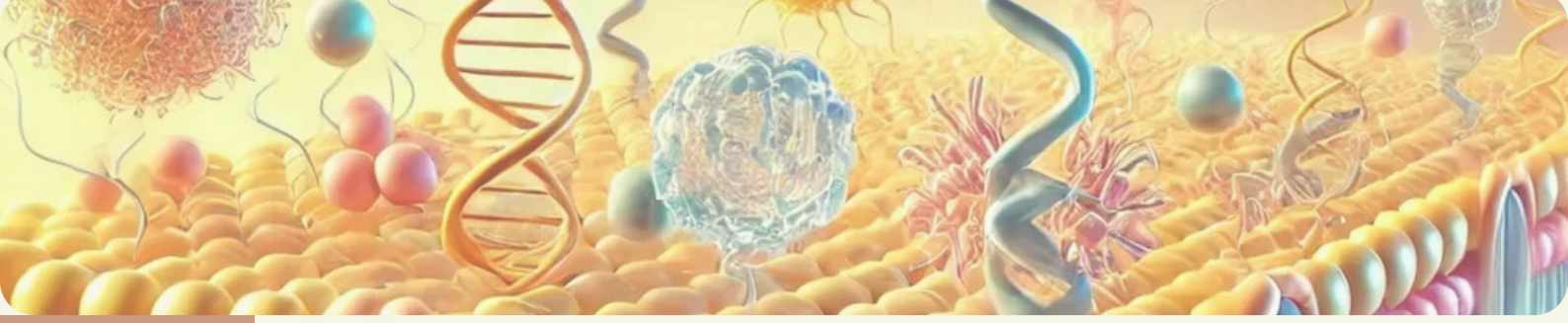

ABSTRACT BOOKLET

FEBS Advanced Course 2025 Biological Surfaces and Interfaces: Biointerfaces at lipids, proteins and polymers

IIS UTOKYO SYMPOSIUM NO 129

8TH-13TH OF JUNE 2025 -HOTEL EDEN ROC, SANT FELIU DE GUIXOLS, CATALONIA, SPAIN

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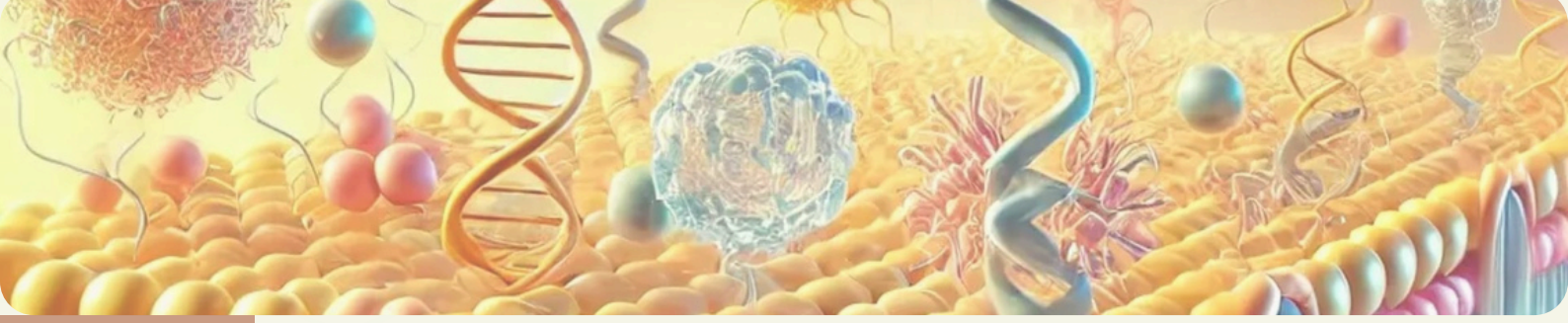

FEBS Advanced Course 2025 Biological Surfaces and Interfaces: Biointerfaces at lipids, proteins and polymers

INVITED SPEAKERS

Tackling the urgent need for a broad spectrum antiviral drug, from lipid interfaces to in vivo activity.

M. Castanho[I]

[I]GIMM, Gulbenkian Institute for Molecular Medicine, Lisbon, Portugal

We are developing peptide-drug conjugates able to inactivate Zika virus, Dengue virus, HIV, and SARSCoV-2, among others, while having the ability to interact with the brain endothelial interfaces and traverse the blood-brain and blood-placenta barriers (BBB and BPB, respectively). Zika is of particular interest because it combines high pandemic potential with severe neurological impairment in newborns when the infection takes place in pregnant women. So far, there is no effective therapy for infection with this virus due to the limited ability of current antiviral drugs to engage interfacial interactions at the brain endothelium with crossing of the BBB and/or the BPB.
Chemically, the drugs underdevelopment consist on the conjugation of an antiviral porphyrin to a trans-BBB carrier peptide. Proprietary trans-BBB peptides were obtained from templates based on domains of the capsid protein of Dengue virus.
The activity, toxicology and brain-targeting efficacy of a panel of conjugates were evaluated both in vitro and in vivo. One of the conjugates is able to perform transcytosis across both the BPB and the BBB, has shown to be effective against Zika Virus (IC50 1.08 μM) and has high serum stability (t1/2 ca. 22 h) without altering cell viability at all tested concentrations. In vivo tests in animals confirm brain penetration and therapeutic action concomitant with reduction of the viral load in the brain.

Related references:
1. Bioconjugate Chem. 2021, 32, 6, 1067– 077; https://doi.org/10.1021/acs.bioconjchem.1c00123
2. Pharmaceutics 2022, 14(4), 738; https://doi.org/10.3390/pharmaceutics14040738
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cell-surface mimics to study virus interactions in the glycocalyx

M. Bally[I]

[I]Umea University, Umea, Sweden

The cell surface is a complex molecular environment consisting of an intricate meshwork of carbohydrates - the glycocalyx - covering the plasma membrane. The plasma membrane itself is a highly dynamic structure made of a lipid bilayer containing a broad variety of (glycol)lipids and (glyco)proteins. Systematic investigations of biological processes occurring at the cell surface require bioanalytical platforms capable of recapitulating in vitro and under well-controlled experimental conditions, the cell surface composition, architecture and physico-chemical properties.
In my presentation, I will highlight the potential of cell-surface mimics of varying compositional complexity, in the context of studying the initial recruitment of a virus to the cell surface. We use single molecule force spectroscopy to characterize individual bonds formed between viral ligand and cellular receptors. Further, we rely on microscopy-based single particle tracking to quantify the interaction kinetics of the whole virus particle with the cell-surface mimic. This allows us to study how biomolecules act in concert to optimize the virus behavior at the cell surface and to determine how virus attachment, detachment and diffusion is modulated in the glycocalyx.
Here, I will focus on unraveling the molecular mechanisms modulating the dynamics of virus particles within the glycocalyx, with special attention to those viruses interacting with the polysaccharide heparan sulfate, a major component of the glycocalyx. Investigated pathogens include filoviruses (Ebola and Marburg), herpes simplex virus, human papilloma virus and SARS-CoV-2. I will further demonstrate how our biophysical experiments uniquely complement cell-based investigations of the infection process.
Taken together, our research contributes to a better understanding of the mechanisms regulating the interaction between a virus and the host surface. Such insights promise to facilitate the design of more efficient antiviral therapies.

Boosting antimicrobial effects of photocatalytic TiO2 nanoparticles by peptide coating

M. Malmsten[I]

[I]University of Copenhagen, Copenhagen, Denmark

Photocatalytic nanoparticles (NPs) offer potent antimicrobial effects under illumination due to the formation of reactive oxygen species (ROS), capable of degrading bacterial membranes. Such nanoparticles may, however, also degrade membranes of human cells and trigger toxicity. Increasing the selectivity between bacterial and human cells is therefore key for their application as therapeutics. Since antimicrobial peptides (AMPs) display excellent selectivity between human cells and bacteria, these may offer opportunities to "target" coated nanoparticles to bacterial membranes. Exploring this, we investigated the interaction of AMP-coated TiO2 NPs with bacteria- and mammalian-like membranes, as well as with bacterial lipopolysaccharides. ROS generation was essentially unaffected by AMP coating, and peptide degradation sufficiently limited to allow peptide-mediated targeting. Consequently, peptide coating promoted membrane binding to bacteria-mimicking membranes and bacterial lipopolysaccharides. Furthermore, membrane degradation during illumination was strongly promoted for bacteria-like membranes, but not for mammalian-like ones. Analogously, AMP coating promoted antimicrobial effects of TiO2 NPs for both Gram-negative and Gram-positive bacteria. In contrast, toxicity against human monocytes remained low. These finding demonstrate that AMP coating of photocatalytic nanoparticles selectively boosts antimicrobial effects at low cell toxicity. In addition, AMP-coated NPs cause co-aggregation and photocatalytic degradation of bacterial lipopolysaccharides, resulting in suppressed and localized inflammation. The dependence of these effects on AMP properties was outlined.

Fluorination effect in self-assembly of amphiphiles and their interaction with biological matter

F. Baldelli Bombelli[I]

[I]Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering G.Natta, Milan, Italy

The functionalization of amphiphiles and polymers with fluorinated moieties is not only a promising strategy for the development of multiscale imaging systems, but also for improving their colloidal stability in the biological environment. Fluorinated compounds are known for their high hydrophobicity and lipophobicity, a phenomenon known as the "fluorous effect", i.e., attitude of fluorinated moieties to preferentially give fluorine-fluorine interactions and segregate. This effect guarantees high colloidal stability of self-assemblies formed by partially fluorinated amphiphiles, mostly in biological environments [1]. Fluorination is also recognized as valuable strategy to modulate the bio-nano interactions of macromolecules and nano-scaled assemblies and promote cytosolic delivery. The exceptional cellular uptake and nucleic acid endosomal escape capabilities of fluorinated delivery vehicles compared to their non-fluorinated counterparts are attributed to the limited interaction of fluorinated vehicles with plasma proteins. The lipophobic nature of fluorinated groups hinders fusion with lipid membranes creating segregated fluorinated domains and destabilizing them. Here we report the development and biological response of amphiphilic and dendrimer nanomaterials functionalized with short-branched fluorinated tags bearing from 9 to 27 fluorine atoms as possible genetic therapeutics [2,3].

References

[1] B.L. Bona, et al. Design of fluorinated stealth poly(ε-caprolactone) nanocarriers. Colloids and Surfaces B 2024, 234, 113730

[2] Deposited Italian Patent application, Non-viral vectors for gene delivery number: 102022000004496

[3] Q.Laurent, et al. Self-Assembly and Biological Properties of Highly Fluorinated Oligonucleotide Amphiphiles. Angew. Chem. Int. Ed. 2024, 64, e202419996.

The shape of the plasma membrane regulates the nanoscale organization, function and pharmacology of transmembrane proteins

D. Stamou[I]

[I]University of Copenhagen, Copenhagen, Denmark

Traditionally, the spatial compartmentalisation of membrane proteins is thought in terms of 2D segregation in plasma membrane (PM) domains enriched in specific lipids. However, the direct observation of such putative domains has been challenging, thus their mechanism of formation remains elusive. Here, 3D imaging of the PM conclusively established the existence of PM domains and revealed a novel general mechanism of 2D spatial organization that is based on energetic coupling of membrane proteins to membrane curvature. Experiments with different families of membrane proteins (including G protein coupled receptors, EGFR, PIEZO1 and H-Ras) revealed this novel mechanism to be general but also protein sequence and, crucially, protein-conformation specific. Thus curvature allosterically modulates also the structure/function and pharmacology of membrane proteins.

Structure and processes at the lipid-aqueous interface - methods and applications

T. Nylander[I]

[I]Division of Physical Chemistry, Chemistry Department, Lund University, Lund, Sweden

The nature of the lipid/water-interphase is key not only in a physiological context, but has implications for applications such as drug delivery and more technical applications such as detergency. The structure of the interface largely depends on the polarity of the lipids involved, but also their molecular shape and packing that both control the self-assembly structures formed. Of particular interest is to understand the processes that can occur during lipolysis that transfer edible oils into polar lipids. Small angle x-ray scattering (SAXS) and cryogenic transmission electron microscopy (cryo-TEM) allowed us to determine the phase behaviour and structure of the formed dispersions. Neutron and x-ray data as well as spectroscopic methods and optical reflectometry techniques, like ellipsometry can give us insight on the nature of the oil/water-interphase and how the processes that can occur during lipolysis can change the structure and composition in the interfacial layer. The structural changes are often controlled by the pH. We will show that a combination of bulk studies using scattering techniques and studies of oil film using surface sensitive techniques will give insights on the structure of the oil-water that provide the basis for a range of applications.

Understanding synergistic antimicrobial peptide combinations though computational approaches

C. Lorenz[I]

[I]King's College London, London, United Kingdom

Synergy between antimicrobial peptides (AMPs) may be the key to their evolutionary success and could be exploited to develop more potent antibacterial agents. One of the factors thought to be essential for AMP potency is their conformational flexibility, but characterising the diverse conformational states of AMPs experimentally remains challenging. We have used molecular scale simulations to investigate the interactions between different synergistic pairs of AMPs used by Winter Flounder fish to combat bacterial infections. In doing so, we aim to elucidate the molecular scale mechanisms which result in certain pairs being more effective than the sum of their parts.

Antimicrobial peptide double cooperative effect towards the development of new antimicrobial agents

K. Sugihara[I]

[I]The University of Tokyo, Tokyo, Japan

Synergy among antimicrobial peptides (AMPs), in which mixing different types of AMPs boosts their antimicrobial efficiency, has garnered attention as a possible approach to improve their potency. Recently, we have reported a unique cooperative function between two well-known antimicrobial peptides (LL-37/HNP1) that kills bacteria more efficiently while minimizing the host damage by suppressing mammalian cell membrane lysis ("double" cooperativity). In this work, we will present our recent efforts in my group to understand its mechanism by exploiting synthetic biophysical assays based on supported, pore-spanning bilayers and vesicles, such as fluorescence recovery after photobleaching (FRAP), electrochemical impedance spectroscopy (EIS), single-channel conductance measurement, atomic force microscopy (AFM), fluorescence resonance energy transfer (FRET), leakage assay, circular dichroism, tryptophan fluorescence assay etc.1-4 Such a double cooperativity may be used in our immune system and may help with developing efficient and safe antimicrobial agents in the future.

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Molecules at the Interface: Modeling Confinement Effects on DNA–Ion Interactions

S. Perepelytsya[I], T. Vasiliu[II], A. Laaksonen[III], L.D.V. Engelbrecht[IV], G. Brancato[V], **F. Mocci**[VI]

[I]Bogolyubov Institute for Theoretical Physics of the National Academy of Sciences of Ukraine, Ukraine, Kiev, Ukraine, [II]Center of Advanced Research in Bionanoconjugates and Biopolymers, "Petru Poni" Institute of Macromolecular Chemistry, Romania, Iasi, Romania, [III]Department of Materials and Environmental Chemistry, Division of Physical Chemistry, Arrhenius Laboratory, Stockholm University, Sweden, Stockholm, Sweden, [IV]Department of Engineering Sciences and Mathematics, Division of Energy Science, Luleå University of Technology, Sweden, Luleå, Sweden, [V]Scuola Normale Superiore and CSGI, Pisa, Italy, Pisa, Italy, [VI]Dipartimento di Scienze Chimiche e Geologiche, Università degli Studi di Cagliari, 09042 Monserrato (CA), Cagliari, Italy

The behavior of biomolecules is strongly influenced by their interactions with surrounding ions, especially in confined environments such as cellular compartments or nanodevices. At the same time, the behavior of ions is also affected by the biomolecules' fine structure when they are at the interface between the molecular surface and the solvent. Understanding these interactions is crucial, as they impact biomolecules' stability, structure, dynamics, and ultimately function.
In this presentation, after a brief introduction to molecular dynamics methods and DNA structure, I will present our work on how spatial confinement and ionic conditions influence DNA-ion interactions. Our research shows that confinement leads to significant changes in ion distribution around DNA, impacting its structural properties and interactions with other molecules. For instance, our simulations show how sequence specificity of ion binding to DNA depends on fine structural details, and is highly affected by the constraints imposed on the structure.
Conversely, the structural and dynamic behavior of natural organic counterions is influenced by the biomolecule's surface, and I will discuss how the structure of a natural DNA counterion can differ in bulk and at the molecular interface.
I will discuss the extension of our DNA-polyamine interaction studies to synthetic systems, focusing on branched polyethyleneimine (b-PEI)–PEG–squalene vectors. Using a two-step MD protocol supported by experimental data, we demonstrate how PEG chain length governs DNA compaction and shielding.
These case studies show how electrostatics, flexibility, hydrogen bonding, and confinement control the binding modes and molecular recognition at the DNA interface. These features are fundamental to understanding DNA condensation, chromatin biology, and in designing non-viral gene delivery systems.
References: 10.1093/nar/gkz434, 10.1039/D1BM00973G, 10.3389/fchem.2022.836994, 10.1016/j.molliq.2023.122828, 10.1063/10.0024969

Can Transition Metal Cations be Screened away from Biomembrane Interfaces?

P. Cremer[I]

[I]Department of Chemistry, Penn State University, State College, United States of America

It has been known for over one hundred years that negatively charged surfaces in aqueous electrolyte solutions concentrate Group I and Group II metal ions into an electrical double layer directly adjacent to the solid/liquid interface. The increased local counterion density leads to the binding of divalent metal cations with anionic membrane lipids at significantly lower ion concentrations than analogous processes taking place in the bulk solution. Moreover, the enhanced binding of divalent cations can typically be screened away by introducing NaCl to the solution. Curiously, these screening effects appear to be far weaker when transition metal counterions, like Cu^{2+} or Ni^{2+}, are used instead. In this presentation it will be shown that transition metal counterions are far less susceptible to electrostatic screening and interfacial potential changes compared to Group I and Group II metal ions. This is because they are typically chelated in the bulk aqueous solution in coordination complexes with a wide variety of net charges. These results have important implications for the trafficking of transition metal ions in vivo.

Instructive soft biomaterials for tissue response by design: steering events at the biointerface

K. Maniura[I]

[I]Empa, Swiss Federal Laboratories for Materials Science and Technology, St. Gallen, Switzerland

Dynamic structural changes in cellular microenvironments are critical factors for development, tissue homeostasis, and disease progression. These changes are enabled by the mechanical properties of the extracellular matrix (ECM) and its susceptibility to cleavage by proteolytic enzymes such as matrix metalloproteinases (MMPs). Instructive hydrogels allow living cells to interact with an ECM-mimicking environment, which has demonstrated the great potential of hydrogels in tissue engineering and regenerative medicine.
Medical challenges in numerous areas can be tackled through the smart design and engineering of hydrogel systems: from implant or deep wound related bacterial infections, tissue regeneration to advanced microphysiological *in vitro* models.
Different hydrogel-based strategies will be discussed.

Soft Materials to Understand Cell-Material Interactions

J. Malmström[I]

[I]The University of Auckland, Auckland, New Zealand

Mechanotransduction plays a crucial role in cell function, differentiation and cancer. The ECM mechanical rigidity and distribution of ligands are sensed and modulated through the contractile and adhesive molecular machinery in the cells. In our research group we are developing materials to achieve spatiotemporal control over mechanical properties. For example, we have developed a projection method to pattern the elastic modulus of GelMA to create models of cardiac fibrosis. We have also combined the GelMA with biocompatible supramolecular fibers made of a small self-assembling sugar-derived molecule (N-heptyl-D-galactonamide, GalC7). The GalC7 fibers were directly grown in the GelMA through a thermal process, and it was shown that the presence of the fibrous network increased the Young's modulus of GelMA. Due to the non-covalent interactions that govern the self-assembly, these fibers were observed to dissolve over time, leading to a dynamic softening of the composite gels. Cardiac fibroblast cells were successfully encapsulated into composite gels for 7 days, showing excellent biocompatibility and fibroblasts extending in an elongated morphology, most likely in the channels left by the fibers after their degradation. These novel composite hydrogels present unique properties and could be used as tools to study biological processes such as fibrosis, vascularization and invasion.
We have also developed a range of conductive hydrogels, that provide tools for both electrical and mechanical stimulation of cells. These conducting hydrogels can be oxidised and reduced, leading to large actuation and a small, but significant, change in the Young's modulus. Such conductive hydrogels also have the potential to be used to encapsulate and release drugs. Our data demonstrates excellent control over the release of a small model drug, upon reduction of the conducting hydrogel. In addition, larger protein drugs can be loaded into the gel for passive release.

Functional surfaces by combining soft polymer membranes with biomolecules and catalytic nanocapsules

C.G. Palivan[I]

[I]University of Basel, Chemistry Department, Basel, Switzerland

Smart surfaces based on flexible membranes combined with biomolecules or active assemblies are in the limelight because they offer the potential to develop solutions for the detection of environment changes or advancement of catalytic reactions on surfaces with time and space precision at the nanoscale. Here we present two approaches to generate functional surfaces: i) one based on insertion of enzymes or catalytic nanocapsules inside soft planar membranes of copolymers and ii) one resulting from the mixture of different types of amphiphilic copolymers inducing the formation of nanotexture for controlled attachment of active molecules.
In the first approach, we introduce hybrid membranes based on the insertion of active compounds (enzymes or functional capsules of self-assembled resocinarene monomers) into synthetic flexible membranes on solid support. We used a solvent-assisted approach for co-deposition of the amphiphilic copolymers and resocinarene monomers to generate functional surfaces. By changing the conditions, we combined soft membranes with enzymes to benefit from the intrinsic activity of the biomolecules to generate active surface. Such hybrid membranes have the ability of specific small molecule uptake and storage while, when decorated with enzymes, they serve for biosensing or degradation of harmful compounds.
In the second approach, we advanced the generation of nano-textured surfaces in a controlled manner, by developing fully synthetic soft planar membranes from binary mixtures of amphiphilic block copolymers. Due to different properties of the selected copolymers, these multicomponent planar membranes undergo separation into domains. The fine tuning of the domains served for specific combination with antimicrobial peptides resulting in efficient surfaces to fight bacteria growth.

From solid supported- to pore-spanning membranes – what do we gain?

C. Steinem[I]

[I]Institute of Organic and Biomolecular Chemistry, University of Göttingen, Tammannstr. 2, Göttingen, Germany

Advances in surface-attached lipid membranes have revolutionized our understanding of membrane-confined processes in a controlled environment. However, traditional supported lipid bilayers come with certain limitations. Their attachment to the surface restricts lateral mobility, a second aqueous compartment is missing, and transmembrane protein reconstitution remains challenging. To overcome these challenges, we and others have developed alternatives comprising tethered lipid bilayers and so-called pore-spanning membranes (PSMs) over the past two decades. In this talk, I will introduce the concept of pore-spanning membranes in more general terms. Specifically, I will focus on PSMs derived from spreading giant unilamellar vesicles on porous substrates with micrometer-sized pores to form model membrane systems or by spreading giant plasma membrane vesicles derived from living cells. I will discuss their unique characteristics including the dynamic mobility of lipids and lipid domains, the establishment of asymmetry in PSMs, and their remarkable mechanical and sealing properties. These properties are essential for investigating complex biological processes, such as membrane fusion, in a highly controlled and physiologically relevant manner.

Immobilisation of enzymes for biocatalysis and biofuel cells

E. Magner[I]

[I]University of Limerick, Limerick, Ireland

The immobilisation of enzymes is used extensively to confer additional stability on enzymes, with additional advantages such as ease of separation, the ability to incorprate the system into flow systems and the potential for reuse. The use of immobilised enzymes has a wide range of applications in biocatalysis, biosensors and biofuel cells. This presentation will describe recent work on the immobilsation of enzymes for use in biocatalysis and in biofuel cells.
The ability to target and directly immobilise enzymes is more complex, the majority of uses have focussed on indirect methods of immobilisation. The targetted immobilisation of enzymes will be described using electrodeposition methods to spatially pattern the adsorption of subsituted alkane thiols that act as attachment points for individual enzymes. Subsequent patterning of additional enzymes can then be performed in the presence of the already attached enzyme. Using this approach, the enzymes alcohol dehydrogenase, formaldehdye dehydrogenase and formaldehyde dehydrogenase have been immobilised with good retention of catalytc activity. Using a similar approach, a flow reactor has been assembled that incorporates a three-enzyme cascade system comprising a hydrogen peroxide generator (glucose oxidase), a hydrogen peroxide dependent enzyme (chloroperoxidase) and a hydrogen peroxide scavenger (catalase) to enable controlled delivery of peroxide. Reactors that generated yields of 90% for the oxidation of indole to 2-oxyindole and of 93 and 91% for the chlorination of thymol and 91% carvacrol, respectively, were successively developed.
The development of biofuel cells will describe the development of highly porous gold electrodes for the immobilisation of enzymes. The development of biofuel cells on contact lenses and on implantable electrodes will be described.

Growth factor affinity binding alginate sulfate substrates for biomedical applications

R. Mhanna[I]

[I]American University of Beirut, beirut, Lebanon

It has been shown that the modification of glycosaminoglycans (GAGs) sulfation codes affect developmental processes and numerous diseases in the brain and other tissues. The effects of GAGs on physiological responses may be linked to their differential binding to growth factors, but the exact mechanisms that regulate their action have not been elucidated. In our lab, we chemically modified alginate with different sulfation densities and then biotinylated alginate sulfates to create hydrogels and nanofilms presenting various sulfation patterns. We also used alginate sulfates in combination with polycaprolactone to create double emulsion nanoparticles with affinity to growth factors. The build-up of the sulfated films and subsequent growth factor binding were assessed using quartz crystal microbalance with dissipation monitoring (QCM-D) and validated via enzyme-linked immunosorbent assay (ELISA) and immunostaining. The morphology, growth and gene expression of adipose derived stem cells, cancer and normal cell lines was assessed. The effects of the sulfated substrates were also studied on epidermal, neural, breast and lung cancer cell lines in 2D and 3D. Alginate sulfation caused drastic changes in cell behavior, where increased sulfation inhibited cell spreading, promoted longer filopodia and maintained stemness of adipose-derived stem cells. Epidermal and neural cell lines showed increased proliferation on highly sulfated substrates. On the other hand, treatment of lung and breast cancer cell lines with alginate sulfates added in solution inhibited cell growth while maintaining relevant differentiation markers for normal healthy cells. Finally, CTGF-loaded alginate sulfate nanoparticles accelerated healing in in vitro and in vivo models. The ability to prepare sulfated substrates and drug delivery systems with controlled sulfation levels has strong implications in the biomedical field.

Tissue as a surface - using soap bubble physics to understand the mechanics of epithelia

A. Janshoff[I]

[I]Georg-August-University of Goettingen, Göttingen, Germany

MDCK cells form multicellular cysts like acini or spheroids, comprised of a closed polarized cell monolayer enclosing a liquid. Tissue tension of these cysts is typically determined using Laplace's law by measuring internal pressure. We conducted force-relaxation experiments on MDCK II cysts and developed a theoretical framework to explain their response to external deformation, considering the potential super-viscoelasticity of the spheroids and volume leakage. Our findings showed that cells reduce tension upon deformation by thinning the cell monolayer, thus providing excess tissue area. This mechanism, which also protects individual cells from lysis, serves as a universal strategy on larger scales to withstand external stress. Using planar monolayers, we also found that the pico-basal polarity play a pivotal role in mechanical resilience and shape restoration.

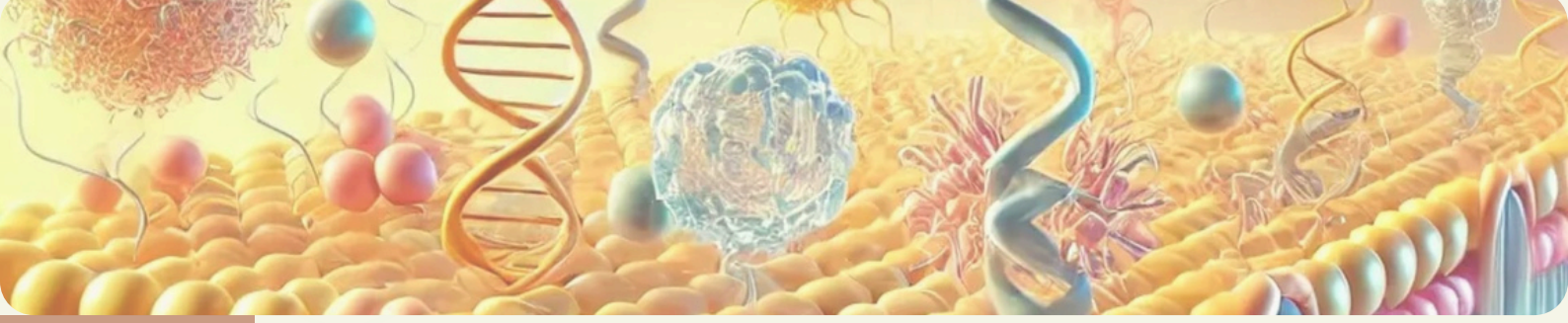
IMPACT ON NANOPARTICLES BIO-BEHAVIOR: PROTEIN CORONA FORMATION

B. Jachimska[I]

[I]Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences, Niezapominajek 8, 30-239, Krakow, Poland

The application of nanoparticles in biology and medicine requires understanding at the molecular level how nanoparticles interact with cells in a physiological environment. Extracellular serum proteins in the blood will adsorb onto the nanoparticle's surface, forming a “protein corona”. In a real system, it is not the nanoparticle itself but rather the nanoparticle-protein system that represents the true identity of the carrier and generates the therapeutic response. Therefore, understanding the mechanisms of nanoparticle-protein complex formation is crucial for predicting and managing the in vivo nanoparticle pathway, including its biodistribution, bioavailability, and toxicity. For this purpose, an effective method was developed to monitor the properties of the protein corona formed on the surface of PAMAM dendrimers. The binding of proteins to dendrimers can alter the dendrimer's structure, mobility, conformation, and functional activity. The compensation of the charge of functional groups in the dendrimer molecule due to the presence of the protein corona affects the effective charge of the complex. The results indicate that electrostatic and hydrophobic forces govern the interactions between proteins and PAMAM dendrimer carriers. The protein corona formed on the carrier surface is very stable, as evidenced by the QCM-D and SPR measurements. On the other hand, the CD spectra indicate a change in the protein's secondary structure. Molecular dynamics simulations reveal that the heterogeneous charge distribution on protein surfaces has a significant influence on their behavior within dendrimer systems. Protein corona formation on the carrier surface is advantageous for the potential use of drug delivery systems, as it inhibits the adsorption of plasma proteins to the dendrimer surface, reduces dendrimer toxicity, and prevents rapid clearance from the bloodstream, thereby increasing circulation time.

Acknowledgments: Supported by Grant NCN OPUS 2021/41/B/ST5/02233



ORAL PRESENTATIONS

FEBS Advanced Course 2025 Biological Surfaces and Interfaces: Biointerfaces at lipids, proteins and polymers

Sulfation patterns of heparan sulfate influence the physicochemical characteristics of papillomavirus interactions for entry

F. Bano *[I,II], L. Soria Martinez *[III,IV], D. van Bodegraven[III,IV], D. Conca[I,II], Y. Abidine[I,II], K. Throsteinsson[I,II], A. Brown[V], I. Fels[III,IV], N. Snyder[VI], M. Schelhaas[III,IV], M. Bally[I,II]

[I]Department of Clinical Microbiology, Umea University, Umea, Sweden, [II]Wallenberg Centre for Molecular Medicine, Umea, Sweden, [III]Institute of Cellular Virology, ZMBE, University of Münster, Münster, Germany, [IV]Research Group "ViroCarb: glycans controlling non-enveloped virus infections" (FOR2327), Coordinating University of Tübingen, Tübingen, Germany, [V]Department of Chemistry, Davidson College, Durham, NC, United States of America, [VI]Department of Chemistry, Davidson College, Davidson, NC, United States of America

Human papillomaviruses (HPVs) are small, non-enveloped DNA viruses, with several types linked to cervical cancer. During the initial stage of infection, HPV16—the most prevalent cancer-causing type—interacts with glycosaminoglycans (highly negatively charged polymers) on the cell surface, particularly heparan sulfate (HS). This interaction is crucial not only for viral attachment to host cells but also for triggering a structural activation in the viral capsid, a key step for entry. However, the specific roles of HS sulfation groups in engaging with the virus capsid and thus enabling the viral entry remain unknown.
Here, we present a comprehensive analysis of HS-HPV16 binding interactions using biochemical and biophysical assays. Our cell-based binding and infection assays reveal that N-sulfation is crucial, but alone insufficient, for binding and structural activation of HPV16 and is likely aided by 6O-sulfation, whereas 2O-sulfation is dispensable. Our biophysical toolbox including atomic force microscopy-based single-molecule force spectroscopy, single-particle tracking by total internal reflection fluorescence microscopy, and surface-bound HS molecules as cell-surface mimics to measure the attachment/detachment of individual virion to HS confirms the crucial role of N sulfation in ensuring stable binding. Moreover, it reveals that the interaction is mechanically strengthened by 6O-sulfation. Finally, our single particle tracking data reveal that HPV16 virions diffuse slowly on HS surfaces with minimal contribution of sulfation types. Altogether, our study highlights the direct involvement of HS sulfation patterns in modulating HPV16 binding and structural activation and reveals how the distinct sulfation groups of HS contribute to viral attachment and entry.

* The authors marked with an asterisk equally contributed to the work.

Variant-specific Interactions at the Plasma Membrane: Heparan Sulfate's Impact on SARS-CoV-2 Binding Kinetics

D.V. Conca[I,II,III], F. Bano[I,II,III], M. Graul[I,II,III], J. von Wirén[I,II,III], L. Scherrer[I,II,III], H. Pace[I,II,III], H. Sharma[II,III,IV,V], M. Bally[I,II,III]

[I]Dept. Clinical Microbiology, Umeå Univerisity, Umea, Sweden, [II]Wallenberg Centre for Molecular Medicine (WCMM), Umeå University, Umea, Sweden, [III]Umeå Centre for Microbial Research (UCMR), Umeå University, Umea, Sweden, [IV]Department of Medical Biochemistry and Biophysics, Umeå University, Umea, Sweden, [V]Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå University, Umea, Sweden

The rapid spread of SARS-CoV-2 has led to the emergence of multiple variants, with mutations primarily concentrated in the spike glycoprotein, responsible for viral attachment and entry. However, how these mutations affect the interaction with the complex host membrane environment remains underexplored. In our study, we employed biomimetic membrane models, including supported lipid bilayers (SLBs) and native cell-derived membranes, to investigate the early-stage binding mechanisms between SARS-CoV-2 virions and the host cell surface.

Unlike conventional ligand-receptor binding studies that isolate individual molecular interactions, we focused on the multivalent nature of viral attachment at the single-virion level. Using biochemical and biophysical techniques, such as single-particle tracking via TIRF microscopy, we characterized the binding kinetics of SARS-CoV-2 variants to the cell membrane, emphasizing the role of membrane-bound coreceptors. We observed a progressive increase in binding affinity among newer variants. By systematically reducing membrane complexity, we identified heparan sulfate (HS) as a key factor in this enhanced interaction. HS role shifted from a weak binder that reduced attachment to the primary receptor in early variants to a high-affinity coreceptor for Omicron BA.1, which exhibits a tenfold higher affinity than the original strain. HS is thus crucial in modulating SARS-CoV-2 adhesion, evolution and possibly tropism.

These findings underscore the critical role of membrane-mimics in studying viral entry mechanisms. SLBs provide a tunable platform to dissect individual, yet multivalent, molecular contributions, while native SLBs retain essential cellular complexity, bridging the gap between reductionist models and physiological conditions. This integrative approach illustrates how biointerface-based assays unravel the synergistic effects of lipids, proteins, and polysaccharides and provide mechanistic insight into viral adhesion and entry.

Dynamic In Situ Synthesis of Molecularly Imprinted Polymeric Nanoparticles via a High-Performance Micro-Reactor Chip

F. Inci[I]

[I]bilkent university, Ankara, Türkiye

The synthesis of molecularly imprinted polymers (MIPs) is often hindered by significant challenges, including protracted processing durations, limited productivity, dependence on expensive and intricate equipment, and constraints in achieving real-time in situ synthesis control. In this study, we unveil a cutting-edge micro-reactor that facilitates the continuous, in situ production of trillions of MIP nanoparticles, encoding with the molecular fingerprints of target proteins, within an impressively short timespan of 5–30 minutes. To optimize the platform's performance, COMSOL Multiphysics simulations were initially conducted to evaluate and refine mixing efficiencies across varied flow rates. Experimental validation demonstrated the successful synthesis of nanoparticles with precisely controlled sizes ranging from 52 to 106 nm. Furthermore, the molecular interactions between monomers and the target protein were elucidated through molecular docking and dynamics simulations. Principal component analysis (PCA) was employed to optimize critical micro-reactor parameters, such as nanoparticle dispersity and polymer concentration. The sensing performance of the synthesized MIPs was rigorously tested using a metamaterial sensor, revealing remarkable precision (81%), exceptional selectivity (a 4.5-fold improvement), and reusability over three successive cycles. Notably, the micro-reactor exhibited unparalleled efficiency, achieving assay speeds 48–288 times faster than conventional methods, while simultaneously halving reagent consumption and producing 1.4–1.5 times more MIPs per synthesis step. This transformative technology enables scalable, rapid (as short as 5 minutes), and cost-effective (approximately $10 per micro-reactor) production of molecularly imprinted nanoparticles, paving the way for advanced sensing applications with unprecedented precision and throughput.

Lipid Nanoparticles and Transethosomes for surface eye pathologies

L. Talarico *[I], I. Clemente *[I], A. Gennari[I], G. Gabbricci[I], S. Pepi[I], G. Leone[I], C. Bonechi[I], C. Rossi[I], S.L. Mattioli[II], N. Detta[II], A. Magnani[I]

[I]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Siena, Italy, [II]Dompè SpA, L'Aquila, Italy

Infectious diseases play a major role among the causes of blindness worldwide. Due to its anatomy and the direct exposure to the environment, the human eye is particularly vulnerable to fungal and parasitic infections, caused e.g. by trauma from penetrating objects, carriage of microorganisms from adjacent structures, use of contact lenses. Negligence in timely treatment often results in vision impairment. Fungal keratitis is a major cause of blindness in corneal diseases and its topical treatment requires several eye-drops applications and may require additional injections or surgery. Natamycin, a tetraene polyene which acts by binding to the main component of fungal walls ergosterol, thus blocking fungal growth, is one of the proposed antifungal drug for topical treatments. However, its low retention at the ocular surface and scarce penetration across inner ocular tissues pose significant challenges. In this work, various lipid nanoparticles (LNP) systems loaded with Natamycin are physicochemically characterized and compared in terms of dimensions and stability studied by Dynamic Light Scattering, morphology investigated by Small Angle X-ray Scattering and Nuclear Magnetic Resonance, and encapsulation efficiency assessed with HPLC-DAD. This work is funded by the European Union—NextGenerationEU within the Italian Piano Nazionale di Ripresa e Resilienza, Missione 4 Componente 2, PNRR M4C2—Investimento 1.5. Creazione e rafforzamento di “ecosistemi dell’innovazione”, costruzione di “leader territoriali R&S”—Progetto THE (Tuscan Health Ecosystem)—Spoke 4—Nanotechnologies for diagnosis and therapy, project code ECS00000017, CUP B63C22000680 007.

* The authors marked with an asterisk equally contributed to the work.

Advanced Dendrimer-Based Nanocarriers for Enhanced and Selective Delivery of 5-fluorouracil or Doxorubicin

M. Goncerz[I], U. Szwedowicz[II], N. Rembiałkowska[II], J. Kulbacka[II], B. Jachimska[III]

[I]Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences, Krakow, Poland, [II]Department of Molecular and Cellular Biology, Wroclaw Medical University, Wroclaw, Poland, [III]Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Science, Cracow, Poland

Nanoparticle-based drug delivery systems offer a promising alternative to conventional cancer treatments. Poly(amidoamine) (PAMAM) dendrimers are characterized by monodispersity and high stability in aqueous solution. Their multifunctional structure allows the binding of active molecules in two ways: by encapsulation in internal spaces and immobilization on the surface. The physicochemical characterization of PAMAM dendrimers in their application as nanocarriers for 5-fluorouracil and doxorubicin is presented.

Analytical and theoretical methods were used to evaluate dendrimer-drug complexes, assessing size, surface charge, accumulation efficiency, and drug localization. PAMAM dendrimers accumulated with the drug's deprotonation while controlling carrier ionization. Process optimization revealed that alkaline conditions favor efficient binding, achieving loading capacities of 18.0% for G4.0 PAMAM-5FU and 39.2% for G4.0 PAMAM-DOX. Due to their chemical properties, the drugs exhibited different affinities and localizations: doxorubicin interacts via hydrophobic and electrostatic forces, while 5-fluorouracil interacts electrostatically with amine groups in the dendrimer structure. The way the drugs are immobilized in the PAMAM structure directly impacts the drug release mechanism from the nanocarriers. Additionally, complementary QCM-D and MP-SPR techniques confirmed the successful functionalization of the gold surface with PAMAM dendrimers. The resulting dendrimer monolayer has been used as a biosensor to detect and monitor the kinetics of interactions with doxorubicin.

In vitro studies demonstrated that the G4.0 PAMAM-5FU complex reduced cell viability, with increased selectivity for glioma (SNB-19), while the G4.0 PAMAM-DOX complex specifically targeted malignant melanoma (A375) and lung cancer (NCI-H23). The findings have confirmed that optimizing dendrimer-based drug delivery systems enhances efficacy and selectivity against cancer cells.

Development of Extracellular Vesicle-Imprinted Biosensors for Elucidating Tumor Microenvironment and Cancer Diagnosis

E.G. Yilmaz[I], F. Inci[I], **Y. Saylan**[II]

[I]Bilkent University, UNAM (National Nanotechnology Research Center), Ankara, Türkiye, [II]Hacettepe University, Ankara, Türkiye

Early detection of breast cancer is paramount for improving treatment options, survival rates, and overall patient outcomes. However, conventional three-dimensional in vitro models face significant limitations in accurately evaluating the role of biophysical stimuli within the tumor microenvironment. This study aims to explore the critical role of extracellular vesicles (EVs) as key biomarkers in cancer progression and to propose an innovative method for their early detection. We present the use of a microfluidic chip that mimics the tumor microenvironment to efficiently isolate EVs secreted by human breast cancer cells (MCF7). By incorporating an extracellular matrix within the microfluidic system, we perform three-dimensional cell cultures to investigate the effects of EVs on normal breast cells (MCF-12A), thus providing valuable insights into the alterations of the cancer microenvironment and the process of tumorigenesis. To address the instability often associated with traditional immunological detection methods, we propose imprinting the molecular architecture of EVs onto nanoparticles. These EV-imprinted nanoparticles will be integrated with advanced optical biosensors designed for precise, selective, and real-time EV detection. This innovative approach aims to significantly enhance the accuracy and reliability of early breast cancer diagnosis, while also elucidating the dynamic interactions within the cancer microenvironment. By combining microfluidic technology, molecular imprinting, and cutting-edge biosensing, this study represents a significant advancement in non-invasive cancer detection. Furthermore, it contributes to a deeper understanding of cancer biology, offering transformative potential for both clinical applications and fundamental research in oncology.

Cell Membrane Derived Supported Lipid Bilayers in Virology Research

H. Pace[I], M. Bally[I]

[I]Umea University, Umea, Sweden

Supported lipid bilayers (SLBs) have a track record spanning more than three decades as model systems for the study of cellular membrane structure and function. While historically SLBs have been composed of simplified lipid mixtures, there has been a surge in compositionally complexity during the last decade to better mimic the natural composition of cellular membranes. To this end, a new class of SLBs derived directly from isolated native membrane preparations, hereforth referred to as native-SLBs (nSLBs), have emerged. This new cell-free platform combines the instrumental accessibility and control of traditional model membrane systems with the complex natural composition of a chosen donor membrane.

As no detergents or organic solvents are used in the production of the nSLBs, the full complement a donor membrane's composition (e.g. lipids, sugars, and proteins) are retained. Once harvested, the native membranes are a snapshot of a membrane's composition that can be used for hundreds of experiments to systematically study the role of different components present therein.

I will present the current state-of-the-art in nSLB production strategies and how this platform can be used to investigate interactions between viruses and the plasma membrane of host cells. I will show how systematic enzymatic treatments can be employed to modify the biomolecules displayed on nSLBs in order to elucidate the roles of different cellular receptors in viral recruitment.

Influence of ethanol on liposome's bilayer properties; systematic comparison between preparation methods

L. Severini[I], S. Sennato[I], C. Bombelli[II], F. D'Acunzo[II], B. Simonis[II], L. Paduano[III], P.G.G. Del Vecchio[IV], M. Campanile[V]

[I]Institute for Complex Systems, National Research Council (ISC-CNR) and Department of Physics, Sapienza University of Rome, Rome, Italy, [II]Institute for Biological Systems, National Research Council (ISB-CNR) and Department of Chemistry, Sapienza University of Rome, Rome, Italy, [III]Department of Chemical Sciences, University of Naples Federico II, 80126, Naples, Italy, [IV]45503 - Department of Chemical Sciences, University of Naples Federico II, 80126, Naples, Italy, [V]45503 - Department of Chemical Sciences, University of Naples Federico II, 80126, Rome, Italy

Over the past decades, various techniques for preparing liposomes have been proposed, with differences in liposome size, dispersity and lamellarity. These methods can be divided into two main categories: bulk and microfluidics. An aspect that is often overlooked is liposome's preparation method, which has a profound influence on their chemical-physical and colloidal properties. This is due to the presence of an "hidden" actor, that is the ethanol chosen for lipid solubilization, which is absent in common bulk method such "thin film (TF) hydration" while it acts as an additional component in microfluidics (μF). Here we perform a systematic comparison between these two preparation methods (TF and μF) considering vesicles formulated with 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) lipid, commonly used in many approved liposomal drugs, and cholesterol as excipient for tuning bilayer fluidity. Detailed studies on the effect of ethanol on liposomes are scarce despite the evidence suggesting that the outcome should be highly dependent on the composition of the system. Alcohol molecules intercalate into membranes and change their structural and/or dynamical properties. Indeed, the presence of short-chain alcohols such as ethanol, strongly promoted the interdigitation of membranes, for saturated phospholipids such as DMPC thus leading to aggregation and/or deformation of liposomes. This, in turn, might affect membrane-bound molecules interaction and result in functional changes. The physico-chemical properties of vesicles were monitored to evaluate their stability over time. Furthermore, the order/fluidity degree of lipid bilayer, induced by ethanol, was investigated through fluorescence anisotropy and DSC measurements. Finally, to achieve specific target product profile, the inclusion in the liposomes' structure of a synthetic molecule, capable to complex trivalent metal ions was performed and its effects studied.

Exploring the Biointerface of Polycrystalline Lysozyme Coatings: Interaction with Biological Fluids and Implications for Antimicrobial Release

N. Kaddour[I,II], K. Roy[I,II], K. O'Dwyer[I,II], C. O'Mahony[I,II], C. Silien[I,II], T. Soulimane[I,II], S.A.M. Tofail[I,II]

[I]University of Limerick, Castletroy, V94 T9PX, Limerick, Ireland, [II]CÚRAM Research Ireland Centre for Medical Devices, Galway, Ireland

The interaction between biological systems and artificial materials remains a key challenge in biomedical engineering, particularly for implantable devices exposed to complex physiological environments. Urinary stents serve as a prime example of these challenges, as they are in prolonged contact with biological fluids and subjected to issues such as encrustation, bacterial colonization, and material degradation. Our study seeks to understand the intricate mechanisms governing the behaviour of our newly developed polycrystalline lysozyme coatings when exposed to biological environments, focusing on their degradation, antimicrobial release profile, and surface charge evolution. By examining how surface charge dynamics and surface composition evolve as a result of material degradation, we aim to understand the role of biological factors—such as pH variations and the presence of compounds like urea and creatinine—in modulating coating performance. Through this investigation, we aspire to predict how structural changes over time influence the coating's antimicrobial efficacy and its ability to resist bacterial adhesion, contributing to the development of next-generation biodegradable antimicrobial coatings. Our work offers insights into the complex interplay between biological systems and medical devices, advancing the field of biomedical coatings and their role in improving the performance of medical devices.

The impact of multivalent phosphate ions on protein self-assembly processes

R. Curtis[I]

[I]University of Manchester, Manchester, United Kingdom

Emerging evidence indicates that the energy metabolite adenosine triphosphate (ATP) plays a central role in modulating protein self-assembly pathways within cellular environments, suggesting that triphosphate molecules could serve as novel stabilizers for protein therapeutics. In this talk, I will discuss our recent research on the weak binding interactions between multivalent phosphate ions and proteins, and how these interactions affect protein-protein interactions, phase behavior, and aggregation. In particular, we find that lysozyme solutions exhibit reentrant condensation in the presence of multivalent phosphate ions. In contrast, these ions are remarkably effective at suppressing heat-induced aggregation of acidic proteins. These effects are closely linked to the ability of multivalent phosphates to tune the range and magnitude of electrostatic interactions between proteins, as revealed through a combination of light scattering measurements and molecular simulations. Finally, I will compare our observations to the behaviour observed in protein solutions containing multivalent cations.

Into the core of Lipid Nanoparticles (LNPs); mRNA transfection efficiency is buffer specific.

C. Carucci *[I], J. Philipp *[II], J.A. Müller[II], A. Sudarsan[III], E. Kostyurina[II], C.E. Blanchet[IV], N. Schwierz[III], D.F. Parsons[I], A. Salis[I], J.O. Rädler[II]

[I]Dipartimento di Scienze Chimiche e Geologiche, Università degli Studi di Cagliari, 09042 Monserrato (CA), Cagliari, Italy, [II]LMU, Faculty of Physics, Munich, Germany, [III]University of Augsburg and University Hospital of Augsburg, Augsburg, Germany, [IV]European Molecular Biology Laboratory, EMBL Hamburg Unit, Hamburg, Germany

Lipid nanoparticles (LNPs) are liposome-like nanosized structures with a complex internal core structure. Recently LNPs have been used to deliver COVID-19 mRNA vaccines [1]. LNPs core consists of a cationic ionizable lipid and cholesterol which self-assemble to create inverse micellar (L_{II}) phases where the mRNA is encapsulated. The core phase is pH responsive, due to the protonation of the cationic ionizable lipid which results in a change of interface curvature corresponding in a phase transition from inverse micellar (L_{II}) at neutral pH, to inverse hexagonal (H_{II}) structure at slightly acidic pH (6.0-5.5). The pH dependent phase transitions have been correlated to the biological effectiveness of the LNP_s in terms of mRNA transfection efficiency, thus playing a key role in mRNA delivery [2]. For mRNA-LNPs preparation a wide range of buffers can be used to set and to maintain the pH but not much attention is paid in the buffer choice. However, buffers influence biological systems in a specific way that strongly depends on their intrinsic identity [3]. In this work the core phase of 3 types of LNPs formulated with 1) DLin-MC3-DMA 2) SM-102 and 3) ALC-305 have been studied as a function of pH and buffer type by means of synchrotron Small Angle X Ray Scattering (SAXS) measurements. The transition of the LNPs core phase, from L_{II} to H_{II} phase is found to be buffer specific, occurring at a pH decreasing along the series citrate > acetate > phosphate. The same buffer sequence was observed for mRNA transfection efficiencies with the fastest gene expression found for LNPs produced in citrate. Future work will be based on customizing the buffer choice and ionic strength to design more effective mRNA-LNPs.

* The authors marked with an asterisk equally contributed to the work.

Bending, crowding and jostling: life is free inside the COVID vaccines.

D. Parsons[I]

[I]Department of Chemical and Geological Sciences, University of CagliarI, Cagliari, Italy

Cationic surfactants form the particles responsible for carrying RNA COVID vaccines. pH-dependent phase transitions of the lipid crystal phases are understood to be responsible for delivery of vaccine molecules inside cells. Understanding the factors controlling them involves a complex combination of disparate factors competing to determine the lowest energy phase at each pH. A bending energy, associated with the curvature of lipid layers and influenced by area per headgroup, tends to favour spherical inverse micelles over the inverse hexagonal (cylindrical) phase at high pH. At low pH, electrostatic interactions associated with headgroup charging tend to favour the hexagonal phase. The specific identity of buffer ions shifts the balance. Nonelectrostatic dispersion interactions enable acetate buffer ions to jostle in close to headgroups, more so than other buffers ions including citrate, shifting the transition pH. At the same time, the high charge environment due to charged headgroups creates a crowded environment similar to the interface of a charged electrode, requiring consideration of steric forces due to finite ion sizes. The background counterion (Cl-) dominates this effect. It is likely that induction forces will be required to provide a complete description of the system, generating a dynamically changing dielectric environment. Lastly, a conventional analysis assumes the aqueous cores
are in equilibrium with an external electrolyte determining the internal pH. This is the thermodynamics of a grand potential, controlled by chemical potentials in the external bulk solution. But after formation, the aqueous core of inverse micelles may be isolated from the external solution, therebye following the thermodynamics of a free energy rather than a grand potential. The impact is analogous to the effect of a Donnan potential. This will require a significant reconfiguration of the assessment of the conditions determining the lowest energy phase at a given external pH.

Advanced Methods to Control and Investigate Inter-Protein Interactions and Energy Transfer in Model Photosynthetic Biomembranes

A. Hancock[I,II], S. Meredith[II], Y. Kusunoki[III], S. Evans[II], K. Morigaki[III], P. Adams[II], N. van Hulst[I], N. Liguori[I]

[I]ICFO-Institute of Photonic Sciences, Castelldefels, Barcelona, Spain, [II]University of Leeds, Leeds, United Kingdom, [III]Graduate School of Agricultural Science and Biosignal Research Center, Kobe University, Kobe, Japan

Light-harvesting (LH) pigment-protein complexes are key to photosynthesis, capturing solar energy and transferring it to reaction centres for energy conversion. The spatial organization of LH proteins is dynamic, and critical for regulating energy transfer and photoprotection. Conventional methods to study these interactions often rely on equilibrium systems like detergent-isolated proteins or supported lipid bilayers (SLBs), which limit manipulation of protein organization and its impact on energy transfer.

Here, I present new approaches to control and study LH protein interactions in model photosynthetic membranes. First, I introduce in-membrane electrophoresis (IME), which applies an electric field in-plane with an SLB to induce migration of charged molecules, generating a continuous concentration gradient within a single membrane. IME enables precise control over local protein density, allowing fluorescence lifetime imaging microscopy (FLIM) to track changes in energy transfer with protein reorganization. Using IME, we successfully guided the migration of the plant LH protein LHCII, creating controlled gradients of protein concentration. Real-time fluorescence intensity imaging confirmed LHCII redistribution, while FLIM showed a significant reduction in fluorescence lifetime in high-concentration regions, indicating energy-dissipative quenching. This method provides insights into dynamic photoprotective mechanisms like non-photochemical quenching (NPQ), which depend on protein-protein interactions and conformational changes.

Additionally, I demonstrate the use of nanofabricated devices to control exciton transfer in SLBs. By combining tuneable nanopatterned substrates with ultrafast spectroscopy and advanced microscopy, we investigate exciton diffusion in membrane-localized LH proteins at high spatial and temporal resolution. These techniques offer new insights into photosynthetic energy transfer and inform the development of bio-hybrid solar energy technologies.

Cross-Linking Effects on Mixed Surfactant Micelles: Insights from Molecular Dynamics Simulations

H. Ishkhanyan[I], J. Lawrence[II], C. Lorenz[III]

[I]Institute for Informatics and Automation Problems of NAS RA, 1 Paruyr Sevak str, Yerevan, Armenia, [II]University of Manchester, Manchester, United Kingdom, [III]King's College London, Strand, London, United Kingdom

Surfactants can be covalently linked to form oligomers, the properties of which differ from their monomeric variants. Surfactant oligomers often possess advantageous qualities, such as lower critical micelle concentrations (CMC) and better wetting properties, which make them better suited for various applications than their monomeric counterparts. Furthermore, such properties can be further enhanced by the introduction of other surfactants. Such mixed surfactant systems can benefit from the functionality provided by both types of surfactant, which can prove advantageous over single surfactant systems. In this work, mixed micelles formed by Triton X-100 and its oligomer, tyloxapol (trimer and heptamer), have been studied. In total, eight systems were investigated that contained different ratios of each surfactant. The size and shape of each micelle were evaluated using various analysis methods. Pure tyloxapol micelles were shown to have the smallest volume and surface area. Additionally, by conducting a neighbour analysis, it has been assessed whether one type of surfactant has affinity for itself or the other. The analysis has shown little or no selectivity between different types of surfactants.

Competitive Behavior of Antibiotics vs. Surfactants on Microplastics

A. Striolo[I]

[I]University of Oklahoma, Norman, United States of America

Microplastics and antimicrobial resistance are two emergent environmental problems. This project is concerned with possible synergistic effects that could magnify the problem. For example, by adsorbing on microplastics, antibiotics, as well as antibiotic-resistant microorganisms could diffuse further in the environment. Surfactants, on the other hand, could be used to remove contaminants from plastic surfaces, although surfactants are also capable of changing the surface features of particles, thereby affecting, and in some cases enhancing, their ability to transport through waterways. To provide a baseline quantification of the multiple phenomena just described, we report here experimental adsorption isotherms for three common surfactants and for the antibiotic novobiocin on polystyrene. The surfactants are chosen to represent anionic, non-ionic, and cationic systems with similar molecular structure. Novobiocin is chosen because preliminary experimental data suggested that a surface-active compound with its charge density could adsorb on polystyrene. Polystyrene was chosen because it is a common plastic material, hence its prevalence in the environment is expected. AFM and XPS were used to characterize the surface features of the solid samples used for the experiments. Experimental adsorption isotherms in water were conducted using the quartz crystal microbalance with dissipation monitoring, The results were interpreted with the help of atomistic molecular dynamics simulations. Results show that CTAB forms a sparse film on polystyrene, while C12E6 forms a complete monolayer and AOT yields a complex multi-layer structure characterized by high water content. Novobiocin is able to remove each of the surfactant films, while the three surfactants are shown to adsorb on novobiocin films previously formed on polystyrene. The implications of these findings for environmental contaminations will be discussed.

Regulation of the photosynthetic AB-GAPDH via self-assembly

A. Del Giudice *[I], R. Marotta *[II], L. Gurrieri[III], S. Fanti[IV], P. Swuec[V], L. Galantini[I], G. Falini[IV], P. Trost[III], S. Fermani[IV], F. Sparla[III]

[I]Sapienza University of Rome. P.le A. Moro 5 00185, Rome, Italy, [II]Istituto Italiano di Tecnologia IIT, Genoa, Italy, [III]Dipartimento di Farmacia e Biotecnologie – FaBiT, University of Bologna, Bologna, Italy, [IV]Dipartimento di Chimica "G. Ciamician," University of Bologna, Bologna, Italy, [V]Biosciences Department, University of Milan, Milan, Italy

Photosynthetic glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a key enzyme of the Calvin-Benson cycle, the "dark" part of photosynthesis in which a fixation of atmospheric carbon dioxide into sugars overall occurs. Through the GAPDH reaction the NADPH produced by the light phase is consumed, leading to the production of the first sugar of the whole photosynthetic process. A tight regulation of GAPDH is therefore necessary to let concurrently proceed the two phases of photosynthesis.

In higher plants two isoforms of GAPDH co-exist in the chloroplast. The hetero-isoform, containing A and B subunits in the active tetramer A2B2, is able to turn off its activity through a self-assembly process that leads to the formation of inactive oligomers, whose stabilization involves the redox-sensitive 30-amino-acid tail called C-terminal extension (CTE) present only in the B subunits. Typically, the fully inactive form is considered an hexadecamer $(A2B2)_4$, generated by the assembly of four A_2B_2-GAPDH tetramers.

With the aim of disclosing the molecular mechanism driving the oligomerization of AB-GAPDH, we performed a structural study of the system in solution by analyzing the purified enzyme from spinach by Small Angle X-ray Scattering coupled with size exclusion chromatography (SEC-SAXS) and single particle high resolution cryo-electron microscopy (cryo-EM). Both experimental approaches highlighted the coexistence of several $(A_2B_2)_n$ oligomerization states, whose relative proportion depended on the solution conditions (activation/inactivation), showing an unexpected dynamicity. The sub-atomic structure obtained by cryo-EM single particle analysis of the most populated oligomers revealed that pairs of B subunits belonging to adjacent tetramers mutually exchange their CTEs, which act as protruding hooks and dock into the active sites of the other subunit substantially blocking the access of the substrate.

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* The authors marked with an asterisk equally contributed to the work.

Tailor-Made Design of Fluorinated Nanotherapeutics for Cardiac Disfunctions

B.L. Bona[I], C. Pagiatakis[II,III], P.M. Lagarrigue[IV], R. Papait[II,III], F. Cellesi[V], P. Metrangolo[V], F. Baldelli Bombelli[V]

[I]Politecnico di Milano, Via Luigi Mancinelli 7, 20131, Milano, Italy, Milano, Italy, [II]IRCCS Humanitas Research Hospital, Via Alessandro Manzoni, 56, 20089-Rozzano MI, Italia, Rozzano, Italy, [III]Department of Biotechnology and Life Sciences, University of Insubria, via J.H. Dunant 3, 21100, Varese, Italy, Varese, Italy, [IV]CIRIMAT, Université Toulouse 3 Paul Sabatier, Toulouse INP, CNRS, Université de Toulouse, 118 Route de Narbonne, 31062 Toulouse cedex 9 - France, Toulouse, France, [V]Department of Chemistry, Materials and Chemical Engineering, Politecnico di Milano, Via Luigi Mancinelli, 7, 20131-Milano, Italia, Milano, Italy

Recent research linked dysregulation of epigenetic modifications, mechanisms controlling gene expression, to the onset of heart failure. Consequently, targeting this imbalance with epigenetic drugs emerged as a promising therapeutic strategy for cardiovascular diseases. However, minimizing the inherent toxicity associated with these drugs necessitates the development of nanoparticles (NPs) able to precisely encapsulate and delivering them to targeted sites.
Simultaneously, considering the influence of protein interaction with NPs on their biological behavior, comprehending NPs biological identity is essential for developing safer and more effective delivery systems, while anticipating potential toxic effects.
Here, we aim to advance cardiovascular nanomedicine by pioneering the development of innovative theranostic NPs tailored for delivering novel epigenetic drugs to treat cardiac pathologies. We developed drug-loaded fluorinated PEG-PCL nanoparticles, detectable via 19F-NMR, which have demonstrated promising therapeutic effects on cardiomyocyte cell lines in vitro. Additionally, we investigated NPs protein corona to understand the impact of NPs composition on their biological identity.

Mitochondriotropic liposomes featuring a triphenylphosphonium bolaamphiphile: development of a protocol for loading antioxidants and evaluation of liposome ability in subcellular targeting

S. Sennato[I], F. Ceccacci[II], G. Bozzuto[III], R. Salvio[IV], V. Moresi[V], A. Calcabrini[VI], A. Ciogli[IV], C. Bombelli[II]

[I]CNR- Institute of Complex Systems, Piazzale Aldo Moro 2, Rome, Italy, [II]CNR, Institute for Biological Systems, Rome, Italy, [III]National Institute of Health, ISS, Rome, Italy, [IV]Sapienza University of Rome, Rome, Italy, [V]CNR, Institute for Nanotechnology, Rome, Italy, [VI]National Institute for Health, ISS, Rome, Italy

Drug delivery to mitochondria represents a promising therapeutic strategy because mitochondrial dysfunctions are associated with many different pathologies, from neurodegenerative diseases to diabetes, obesity, cancer, and physiological disorders related to ageing. Actually, mitocondria targeting is particularly challenging due the inner highly convoluted, densely packed membrane, characterized by a strong negative potential that significantly hinders drug delivery. Cationic amphiphilic structures derived by triphenylphosphonium are planar structures with highly delocalized cationic charge which is driven to mitochondria by the strong negative potential of the inner mitochondrial membrane and can cross the membrane barriers due to the "soft" nature of their cationic headgroups. The inclusion of a triphenylphosphonium bolaamphiphile (TPP3) in liposomes, in mixture with saturated or unsaturated phospatidylcholine and cholesterol, has been explored to realize novel nanocarriers for mitochondrial delivery. We showed that the amount of bolaamphiphile and the lipid nature jointly affect the physicochemical properties of liposomes and the bilayer organization. We explored the ability to reach mitochondria in two different cell lines, the murine skeletal muscle C2C12 and the drug-resistant human breast cancer MDA-MB231, useful to investigate different pathological situations, namely, the oxidative stress associated with ageing and neurodegenerative muscle diseases and the multidrug resistance in cancer. Liposomes with 2.5% of TPP3 were non-toxic and able to target mitochondria, thus resulting good candidates for encapsulation of the antioxidant trans-resveratro, which was loaded in the liposome bilayer but also, for the first time, in the aqueous core of liposomes with high efficiency. Our results support its potential therapeutic use as an adjuvant for a protective activity in neurodegenerative diseases or for a sensitizing activity against drug resistant tumor cells.

Design and investigation of microfluidic-synthetized lipid-based nanocarriers: High-throughput screening and optimization

I. Clemente[I], L. Talarico[I], G. Gabbricci[I], G. Leone[I], A. Magnani[I]

[I]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Siena, Italy

In the past decade, lipidbased nanocarriers have gained prominence as the emergent technology for drug delivery due to their ability to protect bioactive compounds and improve intracellular transport and release. Following their clinical approval for anticancer drug delivery, various lipid nanocarrier formulations have been explored to optimize physicochemical properties and enhance therapeutic efficacy. The choice of lipid components plays a pivotal role in influencing the structure, stability, and intracellular uptake of these carriers. Key components include helper/structural lipids like phospholipids and cholesterol, which provide stability and facilitate membrane fusion. Additionally, ionizable lipids for lipid nanoparticles (LNPs) or solid lipids for solid lipid nanoparticles (SLNs) improve cargo encapsulation and prevent leakage, while surfactant or polyethylene glycollipid coatings are employed to prolong circulation time in vivo. Manipulating lipid composition and relative abundance can dramatically alter the nanocarriers properties and functionality. Microfluidicassisted preparation of lipidbased nanocarriers enables the synthesis of monodisperse and stable formulations with high reproducibility, enhancing rapid screening and optimization for clinical translation. This work presents a highthroughput screening and optimization strategy for three types of lipid nanocarriers—LNPs, SLNs, and transethosomes—as drug delivery candidates. Microfluidic synthesis was used to explore a wide range of lipid components, surface coatings, and compositions. Comprehensive supramolecular characterization techniques such as Dynamic Light Scattering, Zeta potential, Small Angle Xray Scattering, and HighPerformance Liquid Chromatography for drug encapsulation efficiency were employed to select the optimal carrier candidates. This approach allows for the identification of lipid formulations with the desired physicochemical properties for efficient drug delivery.

POSTER PRESENTATIONS

FEBS Advanced Course 2025 Biological Surfaces and Interfaces: Biointerfaces at lipids, proteins and polymers

Counterion Effects on the Amyloid Morphology of a Peptide Gel

M. Acar[I], D. Tatini[II], L. Pacini[III], M. Quagliata[III], F. Nuti[III], A.M. Papini[III], P. Lo Nostro[I]

[I]Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia 3 50019, Sesto Fiorentino (FI), Italy, [II]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Via Aldo Moro, 2 – 53100, Siena, Italy, [III]Department of Chemistry "Ugo Schiff" and PeptLab, University of Florence, Via della Lastruccia 13 50019, Sesto Fiorentino (FI), Italy

Peptide gels offer a versatile tool for designing multiresponsive materials as their physicochemical properties can be tailored through modifications in the peptide sequence. A particular emphasis is placed on amyloid gels, not only for their distinctive material properties but also for their relevance in amyloidogenesis studies, as amyloids bear pathological implications in conditions such as Alzheimer's and Parkinson's diseases.

Advances in solid-phase peptide synthesis (SPPS) largely improved the study of these systems. However, a frequently overlooked aspect is the presence of residual electrolytes, such as trifluoroacetate ($TFAc^-$), in post-synthesis products. These ionic residues can significantly impact the overall properties of a system. In fact, specific ion effects are crucial across a range of systems, *e.g.,* bulk solutions, interfaces and soft matter, making a thorough understanding of them essential.

In this study, we investigated 3% w/v ACP(65-74)-NH_2 amyloid hydrogels in the presence of different counterions ($TFAc^-$, SO_4^{2-}, Cl^-, Br^-, and I^-) using confocal laser scanning microscopy, circular dichroism, Fourier-transformed infrared spectroscopy, and fluorescence spectroscopy to determine their morphology and performed rheological measurements to evaluate their mechanical properties.

The results show how profoundly the nature of the counterion influences the gelation properties, amyloid morphology, and rheological properties of our system and bear an important implication on the choice of the counterion.

Interactions of KSCN on Phospholipid Monolayers at the Air-Water Interface Investigated by Sum Frequency Generation Vibrational Spectroscopy

S. Afroz *[I], B. Mueller *[II], E. Schneck *[II], P.S. Cremer *[I]

[I]The Pennsylvania State University, State College, United States of America, [II]TU Darmstadt, Darmstadt, Germany

Thiocyanate (SCN) is a weakly hydrated anion that can interact relatively strongly with biological interfaces compared to other anions within the Hofmeister series. To date, the specific mechanisms governing these interactions these processes remains poorly characterized. To shed light on ion-lipid membrane interactions, we introduced KSCN into the subphase below 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) monolayers. This saturated lipid was chosen because it has relatively short alkyl chains, while still remaining in the fluid phase at room temperature. Both the two-dimensional pressure of the monolayer and the salt concentration were varied. SFG was used to follow vibrational signals associated with the interfacial water structure, the lipid headgroups, the ordering of the alkyl chains, as well as the SCN anions. The results revealed enhanced ordering of the lipid monolayers at lower thiocyanate concentrations, but disordering as the concentration of the salt was increased. Curiously, the interactions were most pronounced at intermediate pressures with weaker interactions occurring at both lower and higher lateral pressures. Details of these interactions as well as their implications for Hofmeister chemistry will be discussed.

* The authors marked with an asterisk equally contributed to the work.

Double Symmetry Breaking in Filamentous Colloidal Tactoids

M. Almukambetova[I], H. Almohammadi[I,II], F. Schleiffer[I], R. Mezzenga[I,III]

[I]Department of Health Sciences and Technology (D-HEST) ETH Zurich, Zurich, Switzerland, [II]John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, United States of America, [III]Department of Materials (D-MATL) ETH Zürich, Zurich, Switzerland

Understanding dynamics of liquid crystalline tactoids under external forces is of great importance due to their potential applications in optics, medical devices and displays. However, only recently have tactoids started to be studied systematically under external forces, in particular, by extensional flow. Here, we subject tactoids to a shear flow field and study their deformation dynamics upon varying conditions of shear and time scales. Using amyloids and nanocellulose to form tactoids from model filamentous colloids with opposite sequences of chirality amplification (left-handed mesoscopic → right-handed cholesteric for amyloids; right-handed mesoscopic → left-handed cholesteric for nanocellulose), we show a complex deformation mechanism in their shape and internal structure under shear flow. When tactoids deform perpendicularly to their long axis, a double symmetry breaking occurs in both their contour shape, with the emergence of a kink, and their orientation of the nematic field. We further show that the mesoscopic chirality of the building blocks directs the position of the kink, with the macroscopic tactoid asymmetry being mirrored when inverting the mesoscopic chirality of the constitutive filamentous colloids, e.g. from the left-handed amyloids to the right-handed nanocellulose.

Development of nanoforce polydiacetylene-based biosensors and tailoring their properties by integrating additives.

F. Altieri[I], K. Sugihara[I]

[I]The University of Tokyo, Tokyo, Japan

The recent COVID-19 pandemic has prompted and provided a good opportunity for many pharmaceutical companies to develop easy-to-use and low-cost diagnostic tools, such as the rapid antigen detection test (RADT), which requires only saliva or nasal mucosa samples. Nevertheless, most such RADTs are qualitative tests that, as a result, can provide only positive or negative outcomes. Like a weather forecast, which can give a percentage probability that rain will occur, a test that provides information that varies between zero and one is much more informative than just two possible results.

Herein, a potential RADT that can provide a more informative outcome is developed by using the 10,12-tricosadiynoic acid (TRCDA) and biological lipids to build a polydiacetylene (PDA)- based biosensor that is highly tunable, cost-effective, and relatively easy to manufacture.

TRCDA is well-known for its mechanochromic properties: when an external force occurs, it twists the PDA's backbone, causing the blue-to-red color transition and changing from non-fluorescent to fluorescent emission.

By correlating the force with the quantity of energy a compound target exerts on the PDA, the target agent's concentration can be determined. This is possible primarily because the lateral forces (shear) at the nanoscale can be applied, even in liquid conditions, using Nano-Friction Force Microscopy (NFFM), a more advanced and modified version of Atomic Force Microscopy. Simultaneously, fluorescence microscopy detects the fluorescence emission from friction-induced PDA.

Moreover, in the following work, several phosphatidylcholines (PCs) are integrated with TRCDA monomers to tune the fluorescence response and, therefore, the sensitivity. Indeed, it has been observed that PCs significantly affect the distribution, thickness, and organization of the crystalized PDA deposited over the glass coverslip during Langmuir-Blodgett sample preparation.

From reef builders to crack sealers: Characterizing and harnessing photosynthetic bio-calcifiers for cement repair

Y. Weng[I], T. Brueck[I], **D. Awad**[I]

[I]Technical University of Munich, Garching near Munich, Germany

The surfaces of biological organisms offer vast potential in material innovation, yet many remain underexplored. Calcareous red algae (CRA), long known as reef builders, possess a unique bio-calcifying capability-depositing calcite (CaCO) in their cell walls. Here, we investigate these photosynthetic eukaryotes as living bio-calcifiers and explore their potential as functional self-assembling agents in cement repair. As a prerequisite, we established the first lab cultivation of CRA. Our primary focus is on characterizing their cell wall as a biologically active, mineralizing surface. Using Systems Biology approach, we profile fatty acids, sugars, pigments and proteins, complemented by imaging to assess mineralization. This multi-modal approach aims to shed light on CRA's poorly understood calcification mechanisms and to establish foundational aspects of their surface biochemistry. We also explore the potential of CRA integration into inorganic cementitious matrices. Unlike established bacterial self-healing concrete systems, which rely on urea metabolism and external nutrient sources-associated with steel corrosion-CRA offer a photosynthetically driven, nutrient-independent approach. Their autonomous metabolism may enable sealing of larger cracks over time, while contributing to carbon sequestering and storage over the building's lifetime. Preliminary embedding experiments prompt new questions on surface persistence, mineral output, and potential contributions to long-term material durability. Integrating CRA into concrete introduces a novel biogenic strategy for healing, in which the interplay between photosynthesis, calcification, and structural performance is reimagined. By repositioning CRA from reef ecosystems to construction sites, we aim to introduce living, light-powered additives for sustainable construction. This work contributes to the field of bio-based interfaces and offers insight into harnessing biological surface functions for engineered materials.

Structural studies into biofilm formation by the Legionella pneumophila collagen-like protein

M. Baczynska[I], C. Lorenz[I], J. Garnett[I]

[I]King's College London, London, United Kingdom

Legionella pneumophila (*Lp*) is a Gram negative bacterium causing the serious respiratory disease, Legionellosis. *Lp* thrives in water containers due to its ability to form biofilms, complex structures built of bacterial colonies producing molecules that help them aggregate and adhere. *Legionella* collagen-like protein (Lcl) is associated with biofilm formation and host membrane binding. It is made of an N-terminal domain (NTD), a collagen-like domain, and a C-terminal domain. Before helping to facilitate the biofilm formation, Lcl is first secreted via the type II secretion system. We have recently shown that the NTD anchors both the inner and outer bacterial membranes however, the mode of this action remains unclear. We are conducting multiscale molecular dynamics (MD) simulations of Lcl NTD interacting with model lipid membranes of *Lp*, to gain a detailed understanding of the Lcl NTD binding to these membranes. We assist the simulations with wet-lab experiments such as NMR and bacterial assays. The initial simulations of the NTD with the membranes show a unique mode of protein binding. The NTD forms specific contacts and interactions with the membranes, although it does not anchor itself strongly nor facilitate further insertion. Together with the atomistic simulations, the coarse-grained simulations validated that the trimeric NTD structure likely does not dissociate into monomeric helices but rather binds as a whole trimer. It was shown that the three NTD helices are strongly held by specific hydrophobicface residues (e.g., Leu17). To gain a full picture of the NTD peptide-membrane interactions and molecular mechanisms, we are currently performing further MD simulations in parallel with additional wet-lab testing, mainly aimed at understanding the depth of insertion and rotation of the peptide upon membrane binding.

Probing the effect of protein corona on the interaction of RNA-loaded fluorinated dendriplexes with model membranes by QCM and neutron reflectometry

M. Beccalli *[I], L. Fumagalli[I], M. Rosati[I], E. Erba *[II], M. Campana[III], N. Paracini[IV], P. Metrangolo[I], F. Baldelli Bombelli[I], F. Sebastiani[II,V]

[I]Laboratory of Supramolecular and Bio-nanomaterials, Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, Italy, [II]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark, [III]Science and Technology Facilities Council (STFC), ISIS Neutron and Muon Source, Rutherford Appleton Laboratory, Didcot, United Kingdom, [IV]European Spallation Source ERIC Data Management and Software Centre, lyngby, Denmark, [V]Division of Physical Chemistry, Department of Chemistry, Lund University, Lund, Sweden

Dendrimers are considered promising options for efficient nucleic acid (NA) delivery to target cells. However, high production costs for advanced-generation dendrimers may restrict their application in clinical settings. Supramolecular dendrimers constitute an attractive alternative. In this regard, we developed a second-generation Fluorinated Janus-type Dendrimer (FJD_2N) which forms stable dendriplexes with NAs, hence facilitating their cellular uptake. In this study, we investigate the interaction between FJD_2N RNA-loaded dendriplexes and model lipid membranes under varying pH conditions and in presence of serum proteins, aiming to identify the key parameters affecting the interaction.

Quartz-crystal microbalance with dissipation monitoring (QCM-D) and neutron reflectometry (NR) were employed to investigate the interaction between the siRNA-FJD_2N dendriplexes and supported lipid bilayers (SLB) in presence and absence of fetal bovine serum (FBS). 100 mol% DOPC membranes at pH 7.4 were used to represent healthy cell membranes, while 59:23:18 mol% DOPC:DOPE:DOPS SLB at pH 5 were used to mimic endosomal membranes.

Dendrimer aggregates are adsorbed on both types of SLB, and similar results are found when considering siRNA-FJD_2N dendriplexes. On the other hand, the interaction results in larger changes in the SLB and with increasing amount of FBS in solution. Lower dendrimer concentration leads to a decrease in adsorption and slower kinetics.

The QCM-D and NR study of model membranes interacting with siRNA-FJD_2N dendriplexes, both in presence and absence of serum, provided insights into the interaction mechanism. It also demonstrated the significant impact of serum on the interaction of drug delivery systems with membranes.

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* The authors marked with an asterisk equally contributed to the work.

Osmolyte-Mediated Modulation of Protein-Protein Interactions

R. Benani *[I], P. Cremer *[I]

[I]The Pennsylvania State University, State College, United States of America

Organisms such as Escherichia coli initially respond to osmotic stress by taking up inorganic ions, which helps partially restore cytoplasmic water concentration and growth rates. However, long-term survival requires a more effective strategy for re-establishing homeostasis. Nature offers a simple solution: the accumulation of small organic molecules known as osmolytes. Classical models often assume osmolytes are preferentially excluded from protein surfaces, overlooking their interactions with surface charges and their influence on protein-protein interactions (PPIs). We employed a 2D microfluidic assay as well as vapor pressure osmometry to explore this question. Specifically, we attached streptavidin, a negatively charged protein, to two-dimensionally fluid phospholipid membranes composed primarily of phosphatidylcholine lipids. The protein and lipid molecules could be labeled with dyes and studied within polydimethylsiloxane (PDMS) microfluidic devices on glass substrates. Our studies revealed that at least three key factors govern how osmolytes modulate PPIs. While trimethylamine N-oxide (TMAO) is known to counteract urea's denaturing effects, we discovered that the urea-to-TMAO ratio required for this mitigation is not universal. These results challenge traditional views of osmolyte action. Ultimately, our studies provide new insights into the osmolyte-mediated PPIs, with potential implications for protein stability in cellular environments and biotechnological applications.

* The authors marked with an asterisk equally contributed to the work.

End-to-End Rapid Peptide Function Profiling via Polyacetylene Sensors and Deep Learning

J. Chen[I], K. Sugihara[I]

[I]The University of Tokyo, Tokyo, Japan

Peptides have emerged as a cornerstone of therapeutic research, drawing significant attention for their therapeutic potential and biocompatibility. While thousands of peptides occur naturally and many can be synthesized, accelerating their functional discovery remains crucial for healthcare advancement.
Polydiacetylene, a sensing polymer, exhibits a blue-to-red color transition in response to stimuli including heat, light, pH, and biomolecules. This polymer serves as an effective platform for peptide screening through its visible color changes, simple synthesis, and cost-effectiveness. As a lipid, it can mimic biomolecule-membrane interactions, resulting in distinct absorption spectra at the macroscopic level. Using hyperspectral imaging technology, we can visualize spectra at the pixel level, enabling tracking of point-of-contact interactions.
Our research using hyperspectral imaging has revealed that polydiacetylene (PDA) exhibits unique transition patterns beyond the conventional blue-to-red shift in response to various stimuli, driven by different levels of membrane disturbance. We are developing this system for peptide identification by leveraging membrane interaction mechanisms that are specific to each peptide. Our approach utilizes hyperspectral imaging to collect pixel-level spectral data, enabling precise monitoring of molecular–membrane interactions that shed light on peptide functions. For automated analysis and peptide function screening, we have developed a convolutional neural network (CNN) trained on spectral data acquired through hyperspectral microscopy. Users simply input the PDA spectra collected before and after peptide interaction, and the model rapidly analyzes the peptide's membrane activity and determines its mode of action, enabling efficient and high-throughput drug screening for therapeutic applications.

Tuning Molecular Mobility and Polysaccharide Brush Conformation on Synthetic Membrane Platforms

W. Chiang[I,II], O. Kirichuk[I,III,IV], R. Richter[III,IV], G. Dubacheva[II], L. Bureau[I], D. Débarre[I]

[I]LiPhy (Laboratoire Interdisciplinaire de Physique); 140 Rue de la Physique, 38000 Grenoble, France, [II]Département de Chimie Moléculaire, Université Grenoble Alpes, CNRS UMR 5250, 570 rue de la chimie, CS 40700, 38000 Grenoble, France, [III]School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom, [IV]School of Physics and Astronomy, Faculty of Engineering and Physical Sciences, Astbury Centre for Structural Molecular Biology, and Bragg Centre for Materials Research, University of Leeds, Leeds, United Kingdom

The lateral mobility of surface-anchored molecules on cell membranes is critical for cellular processes, such as adhesion and signaling. This study investigates the in-plane diffusion of streptavidin (SAv) and polysaccharide brushes (biotinylated hyaluronic acid, HA) grafted onto a supported lipid bilayer (SLB) to model endothelial glycocalyx dynamics. Through laser scanning confocal microscopy (LSCM) and fluorescence recovery after photobleaching (FRAP), we quantified the diffusion coefficients of SAv under varying surface coverages. Results indicate that molecular crowding significantly restricts SAv mobility, with diffusion coefficients decreasing as SAv density increases. The system was further functionalized with HA chains to form tunable brushes. Reflection interference contrast microscopy (RICM) was used to measure HA brush heights under different conditions. The findings demonstrate that SAv surface density effectively controls HA brush conformation, with denser SAv surfaces promoting extended brushes. This work provides insights into the role of molecular crowding and polymer interactions in regulating membrane-associated molecule mobility, offering potential for applications in synthetic platforms and biomedical research.

Wolbachia-induced Antiviral Protection: What Can We Learn from Drosophila melanogaster and Drosophila C Virus?

Y. Chykunova *[I], E. Chrostek *[I,II]

[I]Molecular Mechanisms of Symbiosis Laboratory, Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Cracow, Poland, [II]Department of Evolution, Ecology and Behaviour, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom

Wolbachia is one of the most widespread symbionts in nature, infecting over 40% of arthropod species, including flies and mosquitoes. What makes this bacterium truly fascinating is its ability to protect its insect hosts from a variety of RNA viruses. While Wolbachia doesn't naturally infect many human disease vectors, like dengue vector Aedes aegypti, researchers have successfully introduced it into this species, using it to reduce the spread of major diseases such as dengue and Zika. Despite this development, the molecular mechanisms behind Wolbachia's antiviral protection remain unclear.
Our work aims to uncover how Wolbachia provides antiviral protection. To achieve this, we use Drosophila melanogaster as a model organism, along with one of its natural pathogens, Drosophila C virus (DCV). Previous studies have demonstrated that Wolbachia disrupts cellular pathways in the host, such as endocytosis. Interestingly, DCV enters host cells through clathrin-mediated endocytosis. By identifying the receptors that DCV uses to infect cells and determining how Wolbachia interferes with this process, we aim to shed light on the molecular mechanisms causing Wolbachia-induced antiviral protection.
Although our work focuses on fruit flies, the implications are far-reaching. Understanding how Wolbachia manipulates host-virus interactions could inspire innovative ways to enhance antiviral immunity in other organisms. We hope this research will contribute to the ongoing efforts to combat vector-borne diseases, offering new tools to reduce their global impact.

* The authors marked with an asterisk equally contributed to the work.

Quantitative detection of pressure distribution on mechanochromic chameleon Packaging

B. Das *[I], Y. Uchikura[I], N. Matsuhisa[I], Y. Oaki[II], M. Penningtone[I], K. Sugihara *[I]

[I]Institute of Industrial Science, The University of Tokyo, Tokyo, Japan, [II]Department of Applied Chemistry, Faculty of Science and Technology, Keio University, Tokyo, Japan

Intelligent packaging system is gaining its popularity for consumer preferences for medicine or food safety. For example,large amount of COVID-19 vaccines that could not be temperature-controlled during the coronavirus pandemic were thrown away mainly due to improper handling (broken vial / syringe), lack of safe packeging, etc. To ensure that goods are handled with care labels or shock detection stickers are used but since there is no spatial resolution, it is difficult to determine specifically where and how much force is applied. Stimuli-responsive materials, i.e, polydiacetylenes (PDAs) is the promising material that has tunable sensitivity and applications in chemo-bio sensing, force-sensing mechanism for designing various sensing and imaging devices [Das, B., et.al., Nanoscale 14, 1670–1678 (2022)., Das, B., et.al., Adv. Mater. Interfaces 11, 2300745 (2024).].

In the present work we demonstrated a mechanochromic chameleon packaging system that quantitatively maps the pressure history experienced during transport with a spatial resolution of a few millimeters based on the polydiacetylene blue-to-red color transition. The key aspects of the development were 1) the adjustment of the sensitivity by adding guest molecules into the PDA matrix, 2) understanding the complex interactions at the interfaces between polymer and guest composites at the molecular level by introducing advanced imaging techniques i.e, dual nano-friction force/fluorescence microscopy, 3) quantitative calibration, and 3) the accurate reading of the pressure from RGB images based on the calibration. Paper-integrated PDA presented a unique speed-dependent force response both characterized at the macro and nanoscale. Finally, we will demonstrate the “Pressure Analysis App”, a mobile application that allows users to easily obtain pressure values applied to damaged packaging areas simply by taking a photo of the red scratches on the package[Das, B.; et.al., ACS Sens. 9, 12, 6844–6851, (2024)] .

* The authors marked with an asterisk equally contributed to the work.

3D Bioprinting of Bioactive and Bioadhesive Materials for Intrinsic Wound Healing Applications

A. El Hajj[I], R. Mhanna[I]

[I]Biomedical Engineering Program, Maroun Semaan Faculty of Engineering and Architecture, American University of Beirut, beirut, Lebanon

Wound healing is the biological response to tissue injury, moving through the phases of hemostasis, inflammation, proliferation, and remodeling. The integration of bioactive materials into wound care has garnered interest in recent years, opening up new possibilities for developing wound dressings that overcome the limitations of traditional options. Our work explores the fabrication and characterization of wound dressings by 3D bioprinting, which is an emerging technology for the precise deposition of bioinks to fabricate complex and functional three-dimensional structures for drug delivery and tissue engineering applications. A preliminary model is designed to serve a possible synergistic wound healing process. It incorporates mushroom-derived chitosan, a polymer recognized for its antimicrobial, bioadhesive, and permeation-enhancing properties, owing to its cationic backbone. It also incorporates Metformin Hydrochloride, a biguanide oral antihyperglycemic drug, typically indicated for patients with type II diabetes mellitus. Recent studies reveal its potential activation of adenosine monophosphate-activated protein kinase, which promotes angiogenesis, inhibits apoptosis, reduces oxidation, and facilitates regeneration. It is thought that drug repurposing may be beneficial in the setting of wound healing. The porous model is physicochemically and mechanically analyzed using scanning electron microscopy, thermogravimetric analysis, X-ray diffraction, drug release testing, and tensile testing. This bioprinted model can potentially support *in vitro* and *in vivo* wound closure and can be adapted with dynamic biomaterials and co-drugs, as well as with bespoke or layered dressings for personalized wound care.

Alginate and Alginate Sulfate/Polycaprolactone Nanoparticles as a Novel Drug Delivery System for the Delivery of Insulin-Like Growth Factor 1 (IGF1) in Cardiac Hypoxia/Reoxygenation

M. FARAJ[I], M. Karam[I], A. Jaffa[I], R. Mhanna[I]

[I]American University of Beirut, beirut, Lebanon

Myocardial infarction (MI) is associated with the highest mortality rates worldwide. Conventional treatments involve clot-dissolving agents and oxygen; however, MI is still linked with cardiac muscle damage. Metabolic molecules such as Insulin-Like Growth Factor 1 (IGF1) provide cardioprotective effects yet, have a short half-life reducing their bioavailability. Nanoparticle (NP)- based delivery systems were established to enhance bioavailability of the delivered IGF1; however, they lacked the growth factor affinity binding and controlled release. In this study, we engineered a novel IGF1 nanocarrier from pure alginate (PA) or growth factor affinity binding sulfated alginate (SA) polymers, with polycaprolactone (PCL), to form double emulsion NPs. This delivery system was assessed in vitro on neonatal rat cardiomyocytes (NCM) after hypoxia/reoxygenation (H/R) injury. Viability and activity were assessed using trypan blue and MTT assays. Protein expression and synthesis were evaluated using polymerase chain reaction (PCR) and western blotting. The NPs had a size of 200-250 nm and a zeta potential of -11 to -13.3 mV, which renders them suitable for drug delivery. The NPs were biocompatible, shown by absent significant cell death upon treatment with NPs (5-100 μg/mL). Additionally, without treatment, a significant 46% ± 10% decrease (P=0.01) in NCM viability was observed after H/R injury with a significant 1.65-fold ± 0.12 increase (P=0.04) in the transcription of cell survival markers. However, treatment of NCM with IGF1 loaded PA and SA NPs post H/R, reduced cell death to only 4% ± 11% (P=0.007) and increased the expression of pAkt cell survival pathway. With the obtained promising results and further studies, IGF1 loaded alginate/PCL delivery system may be adopted as an MI treatment. While the developed nanocarrier was evaluated for MI, it represents a versatile platform that can be adopted for other diseases requiring affinity binding to different growth factors.

Optimizing microfluidic-synthesized solid lipid nanoparticles and nanostructured lipid carriers for dermal delivery: a comparative evaluation

G. Gabbricci[I], I. Clemente[I], L. Talarico[I], A. Magnani[I]

[I]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Siena, Italy

The main challenge in topical delivery is to overcome the skin protective barriers, the outermost being the stratum corneum (SC) which prevents trans-epidermal water loss and the penetration of external substances into the skin. One promising approach for overcoming skin barrier and enhancing the delivery of actives is the use of lipid nanoparticles. Among them, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are suitable for dermal delivery. Their high affinity for the SC allows a higher penetration of the encapsulated molecules[2]. Despite the benefits offered by SLNs, their application remains restricted due to the long preparation times, high concentrations of reagents and significant variability in nanoparticle size and PDI across different batches that characterize the current bulk production methods. However, these limitations can be overcome with innovative microfluidic techniques that enable the reproducible and scalable production of smaller nanoparticles with narrower size distribution, highly desirable for an effectively delivery of bioactives through biological barriers.
In this work, different formulations of SLNs and NLCs were synthetized with a microfluidic apparatus and size, PDI, and encapsulation efficiency of two model molecules were optimized using an experimental design approach. The investigation was focused on the impact of the composition on the crystalline and supramolecular arrangement. The size was measured with Dynamic Light Scattering, the supramolecular and crystalline structure was performed by SAXS/WAXS measurements. Calorimetry was performed to assess the transition of the lattice core and cargo quantification was done with HPLC.

Micromanipulation as a tool to study biological membranes under mechanical stress

Z. Johanovská[I,II], D. Šťastný[III,IV], P. Kapusta[III], P. Jurkiewicz[V], R. Šachl[III], M. Hof[III]

[I]Institute of Physics, Faculty of Mathematics and Physics, Charles University in Prague, Prague, Czech Republic, [II]J. Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences, Dolejškova 3, Prague, Czech Republic, [III]J. Heyrovsky Institute of Physical Chemistry, Dolejškova 2155/3, Praha, Czech Republic, [IV]Charles University in Prague, Faculty of Science, Prague, Czech Republic, [V]BIOCEV, Prumyslová 595, Prague, Czech Republic

In cells, various membrane events are influenced and sometimes even controlled by changing of the tension on the membrane or their curvature (for example movement of the cells, influence on membrane proteins etc.). Micromanipulation technique brings new, so far not fully explored possibilities to this biomembrane research, as it enables direct modifications of membrane mechanical properties of giant unilameral vesicules (GUVs), simulating cell membrane.
The combination of micromanipulation with other methods such as fluorescence microscopy allows us to open the door to many exciting experiments. Especially we are interested in the combination of micromanipulations with our frequently used method MC-FRET, which allows us to detect and characterize membrane nanodomains. Also studying of the protein-membrane interactions and influence of the proteins on mechanical properties of the cells are into our focus.

Exploring Polymer-Protein Interactions: Biochemical Simulations for Inflammatory Bowel Disease

K. kamlesh[I], D. Cheung[I], Y. Rochev[I]

[I]https://curamdevices.ie/our-people/kamlesh-kamlesh/, Galway, Ireland

Targeted and localized drug delivery systems tailored for the colon have garnered considerable attention for delivering therapeutics, including drugs, proteins, and peptides, directly to sites affected by inflammatory bowel disease (IBD). Understanding conformational changes in mucus proteins and polysaccharides is critical for deciphering cellular function regulation and characterizing biomaterial adsorption, which plays a key role in the development of novel IBD therapies. Even minor variations in surface chemistry and protein sequences can substantially influence hyaluronic acid (HA) adsorption onto the Mucin network (comprising both gelling and non-gelling proteins). To investigate this, molecular dynamics (MD) simulations are utilized to identify molecular factors that regulate the behavior of HA and Mucin proteins across varying pH conditions. MD simulations serve as a better complement to experimental assays, offering nanoscale and atomic-level insights into adsorption mechanisms and reaction processes. The results indicate significant alterations in protein-HA comlplex structure at varying pH levels, influenced by interactions such as electrostatic forces. Binding energy calculations were performed to analyze conformational changes over a 400 ns time scale at varying concentrations.

OWLS for online monitoring protein adsorption and cell adhesion

B. Kovacs[I], A. Saftics[II], I. Szekacs[II], H. Jankovics[III], S. Kurunczi[II], F. Vonderviszt[III], R. Horvath[II]

[I]Semilab Semiconductor Physics Laboratory Co. Ltd., Budapest, Hungary, [II]Nanobiosensorics Laboratory, HUN-REN Centre for Energy Research, Institute of Technical Physics and Materials Science, Budapest, Hungary, [III]Bio-nanosystem Laboratory, Research Institute of Biomolecular and Chemical Engineering, University of Pannonia, Budapest, Hungary

During our work, we have characterized genetically modified protein coatings and mammalian cell adhesion in detail using optical waveguide lightmode spectroscopy (OWLS). OWLS is a label-free optical biosensor whose sensing principle is based on the evanescent electromagnetic field of guided light. This measuring system provides not only kinetic information about the layer formation, but also the surface mass density can be calculated. Other advantages are the possibility of chemical surface modification of the sensor surface for different applications and the transparency of the optical chip, which allows the insertion into a microscope immediately after the experiment.
The applied protein was the different variants of flagellin, which is the major building block of bacterial flagellar filaments. Flagellin consists of four domains: D0, D1, D2, and D3, with the D0 domain containing amphipathic helical regions. This part of flagellin is disordered in solution but can be used to anchor the protein to hydrophobic surfaces with the D3 domain pointing towards the solution. The hypervariable D3 is a largely independent part of the flagellin that can be removed or replaced without disturbing filament formation. The adsorption of flagellin was influenced with Hofmeister salts. The monolayer of wild-type flagellin mimics the surface of the bacterial flagellar filament, and we hypothesized that oriented flagellin layers have bacteria-repellent properties. To prove this, we studied the adhesion of bacterial E. coli and human cancer cells on oriented wild-type flagellin layers.
Through genetic modification, specific oligopeptide segments can be also inserted into the D3 domain of flagellin, which can induce cell adhesion through integrin receptors. We studied cancer cell adhesion on this genetically engineered protein layers too. Our results prove, that flagellin can be used in many ways in creating capture layers in biosensors.

Engineering Biomimetic Liposomes: A Bottom-Up Approach

G. Lodigiani[I,II], A. Gori[II], R. Frigerio[II], F. Baldelli Bombelli[I], G. Bergamaschi[II]

[I]Politecnico di Milano, Milano, Italy, [II]SCITEC-CNR, Milano, Italy

Liposomes are synthetic lipid-based nanoparticles, that hold significant potential in drug delivery and therapeutic applications due to their biocompatibility, ability to encapsulate drugs, versatility in functionalization, and scale-up manufacturing. However, the lack of knowledge about the connection between liposomes surface properties and their biological features severely affects their clinical application. On the other hand, extracellular vesicles (EVs) are biogenic nanoparticles naturally designed by cells to navigate through biological fluids. However, their clinical application is hampered by complexity in engineering, low recovery, and cost of production.
Therefore, this study focuses on optimizing liposome formulations to closely mimic EV surface properties, aiming to enhance their targeting and improve cellular uptake. This study aims to optimize liposome formulations to closely mimic EV surface properties, enhancing targeting and cellular uptake. We adopted a bottom-up approach, using EV lipidomic data to define lipid composition and synthesize biomimetic liposomes (EXO). Characterization via NTA, DLS, ζ potential, and membrane rigidity assays confirmed that EXO liposomes exhibit size, morphology, surface charge and membrane stiffness comparable to HEK 293-derived EVs.
EXO biocompatibility was evaluated by measuring cell viability, which remained close to 100% across different liposome concentrations. Additionally, we successfully functionalized the liposome surface with a peptide probe able to target a specific receptor. This was confirmed using a microarray immunoaffinity assay, which demonstrated a strong dose-dependent response across varying liposome concentrations.
These findings highlight the potential of our bottom-up approach to design functionalized liposomes as biocompatible drug delivery systems, advancing their potential for targeted therapy and diagnostic applications.

Modified Glycogen Nanoparticles for Nucleic Acid Delivery Targeting Immune Functions

H. Mårtensson[I], R. Rajgopalan Nair[I], A. Stubelius[I]

[I]Department of Life Sciences, Chalmers University of Technology, Gothenburg, Sweden

Nucleic acid therapies modulate gene expression by delivering nucleic acids including DNA and mRNA to cells with applications in areas of vaccines, gene therapies, and immunotherapies. However, nucleic acids are limited by their instability in biological systems. Efficient delivery systems are essential for the success of nucleic acid therapies, and polymer-based nanoparticles have emerged as promising delivery vectors.
This study develops and evaluates naturally occurring glycogen nanoparticles, as delivery vehicles for nucleic acid therapies. To enable complexation with anionic nucleic acids, the glycogen is modified with different polyamines to introduce cationic properties. The particles are evaluated based on their loading and delivery efficiency of in-house synthesized mCherry-encoding mRNA into cells. Additionally, a polysaccharide coating is added to enhance transfection efficiency and enable targeting of specific immune cells. Improved delivery vehicles could significantly advance nucleic acid therapies, enabling safer and more effective treatments for patients.

The physico-chemical roots of antimicrobial effect of surfactants: interaction with phospholipid membranes

H. Mateos[I], M. Oliver[II], P. Giannone[I], A. Mallardi[III], G. Palazzo[I]

[I]Università di Bari, Bari, Italy, [II]Universitat de les Illes Balears, Palma de Mallorca, Spain, [III]CNR IPCF, Bari, Italy

From the soaps and disinfectants we use in our everyday life to antiseptic solutions in clinical settings, surfactants play a crucial role in disrupting lipid membranes, a key mechanism underlying their antimicrobial action. Despite their widespread use, the fundamental physicochemical interactions between surfactants and phospholipid bilayers remain incompletely understood. In this study, we systematically investigate the effects of surfactants on model membrane systems under diverse ionic strength conditions.

Using liposomes of ~100 nm composed of either pure phosphatidylcholine (PC) or a PC/phosphatidylglycerol (95:5) mixture, we assess the impact of three surfactants —anionic sodium dodecyl sulfate (SDS), cationic dodecyltrimethylammonium bromide (DTAB), and non-ionic octaethylene glycol monodecyl ether ($C_{10}EO_8$). Their effects are studied over a broad concentration range, going from concentrations below and above the critical micelle concentrations (cmc). To probe the extent of bilayer perturbation, we perform calcein leakage assays to quantify membrane permeabilization, Laurdan fluorescence experiments to assess changes in bilayer hydration and packing, and dynamic light scattering (DLS) and zeta potential measurements to monitor vesicle size and surface charge alterations. Experiments are conducted under both low (10 mM phosphate buffer) and high (PBS) ionic strength conditions to explore the role of electrostatic interactions.

These insights have direct implications for optimizing antimicrobial formulations, enhancing drug delivery strategies, and designing more effective surfactant-based applications in healthcare, cosmetics and industry. Beyond its practical significance, this work also contributes to the broader understanding of how amphiphilic molecules interact with biological membranes, a fundamental question in colloid and interface science.

Salivary Histatin 5/6 peptides: adsorption on mesoporous silica nanoparticles

G.M. Meloni[I], C. Carucci[I], C. Contini[II], G. Guadalupi[II], A. Olianas[II], A. Salis[I]

[I]Università di Cagliari, Dipartimento di Scienze Chimiche e geologiche, Cittadella Universitaria di Monserrato SS 554 Bivio per Sestu, 09042, Cagliari, Italy,
[II]Dipartimento di Scienze della Vita e dell'Ambiente, Università di Cagliari, 09042, Monserrato (CA), Cagliari, Italy

Histatin 5 and 6 (H5/H6) peptides have a strong fungicidal activity against the pathogenic yeast *C. albicans*, which is known for causing, among others, skin rushes, itching, blisters and other infections in the gastrointestinal trait and oral cavity. The adsorption on mesoporous silica nanoparticles (MSN) is a promising strategy to exploit the peptide's antifungal properties for drug delivery system development. For this purpose, two types of materials have been tested: MSN and amino functionalized MSN (MSN-NH_2). Those nanoparticles were synthesized and fully characterized through several physicochemical techniques as SAXS, TEM, N_2-adsorption isotherms, ATR-FTIR, DLS, ELS and TGA. The H5/H6 samples were extracted from human saliva and purified by HPLC. The good outcome of purification was qualitatively tested through HPLC-MS technique. Then, the purified peptides were dissolved in a 10 mM Tris-HCl buffer solution at pH 7 and adsorbed both on MSN and MSN-NH_2. The resulting peptide adsorbed amount, quantified by bicinchoninic acid assay, was higher for the bare MSN rather than MSN-NH_2 (8.49 ± 0.08 mg g^{-1} vs. 4.22 ± 0.08 mg g^{-1}, respectively). These results can be rationalized in terms of intermolecular interactions among the peptides and the bare/functionalized MSN surface.

Phosphorene as an Antimicrobial Agent: insights into its interaction with Bacterial Membranes

A. Papalini *[I], G. Brancolini *[I]

[I]Istituto Nanoscienze – CNR-NANO, Center S3, via G. Campi 213/A, MODENA, Italy

Nanomaterials have recently attracted great interest in therapeutic applications against viruses and bacteria as alternatives to classical therapies, such as antibiotics. Among different nanomaterials, phosphorene nanoparticles (BP) stand out for their novel properties, including light absorption and singlet oxygen generation for photothermal and photodynamic antibacterial therapies. This study employs a multilevel computational approach, combining ab initio quantum mechanics and classical molecular dynamics, to investigate the interaction between BP nanoparticles and bacterial/viral biomolecules.
Specifically, the project focused on studying the antimicrobial activity of phosphorene particles by examining their interactions with realistic bacterial membrane models. Bacterial membranes show significant diversity in their lipid composition and external structure and are generally classified into two groups: Gram-positive and Gram-negative bacteria.
To accurately study the differences between the two types of bacteria, two different bacterial membrane models were created using CHARMM-GUI, each incorporating key structural elements characteristic of each type. The Gram-negative model includes an outer layer of lipopolysaccharide (LPS), while the Gram-positive model has a thick layer of peptidoglycan (PG).
It is essential to study the mechanism of interaction with these outer layers because they are the first to encounter antimicrobial agents. To explore the interactions between the BP nanosheets and the two membrane models, atomistic molecular dynamics (MD) simulations were performed, varying the shape and size of the nanoparticles.
The results show how the shape and size of the phosphorene nanoparticles influence membrane interactions, translocation rates, and photokilling effects. Furthermore, we examined the specific interactions between the nanoparticles and distinct molecular components of the membranes, providing insights into their potential as antimicrobial agents.

* The authors marked with an asterisk equally contributed to the work.

The guardian of the cell surface: Towards understanding selective transport across the glycocalyx

J. Pavljuk[I,II], A. Roberts[I,II], S. Jana[I,II], B. Turnbull[III,IV], S. Radford[III], J. Kwok[I,V], R. Richter[I,II]

[I]School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom, [II]School of Physics and Astronomy, Faculty of Engineering and Physical Sciences, Astbury Centre for Structural Molecular Biology, and Bragg Centre for Materials Research, University of Leeds, Leeds, United Kingdom, [III]The Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, United Kingdom, [IV]School of Chemistry, University of Leeds, Leeds, United Kingdom, [V]Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic

The outer layer of all eukaryotic cells is comprised of a complex meshwork rich in sugar polymers, called the glycocalyx. Biological colloids, such as viruses and signalling molecules, need to diffuse through the glycocalyx to reach and interact with their cell surface receptors. Multiple and often transient interactions between the biocolloid and the polymer meshwork of the glycocalyx ensure proper biocolloid distribution in the extracellular matrix and receptor access to trigger downstream cell signalling responses. The same mechanisms, however, are also hijacked by viruses and toxins to gain access to their host cells. The molecular and physical mechanisms that define the rate of diffusion through a sticky glycocalyx are not well understood. To address this question, *in vitro* models of the glycocalyx with a well-defined polysaccharide presentation and tunable interactions between the sugar polymers and the biocolloids are required, as the heterogeneity of real glycocalyces *in vivo* precludes mechanistic analyses.

In this work, a well-defined interaction model is presented, featuring a model glycocalyx film made of high molecular weight hyaluronan (HA) anchored with one end to a planar surface (thus forming a polysaccharide 'brush'), along with fluorescent liposomes as size-defined model colloids. An improved methodology to probe diffusion within model glycocalyces by plane-sphere confinement microscopy (PSCM) and single-particle tracking (SPT) is also presented.

The assay can now be used for a systematic analysis of the diffusion of non-sticky model colloids in HA films depending on the colloid size relative to the mesh size of the HA film, and further expanded to include the effect of interactions between colloids and HA. The resulting data can be used to build and test theoretical models of colloid diffusion in a sticky matrix, and the insights gained can be used to understand transport processes across the glycocalyx and how it can be modulated in disease.

Nanomechanical and surface topography analysis of agarose–silk fibroin hydrogels

T. Plichta[I], V. Richterová[I], M. Khýrová[I,II], J. Lukeš[III], J. Sepitka[III]

[I]Institute of Scientific Instruments of the CAS, v. v. i., Kralovopolska 147, 612 64, Brno, Czech Republic, [II]Faculty of Chemistry, Brno University of Technology, Purkynova 118, 612 00, Brno, Czech Republic, [III]Faculty of Mechanical Engineering, Czech Technical University in Prague, Technicka 4, 166 07, Praha 6, Czech Republic

Naturally, the biological and biochemical characteristics of agarose gels can be altered by adding fibroin, therefore, this study provides a comprehensive characterization of mechanical and surface properties of the prepared agarose–silk fibroin hydrogels by nanoindentation and AFM, respectively. These hydrogels offer a broad range of applications in tissue engineering, controlled release transport, and ECM systems because of these characteristics and the ability to precisely customize them. Gained information improves comprehension of hydrogels' mechanical and surface properties and adhesion energy that can help to their potential of application. Various concentrations of agarose (1 or 2 %) and fibroin (up to 4.5 %) as interpenetrating components were prepared. Performed measurements allowed us to observe the elastic modulus of the hydrogel networks and adhesion energy together with surface topography roughness, therefore study them at the nano-/microscale. The obtained load vs. displacement and load vs. time curves were analysed according to the analytical models of Hertz, DMT, JKR and relaxation theory. Their suitability was assessed and the modulus of elasticity and energy of adhesion were determined. Because of the intrinsic viscoelasticity of the polymer network, hydrogels, like most polymers, show time-dependent mechanical behaviour. However, fluid movement also causes a time-dependent deformation process. As a result, time plays a significant role in the design and implementation of mechanical experiments on hydrogels, which can be described in the frequency or time domain. These gels showed a wide range of elastic modulus values depending on the different crosslinking as indicated by the mesh sizes of these materials, as shown by AFM. The elastic modulus determined using the relaxation model was 108.3 kPa-1.2 kPa. Using nanoindentation data and JKR analysis, the adhesion energy of these gels was also calculated and found to be 0.07 $J \cdot m^{-2}$-0.03 $J \cdot m^{-2}$.

Mechanical and transport properties of collagen modified agarose hydrogels

V. Richterova[I,II], T. Plichta[I], M. Khyrova[I,II], M. Pekar[II]

[I]Institute of Scientific Instruments of the Czech Academy of Sciences, Kralovopolska 62/147, 612 00, Brno, Czech Republic, [II]Institute of Physical and Applied Chemistry, Faculty of Chemistry, Brno University of Technology, Purkynova 464/118, 612 00, Brno, Czech Republic

The extracellular matrix (ECM) is a complex biological structure that combines a fibrous component with a hydrogel-like environment, influencing both mechanical and transport properties. To model these characteristics, agarose hydrogels modified with collagen were studied as simplified ECM-inspired systems. Agarose, a linear polysaccharide, forms biocompatible hydrogels, while collagen, the dominant structural protein in ECM, plays a key role in tissue integrity and molecular transport.
In this study, hydrogels were prepared with three concentrations of agarose (0.5, 1.0, and 2.0 wt. %) and three concentrations of collagen (0.01, 0.05, and 0.1 wt. %). Their rheological properties were characterized using amplitude and frequency sweep tests on a rotational rheometer (Anton Paar MCR 72) to evaluate viscoelastic behavior and mesh size. Transport properties were investigated using a diffusion from a constant source model, where the penetration of positively charged methylene blue and negatively charged eosin B was monitored via UV-VIS spectrophotometry (Cary50, Varian) at defined time intervals for effective diffusive coefficients determination.
Although collagen did not significantly alter the fundamental rheological character of agarose hydrogels, it influenced their viscoelastic moduli and mesh size depending on the concentration of both components. The diffusion coefficients of the oppositely charged dyes revealed the impact of electrostatic interactions on molecular transport. Understanding how collagen incorporation affects agarose hydrogel properties is crucial for designing biomaterials with tunable mechanical and transport characteristics. These findings contribute to the development of hydrogel-based systems for applications in tissue engineering and controlled molecular delivery.

Three-Dimensional In Vitro Blood-Brain Barrier Model Utilizing Hydrogels

J. Saliba[I], N. Ahmad[I], G. Kiriako[I], M. El-Sabban[I], R. Mhanna[I]

[I]American University of Beirut, Hamra, beirut, Lebanon

The central nervous system homeostasis is tightly regulated by the blood-brain barrier (BBB) and its impairment is the hallmark of many neurodegenerative diseases (NDD). Understanding the cellular and molecular mechanisms underlying BBB dysfunction is vital in investigating the progression of NDD. The human brain is highly vascularized and estimated to have a capillary to every neuron with a total surface area of almost $20m^2$. The BBB is mainly comprised of specialized endothelium, and astrocytic foot processes which play a major role in maintaining the BBB characteristics. Herein, we demonstrate biomimetic hydrogels using gelatin methacrylate (GelMA) and polyethylene glycol (PEG) as supporting structures for cell-laden models of the BBB. The BBB models were created by co-culturing endothelial cells (EC) on top of the hydrogels embedded with astrocytes. Embedded astrocytes had high viability (>80%) after 7 days with star-shaped morphology. Gene expression of claudin-5, tight junction protein, was significantly higher in EC cultured on GelMA compared to plastic. The barrier tightness was assessed by transendothelial electrical resistance (TEER) measurements of EC cultured on GelMA hydrogels enbedded with or without astrocytes and were higher to the plastic counterparts. Similarly, TEER measurements of endothelial cells cultured on PEG hydrogels functionalized with both RGD (fibronectin recognition sequence) and IKVAV (laminin recognition sequence) (1:1) with astrocytes had a statistically higher TEER ($55.33\pm1.47\ \Omega.cm^2$ at day 5), compared to the individual peptides and 2D controls. These novel models utilize hydrogels to mimic the basement membrane of the BBB and the ECM of the brain. This allows direct communication between astrocytes and endothelial cells which enhances endothelial barrier function. The hydrogel models allow us to examine the role of ECM and tight junction formation which will provide further insight into BBB function and dysfunction.

Single Amino Acid Drives LL-37 and HNP1 Synergy: Unveiling Antimicrobial Peptide Interactions

A.M. Schwitter[I], K. Sugihara[II]

[I]The University of Tokyo, Tokyo, Japan, [II]Institute of Industrial Science, The University of Tokyo, 4-6-1 Komaba, Meguro City, 153-8505 Tokyo, Tokyo, Japan

Antimicrobial peptides (AMPs) are promising agents against antibiotic-resistant bacterial infections. Unlike traditional antibiotics, AMPs target lipid bilayers, slowing resistance development. The synergy between human AMPs LL-37 and α-defensin HNP1 enhances bacterial killing while reducing host cell damage. This phenomenon, termed double cooperativity, offers the potential to minimize side effects in clinical AMP applications.

The structural basis of this synergy remains unclear. We investigated additional peptide pairs exhibiting double cooperativity and identified critical amino acids involved. The membrane-disruptive activity of LL-37 and its modulation by HNP1-4 and β-defensin hBD1 were studied using POPC model membranes. Fluorescence recovery after photobleaching (FRAP) confirmed the membrane-disruptive action of LL-37 and its suppression by HNP1, HNP3, HNP4, and hBD1. Membrane permeabilization assays using calcein-encapsulated POPC vesicles corroborated these findings, showing the same defensins reduced LL-37-induced dye leakage. Circular dichroism (CD) assessed secondary structure changes of LL-37 and its mixtures with defensins. LL-37 oligomerized in solution, and adding HNP1-4 or hBD1 did not significantly alter LL-37's structure. This suggests hetero-oligomerization does not disrupt LL-37's structure but prevents its dispersion on membranes.

Our findings indicate the first amino acid, the key difference between HNP1 and HNP2, plays a crucial role in double cooperativity. This study provides molecular insights into the synergistic interactions of these AMPs and their effects on lipid membranes.

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The effect of physiological cations on the polydiacetylene mechanochromism and the structures

R. Tamaki[I], E. Schneck[II], K. Sugihara[I]

[I]Institute of Industrial Science, The University of Tokyo, 4-6-1 Komaba Meguro-Ku, Tokyo, Japan, [II]Institute for Condensed Matter Physics, Technical University Darmstadt, Hochschulstrasse 8, Darmstadt, Germany

Polydiacetylene (PDA) is a mechanochromic polymer that detects forces by the twist of its conjugated backbone and changes its color or emits fluorescence. A variety of biosensors have been developed for the detection of temperature, ions, biomolecules etc. using this blue-to-red transition of PDA. In 2018, the Sugihara Group have improved the accuracy of friction force microscopy (FFM), which is a technique to quantify shear forces at nanoscale [1] and have demonstrated the first application of this technique on PDA [2].
X-ray reflectivity (XRR) is extremely sensitive to the thicknesses and electron densities of self-assembled layers, although it cannot provide information about lateral features [3]. Hence, the combination of XRR and FFM, which provides lateral information, can effectively characterize the in situ properties of materials.
Zheng et al. revealed the enhanced force sensitivity of PDA by pH and NaCl [4]. However, the effects of pH and NaCl alone do not allow for perfect control of force sensitivity and are still difficult to develop into a product. Since lipid membranes have a kind of ion selectivity [5], it is necessary to investigate how each ion (K^{+}, Ca^{2+}, Mg^{2+}) affects the PDA property.
Our results will clarify the effect of ions on the PDA force sensitivity and its structure. Four aqueous solutions of NaCl, $MgCl_2$, KCl and $CaCl_2$ under different concentration conditions were used for FFM force sensitivity measurements. The thermochromic temperature was measured by UV-vis spectroscopy under similar conditions. The layer's electron density profile and the density of adsorbed counterions, which inform about the deprotonation degree, were investigated by XRR.

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SAXS insights into protein-protein interactions: effects of ATP and TPP on long-range repulsion of rHSA

S. Tan[I], R. Curtis[II]

[I]Manchester Institute of Biotechnology, 131 Princess Street, Manchester M1 7DN, England, Manchester, United Kingdom, [II]Department of Chemical Engineering and Analytical Science, University of Manchester, Sackville Street, Manchester, M13 9PL, UK, Manchester, United Kingdom

The effective structure factor (S_{eff})as a function of the scattering vector q was obtained from SAXS intensity profiles, revealing that adenosine tripolyphosphate (ATP) and tripolyphosphate (TPP) significantly influence both the range and magnitude of the interparticle potential due to ion-ion correlations. Notably, the observed range is considerably longer than what is predicted by mean-field theories such as DLVO theory. These findings suggest that ATP and TPP effectively mitigate rHSA aggregation and enhance repulsive protein-protein interactions by inducing overcharging through non-specific binding. This study underscores the potential of polyvalent anions in improving the colloidal stability of protein formulations, which is crucial for controlling protein aggregation in biopharmaceutical development.

Multivalent Binding of Antibody-precomplexed Hemagglutinin Constructs at Interfaces

D. Tambuwun[I], M. Rios Carrasco[II], R.P. de Vries[II], J. Huskens[I]

[I]Molecular Nanofabrication, Department of Molecules and Materials, University of Twente, Enschede, Netherlands, [II]Department of Chemical Biology & Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, Netherlands

Infections of influenza viruses begin with the attachment of the virus particles to cell surfaces decorated with glycans using multivalent interactions. Hemagglutinin (HA)-glycan monovalent interactions are weak, typically having a dissociation constant (Kd) in the millimolar range. One way to enhance the affinity is to precomplex multiple HAs together using antibodies (ABs). How exactly this binding enhancement of AB-precomplexed HAs translates from monovalent to multivalent, however, remains elusive. In this work, the binding of AB-precomplexed HA constructs is analyzed on glycan-containing supported lipid bilayers (SLBs) as cell membrane mimics, with controlled surface receptor density. The individual affinity and the overall avidity of multivalent HAs binding to glycan surfaces are determined by titrations performed at varying glycan densities and interpreted using multivalency theory. By comparing the multivalent binding of AB constructs with different valencies, we are able to deduce that additional interactions lead to larger constructs than anticipated, which in turn provide higher activities in hemagglutination and in multivalent binding.

Deformable liposome made with glycine betaine ester surfactants

M. Tozzi[I], C. Carucci[I], A. Salis[I], F. Cesare Marincola[I]

[I]University of Cagliari, Cagliari, Italy

Recently surfactants have been used in combination with lipids to obtain a particular type of liposomes, referred to as deformable liposomes, or transferosomes. Transferosomes are characterized by the presence of an edge activator, a substance, usually a surfactant, that changes important features of liposomes, such as hydrodynamic diameter, zeta potential, colloidal stability and deformability. Among the above features, deformability has been found to be favorable for transdermal drug delivery.
Glycine betaine ester surfactants (GBOCn) have been described in the literature as cleavable surfactants, that is, surfactants that are easily hydrolyzed, making them more biocompatible compared to traditional cationic surfactants.
GBOCn are readily synthesized by an esterification reaction starting from natural origin reactants such as glycine betaine, a molecule derived from amino acids, and heavily present in foods such as beetroot, and long chain fatty alcohols.
In this work different fatty alcohols have been used to synthesize various GBOCn, namely 1-decanol (GBOC10), 1-dodecanol (GBOC12), 1-tetradecanol (GBOC14) and 1-hexadecanol (GBOC16). The final products have been characterized using NMR and FTIR spectroscopy.
GBOCn have been combined with 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) to obtain deformable liposomes. The systematic study of the effects of chain length, ionic strength of the dispersant solution and surfactant molar percentage on the size and zeta potential has been carried out by means of Dynamic Light Scattering (DLS) and Electrophoretic Light Scattering (ELS) measurements.
In addition, hydrolysis kinetics of the GBOCn alone and in the liposomes are being carried out using NMR spectroscopy, together with Krafft point measurements and CMC determination of the surfactants.
Future work includes entrapment of a hydrophilic small molecule together with in vitro measurements of transferosome effects on human cells.

Examining Multi-Component Lipid Membranes for Controlled Interaction with Nanocarriers

K. VAID[I], M. Goncerz[I], A. Kamińska[I], B. Jachimska[I]

[I]IKIFPAN, Krakow, Poland

The aim of the study was to comprehensively characterize lipid membranes with variable architecture and composition in the context of their interactions with dendrimer nanocarriers. The viscoelastic properties of these membranes were monitored using a quartz crystal microbalance with energy dissipation (QCM-D). The high dissipation energy of lipid membranes is related to the elastic nature of the layers formed. Notably, the tested dendrimer carriers exhibit irreversible adsorption on the lipid surface. Understanding the interaction mechanism between lipid membranes and dendrimers is crucial for deciphering how these polymeric carriers are internalized into living cells. To determine the nature of these interactions, Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR), and electrophoretic mobility measurements were employed. Spectroscopic techniques provided detailed information on alterations in the structure of the membrane, while changes in the zeta potential of the initial lipid systems indicate the dominant role of electrostatic interactions on the adsorption efficiency of dendrimer molecules on the lipid surface. The conducted research may prove helpful in designing Drug Delivery Systems (DDS), taking into account the effectiveness of their internalization in biological systems.
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The Relationship Between Structural Components in MUC5B Lubrication

A. Weston[I]

[I]King's College London, London, United Kingdom

Salivary mucin MUC5B plays a crucial role in oral lubrication, yet the molecular determinants underlying its function remain incompletely understood. This study investigates the contribution of specific structural domains and glycan moieties to the lubricating properties of MUC5B, focusing on the role of sodium ions in modulating these effects. Recombinant expression of MUC5B terminal end peptides revealed that these domains, while inherently lubricating, exhibit limited responsiveness to sodium, suggesting they contribute primarily to mucin-surface adsorption rather than hydration lubrication. Enzymatic desialylation of native MUC5B reduced lubrication marginally under physiological conditions, but in the presence of 200 mM NaCl, both native and desialylated MUC5B displayed a dramatic (>95%) decrease in friction. This underscores a sodium-dependent lubrication mechanism that is independent of sialic acids. SAXS and TEM analyses suggest that sodium ions induce conformational condensing of the mucin polymer, likely by screening electrostatic repulsions between glycans. Interestingly, desialylated MUC5B showed reduced conformational adaptability in response to salt, highlighting sialic acids' role in structural flexibility, though not directly in lubrication. A computational model revealed that sodium ions bind broadly across MUC5B glycans and backbone, including to sialic acid, galactose, GalNAc and hydroxylated amino acids. Collectively, these findings support a multifaceted lubrication mechanism for MUC5B involving both structural condensation and hydration shell formation, mediated primarily by sodium interactions across diverse molecular moieties rather than through electrostatics alone. These insights advance our understanding of mucin function and have potential implications for addressing developing mucin-mimetic therapeutics.

Lipid metabolism as a mediator of sleep disturbances in Alzheimer's disease

D. Youngstrom[I], A. Sehgal[I]

[I]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States of America

Alzheimer's disease (AD) is a devastating progressive neurodegenerative disease that leads to memory loss and is becoming increasingly common with limited disease-modifying treatments. Sleep disturbances are frequently seen prodromally and throughout AD and other neurodegenerative diseases, where pathological features—including tau and amyloid-beta (Aβ)—shorten and fragment sleep, and chronic sleep disruption accelerates neurodegeneration in humans, mice, and Drosophila. Despite growing evidence for the bidirectional relationship between sleep and neurodegeneration, the underlying mechanisms remain poorly understood. We hypothesize that lipid metabolism alterations could be the missing link mediating the relationship between sleep and neurodegeneration. Our lab recently demonstrated the essential role of sleep in clearing glial lipid droplets (LDs) that accumulate following wake to mitigate oxidative damage in neurons. Furthermore, many top AD genetic risk factors identified through GWAS are involved in lipid metabolism, and early lipid alterations—such as lipid peroxidation and dysregulated sphingolipid metabolism—are implicated in AD onset and progression. We find that Drosophila models of AD recapitulate mammalian sleep loss phenotypes and have increased lipid droplets (measured by BODIPY staining) and elevated lipid peroxidation (measured by MDA staining). We aim to further dissect the mechanistic relationship between lipid alterations and sleep disturbances in AD flies, leveraging the strong genetic tools available in Drosophila. Attending this course will provide essential training in lipid biology and lipid-protein interactions, equipping me with the necessary expertise to advance this project and elucidate the role of lipid metabolism in AD-associated sleep disturbances.

DNA Barcoding for High-Precision Purification and Detection of Extracellular Vesicles

L. Zarini[I], P. Bergese[I], D. Brambilla[II], M. Chiari[II]

[I]Università degli Studi di Brescia, Viale Europa 11, Brescia, Italy, [II]SCITEC-CNR , via privata mario bianco, 9, Milan, Italy

Extracellular vesicles (EVs) hold great promise for diagnostic and therapeutic applications, particularly in cancer, where tumor-derived EVs serve as biomarkers for liquid biopsy. However, isolating specific EV subpopulations remains challenging due to their low abundance in body fluids. Conventional methods such as ultracentrifugation and size exclusion chromatography lack the ability to distinguish EV subtypes with similar physical properties.
To address this limitation, we developed a next-generation system for high-precision purification and detection of specific EV subpopulations. Immunoaffinity-based methods have been explored for capturing EVs based on surface markers, but these approaches often struggle with efficient release for downstream analysis. To overcome this challenge, we employed DNA-directed immobilization (DDI), in which antibodies are conjugated to single-stranded DNA (ssDNA). This allows target EVs to be captured via antibody binding, while complementary DNA strands facilitate their controlled release.
Furthermore, integrating DNA barcodes into this system enables multiplexed analysis of different EV targets within a single sample. Originally developed for species identification, DNA barcoding can be adapted using synthetic DNA strands, where each antibody is linked to a unique DNA sequence. This effectively "tags" specific EV subpopulations, allowing their simultaneous detection. This approach, which has already demonstrated success in detecting circulating tumor cells, is expected to enhance the sensitivity and specificity of EV analysis. Detection is achieved using Single-Particle Interferometric Reflectance Imaging Sensing (SP-IRIS), which enables single-EV resolution. By spotting different complementary DNA sequences onto a sensor surface, distinct regions can be assigned to various targets, facilitating precise and high-throughput EV profiling.

Double Cooperative Effects between Amphotericin B and LL-37

J. Zhang[I], K. Sugihara *[I]

[I]?153-8505 ????????4-6-1 Fe402, Tokyo, Japan

Amphotericin B (AmB) is a vital medication for treating severe systemic fungal infections. However, its notable side effects and toxicity can sometimes necessitate discontinuing treatment, even in the face of life-threatening fungal infections. Over the past 30 years, research has focused on developing AmB lipid formulations, which are now considered the "gold standard" in polyene therapy. The cytotoxic mechanism of AmB primarily involves its binding to cholesterol in the eukaryotic cell membrane, forming aggregates that create transmembrane channels. These channels allow cytoplasmic contents to leak out, ultimately leading to cell death. LL-37 is a 37-residue α-helical antimicrobial peptide derived from the human body, exhibiting broad-spectrum antifungal, antiviral, and immunomodulatory activities. However, its further clinical application is limited by its inherent cytolytic toxicity.
Inspired by the "Double Cooperative effects" proposed by Sugihara et al., we discovered that when Amphotericin B and LL-37, both of which possess significant cytotoxicity, are combined, the toxicity of each component in the mixture is attenuated. This results in enhanced safety for mammalian cells, while also yielding augmented antifungal efficacy. This dual-pronged synergistic strategy, which ensures both safety and effectiveness, offers a viable approach for the development of novel drugs against resistant fungi.

* The authors marked with an asterisk equally contributed to the work.

Polydiacetylene high-throughput assay for peptide screening

Q. zhu[I], K. Sugihara[I]

[I]The University of Tokyo, Tokyo, Japan

The objective of this research project is to tunning the electric charge, aggregate size, and polymerization efficiency of the PDA assay. This optimization is achieved by systematically varying the lipid composition in two distinct PDA systems. Aiming pinpoint the critical balance—referred to as the “sweet spot”—during the development of the PDA assay.[1]

Membrane-active peptides, including antimicrobial peptides, cell-penetrating peptides, and ionophores, often require time-consuming and costly techniques for functional characterization, creating a bottleneck in drug screening. In this study, we present a high-throughput, high-sensitivity peptide functional assay utilizing the mechanochromic polymer polydiacetylene (PDA). We demonstrate that optimizing the composition of PDA can significantly enhance its assay performance, increasing reaction speed by 4-fold and sensitivity by 2.6-fold. Moreover, the optimized PDA assay effectively captured the cooperative effect, offering superior performance compared to conventional assays.

Reference

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