fluctuations and tube formation in giant unilamellar vesicles. Surprisingly, however, despite a 75% loss of elastic modulus, even fully hydroperoxidized membranes can remain effective barriers against the small molecule diffusion1, contrary to membranes including other downstream oxidation products2,3,4. We were therefore interested to ascertain whether membrane permeability to ions of pure lipid bilayers is affected by lipid hydroperoxidation. Using a chip-based device for automated bilayer formation and parallel recording from suspended synthetic lipid bilayers (Orbit-16, Nanion), we compared responses to positive and negative voltages in the range of 50 to 200 mV of POPC- and 100% hydroperoxidized POPC (POPC-OOH)1 membranes formed on 50 µm-diam. microelectrode cavities of the MECA16 chip (Ionera). While control POPC membranes showed a stable conductance in the range of a few pS without evidence for pore-formation, POPC-OOH membranes responded with fluctuating conductance increases of conductance already at 50 mV. This conductance increased in a voltage and time-dependent manner, reaching values of several nS at +/-150 mV. Remarkably, the voltage induced conductance fluctuations were stable over time and did not recover during a 1 s waiting period between pulses. Conductance fluctuations were characterized by multiple preferred conductance levels separated by several tens of pS without evident equidistant periodicity. Taken together, these findings suggest that in response to voltage, hydroperoxidized POPC membranes form stable voltage-dependent, ion-permeant pores with conductances similar to protein ion channels.

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Photo-Induced Lipid Oxidation Studies by High-Resolution NMR Spectroscopy

A poster by Yu. E. Moskalenko, M. S. Baptista, C. M. Marques

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Lipid oxidation plays a central role in the life of the eukaryotic cells [1]. The products of lipid oxidation, if uncontrolled, can have a deleterious effect on the functioning of the cell, and are known to be involved in a variety of diseases. Photosensitization reactions producing excited triplet oxygen species cause the chemical transformation of biological tissues and have been practically employed in photo-dynamic therapy, PDT. This work aims at understanding chemical and structural changes in phos-pholipids caused by photo-induced oxidation and seen in 1H, 13C, 2H and 31P NMR spectra.
We study mono-unsaturated phospholipids in isotropic solutions, liposome suspensions and lamellar phases being subjected to Methylene Blue (MB) photosensitization under red light illumination (630 nm). Under irradiation MB can interact with the molecular O in the solution and generate singlet oxygen 1O2 species with high quantum yield. The reactive 1O2 species can further react with unsaturated bonds of phospholipids leading to a number of products [2,3,4]. As we show in this work, the type of oxidation products and their relative amount is regulated by the medium and by lipid organisation.

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Massive release of extracellular vesicles: a major side effect of photodynamic therapy

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Photodynamic therapy (PDT) is an emerging cancer treatment that is particularly adapted for localized malignant tumor. Photo-activation of the photosensitive drug induces a massive production of reactive oxygen species, which are cytotoxic. In the clinical workflow, the phototherapeutic agent is generally injected in the bloodstream and circulates in the whole organism as a chemotherapeutic agent. Once activated through light triggering, localized therapeutic effects are induced. In addition to cytotoxic effect, we found that photodynamic therapy activated a massive production and emission of extracellular vesicles (EVs) from cancer cells. Only 1 hour after the photo-activation of meso-tetrahydroxyphenylchlorin (m-THPC or Foscan ®) photosensitizer, thousands of vesicles per cell were emitted in vitro, in the extracellular medium. Furthermore, we found that the released EVs could transfer extracellular membrane components, drugs and even intracellular objects to naive target cells. In vivo, photodynamic treatment increased the levels of circulating EVs in the bloodstream several fold, confirming the vast induction of cancer cell vesiculation triggered by this cancer therapy. EV is the main messenger for cell- cell communication and exchange between intracellular and extracellular compartments. In the context of cancer, EVs are known to be involved in the onset of metastasis, in particular by shaping pro-metastatic niches. Therefore, this massive release of cancer extracellular vesicles could have a dramatic impact of PDT-treated patient's clinical outcome.