Book of Abstracts

Bilbao, Spain: 25th-27th July, 2019

PROTEIN-LIPID NANOSTRUCTURES: FROM DOMAINS TO DEVICES

Abstracts of papers presented at the

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PROTEIN-LIPID NANOSTRUCTURES: FROM DOMAINS TO DEVICES

Bilbao, Spain, 25-27 July 2019

Scientific Organizers:

Vadim A. Frolov, Biofisika Institute (CSIC, UPV/EHU), Spain
Anna V. Shnyrova, Biofisika Institute (CSIC, UPV/EHU), Spain
Sergi Padilla-Parra, University of Oxford, United Kingdom
Aitor Hierro, CIC bioGUNE, Spain
David Rodriguez-Larrea, Biofisika Institute (CSIC, UPV/EHU), Spain

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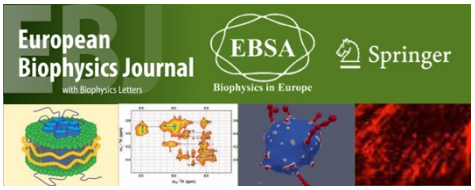

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Charles Coomer — *University of Oxford, UK*
Tetiana Mukhina — *Institute Laue-Langevin, FR*

Table of Contents

Oral

Poster

Oral

Binding affinity of membrane-inserted epitope to HIV-1 antibody and its dependency on lipids quantified by fluorescence correlation spectroscopy

Oral

Mr. Arturo García [1], ***Mrs. Johana Torralba*** [1], ***Dr. Pablo Carravilla*** [1], ***Dr. Beatriz Apellaniz*** [1], ***Dr. Jose Requejo-Isidro*** [2], ***Prof. Jose Luis Nieva*** [1]

1. *University of the Basque Country,* ***2.*** *CNB/CSIC*

Abstract

Broadly neutralising HIV antibodies (bnAbs) are defined by their ability to neutralise diverse HIV isolates. Among all described bnAbs, the ones that target the membrane-proximal external region (MPER) show the highest breadth (98% of tested isolates neutralised). MPER is localised in the interface of the viral membrane and thus, anti-MPER bnAbs have evolved to include membrane-interacting regions that permit MPER recognition in a lipid environment. Moreover, these antibody (Ab)-lipid interactions seem to be essential for the neutralizing activity of anti-MPER bnAbs, although their contribution to epitope binding is not fully understood.

In this work, we quantitatively study Ab-MPER interaction in its membrane context by means of fluorescence correlation spectroscopy (FCS). We have quantified the partition coefficient (K_p) of several 10E8 variants bound to MPER-bearing large unilamellar vesicles (LUV) of different compositions through the evaluation of the Ab diffusion regime. Our work builds on previously reported methodology used to assess peptide-membrane partitioning [1, 2], adding to it the complexity of a third component. Reliable K_p values were obtained upon careful quantification of the amount of accessible lipid on the vesicles. The uncertainty of the determined K_p was computed through support-plane analysis.

In conclusion, our measurements set a robust method for the quantitative determination of membrane partitioning in intact systems. We foresee that its application will shed light over the functional relevance of interactions between bnAbs and MPER in a lipid membrane environment.

References:

[1] Rusu L, Gambhir A, McLaughlin S, Rädler J. Fluorescence correlation spectroscopy studies of peptide and protein binding phospholipid vesicles. Biophys J. 2004

[2] Melo AM, Prieto M, Coutinho A. The effect of variable liposome brightness on quantifying lipid-protein interactions using fluorescence correlation spectroscopy. Biochim Biophys Acta - Biomembr. 2011

Keywords

Optical microscopy, FCS, HIV

Characterizing folded, unfolded, and oligomeric proteins on a single molecule level

Oral

Dr. Michael Mayer [1]

1. *Adolphe Merkle Institute*

Abstract

This talk will demonstrate the usefulness of nanopores in solid state membranes to characterize individual proteins and protein complexes. Specifically, this approach determines the volume, shape and dipole of protein particles as they translocate and rotate through an electrolyte-filled nanopore. To do so, we take advantage of modulations in electrical resistance of the pore as a function of the orientation of the protein particle relative to the electric field in the pore. We demonstrate the usefulness of this approach with three sets of experiments: 1) We estimate the most probable shape of intrinsically disordered proteins in aqueous solution. 2) We compare the shape of individual folded and unfolded proteins. And 3) we estimate the volume, shape and dipole moment of oligomeric aggregates of proteins that form amyloids and are involved in neurodegenerative diseases such as Alzheimer's disease. We suggest that the multiparametric characterization of individual protein particles in nanopores provides a fresh approach to rapid and solution-based routine protein analysis, biomarker detection, and fundamental biophysics studies on single proteins.

Keywords

nanopores in solid state membranes

Computing free-energy landscapes of co-operative changes of membrane topology

Oral

Prof. Marcus Mueller [1]

1. *Georg-August-University Göttingen*

Abstract

Using computer simulation and self-consistent field theory of coarse-grained models for lipid membranes, we study the free-energy landscape of collective phenomena that alter the topology of lipid membranes. These basic processes – pore formation, fusion and fission – often involve time scales of tens of nanometers and milliseconds that are large for atomistic simulation. Frequently, they involve transition states with high curvatures that are difficult to describe by Helfrich-like models. Coarse-grained models can access the relevant time and length scales, allow for a systematic exploration of parameters like the lipid architecture or membrane tension, and they are well suited to study collective phenomena that alter the topology of membranes.

The talk will discuss different computational techniques – Wang-Landau sampling, field-theoretic umbrella sampling, and the string method – to investigate metastable intermediates (like the stalk in the course of membrane fusion) and transition states of pore formation, membrane fusion and fission. Using coarse-grained models, we explore the universal aspects of topology-altering processes in membranes and comment on the extent, to which coarse-grained model capture specific effects of protein-mediated processes.

Keywords

pore formation, fusion, fission, simulation

Dynamic constriction and fission of ER membranes by reticulon

Oral

Dr. Andrea Daga [1], ***Mr. Javier Espadas*** [2], ***Dr. Diana Pendin*** [3], ***Dr. Rebeca Bocanegra*** [4], ***Dr. Artur Escalada*** [5], ***Dr. Borja Ibarra*** [4], ***Dr. Ariana Velasco*** [2], ***Dr. Anna Shnyrova*** [2], ***Dr. Vadim Frolov*** [6]

1. *IRCCS E. Medea,* ***2.*** *Biofisika Institute (UPV/EHU, CSIC); Department of Biochemistry and Molecular Biology, University of the Basque Country,* ***3.*** *CNR Italy,* ***4.*** *IMDEA Nanociencia,* ***5.*** *Biofisika Institute (UPV/EHU, CSIC),* ***6.*** *Biofisika Institute (UPV/EHU, CSIC); Department of Biochemistry and Molecular Biology, University of the Basque Country; Ikerbasque, Basque Foundation for Science*

Abstract

How ER morphology is established and maintained remains an open question. Tubule-to-tubule membrane fusion is known to occur and is crucial for ER maintenance, however, the molecular mechanism/s counterbalancing fusion to keep ER network morphology largely stable have long remained a lingering puzzle. In the search for this mechanism we focused on the reticulons, a protein family implicated in controlling ER morphology by stabilizing ER tubule formation through their intrinsic ability to create widespread membrane curvature. The starting point of this work was the discovery in Drosophila of a surprisingly robust antagonistic genetic interaction between atlastin and reticulon, suggesting the possibility that Rtnl1 may be involved in membrane fission. Building up on this finding, we demonstrate that Rtnl1 utilizes two synergistic modes of membrane curvature, static and dynamic, whose combination can produce either stable membrane curvature or fission, depending on intrinsic membrane dynamics. Our findings imply that in a living cell, the ability of Rtnl1 to produce membrane constriction is inseparable from its ability to produce fission. The intrinsic sensitivity to membrane movement suggests a novel paradigm of dynamic regulation of ER topology, linking membrane fission to membrane motility.

Keywords

reticulon drosophila fission membrane

Dynamic membrane remodelling by the ESCRT-III complex

Oral

Prof. Aurelien Roux [1]

1. *University of Geneva*

Abstract

The Endosomal Sorting Complex Required for Transport-III (ESCRT-III) is the ubiquitous complex in the cytosol that catalyzes membrane fission from inside of membrane necks via a yet unknown mechanism. Using a variety of in vitro reconstituted systems, we describe the full reconstitution of the ESCRT-III complex at the membrane with six proteins: Snf7, Vps2, Vps24, Did2, Ist1 and Vps4. We show that because of the variable affinities of each of these subunits toward Vps4, and between them, the subunits are recruited in a Vps4-driven sequence that begins with Snf7/Vps24 and ends with Did2/Ist1. In this sequence, Vps2 is pivotal, as it stabilizes Vps24, that then is replaces by Did2. All subunits are in a final step exchange or depolymerized by the action of Vps4 allowing recycling of the ESCRT-machinery. We further show that this sequence is accompanied by membrane protrusion that are later constricted and fissioned.

Keywords

ESCRT-III

membrane deformation

membrane fission

Extracellular vesicles interaction with model membranes

Oral

Dr. Pietro Parisse [1], ***Dr. Fabio Perissinotto*** [1], ***Dr. Loredana Casalis*** [1], ***Dr. Valeria Rondelli*** [2]

1. *Elettra Sincrotrone Trieste S.C.p.A.,* ***2.*** *University of Milan*

Abstract

Extracellular vesicles (EVs) are a potent intercellular communication system. Within a lipidic bilayer, such small vesicles (diameter ranging from 30 to few hundreds nanometers) transport biomolecules between cells and throughout the body, strongly influencing the fate of recipient cells. They have been proposed as biomarkers for several diseases and as optimal candidates for therapeutic applications, due to their small size and specific biological functions. Nonetheless, since their isolation, quantification and biophysical and biochemical characterization are challenging tasks, the understanding of the complex network of EVs/cell interaction is still incomplete. Here we propose a combination of Atomic Force Microscopy and Small Angle X-Ray and Neutron Scattering (SAXS and SANS)) for the careful characterization of isolated vesicles and for the analysis of their interaction with model membranes, in form of liposomes and supported lipid bilayers. Our analysis reveals a strong interaction of EVs with model membranes mimicking lipid rafts, pointing out the importance of rafts-like structure in the uptake processes.

Keywords

Extracellular Vesicles, AFM, SAXS, Lipid-bilayers

Functional nanostructure of NhaA protein in tethered lipid bilayer membranes

Oral

Mr. Sebastian Köhler [1], ***Dr. Marco Maccarini*** [2], ***Dr. Giovanna Fragneto*** [1], ***Dr. Jean-Pierre Alcaraz*** [2], ***Prof. Donald Martin*** [2]

1. *Institut Laue-Langevin,* ***2.*** *Université Grenoble Alpes*

Abstract

The study of active membrane proteins requires an environment which is as close as possible to their natural environment to retain protein function, while at the same time keeping the system as simple as possible to allow for an experimental characterization and to be able to identify factors which influence the system. Tethered lipid bilayers (tBLMs) represent an experimentally accessible and stable model for biological membranes that offers a high level of control over the structure and can form a more natural environment for membrane protein incorporation than the widely used solid supported bilayers [1]. We report the use of a tBLM system to investigate how the structural factors of the surrounding membrane influence the incorporation and subsequently the activity of the NhaA protein, which is the main sodium proton antiporter of *Escherichia coli*. NhaA serves as the means for *E. coli* to maintain sodium homeostasis and for pH control [2]. Here we present a study on the incorporation of NhaA into PEG-tBLM on gold surfaces. We show the nanostructural characterization of highly covering tBLMs of different lipid composition with large fractions of incorporated NhaA by neutron reflectometry (NR) and how electrochemical impedance spectroscopy (EIS) can be used to investigate its activity. NR allowed us to determine the structure of the membrane/protein system to monitor bilayer dimensions, completeness and to precisely determine the amount of incorporated NhaA protein. EIS provided functional characteristics like electrophysiological properties related mainly to ion permeability and indicated NhaA activity, as this is associated to an ionic current across the bilayer.

The combination of these two methods enables us to correlate structural and functional information of the NhaA-membrane system in order to understand the mechanisms behind these dependencies.

This study provides the information to optimize the membrane/protein system regarding protein activity to enable further optimization of our biomimetic fuel cells, which take advantage of NhaAs electrogenic properties.

[1] M. Maccarini et al., *Langmuir*, **2017**, *33*, 9988-9996

[2] Etana Padan, *BBA-Bioenergetics*, **2014**, 1837. Jg., Nr. 7, S. 1047-1062

Keywords

tethered membranes, neutron reflectometry, antiporters

High-Throughput Superresolution Microscopy of Endocytosis - Linking Molecular Architecture and Mechanics of a Protein Machinery

Oral

Dr. Markus Mund [1], ***Prof. Marko Kaksonen*** [2], ***Dr. Jonas Ries*** [1]

1. *EMBL Heidelberg,* ***2.*** *University of Geneva*

Abstract

Clathrin-mediated endocytosis is an essential cellular functionof all eukaryotes. Itrelies on a self-assembled macromolecular machine of over 50 different proteins in tens to hundreds of copies that mediate vesicle formation. How so many proteins can be organized to produce endocytic vesicles with high precision and efficiency is not understood. To address this gap, we developed high-throughput superresolution microscopy to reconstruct the nanoscale structural organization of 23 endocytic proteins from over 100,000 endocytic sites in yeast. This allowed us tovisualize where individual proteins are localized within the machinery throughout the endocytic process.

By combining superresolution imaging, live-cell microscopy and Brownian dynamics simulations, we aim to identify the architectural features that allow the endocytic machinery to create vesicles with high efficiency and robustness. We found that actin filament nucleation is pre-patterned by a nucleation nanotemplate, which directly links molecular organization to the mechanics of endocytosis, and might represent a general design principle for directional force generation in other membrane remodeling processes such as during cell migration and division.

I will present first results on a dynamic reconstruction of the yeast endocytic machinery from thousands of images of fixed structures.

Mund et al. “Systematic analysis of the molecular architecture of endocytosis reveals a nanoscale actin nucleation template that drives efficient vesicle formation,” *Cell*, (2018).

Keywords

Superresolution microscopy, high-throughput, endocytosis, yeast.

Influence of bacteriorhodopsin activity on the structure and fluctuations of a floating lipid bilayer

Oral

Mrs. Tetiana Mukhina [1], ***Dr. Yuri Gerelli*** [1], ***Dr. Giovanna Fragneto*** [1], ***Dr. Thierry Charitat*** [2]

1. *Institut Laue-Langevin,* ***2.*** *University of Strasbourg*

Abstract

Cell membranes exhibit thermal fluctuations that can be enhanced by transmembrane protein activity leading to out-of-equilibrium fluctuations. Active fluctuations have been widely described theoretically [1], but to a lesser extent experimentally.

We will present our recent results on the investigation of out-of-equilibrium fluctuations of phospholipid membranes induced by active transmembrane protein. In this context, bacteriorhodopsin (BR) was used as a light-driven proton pump, whose activity can be triggered by visible light. Model systems such as solid-supported single and floating phospholipid bilayers are suitable to study phospholipid membranes and their interactions. A detergent-mediated incorporation method [2] was adapted to perform the insertion of BR into the phospholipid bilayer at the interfaces, using a sugar- based detergent such as DDM.

The combination of neutron reflectometry, QCM-D, fluorescence microscopy and AFM demonstrated that it is possible to insert BR in model bilayer systems without losing their structural integrity.

Recent specular and off-specular X-ray reflectometry experiments showed an activity of the incorporated proteins through its effect on the structure and on the fluctuations of a double bilayer system. These results open the way to investigate, for the first time, the fluctuation spectrum of a planar membrane-protein system at the nanoscale and to access the physical properties of the system such as bending modulus, surface tension and interaction potential between adjacent membranes.

References:

[1] Prost J., Manneville J.-B., and Bruinsma R., EPJ B, 1:465–480 (1998).

[2] Dezi M., Di Cicco A., Bassereau P. and Lévy D., PNAS, 7276, 110727 (2013

Keywords

membranes, proteins, fluctuations, neutron reflectometry

Lipid and proteo-lipid nanotubes with adjustable physicochemical and geometrical parameters.

Oral

Mr. Sergey Romanov [1], ***Dr. Timur Galimzyanov*** [2], ***Mr. Peter Kuzmin*** [2], ***Dr. Pavel Bashkirov*** [1]

1. *Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency,* ***2.*** *A.N. Frumkin Institute of Physical Chemistry and Electrochemistry of Russian Academy of Sciences*

Abstract

Functionalization of nanopores with lipid bilayers (lipid bilayer coating) has already attracted growing interest going far beyond merely imparting biomimetic properties to them. Lipid bilayer coated nanopores appear to be a novel platform for applications of controlled transport of ions and nanoparticles/macromolecules. To improve these emerging applications, we study for the first time the possibility to use 2D fluidity of the lipid bilayer for creating a nanopore with adjustable physicochemical properties and luminal radius. We pulled lipid nanotubes (NT) from planar reservoir membranes to obtain biomimetic elastic nanopores, the lumen radius of which could vary from 10 to 2 nm depending on the proteo-lipid composition of the NT membrane. We showed that the net surface charge inside the NT could be adjusted through accumulation or depletion of charged lipids in the inner monolayer of electrically biased NT. Electric field acts as regulator of ionic selectivity of the NT. Moreover, by conjugating electromigration with curvature-driven distribution of conical lipids, we demonstrated the possibility of making elastic nanopores with voltage-controlled diameters – an effect similar to piezoelectricity. Taking into account that geometrical parameters of NTs allow detecting single macromolecule transport, simultaneous regulation of both the surface charge and the luminal radius of such nanostructures significantly improves their dynamic functionality and suggests proteo-lipid nanotubular structures as promising tools for single molecule research/detection.

Keywords

biomimetic nanopores, proteo-lipid nanotubes

Lipid domain boundary as universal attractor for various membrane inclusions

Oral

Mr. Konstantin Pinigin [1], ***Mr. Oleg Kondrashov*** [1], ***Dr. Oleg Batishchev*** [1], ***Dr. Sergey Akimov*** [1], ***Dr. Timur Galimzyanov*** [2]

1. *A.N. Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences,* ***2.*** *A.N. Fumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences*

Abstract

Cell membranes are nonuniform multicomponent systems comprising various types of lipids and membrane proteins interacting with each other. Lipid diversity results in phase separation and formation of liquid-ordered domains also called lipid rafts. They are assumed to be important actors in diverse cellular processes, mainly signal transduction and membrane trafficking. Rafts are thicker than the disordered part of the membrane, thus compensating the hydrophobic mismatch between transmembrane proteins and the disordered lipid environment. That leads to the main cause of the boundary energy, a strained structure of the boundary. Membranes may also contain a wide variety of inclusions, inducing elastic stress: peripheral proteins, nonlamellar lipids, transmembrane proteins, etc. Deformations, induced by these inclusions, can interfere with raft boundary deformations, leading to the specific arrangement of these impurities around the domain edge, relaxing the membrane strain. Using elasticity theory approach we revealed that the domain boundary serves as an attractor for almost all types of membrane inclusions developed for lipid membranes. We have shown that various types of impurities tend to distribute to the narrow intermediate region at the liquid-ordered domain boundary, falling to the elastic energy well. The magnitude of the potential well equals to 0.4–0.5 k_BT per 1 nm of the boundary for membrane proteins, amphipathic peptides and hydrophobic molecules. For nonlamellar lipids, possessing spontaneous curvature of ±0.25 nm^{-1}, the depth of the energy well equals to 0.15–0.2 k_BT/nm. Moreover, the redistribution of these components dramatically varies the morphology and size of liquid-ordered domains, which is achieved by changing the domain boundary energy.Thus, we have shown that the boundary of the liquid-ordered domains can act as the universal attractor for a wide variety of membrane minor components, such as various peptides and non-bilayer lipids. Such attractive activity of the domain boundary can explain the mechanisms and suggest the new pathways of the strong influence of the low concentration of membrane impurities on various physiological processes involving rafts and may have an impact on cell signal transduction pathways, viral-induced membrane fusion, aggregation of amphipathic peptides.

This work is supported by the Grant MK 3119.2019.4

Keywords

lipid-protein interaction, rafts, amphipathic peptides

Lipid dynamics in nanodiscs probed by solid-state NMR

Oral

Dr. Ahmad Saad [1], ***Dr. Denis Martinez*** [1], ***Dr. Lukas Frey*** [2], ***Dr. Erick Dufourc*** [1], ***Dr. Birgit Habenstein*** [1], ***Dr. Stefan Bibow*** [3], ***Prof. Roland Riek*** [2], ***Dr. Antoine Loquet*** [1]

1. *CBMN, CNRS, University of Bordeaux, IECB,* ***2.*** *Laboratory for Physical Chemistry, ETH Zürich,* ***3.*** *Biozentrum, University of Basel*

Abstract

Nanodiscs provide a novel tool to embed membrane proteins into native-like lipid bilayers environment allowing high-resolution biophysical studies while maintaining their structure and function. Nanodiscs are composed of a small patch of a lipid bilayer surrounded by a membrane scaffold protein (MSP) creating a belt like shape. Different scaffold proteins can be used resulting in nanodiscs of various diameters and properties. It still remains unclear how lipids dynamically organize inside a nanodisc scaffold compared to their behavior in cellular membranes.

Recently, we compared lipid dynamics in nanodiscs and in liposome [1]. In the present work, we use deuterium solid-state NMR spectroscopy to investigate the organization and dynamic of lipids in various nanodisc constructs with an average size from 8 to 13 nm, assembled in different lipids composition (DMPC, DPPC). The thermotropic behavior, ordering and thickness of lipid membrane in nanodiscs were assessed. The results point out highly variable gel-to-fluid phase transitions and lipid ordering as a function of the nanodisc diameter, suggesting that the scaffold design have a profound impact of the dynamic organization of the lipid bilayer.

Keywords

Nanodisc, ssNMR, MSP, lipid dynamic

Lipidic Cubic Phase for Structural Studies of Membrane Proteins

Oral

Prof. Vadim Cherezov [1]

1. *University of Southern California*

Abstract

Lipidic cubic phase (LCP) is a membrane-mimetic matrix that serves as a convenient tool for stabilization of membrane proteins for biophysical studies and support crystallization directly from membrane-like environment. First applied to microbial rhodopsins, LCP crystallization gained its wider acceptance for enabling crystallization of challenging membrane proteins, such as G protein-coupled receptors (GPCRs), the structure of which is difficult to obtain by other methods. Multiple advancements in the development of protocols, tools, and instruments for LCP crystallization over the last two decades have facilitated the research in this field. As a result, LCP technology has come of age, with over a quarter of all new membrane protein structures currently being produced by this method. A most recent successful application of LCP as a growth and delivery matrix for microcrystals of membrane and soluble proteins for room temperature structure determination by Serial Femtosecond Crystallography (SFX) at X-ray Free Electron Lasers (XFELs) further illustrates uniqueness and versatility of this biomaterial.

Keywords

Lipidic Cubic Phase, Membrane proteins,

Mechanism of passive translocation of nano-objects through lipid membranes

Oral

Dr. Vladimir Baulin [1], ***Dr. Yachong Guo*** [2], ***Dr. Marco Werner*** [3], ***Dr. Jean-Baptiste Fleury*** [4]

1. *Universitat Rovira i Virgili,* ***2.*** *Department of Physics, Nanjing University, Nanjing,* ***3.*** *Leibniz-Institut für Polymerforschung Dresden,* ***4.*** *Universitat des Saarlandes, Saarbruecken*

Abstract

Design of nanomaterials able to cross lipid bilayers is a challenging task in nanotechnology. Large variety of shapes, sizes and surface coatings are used for the design of nanomaterials to overcome this barrier. However, the potential barrier to cross the lipid bilayer only by thermal motion is too high even for nano-objects with size > 5nm. It is generally accepted that small hydrophobic nanoparticles are blocked by lipid bilayers and accumulate in the bilayer core, while nanoparticles with sizes larger than 5 nm can only penetrate cells through a slow energy-dependent processes such as endocytosis, lasting minutes.

In one example, we show how variation of hydrophobicity of the nanoparticles can lead to passive translocation of nanoparticles through the lipid bilayer. This adsorption transition through reversible destabilization of the structure of the bilayer induces enhanced permeability for water and small solutes.

In another example, we demonstrate that lipid-covered hydrophobic nanoparticles may translocate through lipid membranes by direct penetration within milliseconds. We identified the threshold size for translocation: nanoparticles with diameters smaller than 5 nm stay trapped in the bilayer, while nanoparticles larger than 5 nm insert into bilayer, open transient pore in the bilayer. Using the Single Chain Mean Field (SCMF) theory a mechanism of passive translocation through lipid bilayers is proposed. Observing individual translocation events of gold nanoparticles with 1-dodecanethiol chains through DMPC bilayers we confirm the particle translocation and characterize the kinetic pathway in agreement with our numerical predictions. Mechanism relies on spontaneous pore formation in the lipid bilayer. The observed universal interaction behaviour of neutral and chemically inert nanoparticles with the bilayer can be classified according to their size and surface properties.

Keywords

lipid membrane, nanoparticle, membrane, translocation

Membrane remodeling during selective ER-phagy

Oral

Dr. Ramachandra M Bhaskara [1], ***Dr. Paolo Grumati*** [2], ***Dr. Javier Garcia-Padro*** [2], ***Dr. Sissy Kalayil*** [2], ***Dr. Adriana Covarrubais-Pinto*** [2], ***Mr. Wenbo Chen*** [1], ***Dr. Misha Kudryashev*** [1], ***Prof. Ivan Dikic*** [2], ***Prof. Gerhard Hummer*** [1]

1. *Max-Planck-Institut für Biophysik,* ***2.*** *Goethe University*

Abstract

The size and shape of the endoplasmic reticulum is regulated by FAM134B, a selective ER-phagy receptor. The structure of its reticulon-homology domain (RHD), an element shared with other ER-shaping proteins, and the mechanism of membrane shaping remain poorly understood. Using molecular modeling and molecular dynamics (MD) simulations, we assemble a structural model for the RHD of FAM134B. Through MD simulations of FAM134B in flat and curved membranes, we relate the dynamic RHD structure with its unique topology to membrane-curvature induction and curvature-mediated protein sorting functions. Receptor clustering amplifies the membrane-shaping effects. Electron microscopy of in-vitro liposome remodeling experiments support the membrane remodeling functions of the different RHD structural elements. Disruption of the RHD structure affects selective autophagy flux and leads to disease states.

[1]. Bhaskara, R.M., Grumati, P., Garcia-Padro, J., Kalayil, S., Covarrubais-Pinto., A., Chen, W., Kudrayshev, M., Dikic, I., Hummer, G. (2019) Curvature induction and membrane remodelling by FAM134B reticulon homology domain assist selective ER-phagy. (in press) *Nat. Commun.*

Keywords

Curvature-induction, Sensing, ER-phagy, Remodeling

Mixing water, transducing energy, shaping membranes: Autonomously self-regulating giant vesicles

Oral

Prof. Atul Parikh [1]

1. *University of California, Davis*

Abstract

A solute, excluded from or confined within a spatial “compartment” embedded in an aqueous continuum, creates a gradient in the chemical activity of water. This in turn prompts a directed flow of water pushing it into the solute-laden compartment and out of the solute-starved one. Serving as a non-specific entropic force, this osmotic stress acts on the vesicular boundaries producing long-lived out-of-equilibrium morphologies and cooperative behaviors.

Drawing from recent experiments in our lab employing giant vesicles containing (or excluding) molecular (e.g., sugars) and colligatively non-ideal macromolecular (e.g., PEG and Dextran) osmolytes, this talk considers how the osmotic activity of water dynamically remodels the membrane, inducing membrane shapes (including protrusions, invaginations, and buds), driving topological transitions (producing colonies of daughter vesicles), and orchestrating liquid-liquid phase separations - all while dissipating the osmotic energy in theoretically predictable manners.

Keywords

vesicles, osmotic stress, phase separation

Neural guidance: dissecting molecular interactions in the context of the plasma membrane.

Oral

Prof. E. Yvonne Jones [1]

1. *Wellcome Trust Centre for Human Genetics, Cellular Imaging, University of Oxford*

Abstract

Neural guidance cues are secreted or cell surface attached proteins that interact with receptors to trigger cell attraction or repulsion. Although neural guidance cues were first characterized by their role in the development of the nervous system they are ubiquitous, functioning both during embryogenesis and in adult tissue homoeostasis. I will discuss how we are using a combination of structural and biophysical analyses in vitro and advanced light microscopy techniques in cellulo to reveal the mechanisms at work in neural guidance.

Keywords

Structure, advanced light-microscopy, neural guidance,

Protein scaffolds in endosomal cargo retrieval

Oral

Mr. Carlos Lopez Robles [1], ***Dr. Soledad Baños-Mateos*** [1], ***Dr. David Gil-Cartón*** [1], ***Dr. Miguel Romano-Moreno*** [1], ***Mr. Miguel Romero-Durana*** [2], ***Dr. Juan Fernandez-Recio*** [2], ***Dr. Adriana L Rojas*** [1], ***Dr. Aitor Hierro*** [1]

1. *CIC bioGUNE,* ***2.*** *Barcelona Supercomputing Center, ICVV*

Abstract

The **endolysosomal system** is a highly dynamic network of membranes for degradation and **recycling**. During endosomal maturation, cargo molecules destined for lysosomal degradation are progressively concentrated through continuous rounds of fusion and fission reactions concomitant with inbound and outbound membrane fluxes. Of the cargo molecules delivered to endosomes, about two-thirds are rescued from degradation and recycled for reuse. This balance between degradation and recycling is essential to preserve the proteostatic plasticity of the cell under variable physiological demands. **Cargo retrieval** from endosomes involves several sorting complexes that associate with multidomain regulatory proteins displaying complex interaction networks. The most studied recycling machinery is the retromer complex that, in combination with distinct **sorting nexin** (SNX) proteins, is recruited to endosomal membranes to mediate the retrieval of cargo to the plasma membrane, the TGN or other specialized organelles. Retromer associates with at least six different SNXs (SNX3, SNX27 and SNX1/2-SNX5/6 heterodimers) to form functionally distinct complexes. The current hypothesis is that the more complex and transient interactions between SNX proteins and retromer in higher metazoans might involve different modular scaffolds and architectural organizations to bridge cargo and coat assembly. Our goal is to establish a detailed understanding at the molecular level of this modular design and stoichiometric architecture.

Keywords

endosomes, recycling,sorting nexin

Quantitative analysis of molecular interactions between proteins and biomimetic lipid systems

Oral

Prof. Catherine PICART [1]

1. *Université Grenoble Alpes*

Abstract

In this talk, I will first present the different types of biomimetic lipid membranes containing specific lipids of the phosphoinositide family that can be used to *study in vitro* the molecular interactions between proteins and lipid membranes: large unilamellar vesicles (LUVs) [1], giant unilamellar vesicles (GUVs) [2]and supported lipid bilayers (SLBs) [3]. Using these model membranes, it is possible to decorticate the different steps of the protein interactions with the membranes and to simplify the complexity of the biological environments in mammalian cells. I will then show examples of quantitative analysis of protein/lipid membrane interactions [4] for two important kinds of proteins that have important and different physiological roles: i) the ezrin/radixin/moesin (ERM) proteins that play a key role in morphogenetic, cancer metastasis [5]; ii) the self-assembly of HIV-1 Gag polyprotein at the inner leaflet of the cell host plasma membrane that is the key orchestrator of virus assembly [6].

The well-defined biomimetic membranes enable to get insight into the molecular mechanisms of interactions, revealing the importance of phosphorylation sites and protein domains as well as the impact of protein interaction on lipid reorganization.

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Keywords

biomimetism, Lipid membranes, proteins, interactions

Single-cell resolution of metabolic control over HIV-1 entry and a role for membrane lipid order and tension

Oral

Mr. Charles Coomer [1], ***Dr. Irene Carlon-Andres*** [1], ***Ms. Maro Iliopoulou*** [1], ***Dr. Michael Dustin*** [2], ***Dr. Ewaldus Compeer*** [2], ***Dr. Alex Compton*** [3], ***Dr. Sergi Padilla-Parra*** [1]

1. *Wellcome Trust Centre for Human Genetics, Cellular Imaging, University of Oxford,* ***2.*** *Kennedy Institute for Rheumatology, University of Oxford,* ***3.*** *HIV Dynamics and Replication Program, National Cancer Institute*

Abstract

Recent studies have highlighted cellular metabolic activity as a critical factor driving HIV-1 infection in T cells. However, deciphering how the metabolic state of single cells affects virus entry remains to be fully characterised. We developed an assay utilising FRET-based biosensors of various metabolites to evaluate the influence of global metabolic processes on the success rate of virus entry in single cells. Lifetime fluorescence images of single cells were recorded immediately before and after addition of HIV-1 pseudovirions (i.e. HIV-1_{JR-FL}) or non-enveloped HIV-1 with incorporated BLaM-Vpr. Lifetime measurements of cells expressing biosensors for ATP:ADP ratio or lactate were utilised to determine relative metabolite concentrations before and during entry. The same cells were subsequently screened for fusion and productive infection to determine whether baseline intracellular metabolite concentrations were correlated with these processes. Interestingly, cells with a lower ATP:ADP ratio prior to virus addition were less permissive to virus fusion and infection. These results indicated a relationship between host metabolic state and the likelihood for virus-cell fusion to occur. To confirm this, we show that cells treated acutely with 2-deoxy-d-glucose (2-DG), an inhibitor of glycolysis, permitted substantially fewer fusion events. Single particle tracking (SPT) revealed that virions were arrested at hemifusion in 2-DG-treated cells. Interestingly, cells treated with 2-DG also possessed less surface membrane cholesterol, while the addition of cholesterol to the plasma membrane rescued the block to fusion. Further investigation with additional reporters revealed a link between host glycolytic activity and membrane tension and order, with cells treated with 2-DG exhibiting lower plasma membrane lipid order and higher tension values. These data suggest that low glycolytic activity results in a deficiency of membrane cholesterol. Finally, SPT illustrated that virions were less likely to enter cells at areas of high membrane tension. We are currently performing similar experiments in T cells. We have identified a connection between host glycolytic activity and membrane tension which may influence HIV-1 fusion at the single-cell level. Our results indicate that HIV-1 fuses with glycolytically-active cells and that this activity is linked to cell surface membrane cholesterol and membrane tension.

Keywords

HIV-1 entry, FRET/FLIM, membrane tension

Solid-state NMR studies of dynamic mitochondrial protein-lipid nanocomplexes.

Oral

Prof. Patrick van der Wel [1], ***Dr. Mingyue Li*** [2], ***Dr. Abhishek Mandal*** [2], ***Ms. Maria DeLucia*** [2], ***Prof. Jinwoo Ahn*** [2], ***Prof. Rajesh Ramachandran*** [3]

1. *University of Groningen,* ***2.*** *University of Pittsburgh,* ***3.*** *Case Western Reserve University*

Abstract

Organelle-specific lipid species play critical roles in cellular signalling events as well as the trafficking and targeting of numerous proteins. Frequently, these processes are mediated by lipid-binding or lipid-recognition domains that are soluble and non-membrane bound in absence of their lipid targets. The “peripheral” interactions between lipid bilayers and such conditional membrane-associated proteins remain difficult to probe on the molecular level, even as huge progress is made in tackling integral membrane proteins. One primary challenge stems from the dynamics and heterogeneity inherent in many of these nano-scopic protein-lipid complexes. In recent published and unpublished work we employ advanced solid-state NMR (ssNMR) methods to probe the structure and dynamics of two different cardiolipin-protein complexes involved in mitochondrial fission and apoptosis. Using multinuclear ssNMR we observe the lipid-specific membrane binding of the dynamic and disordered “variable domain” of dynamin-related protein Drp1. Membrane curvature and non-bilayer phases are instrumental for the function of this mitochondrial protein, which mediates key molecular events in mitochondrial membrane fission and mitochondrial dynamics. We use ssNMR’s ability to detect and characterise the structure and dynamics of the lipid bilayer membrane, and especially non-bilayer phases, to experimentally probe for curvature changes in the protein-bound membrane.

In parallel, we have been using ssNMR to probe how mitochondrial cytochrome c binds cardiolipin (CL) and facilitates its peroxidation in presence of mitochondrial reactive oxygen species (ROS). This process of great interest due to the fact that exposure and peroxidation of cardiolipin are pivotal signals in mitochondrial apoptosis, with implications for cancer treatments and neurodegenerative diseases. With the aid of ssNMR and complementary biophysical studies, we gain new insights into the way that the peripherally bound protein engages nanoclusters of CL lipids, which in turn act as dynamic regulators that control the CL-specific lipid oxidation activity of this pro-apoptotic protein-lipid complex. In both cases, a toolkit of advanced ssNMR methods is deployed to access unique structural and dynamical information for both the fluid membrane and the surface-bound protein, despite the dynamics and disorder that is inherent in these mitochondrial protein-lipid nanocomplexes.

Keywords

Mitochondria, cardiolipin, NMR, protein-lipid complexes

Sphingomyelin metabolism controls the shape, function and organization of the Golgi membranes

Oral

Dr. Pablo Lujan [1], ***Prof. Vivek Malhotra*** [2], ***Prof. Maria Garcia-Parajo*** [1], ***Dr. Felix Campelo*** [1]

1. *ICFO-Institut de Ciencies Fotoniques,* ***2.*** *CRG-Centre for Genomic Regulation*

Abstract

The flat Golgi cisterna is a highly conserved feature of all eukaryotic cells, but how is this cisterna morphology achieved and maintained? I will present a biophysical model of cisterna morphology, which led us to propose that sphingomyelin (SM) synthesis at the *trans*-Golgi membranes essentially controls the structural features of a Golgi cisterna. SM laterally controls membrane rigidity by generating nanodomains and its metabolism regulates the association of curvature-generating proteins. An experimental test of this hypothesis revealed that affecting SM homeostasis converted flat cisternae into highly curved membranes with a concomitant dissociation of membrane curvature-generating proteins. These data lend support to our hypothesis that SM metabolism controls the structural organization of Golgi cisternae. Together with our data on the role of SM in controlling the lateral sorting of transmembrane proteins, our data reveal the significance of SM metabolism in the structural organization and function of the Golgi complex.

Keywords

Protein-sorting

Membrane-curvature

Lipid-domains

Structural and mechanistic bases of Drp1-CL interactions in mitochondrial fission.

Oral

Dr. Mukesh Mahajan [1], ***Dr. Nikhil Bharambe*** [1], ***Dr. Matthias Buck*** [1], ***Dr. Xin Qi*** [1], ***Prof. Patrick van der Wel*** [2], ***Prof. Rajesh Ramachandran*** [1]

1. *Case Western Reserve University,* ***2.*** *University of Groningen*

Abstract

Mitochondria form tubular networks that undergo coordinated cycles of fission and fusion. Emerging evidence suggests that a direct yet unresolved interaction of the mechanoenzymatic GTPase dynamin-related protein 1 (Drp1) with mitochondrial surface-localized cardiolipin (CL) catalyzes mitochondrial fission. Here, using a comprehensive set of structural, biophysical and cell biological tools, we have uncovered a CL-binding motif (CBM) conserved between the Drp1 variable domain (VD) and the unrelated ADP/ATP carrier that intercalates into the hydrocarbon membrane core via a disorder-to-order helical structural transition to effect CL acyl chain interactions. Yet, membrane insertion and GTP-dependent conformational rearrangements induce only transient CL nonbilayer topological forays, which favor high local membrane constriction but not fission. A conservative CBM mutation that weakens CL interactions induces ‘donut’ mitochondria formation stimulated by impaired Drp1-dependent fission coupled to excessive mitofusin-enabled fusion. Our studies, for the first time, firmly establish an indispensable role for Drp1 VD-CL interactions in productive physiological mitochondrial fission.

Keywords

NMR, nanodiscs, IDP, Drp1, fission

Structural basis for membrane tethering by a bacterial dynamin-like pair

Oral

Dr. Harry Low [1], ***Dr. Jiwei Liu*** [2]

1. *Imperial College,* **2.** *University of Oxford*

Abstract

Dynamin-like proteins (DLPs) are large GTPases that restructure membrane. DLPs such as the mitofusins form heterotypic oligomers between isoform pairs that bridge and fuse opposing membranes. In bacteria, heterotypic oligomerisation may also be important for membrane remodelling as most DLP genes are paired within operons. How DLPs tether opposing membranes is unknown. Here we show the crystal structure of a DLP heterotypic pair from the pathogen Campylobacter jejuni. A 2:2 stoichiometric tetramer is observed where heterodimers, conjoined by a random coil linker, assemble back-to-back to form a tripartite DLP chain with extreme flexibility. In vitro, tetramerisation triggers GTPase activity and induces lipid binding. Liposomes are readily tethered and form tubes at high tetramer con- centration. Our results provide a direct mechanism for the long-range binding and bridging of opposing membranes by a bacterial DLP pair. They also provide broad mechanistic and structural insights that are relevant to other heterotypic DLP complexes.

Keywords

Bacteria, dynamin, mitofusins, membrane, tethering

The GDP-bounded state of Mfn1 induces membrane adhesion of apposing lipid vesicles

Oral

Mr. Andrés Tolosa-Diaz [1], ***Dr. Victor Almendro-Vedia*** [1], ***Dr. Paolo Natale*** [1], ***Dr. Ivan Lopez-Montero*** [1]

1. *Universidad Complutense de Madrid*

Abstract

Mitochondria form a highly dynamic network that constantly fuse and divide for an optimal function. Fusion processes create interconnected mitochondrial network whereas fission promotes ball-shaped mitochondria. Several proteins are the central players that regulate both fusion and fission events. In mammalian cells, the fusion lies on the GTP-dependent assembly of Mitofusin 1 (Mfn1), Mitofusin 2 (Mfn2) and the optic atrophy 1 protein (Opa1). Mfn1 and Mfn2 promote the fusion of the outer mitochondrial membranes (OMM) whereas Opa1 is responsible of the inner mitochondrial membrane (IMM) fusion. The detailed mechanism of the mitofusin-promoted membrane fusion is currently not known. Genetics indicate that the presence of a functional GTPase domain is mandatory. The crystal structures of truncated Mfn indicate that GTP hydrolysis produces a large conformational change from an extended four-helix bundle arrangement to a compact fold-knife like closed conformation. This power stroke is hypothesized to bring adjacent Mfn-containing membranes into proximity promoting membrane tethering as well as their fusion. Here we cloned and produced the N-terminal histidine tagged variant of the full-length human Mfn1 (his-hs-Mfn1) protein in *E. coli*. inner membrane vesicles. After incorporation into giant vesicles, we evidence that Mfn1 mediates the adhesion of lipid bilayers through the GDP-bound state of the protein. The adhesion strength was weak, $E_{adh} \approx 10^{-7}$ J/m^2 but strong enough to closely dock the apposing lipid bilayers. Our results are relevant for dissection of the Mfn mediated membrane fusion and to understand the series of events that finally produce mitochondrial membrane fusion.

Keywords

Mitofusin, mitochondria, GUVs, membrane adhesion

The role of dynamin twist in membrane fission

Oral

Dr. Martina Pannuzzo [1], ***Dr. Zachary McDargh*** [2], ***Prof. Markus Deserno*** [3]

1. *Istituto Italiano di Tecnologia,* ***2.*** *Columbia University,* ***3.*** *Carnegie Mellon University*

Abstract

The final step of many biological membrane fission events involves the GTPase dynamin, which assembles into a helical filament around the neck of a nascent vesicle and somehow severs this remaining connection. But despite about two decades of research, the actual physical processes that lead to fission are still a matter of debate. Dynamin's action occurs on the scale of a few tens of nanometers over just a few milliseconds, which is too small and fast for many experimental techniques, but too large and slow for atomistic simulations. In this talk I explore this problem using both continuum theory and coarse-grained simulations specifically designed to capture the interplay of geometry and elasticity. I show that within reasonable experimental limits, the two widely discussed conformational changes of shrinking the radius or increasing the pitch of a dynamin helix are insufficient to trigger fission. However, a third change, reminiscent of an effective twist of the filament, turns out to efficiently drive the neck into the hemifission state. This motion mirrors the experimentally observed asymmetric tilting of dynamin's PH-domains. Following the retraction of the substrate, the remaining dynamin coat can unbind, and the tensile force in the connecting micellar string draws the almost severed membranes together one more time, until bilayer contact catalyzes the scission of the micelle.

Keywords

membrane
fission
dynamin
theory
simulation

Using DNA to Cross Membrane Barriers

Oral

Prof. Stefan Howorka [1]

1. *University College London*

Abstract

Semifluid membranes enclose biological cells and drug delivery vehicles. Crossing the membrane barrier enables essential transport of molecular cargo. My talk presents routes to cross the barrier with synthetic transport channels made from DNA. Nucleic acids are easier to engineer than proteins of biological channels(1). The artificial DNA channels are composed of interlinked duplexes. Attached lipid anchors hold the negatively charged structures in the membrane(2,3,4)based on rational design rules(5). The DNA channels open and close in response to physical voltage stimuli, like natural templates(3,4,6). The DNA versions can also mimic ligand-gated(3)and temperature-gated channels(7)to help release drugs or build cell-like networks. The artificial pores can be programmed into cytotoxic agents to kill cancer cells(8), or to create porous bionanoreactors(9). Other rationally designed DNA nanostructures extend the functional range and can control, for example, bilayer shape(10). The presentation concludes with an outlook of how DNA nanotechnology at membrane interfaces can help replicate biological functions to open up new applications in nanobiotechnology and synthetic biology.

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(7) *ACS Nano 2019*
(8) *Angew. Chem. Int. Ed. 2014*53 12466; *Nat. Chem. 2014*7 17;
(9) *Angew. Chem. Int. Ed. 2016*55 11106;
(10) *Science 2016*352 890; *Nat. Chem. 2017*9 611; *Nat. Commun. 2018*9 1521;

Keywords

DNA, nanotechnology, membrane, nanopores, biomimetics

Poster

CD300a Receptor Promotes HIV-Cell Fusion Through The Interaction With Phosphatidylserine And Phosphatidylethanolamine

Poster

Ms. Joana Vitalle [1], Dr. Irene Carlon-Andres [2], Dr. Olatz Zenarruzabeitia [1], Dr. Sergi Padilla-Parra [2], Prof. Francisco Borrego [1]

1. *Biocruces Bizkaia Health Research Institute,* ***2.*** *Wellcome Trust Centre for Human Genetics, Cellular Imaging, University of Oxford*

Abstract

The CD300a surfacereceptor promotes the infection of host cells by several enveloped viruses,such as Dengue, West Nile, Yellow Fever and Chikungunya viruses. The ligands ofCD300a, phosphatidylserine (PS) and phosphatidylethanolamine (PE), are exposed in the outer leaflet of the plasma membrane of HIV-infected cells and they are also present in the HIV envelope. It has been described that mutations in the IgV domain of the receptor prevent the binding to PS and PE and abrogate the increase of CD300a-mediated Dengue virus infection. We have previously observed that CD4+ T lymphocytes from healthy donors expressing CD300a were significantly more susceptible to HIV *in vitro*infection.The objective of this work was to investigate the specific role of CD300a and its ligands PS and PE in HIV infection, mainly in the fusion stage of viral entry. Accordingly, we first infected CD300a-transfected TZM-bl cells with $HIV_{JR\text{-}FL}$ expressing Gag-GFP for 48 hours, and subsequently measured the infection by fluorescence microscopy. In this assay, we used three compounds: A01, an inhibitor of phospholipid scrambling, and MFG-E8 and cinnamycin, which sequester PS and PE respectively from receptor binding. We have observed a decrease in the number of HIV-infected cells after the addition of these three compounds, in particular in cinnamycin-treated cells. Furthermore, we also transfected TZM-bl cells with wild type and CD300a mutants (D95A and F39A) unable to engage with PS and PE or both.Then, we infected these cells with $HIV_{JR\text{-}FL}$ during 90 min and we performed a β-lactamase assay in order to study the level of HIV-cell fusion. Our results showed a higher level of HIV-cell fusion in cells expressing wild type CD300a than in non-transfected cells or cells expressing mutated CD300a. We conclude that CD300a promotes HIV-cell fusion, which depends on the interaction with its ligands PS and PE. Therefore,CD300a could be a potential therapeutic target in HIV infection.

Keywords

CD300a, phosphatidylserine, phosphatidylethanolamine, HIV, fusion

Characterization of nuclear pore membrane proteins and FG nucleoporins by high-speed atomic force microscopy

Poster

Mr. Toshiya Kozai [1], ***Dr. Radhakrishnan Panatala*** [1], ***Dr. Larisa E. Kapinos*** [1], ***Prof. Roderick Y. H. Lim*** [1]

1. *Biozentrum and the swiss nanoscience institute, University of Basel*

Abstract

Nuclear pore complexes (NPCs) mediate the selective transport of biomolecules between the cytoplasm and nucleus in eukaryotic cells. However, its molecular mechanism remains disputed. This is because the NPC barrier is comprised of numerous intrinsically disordered phenylalanine-glycine nucleoporins (FG Nups) that are extremely difficult to visualize, not least at transport-relevant timescales (~10 ms). Recently, we used high-speed atomic force microscopy (HS-AFM) to resolve the dynamic behavior of FG Nups inside NPCs at ~200 ms timescales. Meanwhile, structural changes of FG Nups under transporting conditions are still unclear. Here, we have used HS-AFM to characterize the dynamic behavior of different FG Nups tethered to a lipid bilayer. We observe that the FG Nups form extended conformations that fluctuate rapidly on a lipid membrane. In parallel, we have successfully reconstituted “pre-NPCs” from two NPC-associated transmembrane nucleoporins (TM Nups; i.e., Pom121 and Ndc1) in lipid bilayers as resolved by HS-AFM. Besides providing insight as to how TM Nups self-assemble to form the NPC channel, we envisage that pre-NPCs may be used as templates to build on other NPC components. Further, the function of Pom121-Ndc1 pores to function as a NPC mimic is the subject of ongoing work.

Keywords

NPC

Nucleoporin

HS-AFM

Comparative analysis of membrane constriction by dynamin isoforms

Poster

Dr. Rebeca Bocanegra [1], ***Dr. Ariana Velasco*** [2], ***Dr. Sara De Lorenzo*** [1], ***Dr. Julene Ormaetxea*** [2], ***Dr. José L. Carrascosa*** [3], ***Dr. Anna Shnyrova*** [2], ***Dr. Borja Ibarra*** [1], ***Dr. Vadim Frolov*** [4]

1. *IMDEA Nanociencia,* ***2.*** *Biofisika Institute (UPV/EHU, CSIC); Department of Biochemistry and Molecular Biology, University of the Basque Country,* ***3.*** *CNB/CSIC,* ***4.*** *Biofisika Institute (UPV/EHU, CSIC); Department of Biochemistry and Molecular Biology, University of the Basque Country; Ikerbasque, Basque Foundation for Science*

Abstract

The proteins of dynamin superfamily are large GTPases widely implicated in fission and fusion of endomembranes. Their activity is necessary for internalizing essential nutrients, organelle transformations and maintenance, dynamins are intimately involved in signalling and membrane trafficking networks in the cell, in life and pathology. The superfamily founding members, dynamins 1 and 2 (Dyn1 and 2), remain the most characterized dynamins primarily involved in orchestrating membrane fission in the clathrin dependent endocitosis. As an endocytic vesicle buds, dynamin molecules are recruited to its neck, where it self-assembles a helical coat generating a dynamin-lipid tube. The helix constriction driven by GTP hydrolysis promotes fission of the neck and release of the vesicle. While the above patterns of dynamin activities common for Dyn1 and 2 are well understood, *in vitro* analyses revealed important functional differences between neuron-specific Dyn1 and ubiquitous Dyn2 isoforms. Here we performed systematic mechanistic comparison of membrane remodelling activities of Dyn1 and 2 reconstituted using lipid membrane nanotubes. By combining fluorescence microscopy and optical tweezers approaches, we quantified the nanotube constriction by dynamin isoform in apo state and in the presence of different nucleotide. Our analyses revealed significant differences between membrane constriction and curvature stabilization activities of Dyn1 and 2. We discuss possible relevance of these differences to physiological functions of the proteins.

Keywords

Dynamin

Membrane remodelling

Optical Tweezers

Constriction-by-friction mechanism of membrane fission by reticulon

Poster

Mr. Javier Espadas [1], ***Dr. Rebeca Bocanegra*** [2], ***Dr. Artur Escalada*** [3], ***Ms. Julene Ormaetxea Gisasola*** [1], ***Dr. Borja Ibarra*** [2], ***Dr. Anna Shnyrova*** [1], ***Dr. Andrea Daga*** [4], ***Dr. Vadim Frolov*** [5]

1. *Biofisika Institute (UPV/EHU, CSIC); Department of Biochemistry and Molecular Biology, University of the Basque Country,* ***2.*** *IMDEA Nanociencia,* ***3.*** *Biofisika Institute (UPV/EHU, CSIC),* ***4.*** *IRCCS E. Medea,* ***5.*** *Biofisika Institute (UPV/EHU, CSIC); Department of Biochemistry and Molecular Biology, University of the Basque Country; Ikerbasque, Basque Foundation for Science*

Abstract

Reticulons are the protein family creating and supporting the membrane curvature in the endoplasmic reticulum (ER). Paradoxically, reticulons have been also implicated in the ER fragmentation. Here we reveal how these seemingly opposed creative and destructive functionalities can coexist in reticulons operating in dynamic tubular ER network. We reconstituted *Drosophila*reticulon (Rtnl1) into biomimetic planar reservoir membranes and pulled nanotubes from this reservoir. We showed that Rtnl1 produced stable membrane curvature in static nanotubes and related the nanotube stabilization to curvature-driven sorting of Rtnl1 towards the nanotube. Crucially, we found that membrane constriction is increased in elongating nanotubes leading to stochastic scission via the hemifission route. The dynamic constriction is driven by an increased pulling force acting against the viscose drug imposed by Rtnl1. Hence, while Rtnl1 acts as stabilizers of membrane curvature of static ER tubules, it can also produce stochastic scission of the tubules upon their movement, thus mediating the ER fragmentation.

Keywords

Reticulon, ER, membrane fission

Dynamins in double membrane fission

Poster

Mr. Juan Manuel Martínez Gálvez [1], ***Ms. Maite Garcia*** [2], ***Ms. Julene Ormaetxea Gisasola*** [1], ***Dr. Fernando Benito López*** [2], ***Dr. Lourdes Basabe Desmonts*** [2], ***Dr. Anna Shnyrova*** [1]

1. *Biofisika Institute (UPV/EHU, CSIC); Department of Biochemistry and Molecular Biology, University of the Basque Country,* ***2.*** *Microfluidics and Biomics Cluster, University of the Basque Country*

Abstract

Mitochondria appear in cells as a network showing a pronounced dynamic behaviour where their double lipid bilayer continuously divide and/or merge together. Mitochondrial division (MD), i.e. the splitting of a mitochondrion into two, has been reported to be critical both for cellular life and for cellular death. A failure in some point of MD has implications in aging, neurogenerative diseases and acute brain injury among other pathologies Double lipid bilayer reorganization during MD is orchestrated by proteins from the dynamin superfamily of large GTPases universally involved in membrane fission. Surprisingly, while the interplay between the membrane and the dynamins during single membrane fission has been wildly studied, no much is known about such interplay during the membrane rearrangements taking place in double membrane fission.

Here, by combining microfluidic, soft-lithography and microscopy techniques, we have developed an *in vitro* system that allows studying the double membrane remodelling resulting from fission forces, either from dynamins involved in MD or from external bulks forces. We observed how dynamin 2 assembles a “soft” scaffold which is sufficient for fission of double membrane necks with composition mimicking that of mitochondrial outer membranes. Drp1, the protein universally involved in MD, does not have such effect, further stabilizing fission necks with extreme membrane curvatures. We further expect that our in vitro system will help clarifying the role of these two dynamins in mitochondrial fission.

Keywords

Mitochondrial division, dynamins, fission, nanotubes

Fluid mechanics near lipid bilayers

Poster

Dr. Guillermo Amador [1], ***Mr. Dennis van Dijk*** [1], ***Dr. Marie-Eve Aubin-Tam*** [1], ***Dr. Daniel Tam*** [1]

1. *Delft University of Technology*

Abstract

The mechanical and chemical interactions between cells and their surroundings dictate their behavior, such as motion, growth, and proliferation. Such interactions are mediated by the cell's membrane, a thin layer composed of a lipid bilayer and embedded proteins. The membrane's functions include the transport of molecules and ions, and sensing (or signaling); therefore, it is crucial for homeostasis, or the maintenance of a stable internal state. Controlled studies into membrane mechanics have been limited by complexities in geometry and local detection of forces at the scale of pico-Newtons.

To address these challenges, we use novel techniques with optical tweezers to probe the hydrodynamic flow around free-standing lipid bilayers within microfluidic channels. The planar geometry of the lipid bilayer facilitates interpretation of measurements using physical models. This technique is the first to combine multiple optical tweezers probes with planar free-standing lipid bilayers accessible on both sides of the bilayer. The aims of these measurements are to quantify fluid slip close to and transmission of hydrodynamic forces across the bilayer surface, building towards a fundamental understanding of the physical principles governing the hydrodynamics around and through membranes. Such findings may contribute to understanding how cells generate and detect forces, as well as providing a tool for designing and optimizing vesicles for drug delivery.

Keywords

optical tweezers, microfluidics, surface viscosity

From sensing to stabilization of membrane curvature by HIV Gag

Poster

Dr. Artur Escalada [1], ***Dr. Anna Shnyrova*** [2], ***Mrs. Hang Waters*** [3], ***Dr. Joshua Zimmerberg*** [3], ***Dr. Vadim Frolov*** [4]

1. *Biofisika Institute (UPV/EHU, CSIC),* ***2.*** *Biofisika Institute (UPV/EHU, CSIC); Department of Biochemistry and Molecular Biology, University of the Basque Country,* ***3.*** *Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health,* ***4.*** *Biofisika Institute (UPV/EHU, CSIC); Department of Biochemistry and Molecular Biology, University of the Basque Country; Ikerbasque, Basque Foundation for Science*

Abstract

The geometry of HIV particles is supported by a protein shell formed by the polyprotein Gag beneath the membrane of the virus envelope. Oligomerization of Gag on the inner leaflet of the host plasma membrane is coupled to membrane curvature creation via yet undetermined mechanism. Here we report that small oligomers of Gag discriminate negative membrane curvature. Furthermore, the curvature intrinsic for viral particles promote rapid and reversible condensation of Gag into fluid-like domains on the membrane surface. With time, these domains acquire intrinsic spherical shape and rigidity sufficient to support the shape. Theoretical analysis identifies condensation-polymerization mechanism as responsible for progressive conversion of the Gag activity from curvature sensing to creation.

Keywords

HIV Gag, Curvature, Membrane domain

Hijacking the Retrograde trafficking by the L. Pneumophila effector RidL

Poster

Dr. Miguel Romano-Moreno [1], ***Dr. Adriana L Rojas*** [1], ***Dr. Maria Lucas*** [2], ***Dr. Aitor Hierro*** [1]

1. *CIC bioGUNE,* ***2.*** *Universidad de Cantabria*

Abstract

Microbial pathogens employ sophisticated virulence strategies to cause infections in humans. The intracellular pathogen *Legionella pneumophila* encodes RidL to hijack the host cell retromer, a membrane coat complex composed of VPS26, VPS29 and VPS35 that is critical for endosomal cargo recycling. Here, we determined the crystal structure of *L. pneumophila* RidL in complex with the human VPS29-VPS35 retromer subcomplex. A hairpin loop protruding from RidL inserts into a conserved pocket on VPS29 that is also used by cellular ligands such as TBC1D5 for VPS29 binding. Consistent with the idea of molecular mimicry in protein interactions, RidL out-competed TBC1D5 for binding to VPS29. Furthermore, the interaction of RidL with retromer did not interfere with retromer dimerization and was essential for association of RidL with retromer-coated vacuolar and tubular endosomes. Our work provides structural and mechanistic evidence into how RidL is targeted to endosomal membranes to co-opt retromer function.

Keywords

X-ray crystallography, endosomal transport, Pathogens

HIV-1 Gag protein sensitivity to membrane curvature and lipid environment

Poster

Dr. Natalia Kuzmina [1], ***Dr. Timur Galimzyanov*** [1], ***Mr. Peter Kuzmin*** [1], ***Dr. Oleg Batishchev*** [1]

1. *A.N. Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences*

Abstract

Among other enveloped viruses, human immunodeficiency viruses (HIV) attracts the most efforts to elucidate molecular mechanisms of its infectivity, mutagenesis and reproduction activity. Lack of vaccine candidates against HIV drive attention to the self-assembly of the virion in order to disturb it. Group-specific antigen (Gag) polyprotein, interacting both viral RNA and plasma (or, later, viral) membrane, is believed to play a pivotal role in the process of HIV assembly. Moreover, this protein alone can produce virus-like particles from infected cells. Despite a loan of studies of Gag interaction with lipid membranes, there are still open questions about the main partners of such interactions among lipids and cell and viral proteins, as well as about the mechanism of viral protein envelope self-organization during budding of progeny virions from the infected cell. In the present study, we focused on the effect of membrane curvature, lipid composition and pH of the environment on self-assembly of Gag protein on the lipid bilayer. Using atomic force microscopy (AFM), we characterized the Gag adsorption in physiological conditions on lipid membranes of various composition, as well as its sensitivity to membrane curvature. We presented a theoretical model describing the protein rearrangements between flat and curved membrane regions. Moreover, we demonstrated the effect of low pH environment on Gag self-assembly to clarify the possible outcomes of the HIV infectivity through endocytic pathway.

This work was supported by the Russian Foundation of Basic Research (grant #17-54-30022).

Keywords

HIV-1, Gag, self-assembly, protein-lipid interactions

Induction of a local phase transition in lipid membranes by monolayer domains of an ordered phase and protein inclusions

Poster

Dr. Rodion Molotkovskiy [1], ***Dr. Sergey Akimov*** [1], ***Dr. Timur Galimzyanov*** [2]

1. *A.N. Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences,* ***2.*** *A.N. Fumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences*

Abstract

Cell membranes are structurally inhomogeneous systems with separate domains of the liquid-ordered phase, called rafts. Cell membranes and its organelles are asymmetric in the composition of the outer and inner monolayers. Experimental data on asymmetric bilayers are scarce; the effect of asymmetry on the structure and physical properties of the membrane remains insufficiently studied. It is known that the formation of bilayer domains in asymmetric membranes in some cases may be thermodynamically disadvantageous. At the same time, experimental data indicate the mutual influence of opposite monolayers, leading to averaging degrees of the order of monolayers. Using the available data on the structure of the raft boundary, we have investigated the mechanism of the formation of ordered domains under thermodynamically unfavourable conditions induced by the monolayer domain of the ordered phase or protein inclusion. We determine the finite critical size inclusion or monolayer domain that can induce the transition from monolayer to bilayer domain configuration. We find the dependence of this size on the geometry of protein inclusion. We have also investigated the effect of inter-layer coupling and spontaneous curvature of monolayers on this process. We show that under certain conditions a monolayer domain will tend to grow limitlessly without the formation of a bilayer structure. For calculations, we use the theory of elasticity of a continuous liquid crystal medium, adapted to lipid membranes. When calculating the elastic deformations of the membrane, we utilized the fact that the thicknesses of the raft and the surrounding membrane are different. The energy of elastic deformations caused by smoothing the thickness jump at the boundary with the liquid-disordered phase is shown to be the main driving force for the formation of ordered domains under thermodynamically unfavourable conditions.

This work is supported by the Grant MK 3119.2019.4

Keywords

lipid membranes, rafts

Interaction of the Lactobacillus surface-layer array to the bacterial cell

Poster

Ms. Djenana Vejzovic [1], ***Mr. Markus Eder*** [1], ***Ms. Elisabeth Damisch*** [1], ***Mr. Theo Sagmeister*** [1], ***Ms. Francesca Berni*** [2], ***Prof. Jeroen Codee*** [2], ***Prof. Tea Pavkov-Keller*** [1]

1. *University of Graz,* ***2.*** *Institute of Chemistry, Leiden University*

Abstract

Surface layers (S-layers) are 2D paracrystalline lattices of proteins or glycoproteins which cover the whole cell surface of many Archaea and Bacteria. Since these proteins are in close contact with their habitat they fulfil many vital tasks like bacterial adherence to other cells or substrates, protection against life-threatening conditions and maintenance of the cell shape.

S-layer proteins of *Lactobacilli* species have a highly basic pI and are between 25-71 kDa in size. They are attached to the cell wall by interaction with lipoteichoic acids (LTA). It is reported that they are involved in auto-coaggregation and adherence and therefore are significant for the stimulation of gut dendritic cells by interacting with specific receptors.

Our goal is to characterize the surface layer proteins of *Lactobacilli* at atomic scale. For structure-function characterization, we designed several protein fragments. Soluble fragments were purified and subjected to crystallization. Optimized crystals of the C-terminal fragments, containing the LTA-binding domain, diffracted to 1.8 and 2.2 Å. Crystal structures were solved by SeMet-SAD and the later by molecular replacement. To further characterize the binding of the S-layer to bacterial cell we performed microscale thermophoresis and isothermal titration calorimetry experiments with the C-terminal fragment of the protein.

Keywords

S-layer

Lactobacilli

Lipoteichoic acid

cell-wall-binding

Interactions through the membrane-accommodation surface improve the biological function of broadly neutralizing HIV-1 antibody 10E8

Poster

Mrs. Sara Insausti Gonzalez [1], ***Dr. Edurne Rujas*** [1], ***Dr. Daniel P. Leaman*** [2], ***Dr. Pablo Carravilla*** [1], ***Dr. Jakub Chojnacki*** [3], ***Mr. Lei Ortigosa*** [1], ***Mr. Lei Zhang*** [2], ***Dr. Christian Eggeling*** [3], ***Dr. Michael Zwick*** [2], ***Prof. Jose Luis Nieva*** [1]

1. *Biofisika Institute (UPV/EHU, CSIC); Department of Biochemistry and Molecular Biology, University of the Basque Country,* ***2.*** *Scripps Reserach Institute,* ***3.*** *University of Oxford*

Abstract

The 10E8 antibody targets a helical epitope in the membrane-proximal external region (MPER) and transmembrane domain (TMD) of the envelope glycoprotein (Env) subunit gp41, and is among the broadest known neutralizing antibodies against HIV-1. Accordingly, this antibody and its mechanism of action valuably inform the design of effective vaccines and immunotherapies. 10E8 exhibits unusual adaptations to attain specific, high-affinity binding to the MPER at the viral membrane interface. Here, we demonstrate that by increasing the net positive charge of the polar surface-patch in contact with the viral membrane, the neutralization potency of the antibody may be significantly enhanced. We found that the optimized 10E8 could interact spontaneously with synthetic-fluid membranes, but did not gain any observable polyreactivity. Binding analyses, including single virion STED microscopy, revealed that the increase in neutralization potency correlated with higher affinity for Env spikes inserted into the rigid viral membrane. Overall, our data provide a proof-of-principle for rational optimization of 10E8 via manipulation of its interaction with the membrane element of its epitope. Moreover, our results emphasize the crucial role played by the viral membrane in the antigenicity of the MPER-TMD of HIV-1.

Keywords

HIV-1, antibody, 10E8, neutralization, phophatidylserine

LC3/GABARAP protein family in mitophagy

Poster

Ms. Uxue Ballesteros [1], ***Dr. Asier Etxaniz*** [1], ***Ms. Marina Iriondo*** [1], ***Prof. Alicia Alonso*** [1]

1. *Biofisika Institute (UPV/EHU, CSIC)*

Abstract

Mitophagy is a selective mitochondrial autophagy in which the phospholipid cardiolipin (CL) has been proposed to play a role. CL externalization to the outer mitochondrial membrane would act as a signal for the autophagic machinery to degrade damaged mitochondria. LC3/GABARAP protein family could mediate both mitochondrial recognition and autophagosome formation, ultimately leading to removal of damaged mitochondria. Previous studies have shown that LC3B binds preferentially to CL enriched membranes. The objective of this project is to understand this interaction in different members of the LC3/GABARAP protein family. Even though LC3/GABARAP family members have similar structures, they show different binding affinities to CL liposomes in our experiments. With the aim of validating these result in eukaryotic cells we have performed binding assays in isolated mitochondria and co-localization assay in SH-SY5Y cells by confocal microscopy. Differences have been observed between the different family members. Our results support the hypothesis that the interaction between LC3/GABARAP protein family and CL plays a role in the recognition of mitophagy cargo.
Keywords:LC3/GABARAP, Cardiolipin, mitophagy.

Keywords

LC3/GABARAP, Cardiolipin, mitophagy.

Lipid bilayer stabilization for the correct exposure of MPER epitope in peptide-liposome vaccines targeting the gp41 subunit of HIV-1 Env

Poster

Mrs. Johana Torralba [1], ***Dr. Igor de la Arada*** [1], ***Dr. Eneko Largo*** [1], ***Dr. Edurne Rujas*** [1], ***Mrs. Sara Insausti*** [1], ***Dr. Pablo Carravilla*** [1], ***Mr. Miguel García-Porras*** [1], ***Prof. José Luis Rodriguez Arrondo*** [1], ***Prof. Jose Luis Nieva*** [1], ***Dr. Beatriz Apellaniz*** [1]

1. *University of the Basque Country*

Abstract

Antibodies targeting the C-terminal subdomain of the membrane-proximal external region (C-MPER), which is connected to the transmembrane domain (TMD) of the envelope glycoprotein (Env), achieve near-pan neutralization of HIV-1. Thus, recreating the structure that generates C-MPER-targeting antibodies is a major goal of the rational development of HIV vaccines. Here, to assess the TMD section as a potential membrane integral scaffold for the MPER epitope, we first attempted to reconstitute two sequences into lipid bilayers, namely, MPER-TMD1 (gp41 residues 671-700) and MPER-TMD2 (gp41 residues 671-709). Infrared spectroscopy determinations as a function of the cholesterol content, and immunochemical assays revealed that, following our method, MPER-TMD1, but not MPER-TMD2, could be effectively reconstituted adopting a main helical conformation, and exposing the MPER epitope on the surface of phospholipid bilayers. The structural features including the angle of insertion and the effects on membrane order and architecture were further correlated with antigenicity and immunogenicity of liposome formulations containing MPER-TMD1 transmembrane helices. Overall, our results suggest new approaches to the design of effective immunogens directed against MPER.

Keywords

HIV-1, MPER, peptide-liposome vaccines

Lipid-gold clusters (AuroraTM): a membrane-friendly form of gold nanoparticles

Poster

Dr. Aritz García [1], ***Dr. Jesús Sot*** [1], ***Dr. Sebastiao Mendanha*** [2], ***Dr. Jon Busto*** [1], ***Dr. Shengrong Li*** [3], ***Dr. Stephen Burgess*** [3], ***Dr. Walter Shaw*** [3], ***Dr. David Gil-Cartón*** [4], ***Prof. Félix Goñi*** [1], ***Prof. Alicia Alonso*** [1]

1. *Biofisika Institute (UPV/EHU, CSIC),* ***2.*** *Federal University of Goiás,* ***3.*** *Avanti Polar Lipids, Inc.,* ***4.*** *CIC bioGUNE*

Abstract

Lipid model membranes in lamellar phases (bilayers) of different phospholipid compositions have been prepared, in the form of vesicles, or of supported lipid bilayers, and doped with Aurora^{TM} at 0.1 mol%. Aurora^{TM} consists of an Au_{55} gold nanoparticle (about 1.4 nm in diameter) capped with triphenylphosphine ligands and a single diglyceride (distearoyl glycerol) ligand. Gold nanoparticles have been incorporated in the past inside liposomes, or grafted onto their surfaces, with diagnostic or therapeutic aims. Including the gold nanoparticles in a stable form within the lipid bilayers has serious technical difficulties. We have tested the hypothesis that, because of the diglyceride ligand, Aurora^{TM} would allow the easy incorporation of gold nanoclusters into cell membranes or lipid bilayers without significant effects in their biophysical properties. Our results show that Aurora^{TM} readily incorporates into lipid bilayers, particularly when they are in the fluid phase, i.e. the state in which cell membranes exist. Calorimetric, fluorescence polarization or fluorescence confocal microscopy concur in showing that bilayer-embedded Aurora^{TM}hardly changes the physical properties of the bilayers, nor does it perturb the phase equilibrium in lipid mixtures giving rise to lateral phase separation in the plane of the membrane. Atomic force microscopy shows, in fluid bilayers, well-resolved particles, 1.2 – 2.9 nm in height, that are interpreted as single Aurora^{TM}conjugates. Cryo-transmission electron microscopy allows the clear observation of lipid bilayers with an enhanced contrast due to the Aurora^{TM} gold nanoparticles; the single particles can be resolved at high magnification. Our studies support the applicability of Aurora^{TM} as a membrane-friendly form of nano-gold particles for biological research or clinical applications.

Keywords

Lipid, gold, nanoparticle, membrane, phase

Mechanism for membrane association of the Legionella pneumophila deAMPylase SidD

Poster

Dr. Igor Tascon [1], ***Dr. Adriana L Rojas*** [1], ***Dr. Maria Lucas*** [2], ***Dr. Aitor Hierro*** [1]

1. *CIC bioGUNE,* ***2.*** *Instituto de Biomedicina y Biotecnología de Cantabria, IBBTEC*

Abstract

Microbial pathogens have developed a diverse spectrum of mechanisms to manipulate the human host and cause disease. Many bacterial proteins post-translationally modify host factors in order to alter their function. SidM (DrrA) and SidD from *L. penumophila* AMPylates and de-AMPylates mammalian Rab1 in a sequence manner to exploit intracellular vesicle trafficking routes. While Rab1 de-AMPylation is catalyzed by the N-terminal phosphatase-like domain of SidD, the C-terminal domain is responsible for membrane targeting. Here, we present the crystal structural of full length SidD, including the less-well characterized C-terminal domain (CTD). We found that a flexible loop rich in aromatic residues is the primary membrane targeting of SidD. SAXS analysis indicates inter-domain flexibility thereby expanding the sampling space accessible to the catalytic domain.

Keywords

*L. pneumophila e*ffector, membrane binding

Mechanism of lipidic pore formation by helical amphipathic peptides

Poster

Dr. Timur Galimzyanov [1], ***Mr. Oleg Kondrashov*** [1], ***Dr. Oleg Batishchev*** [1], ***Dr. Sergey Akimov*** [1]

1. *A.N. Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences*

Abstract

Amphipathic alpha-helical peptides are promising antimicrobial agents. Positively charged peptides can selectively recognize and bind outer monolayer of bacterial membranes that bears negative charge. Interaction of peptides with the bacterial membrane results in formation of through pore. When bound to the membrane, the peptide helix exposes hydrophobic residues on its side-surface, and polar or charged residues on the opposite side-surface. The tendency to hide hydrophobic residues from contact with water leads to partial incorporation of the peptide into the lipid monolayer. The incorporation induces elastic deformations around the peptide. When two peptides are far separated, these deformations are independent. Upon approaching, the deformations overlap leading to effective lateral interaction of the peptides. In the framework of theory of elasticity of lipid membranes, we obtained that the total elastic energy of the membrane is minimal when two peptides are in register, parallel to each other, and the distance between their longitudinal axes is about 5 nm. At such separation the electrostatic repulsion of the peptides is negligible. The most stressed region is situated in the middle between amphipathic alpha-helical peptides. Formation of through pore in this region seems to be the most probable. As the equilibrium distance between peptides is about 5 nm, the initial pore should be purely lipidic. We obtained that in the presence of peptides the energy barrier of pore formation decreases by ten-tens of k_BT. After pore formation, the peptides tend to arrange to its equatorial plane: the total elastic energy has local minimum in such configuration. The depth of the minimum (and the life-time of the pore) is influenced by electrostatic repulsion of charged peptides; stronger repulsion results in shallower minima and shorter life-times. Long-living pore can be further stabilized by recruiting additional peptides from the membrane surface. Highly charged peptides rapidly escape from the edge of short-living pore either to the initial or to the opposite side of the membrane. This provides the mechanism of membrane penetration by charged substances without formation of long-living pores.

The work was supported by Russian Foundation for Basic Research grants ## 18-54-74001, 17-04-02070.

Keywords

amphipathic peptides, pore, lipid membrane

Mechano-chemistry of small oligomers of Dynamin1

Poster

Mr. Javier Vera Lillo [1], ***Mr. Juan Manuel Martínez Gálvez*** [1], ***Ms. Julene Ormaetxea Gisasola*** [1], ***Dr. Anna Shnyrova*** [1], ***Dr. Vadim Frolov*** [2]

1. *Biofisika Institute (UPV/EHU, CSIC); Department of Biochemistry and Molecular Biology, University of the Basque Country,* ***2.*** *Biofisika Institute (UPV/EHU, CSIC); Department of Biochemistry and Molecular Biology, University of the Basque Country; Ikerbasque, Basque Foundation for Science*

Abstract

Dynamins are a superfamily of large mechano-chemical enzymes deeply implicated in membrane remodeling processes inside the cell. Though it has been established that dynamins use the energy of GTP hydrolysis for membrane deformations, the mechanism(s) of the energy transduction remains highly controversial. To resolve the dynamic coupling between the GTP hydrolysis and membrane deformation by Dynamin1 (Dyn1), the founding member of the superfamily, we analyzed the dynamic oligomerization of Dyn1-eGFP on different membrane templates. We found that small Dyn1-eGFP clusters respond to GTP addition and related the observed changes in the cluster mobility and interactions to local membrane deformations.

Keywords

Dynamin1, GTP hydrolysis, cluster mobility

MERLIN: Mitochondria-ER Length Indicative Nanosensor

Poster

Dr. Hector Flores Romero [1], ***Ms. Vanessa Hertlein*** [1], ***Dr. Ana J. Garcia-Saez*** [1]

1. *University of Tübingen*

Abstract

The contacts between the endoplasmic reticulum (ER) and mitochondria play an important role in multiple cellular functions like the exchange of lipids and calcium between both organelles, as well as in apoptosis and autophagy signaling. The molecular architecture and spatiotemporal regulation of these distinct contact regions remain obscure and there is a need for new tools that enable tackling these questions. Here we present a new Bioluminescence Resonance Energy Transfer (BRET)-based biosensor for the quantitative analysis of distances between ER and mitochondria that we call MERLIN (Mitochondria ER Length Indicative Nanosensor). The main advantages of MERLIN compared to available alternatives are that it does not rely on the formation of artificial physical links between the two organelles, which could lead to artifacts, and that it allows to study contact site reversibility and dynamics. We show the applicability of MERLIN by characterizing the role of the mitochondrial dynamics machinery on the contacts of this organelle with the ER.

Keywords

Biosensor, MERCs, BRET

Molecular determinants of lung surfactant function from the study of surfactant purified from human amniotic fluid

Poster

Mr. Jose Carlos Castillo Sánchez [1], ***Dr. Barbara Eicher*** [2], ***Dr. Emma Batllori-Badia*** [3], ***Prof. Alberto Galindo*** [4], ***Prof. Georg Pabst*** [2], ***Prof. Antonio Cruz*** [1], ***Prof. Jesús Pérez-Gil*** [1]

1. *Biochemistry and Molecular Biology Department, Faculty of Biology, Complutense University, Madrid and Research Institute Hospital Universitario "12 de Octubre" (imas12), Madrid,* ***2.*** *Biophysics Division of the Institute of Molecular Biosciences, University of Graz, Graz,* ***3.*** *Department of Obstetrics and Gynecology, Hospital Universitario 12 de Octubre, Madrid,* ***4.*** *Research Institute Hospital Universitario "12 de Octubre" (imas12) and Complutense University, Madrid and Department of Obstetrics and Gynaecology. Hospital Universitario 12 de Octubre, Madrid*

Abstract

Alveolar type-II cells synthesize and secrete the membranous lipid-protein complexes of lung surfactant to the alveolar air-liquid interface in order to overwhelm surface tension forces that otherwise, would hinder respiratory dynamics. From its secretion to recycling, surfactant membranes undergo profound structural changes with functional impact. That is the reason why obtaining a material that preserves the molecular determinants of pristine lipid-protein complexes, as they are assembled and packed into lamellar bodies, is a major goal. Standard surfactant preparations, typically obtained from bronchoalveolar lung lavages, are useless since these materials have been modified and partly inactivated during respiratory dynamics. Purifying surfactant as it is freshly secreted by primary cultures of type-II pneumocytes has been usually considered the best approach to obtain a pristine surfactant, assembled into true lamellar body-like particles. However, the amount of surfactant that can be obtained by this methodology is limited, which prevents its use in clinical applications or to understand the molecular determinants underlying its actual interfacial behavior. In this context, a material obtained from human amniotic fluid, before air-breathing could have been established by the baby lungs, has been recently proposed as a potential source of genuine freshly secreted surfactant.

In the present work, we have performed Small and Wide Angle-X Scattering experiments to discern the structural determinants of lung surfactant membranes at the nanoscale. Specifically, we have compared structural and functional properties of a surfactant purified from porcine lungs with those of a surface-active material isolated from human amniotic fluid. We observed meaningful temperature-dependent differences in terms of lamellar repeat distances and phase coexistence among both surfactants, which could be crucial to understand the type of structure established by surfactant once it reaches the respiratory surface.

Keywords

surfactant, lamellar body-like particles, SAXS

Molecular mechanisms behind remorin nanodomain formation by solid-state NMR

Poster

Mr. Anthony Legrand [1], ***Dr. Denis Martinez*** [1], ***Dr. Julien Gronnier*** [2], ***Mrs. Mélanie Berbon*** [1], ***Dr. Marion Decossas-Mendoza*** [1], ***Dr. Paul Gouguet*** [3], ***Dr. Véronique Germain*** [4], ***Mrs. Axelle Grélard*** [1], ***Dr. Olivier Lambert*** [1], ***Dr. Antoine Loquet*** [1], ***Dr. Sébastien Mongrand*** [4], ***Dr. Birgit Habenstein*** [1]

1. *CBMN, CNRS, University of Bordeaux, IECB,* ***2.*** *ETH Zurich,* ***3.*** *Universität Tübingen,* ***4.*** *Laboratoire de biogenèse membranaire, UMR5200, CNRS, University of Bordeaux*

Abstract

Protein and lipid components in biological membranes act as a dynamic network of subtle molecular interactions segregating the membrane into particular regions called nanodomains. Nanodomains act as functional platforms enriched in specific lipids (such as sterols and phosphoinositides) and proteins to perform their diverse activities. Remorins (REMs) are plant proteins and well-established nanodomain markers and, as such, they can be considered as paradigm to provide a mechanistic description of membrane organisation into functional nanodomains. Using solid-state nuclear magnetic resonance (ssNMR) and building upon our initial knowledge of *St*REM1.3 and its C-terminal membrane anchor, we reveal the delicate balance between hydrophobic and electrostatic effects leading up to the protein's characteristic affinity for negatively charged phospholipids. In a divide-and-conquer approach, we describe the impact of *St*REM1.3's C-terminal anchor, its oligomerisation domain and intrinsically disordered region on membrane structure and dynamics. Furthermore, we tackle the structural features of *St*REM1.3 when associated to nanodomain-mimicking membranes. We reveal that *St*REM1.3 drives nanodomain organisation by concerted lipid-protein and protein-protein interactions.

Keywords

Solid-state NMR
Membrane
Nanodomains
Remorin

Peptide nanofibrils as enhancers of viral infection

Poster

Mr. Kai Stroh [1], ***Dr. Jelger Risselada*** [2]

1. *Georg-August-University Göttingen,* ***2.*** *Leiden University*

Abstract

It has been shown experimentally that amyloid fibrils, which are naturally present in seminal fluid, may increase the infectious viral titer of HIV-1 by four to five orders of magnitude [1]. These fibrils termed SEVI (semen-derived enhancer of viral infection) interact with HIV-1 virions and promote their attachment to target cells, resulting in accelerated fusion. While the inhibition of this process would be of interest in the context of HIV, fusion enhancement is desirable for targeted retroviral gene transfer and drug delivery.

The infection enhancement mechanism is not yet fully understood. While the positive surface charge of the amyloid fibrils evidently plays a role in bringing the negatively charged viruses and cells in contact, the increased infectivity also appears to depend on the intrinsic properties of the fibril structures [2], such as fibril stiffness and fibril length. To reconstruct the interaction landscape that amyloid fibril covered viruses face when undergoing fusion with a host cell, we employ elastic continuum models in conjunction with coarse grained molecular dynamics simulations.

References

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Keywords

amyloid-fibrils

MD simulations

viral infection

Proteins Interacting with the Phosphopholipid Monolayer of Lipid Droplets Regulate Lipid Hydrolysis

Poster

Dr. Natalia Kulminskaya [1], ***Mr. Marton Liziczai*** [1], ***Dr. Andras Boeszoermenyi*** [2], ***Dr. Roland Viertlmayr*** [1], ***Dr. Krishna Mohan Padmanabha Das*** [2], ***Prof. Tea Pavkov-Keller*** [1], ***Dr. Monika Oberer*** [1]

1. *University of Graz,* ***2.*** *Harvard Medical School*

Abstract

Intracellular lipid droplets (LD) are ubiquitous organelles with the characteristic features that the hydrophobic core composed of neutral lipids (triacylglycerols and sterol esters) is shielded from the outer, aqueous phase with a phospholipid monolayer. Often, different proteins regulating access to the interior are bound to this phospholipid monolayer. The size of LDs size varies greatly, ranging from the nanometer to micrometer scale. LDs play important roles in energy homeostasis and lipid metabolism, e.g. they act as storage depots for neutral lipids that can be mobilized upon demand. In mammals, energy mobilization from LDs is a highly conserved process. It is mediated by the action of lipases, which degrade the triacylglycerols step-by-step to molecules of glycerol and three fatty acids. Adipose triglyceride lipase (ATGL) localizes to the LD and hydrolyzes triacylglycerol into diacylglycerol and fatty acid. On a protein level, the activity of ATGL is regulated by interaction of ATGL with the stimulatory proteins “alpha/beta hydrolase domain containing 5” (ABHD5) and the inhibitory proteins termed “G0/G1 switch gene 2” (G0S2) and “hypoxia inducible lipid droplet associated (HILPDA)”. Our goal is to understand the activating and inhibitory mechanism of these protein-protein complexes at the LD-water interphase at molecular detail. Therefore, we apply biophysical, structural and biochemical approaches with native LDs or different nano-structures as LD mimic. We will present ongoing structural characterization of the inhibitory protein G0S2 in dodecylphosphocholine (DPC) micelles and circularized nano-discs as LD mimics. Furthermore, we present the solution-state NMR structure of a peptide derived from the co-activator ABHD5 bound to (DPC) micelles. This peptide anchors ABHD5 to the LD, which is strictly required for co-activation of ATGL.

Keywords

lipid-droplet, nano-discs, lipolysis, NMR, interaction

Pulmonary surfactant protein SP-C membrane fragmentation: an emerging role in lung defense and homeostasis.

Poster

Dr. Alejandro Barriga [1], ***Ms. Michelle Juranny Morán-Lalangui*** [1], ***Prof. Ismael Mingarro*** [2], ***Prof. Jesús Pérez Gil*** [1], ***Dr. Begoña García Álvarez*** [1]

1. *Biochemistry and Molecular Biology Department, Faculty of Biology, Complutense University, Madrid,* ***2.*** *Universidad de Valencia*

Abstract

Pulmonary surfactant (PS) is a lipid/protein complex located at the surface of the alveolar epithelium that fulfills two major roles. On the one hand, it prevents the collapse of lungs during breathing cycles due to its ability to reduce surface tension of the respiratory air-liquid interface to very low levels. Additionally, PS is involved in lung defense as it is the first physical barrier that every inhaled particle or microorganism, harmful or not, should overcome to reach the epithelium and the systemic circulation. This latest function is strictly coordinated with alveolar macrophages by a combination of mechanisms that are still being explored.

Surfactant protein C (SP-C) is a very small and hydrophobic transmembrane protein whose role has been classically associated to the biophysical function of PS. However, several studies have reported the implication of SP-C in the immunity of the lung, since this protein can interact with both the bacterial lipopolysaccharide (LPS) and the macrophage receptor of this molecule, CD14.

The link between these functions could rely on the capacity of SP-C to cause fragmentation and vesicle formation in phospholipid-based membranes. We hypothesize that this process emerges from the interaction between different SP-C molecules through putative oligomerization motifs within the protein. This would lead to the generation of stiff SP-C clusters which could impose a given membrane curvature, generating a stress in the bilayer that would be eventually resolved through the fragmentation of the membrane and the formation of smaller vesicles. These SP-C-enriched nanoparticles could constitute a genuine vehicle to transfer wasted or harmful material from PS to phagocytic cells such as macrophages.

To test this hypothesis, we have carried out a set of experiments including tunable resistive pulse sensing (TRPS) setups, bimolecular fluorescence complementation (BiFC) techniques, flow cytometry and fluorescence microscopy. Our results point out towards a close relation between SP-C oligomers formation, membrane fragmentation, nanovesicles formation and macrophage uptake: a bridge between the biophysics and the immunity worlds.

Keywords

Pulmonary surfactant, macrophages, SP-C, biophysics

Reconstitution of aromatic foldamer rods into lipid nanotubes

Poster

Mr. Pedro Arrasate [1], ***Ms. Kathrin Aftahy*** [2], ***Dr. Joan Atcher*** [2], ***Prof. Ivan Huc*** [2], ***Dr. Vadim Frolov*** [3]

1. *Biofísika Institute (CSIC, UPV/EHU); Department of Biochemistry and Molecular Biology, University of the Basque Country,* ***2.*** *Ludwig-Maximilians-Universität München,* ***3.*** *Biofísika Institute (CSIC, UPV/EHU); Department of Biochemistry and Molecular Biology, University of the Basque Country; Ikerbasque, Basque Foundation for Science*

Abstract

Lipid membrane nanotubes (NTs) are widely used synthetic membrane templates. The NT lumen can be constricted to molecular dimensions thus enabling detection of single molecules passing through the NT lumen and/or deforming the NT membrane. However, the constriction causes curvature instabilities interfering with molecular sensing. In the search for improved stability and mechanical versatility of the NT template we explored composite NTs containing synthetic bola-amphiphiles molecules, rod-like aromatic oligoamide foldamers. The rigid foldamer rods designed to span the lipid bilayer are expected to change its elastic behavior and increase the NT stability at high curvatures. We found that foldamers of different molecular architecture could be mixed with lipids to form giant foldamer-lipid vesicles. We quantified the foldamer incorporation into the vesicle membrane and further analyzed how foldamers affect the stability and mechanical properties of the NTs pulled from the foldamer-lipid vesicles.

Keywords

lipid nanotubes, foldamers

Targeting retromer complex with stabilizing molecules to promote neuroprotection

Poster

Ms. Elsa Astorga [1], ***Dr. Adriana L Rojas*** [1], ***Dr. Estibaliz Capetillo-Zarate*** [2], ***Dr. Aitor Hierro*** [1]

1. *CIC bioGUNE,* ***2.*** *Achucarro Basque Center for Neuroscience*

Abstract

Deregulation of protein homeostasis is a common feature of aging, that underlines the multifactorial nature of many neurodegenerative disorders including Alzheimer's disease (AD). Endosomes are key intracellular recycling compartments where the biosynthetic and endocytic pathways intersect, thus playing a major role in protein homeostasis. Not surprisingly, endosomal dysfunction is a convergent point in multiple age-related neurodegenerative disorders. Indeed, reduced levels of retromer, a protein complex that plays a central role in protein recycling at endosomes, have been found in various neurodegenerative conditions including Alzheimer's and Parkinson's diseases. We have used Nuclear magnetic resonance and thermal stability shift analysis to perform a high-throughput screening of natural compounds to identify molecular chaperones that stabilize retromer.

Keywords

Alzheimer´s disease, endosomes, retromer, trafficking

The interplay between protein and lipid aggregation in supported membranes: experiments and modeling

Poster

Dr. Marisela Velez [1], ***Prof. Pedro Tarazona*** [2]

1. *ICP-CSIC,* ***2.*** *UAM*

Abstract

The interplay between protein and lipid aggregation in supported membranes: experiments and modeling
Experimental results performed on a well-defined system in which a self-aggregating soluble bacterial cytoskeletal protein polymerizes on a lipid bilayer containing two lipid components has shown that the interplay between lipids and the self-aggregation of lipid-attached proteins is complex. We present results under different conditions in which we observe that the lipid bilayer affects the protein assemblies.
We present a theoretical model that can explain some of the observations. The model, in contrast to previous ones that consider proteins only as passive elements affecting the lipid distribution, describes the system including three terms: the dynamic interactions between protein monomers, the interactions between lipid components, and a mixed term considering protein–lipid interactions. It explains experimental results performed on a well-defined system under certain conditions. All the elements considered in a previously described protein model, including torsion of the monomers within the filament, are needed to account for the observed filament shapes.
The model also points out that lipid segregation can affect the length and curvature of the filaments and that the dynamic behavior of the lipids and proteins can have different time scales, giving rise to memory effects. This simple model that considers a dynamic protein assembly on a fluid and active lipid surface can be easily extended to other biologically relevant situations in which the interplay between protein and lipid aggregation is needed to fully describe the system.
Chemistry and Physics of Lipids 185 (2015) 141–152 “Modeling the interplay between protein and lipid aggregation in supported membranes” Pablo González de Prado Salas , Mario Encinar , Alvaro Alonso , Marisela Vélez, Pedro Tarazona

Keywords

Theory, Dynamic-filaments, Protein–lipid-interactions, AFM, bilayers

The N-terminal Domain of Lactobacillus acidophilus SlpA promotes Self-Assembly of the S-Layer Array

Poster

Mr. Theo Sagmeister [1], ***Mr. Markus Eder*** [1], ***Ms. Elisabeth Damisch*** [1], ***Ms. Djenana Vejzovic*** [1], ***Dr. Andela Dordic*** [1], ***Dr. Janet Vonck*** [2], ***Dr. Claudia Millán*** [3], ***Prof. Isabel Usón*** [3], ***Prof. Tea Pavkov-Keller*** [1]

1. *University of Graz,* ***2.*** *Max-Planck-Institut of Biophysics,* ***3.*** *The Molecular Biology Institute of Barcelona*

Abstract

Surface layer proteins (S-layers) represent the outermost cell envelope in many bacteria and archaea. They assemble into highly regular 2D crystalline arrays composed of mostly single protein or glycoprotein species. These arrays are in close contact with their surrounding and fulfill various functions like bacterial adherence to other cells or substrates, protection against life-threatening conditions and maintenance of the cell shape.

The S-layer of *L. acidophilus*consists of two proteins. SlpA is mainly expressed under normal physiological conditions, whereas SlpX expression is increased under osmotic stress. Both proteins have a highly basic pI and are 47 kDa and 54 kDa in size. They have two functional regions: the C-terminal region that is important for the attachment to the cell wall and the N-terminal region for the self-assembly of the S-layer array.

Our goal is to structurally characterize the S-layer protein SlpA of *L. acidophilus* and further understand the mechanism of the self-assembly. Since full length S-layers form insoluble 2D crystal we designed three functional protein fragments. We obtained crystals and determined the atomic structures of fragments containing the domains involved in the self-assembly. Both structures together suggests the mode of action how the self-assembly of the SlpA protein occurs. Furthermore, the putative exposed areas of the S-layer, which are important for potential interactions with the environment are analysed.

Keywords

S-Layer

Lactobacillus

Self-Assembly

X-ray-crystallography

Towards simulating relevant liposomes for drug delivery

Poster

Ms. Rianne van der Pol [1], ***Ms. Panagiota Papadopoulou*** [1], ***Ms. Gabriela Arias Alpizar*** [1], ***Dr. Fred Campbell*** [1], ***Dr. Alexander Kros*** [1], ***Dr. Geert Jan Agur Sevink*** [1]

1. *Institute of Chemistry, Leiden University*

Abstract

Targeted drug delivery increasingly benefits from the design of nanoparticles with distinct properties. In our group, we are experimentally focused on unraveling the structure-function relationship of novel, phase separated liposomes capable of specifically targeting (>50% I.D.) the blood-brain barrier *in vivo*. In silico methods can be employed to enhance understanding of this phenomenon and important factors in terms of structural properties, lipid mixing and phase coexistence, protein binding and dynamics, etcetera.

In silico experiments can be performed at different resolutions in time and space, from the electronic (QM) to the molecular (coarse-grained molecular dynamics (CGMD)) to the macroscopic scale (field models). Since interactions in field models are mean-field and soft, and the number of degrees of freedom are considerably reduced compared to methods with molecular resolution, models like self-consistent field (SCF) are suited for simulating liposomes at relevant scales. CGMD models have the advantage of retaining molecular resolution, but are limited in time and length scales.

In recent years, a number of groups have proposed hybrid approaches that aim at combining CGMD and SCF into a description that merges molecular detail with the potential to go to experimentally relevant time and length scales. While one of these, MD-SCF,[1] has been proven to reproduce equilibrium CGMD membrane profiles for unsaturated lipids,[2] which in theory enables simulation of realistic ~100nm diameter liposomes for several microseconds, there are still several challenges that have to be resolved before this method can be generally applied.

Recently, we have proposed an efficient approach to include long-range hydrodynamic interactions via momentum conservation into the MD-SCF framework.[3] Currently, we are working on including different equations of state into the MD-SCF framework, which will allow us to simulate gel phases of saturated lipids as well as liquid-gel phase transitions in lipid membranes. In this contribution, I will focus on these new developments via several preliminary results, highlighting their potential.

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Keywords

Drug delivery, MD-SCF,

Unraveling the mechanism of IFITM-mediated viral fusion inhibition

Poster

Mr. Niek van Hilten [1], ***Dr. Jelger Risselada*** [1]

1. *Leiden University*

Abstract

Interferon-induced transmembrane (IFITM) proteins inhibit the fusion between the viral envelope and the endosomal membrane of the host cell, thereby preventing infection. Evidently, it does so at a late fusion stage while leaving the host's innate functional fusion processes (like SNARE-mediated fusion) unaffected. Therefore, some mechanism must be in place for the IFITM proteins to specifically sense the looming presence of the virus before the opening of the viral fusion pore. In our research, we use coarse grained molecular dynamics to simulate the inhibitory effect of IFITMs on viral fusion with the ultimate aim to unravel this mechanism.

Keywords

Protein Membranes Virus Fusion Simulations

Authors Index