Année de publication : 2019

Beber A, Taveneau C, Nania M, Tsai FC, Di Cicco A, Bassereau P, Lévy D, Cabral JT, Isambert H, Mangenot S*, Bertin A* (2019 Jan 24)

Membrane reshaping by micrometric curvature sensitive septin filaments

Nature communications : DOI : 10.1038/s41467-019-08344-5

Résumé

Septins are cytoskeletal filaments that assemble at the inner face of the plasma membrane.They are localized at constriction sites and impact membrane remodeling. We report in vitro tools to examine how yeast septins behave on curved and deformable membranes. Septins reshape the membranes of Giant Unilamellar Vesicles with the formation of periodic spikes, while flattening smaller vesicles. We show that membrane deformations are associated to preferential arrangement of Septin filaments on specific curvatures. When binding to bilayers supported on custom-designed periodic wavy patterns displaying positive and negative micrometric radii of curvatures, septin filaments remain straight and perpendicular to the curvature of the convex parts, while bending negatively to follow concave geometries. Based on these results, we propose a theoretical model that describes the deformations and micrometric curvature sensitivity observed in vitro. The model captures the reorganizations of septin filaments throughout cytokinesis in vivo, providing mechanistic insights into cell division.

Année de publication : 2018

Feng-Ching Tsai*, Aurelie Bertin*, Hugo Bousquet, John Manzi, Yosuke Senju, Meng-Chen Tsai, Laura Picas, Stephanie Miserey-Lenkei, Pekka Lappalainen, Emmanuel Lemichez, Evelyne Coudrier*, Patricia Bassereau* (2018 Sep 30)

Ezrin enrichment on curved membranes requires a specific conformation or interaction with a curvature-sensitive partner.

elife : 7 : e37262 : DOI : 10.7554/eLife.37262

Résumé

One challenge in cell biology is to decipher the biophysical mechanisms governing protein enrichment on curved membranes and the resulting membrane deformation. The ERM protein ezrin is abundant and associated with cellular membranes that are flat, positively or negatively curved. Using *in vitro* and cell biology approaches, we assess mechanisms of ezrin's enrichment on curved membranes. We evidence that wild-type ezrin (ezrinWT) and its phosphomimetic mutant T567D (ezrinTD) do not deform membranes but self-assemble anti-parallelly, zipping adjacent membranes. EzrinTD's specific conformation reduces intermolecular interactions, allows binding to actin filaments, which reduces membrane tethering, and promotes ezrin binding to positively-curved membranes. While neither ezrinTD nor ezrinWT senses negative curvature alone, we demonstrate that interacting with curvature-sensing I-BAR-domain proteins facilitates ezrin enrichment in negatively-curved

membrane protrusions. Overall, our work demonstrates that ezrin can tether membranes, or be targeted to curved membranes, depending on conformations and interactions with actin and curvature-sensing binding partners.

Patricia Bassereau, Pierre Sens (2018 Sep 11)

Physics of Biological Membranes

Physics of Biological Membranes : DOI : 10.1007/978-3-030-00630-3

Résumé

Preface
In searching for optimal boundaries to separate living cells from their environment and to compartmentalize eukaryotic cells into regions of different properties, evolution has selected a design that appears universal: a bilayer made of lipid molecules. This design has physical properties that are particularly advantageous for the versatile boundary of a highly dynamical system that is in constant exchange with its environment. Lipid bilayers spontaneously self-assemble, owing to the amphiphilic nature of the lipids, and are rather impermeable to ions and large macromolecules. At physiological temperatures, they are fluid and deformable, allowing for large shape changes, and they are able to undergo fusion and scission without leakage. According to the fluid mosaic model of Singer and Nicholson (1972), the lipid bilayer provides the membrane with fluidity and elasticity, while most of the biological functions are performed by membrane-associated proteins. Since then our view of biomembranes has greatly evolved, and lipids themselves are now known to actively participate in many biological functions, either by directed interaction with other cellular components or by providing particular micro-environments for the proper functioning of proteins…

Nicola De Franceschi, Maryam Alqabandi, Nolwenn Miguet, Christophe Caillat, Stephanie Mangenot, Winfried Weissenhorn*, Patricia Bassereau* (2018 Aug 3)

The ESCRT protein CHMP2B acts as a diffusion barrier on reconstituted membrane necks.

Journal of Cell Science : 132 : jcs217968 : DOI : 10.1242/jcs.217968

Résumé

Endosomal sorting complexes required for transport (ESCRT)-III family proteins catalyze membrane remodeling processes that stabilize and constrict membrane structures. It has been proposed that stable ESCRT-III complexes containing CHMP2B could establish diffusion barriers at the post-synaptic spine neck. In order to better understand this process, we developed a novel method based on fusion of giant unilamellar vesicles to reconstitute ESCRT-III proteins inside GUVs, from which membrane nanotubes are pulled. The new assay ensures that ESCRT-III proteins polymerize only when they become exposed to physiologically relevant membrane topology mimicking the complex geometry of post-

synaptic spines. We establish that CHMP2B, both full-length and with a C-terminal deletion (ΔC), preferentially binds to membranes containing phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2]. Moreover, we show that CHMP2B preferentially accumulates at the neck of membrane nanotubes, and provide evidence that CHMP2B-ΔC prevents the diffusion of PI(4,5)P2 lipids and membrane-bound proteins across the tube neck. This indicates that CHMP2B polymers formed at a membrane neck may function as a diffusion barrier, highlighting a potential important function of CHMP2B in maintaining synaptic spine structures.

Patricia Bassereau, Rui Jin, Tobias Baumgart, Markus Deserno, Rumiana Dimova, Vadim A. Frolov, Pavel V. Bashkirov, Helmut Grubmüller, Reinhard Jahn, H. Jelger Risselada, Ludger Johannes, Michael M. Kozlov, Reinhard Lipowsky, Thomas J. Pucadyil, Wade F. Zeno, Jeanne C. Stachowiak, Dimitrios Stamou, Artù Breuer, Line Lauritsen, Camille Simon, Cécile Sykes, Gregory A. Voth, Thomas R Weikl (2018 Jul 20)

The 2018 biomembrane curvature and remodeling roadmap.

Journal of Physics D: Applied Physics : 51 : 343001 : DOI : 10.1088/1361-6463/aacb98

Résumé

The importance of curvature as a structural feature of biological membranes has been recognized for many years and has fascinated scientists from a wide range of different backgrounds. On the one hand, changes in membrane morphology are involved in a plethora of phenomena involving the plasma membrane of eukaryotic cells, including endo- and exocytosis, phagocytosis and filopodia formation. On the other hand, a multitude of intracellular processes at the level of organelles rely on generation, modulation, and maintenance of membrane curvature to maintain the organelle shape and functionality. The contribution of biophysicists and biologists is essential for shedding light on the mechanistic understanding and quantification of these processes.

Given the vast complexity of phenomena and mechanisms involved in the coupling between membrane shape and function, it is not always clear in what direction to advance to eventually arrive at an exhaustive understanding of this important research area. The 2018 Biomembrane Curvature and Remodeling Roadmap of *Journal of Physics D: Applied Physics* addresses this need for clarity and is intended to provide guidance both for students who have just entered the field as well as established scientists who would like to improve their orientation within this fascinating area.

Alexandre Beber, Maryam Alqabandi, Coline Prevost, Fanny Viars, Daniel Levy, Patricia Bassereau, Aurélie Bertin*, Stéphanie Mangenot* (2018 Jul 16)

Septin-based readout of PI(4,5)P2 incorporation into membranes of giant unilamellar vesicles

Cytoskeleton : DOI : 10.1002/cm.21480

Résumé

Septins constitute a novel class of cytoskeletal proteins. Budding yeast septins self-assemble into non-polar filaments bound to the inner plasma membrane through specific interactions with L- α-phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2). Biomimetic in vitro assays using Giant Unilamellar Vesicles (GUVs) are relevant tools to dissect and reveal insights in proteins-lipids interactions, membrane mechanics and curvature sensitivity. GUVs doped with PI(4,5)P2 are challenging to prepare. This report is dedicated to optimize the incorporation of PI(4,5)P2 lipids into GUVs by probing the proteins-PI(4,5)P2 GUVs interactions. We show that the interaction between budding yeast septins and PI(4,5)P2 is more specific than using usual reporters (phospholipase Cd1). Septins have thus been chosen as reporters to probe the proper incorporation of PI(4,5)P2 into giant vesicles. We have shown that electro-formation on platinum wires is the most appropriate method to achieve an optimal septin-lipid interaction resulting from an optimal PI(4,5)P2 incorporation for which, we have optimized the growth conditions. Finally, we have shown that PI(4,5)P2 GUVs have to be used within a few hours after their preparation. Indeed, over time, PI(4,5)P2 is expelled from he GUV membrane and the PI(4,5)P2 concentration in the bilayer decreases .

Begoña Ugarte-Uribe, Coline Prévost, Kushal Kumar Das, Patricia Bassereau, Ana J. García-Sáez (2018 Apr 19)

Drp1 polymerization stabilizes curved tubular membranes similar to those of constricted mitochondria.

Journal of Cell Science : 132 : jcs208603 : DOI : 10.1242/jcs.208603

Résumé

Dynamin-related protein 1 (Drp1), an 80 kDa mechanochemical GTPase of the dynamin superfamily, is required for mitochondrial division in mammals. Despite the role of Drp1 dysfunction in human disease, its molecular mechanism remains poorly understood. Here, we examined the effect of Drp1 on membrane curvature using tubes pulled from giant unilamellar vesicles (GUVs). We found that GTP promoted rapid rearrangement of Drp1 from a uniform distribution to discrete foci, in line with the assembly of Drp1 scaffolds at multiple nucleation sites around the lipid tube. Polymerized Drp1 preserved the membrane tube below the protein coat, also in the absence of pulling forces, but did not induce spontaneous membrane fission. Strikingly, Drp1 polymers stabilized membrane curvatures similar to those of constricted mitochondria against pressure changes. Our findings support a new model for mitochondrial division whereby Drp1 mainly acts as a scaffold for membrane curvature stabilization, which sets it apart from other dynamin homologs.

Mijo Simunovic, Patricia Bassereau, Gregory A. Voth (2018 Mar 30)

Organizing membrane-curving proteins: the emerging dynamical picture.

Current Opinion in Structural Biology : 51 : 99-105 : DOI : 10.1016/j.sbi.2018.03.018

Résumé

Lipid membranes play key roles in cells, such as in trafficking, division, infection, remodeling of organelles, among others. The key step in all these processes is creating membrane curvature, typically under the control of many anchored, adhered or included proteins. However, it has become clear that the membrane itself can mediate the interactions among proteins to produce highly ordered assemblies. Computer simulations are ideally suited to investigate protein organization and the dynamics of membrane remodeling at near-micron scales, something that is extremely challenging to tackle experimentally. We review recent computational efforts in modeling protein-caused membrane deformation mechanisms, specifically focusing on coarse-grained simulations. We highlight work that exposed the membrane-mediated ordering of proteins into lines, meshwork, spirals and other assemblies, in what seems to be a very generic mechanism driven by a combination of short and long-ranged forces. Modulating the mechanical properties of membranes is an underexplored signaling mechanism in various processes deserving of more attention in the near future.

Eran Agmon, Jérôme Solon, Patricia Bassereau, Brent R. Stockwell (2018 Mar 26)

Modeling the effects of lipid peroxidation during ferroptosis on membrane properties.

Scientific Reports : 8 : 5155 : DOI : 10.1038/s41598-018-23408-0

Résumé

Ferroptosis is a form of regulated cell death characterized by the accumulation of lipid hydroperoxides. There has been significant research on the pathways leading to the accumulation of oxidized lipids, but the downstream effects and how lipid peroxides cause cell death during ferroptosis remain a major puzzle. We evaluated key features of ferroptosis in newly developed molecular dynamics models of lipid membranes to investigate the biophysical consequences of lipid peroxidation, and generated hypotheses about how lipid peroxides contribute to cell death during ferroptosis.

Année de publication : 2017

Win Pin Ng, Kevin D. Webster, Caroline Stefani, Eva M. Schmid, Emmanuel Lemichez, Patricia Bassereau, Daniel A. Fletcher (2017 Oct 2)

Force-induced transcellular tunnel formation in endothelial cells

Molecular Biology of the Cell : 28 : 2650-2660 : DOI : 10.1091/mbc.E17-01-0080

Résumé

The endothelium serves as a protective semipermeable barrier in blood vessels and lymphatic vessels. Leukocytes and pathogens can pass directly through the endothelium by opening holes in endothelial cells, known as transcellular tunnels, which are formed by contact and self-fusion of the apical and basal plasma membranes. Here we test the

hypothesis that the actin cytoskeleton is the primary barrier to transcellular tunnel formation using a combination of atomic force microscopy and fluorescence microscopy of live cells. We find that localized mechanical forces are sufficient to induce the formation of transcellular tunnels in human umbilical vein endothelial cells (HUVECs). When HUVECs are exposed to the bacterial toxin called epidermal cell differentiation inhibitor (EDIN), which can induce spontaneous transcellular tunnels, less mechanical work is required to form tunnels due to the reduced cytoskeletal stiffness and thickness of these cells, similarly to the effects of a Rho-associated protein kinase (ROCK) inhibitor. We also observe actin enrichment in response to mechanical indentation that is reduced in cells exposed to the bacterial toxin. Our study shows that the actin cytoskeleton of endothelial cells provides both passive and active resistance against transcellular tunnel formation, serving as a mechanical barrier that can be overcome by mechanical force as well as disruption of the cytoskeleton.

Caroline Stefani, David Gonzalez-Rodriguez, Yosuke Senju, Anne Doye, Nadia Efimova, Sébastien Janel, Justine Lipuma, Meng Chen Tsai, Daniel Hamaoui, Madhavi P. Maddugoda, Olivier Cochet-Escartin, Coline Prévost, Frank Lafont, Tatyana Svitkina, Pekka Lappalainen, Patricia Bassereau, Emmanuel Lemichez (2017 Jun 23)

Ezrin enhances line tension along transcellular tunnel edges via NMIIa driven actomyosin cable formation.

Nature Communications : 8 : 15839 : DOI : 10.1038/ncomms15839

Résumé

Transendothelial cell macroaperture (TEM) tunnels control endothelium barrier function and are triggered by several toxins from pathogenic bacteria that provoke vascular leakage. Cellular dewetting theory predicted that a line tension of uncharacterized origin works at TEM boundaries to limit their widening. Here, by conducting high-resolution microscopy approaches we unveil the presence of an actomyosin cable encircling TEMs. We develop a theoretical cellular dewetting framework to interpret TEM physical parameters that are quantitatively determined by laser ablation experiments. This establishes the critical role of ezrin and non-muscle myosin II (NMII) in the progressive implementation of line tension. Mechanistically, fluorescence-recovery-after-photobleaching experiments point for the upstream role of ezrin in stabilizing actin filaments at the edges of TEMs, thereby favouring their crosslinking by NMIIa. Collectively, our findings ascribe to ezrin and NMIIa a critical function of enhancing line tension at the cell boundary surrounding the TEMs by promoting the formation of an actomyosin ring.

David Saletti, Jens Radzimanowski, Gregory Effantin, Daniel Midtvedt, Stéphanie Mangenot, Winfried Weissenhorn, Patricia Bassereau, Marta Bally (2017 Jan 26)

The Matrix protein M1 from influenza C virus induces tubular membrane invaginations in an in vitro cell membrane model.

Scientific reports : 40801 : DOI : 10.1038/srep40801

Résumé

Matrix proteins from enveloped viruses play an important role in budding and stabilizing virus particles. In order to assess the role of the matrix protein M1 from influenza C virus (M1-C) in plasma membrane deformation, we have combined structural and in vitro reconstitution experiments with model membranes. We present the crystal structure of the N-terminal domain of M1-C and show by Small Angle X-Ray Scattering analysis that full-length M1-C folds into an elongated structure that associates laterally into ring-like or filamentous polymers. Using negatively charged giant unilamellar vesicles (GUVs), we demonstrate that M1-C full-length binds to and induces inward budding of membrane tubules with diameters that resemble the diameter of viruses. Membrane tubule formation requires the C-terminal domain of M1-C, corroborating its essential role for M1-C polymerization. Our results indicate that M1-C assembly on membranes constitutes the driving force for budding and suggest that M1-C plays a key role in facilitating viral egress.

Garten M., Mosgaard L.D., Bornschlögl T., Dieudonné S., Bassereau P., Toombes G.E.S. (2017 Jan 1)

Whole-GUV patch-clamping

Proceedings of the National Academy of Sciences : 114 : 328-333 : DOI : 10.1073/pnas.1609142114

Résumé

Studying how the membrane modulates ion channel and transporter activity is challenging because cells actively regulate membrane properties, whereas existing in vitro systems have limitations, such as residual solvent and unphysiologically high membrane tension. Cell-sized giant unilamellar vesicles (GUVs) would be ideal for in vitro electrophysiology, but efforts to measure the membrane current of intact GUVs have been unsuccessful. In this work, two challenges for obtaining the “whole-GUV” patch-clamp configuration were identified and resolved. First, unless the patch pipette and GUV pressures are precisely matched in the GUV-attached configuration, breaking the patch membrane also ruptures the GUV. Second, GUVs shrink irreversibly because the membrane/glass adhesion creating the high-resistance seal (>1 GΩ) continuously pulls membrane into the pipette. In contrast, for cell-derived giant plasma membrane vesicles (GPMVs), breaking the patch membrane allows the GPMV contents to passivate the pipette surface, thereby dynamically blocking membrane spreading in the whole-GMPV mode. To mimic this dynamic passivation mechanism, beta-casein was encapsulated into GUVs, yielding a stable, high-resistance, whole-GUV configuration for a range of membrane compositions. Specific membrane capacitance measurements confirmed that the membranes were truly solvent-free and that membrane tension could be controlled over a physiological range. Finally, the potential for ion transport studies was tested using the model ion channel, gramicidin, and voltage-clamp fluorometry measurements were performed with a voltage-dependent fluorophore/quencher pair. Whole-GUV patch-clamping allows ion transport and other voltage-dependent processes to be studied while controlling membrane composition, tension, and shape.

Année de publication : 2016

Mijo Simunovic, Coline Prévost, Andrew Callan-Jones, Patricia Bassereau (2016 Jun 15)
Physical basis of some membrane shaping mechanisms.
Philosophical transactions. Series A, Mathematical, physical, and engineering sciences : DOI : 10.1098/rsta.2016.0034

Résumé

In vesicular transportation pathways, membrane lipids and proteins are internalized, externalized or transported Within cells, not by bulk diffusion of single molecules, goal embedded in the membrane of small vesicles or thin tubules. The formation of These 'transportation carriers' Follows sequential events: bending membrane fission from the donor compartment, and transportation Eventually fusion with the acceptor membrane. A similar sequence is Involved During the internalization of drug or gene carriers inside cells. These membrane-shaping events are mediated by proteins Generally binding to membranes. The thesis Mechanisms behind biological processes are Actively Studied Both in the context of cell biology and biophysics. Bin / Amphiphysin / Rvs (BAR) domain proteins are Ideally suited for single Illustrating how soft matter principles can account for deformation by membrane proteins. We review here Some experimental methods and theoretical models to measure Corresponding thesis how proteins affect the mechanics and the shape of membranes. In more detail, we show how an experimental method Employing optical tweezers to pull a tube from a giant vesicle May give significant quantitative insights into the mechanism by which proteins sense and generate membrane curvature and the mechanism of membrane scission.This article is share of the themed issue 'Soft interfacial materials: from fundamentals to formulation'.

Alice Berthaud, François Quemeneur, Maxime Deforet, Patricia Bassereau, Françoise Brochard-Wyart, Stéphanie Mangenot (2015 Dec 15)
Spreading of porous vesicles subjected to osmotic shocks: the role of aquaporins.
Soft matter : 12 : 1601-1609 : DOI : 10.1039/c5sm01654a

Résumé

Aquaporin 0 (AQP0) is a transmembrane protein specific to the eye lens, Involved as a water carrier across the lipid membranes. During maturation eye lens, AQP0s are truncated by proteolytic cleavage. We Investigate this work in the capability of truncated AQP0 to conduite water across membranes. We Developed a method to Accurately determine water permeability across lipid membranes and proteins across from the deflation under osmotic pressure of giant unilamellar vesicles (GUVs) Deposited adhesive substrate on year. Using reflection interference contrast microscopy (RICM), we measure the spreading area of GUVs During deswelling. We interpret thesis results using a model based on hydrodynamic, binder diffusion Reviews towards the touch area, and Helfrich's law for the membrane voltage, qui allows us to spread recounts the area to the internal vesicle volume. We first study the

specific adhesion of vesicles coated with biotin was spreading streptavidin substrate. Then we determine the permeability of a single functional AQP0 and Demonstrate That truncated AQP0 is no more a water channel.