
**The 2018 biomembrane curvature and remodeling roadmap.**


**Summary**

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.

Weria Pezeshkian, Haifei Gao, Senthil Arumugam, Ulrike Becken, Patricia Bassereau, Jean-Claude Florent, John Hjort Ipsen, Ludger Johannes, Julian C Shillcock (2017 Jan 24)

**Mechanism of Shiga Toxin Clustering on Membranes**


**Summary**

The bacterial Shiga toxin interacts with its cellular receptor, the glycosphingolipid globotriaosylceramide (Gb3 or CD77), as a first step to entering target cells. Previous studies have shown that toxin molecules cluster on the plasma membrane, despite the apparent lack of direct interactions between them. The precise mechanism by which this clustering occurs remains poorly defined. Here, we used vesicle and cell systems and computer models to investigate the mechanism of clustering.
simulations to show that line tension due to curvature, height, or compositional mismatch, and lipid or solvent depletion cannot drive the clustering of Shiga toxin molecules. By contrast, in coarse-grained computer simulations, a correlation was found between clustering and toxin nanoparticle-driven suppression of membrane fluctuations, and experimentally we observed that clustering required the toxin molecules to be tightly bound to the membrane surface. The most likely interpretation of these findings is that a membrane fluctuation-induced force generates an effective attraction between toxin molecules. Such force would be of similar strength to the electrostatic force at separations around 1 nm, remain strong at distances up to the size of toxin molecules (several nanometers), and persist even beyond. This force is predicted to operate between manufactured nanoparticles providing they are sufficiently rigid and tightly bound to the plasma membrane, thereby suggesting a route for the targeting of nanoparticles to cells for biomedical applications.

Year of publication 2016

Ludger Johannes, Christian Wunder, Massiullah Shafaq-Zadah (2016 Dec 17)
**Glycolipids and Lectins in Endocytic Uptake Processes.**

**Summary**

A host of endocytic processes has been described at the plasma membrane of eukaryotic cells. Their categorization has most commonly referenced cytosolic machinery, of which the clathrin coat has occupied a preponderant position. In what concerns intra-membrane constituents, the focus of interest has been on phosphatidylinositol lipids and their capacity to orchestrate endocytic events on the cytosolic leaflet of the membrane. The contribution of extracellular determinants to the construction of endocytic pits has received much less attention, despite the fact that (glyco)sphingolipids are exoplasmic leaflet fabric of membrane domains, termed rafts, whose contributions to predominantly clathrin-independent internalization processes is well recognized. Furthermore, sugar modifications on extracellular domains of proteins, and sugar-binding proteins, termed lectins, have also been linked to the uptake of endocytic cargoes at the plasma membrane. In this review, we first summarize these contributions by extracellular determinants to the endocytic process. We thus propose a molecular hypothesis – termed the GL-Lect hypothesis – on how GlycoLipids and Lectins drive the formation of compositional nanoenvironments from which the endocytic uptake of glycosylated cargo proteins is operated via clathrin-independent carriers. Finally, we position this hypothesis within the global context of endocytic pathway proposals that have emerged in recent years.

Weria Pezeshkian, Haifei Gao, Senthil Arumugam, Ulrike Becken, Patricia Bassereau, Jean-Claude Florent, John Hjort Ipsen, Ludger Johannes, Julian C Shillcock (2016 Dec 13)
**Mechanism of Shiga Toxin Clustering on Membranes.**
ACS nano
Summary

The bacterial Shiga toxin interacts with its cellular receptor, the glycosphingolipid globotriaosylceramide (Gb3 or CD77), as a first step to entering target cells. Previous studies have shown that toxin molecules cluster on the plasma membrane, despite the apparent lack of direct interactions between them. The precise mechanism by which this clustering occurs remains poorly defined. Here, we used vesicle and cell systems and computer simulations to show that line tension due to curvature, height, or compositional mismatch, and lipid or solvent depletion cannot drive the clustering of Shiga toxin molecules. By contrast, in coarse-grained computer simulations, a correlation was found between clustering and toxin nanoparticle-driven suppression of membrane fluctuations, and experimentally we observed that clustering required the toxin molecules to be tightly bound to the membrane surface. The most likely interpretation of these findings is that a membrane fluctuation-induced force generates an effective attraction between toxin molecules. Such force would be of similar strength to the electrostatic force at separations around 1 nm, remain strong at distances up to the size of toxin molecules (several nanometers), and persist even beyond. This force is predicted to operate between manufactured nanoparticles providing they are sufficiently rigid and tightly bound to the plasma membrane, thereby suggesting a route for the targeting of nanoparticles to cells for biomedical applications.


Spatiotemporal control of interferon-induced JAK/STAT signalling and gene transcription by the retromer complex.

Nature communications : 13476 : DOI : 10.1038/ncomms13476

Summary

Type-I interferons (IFNs) play a key role in the immune defences against viral and bacterial infections, and in cancer immunosurveillance. We have established that clathrin-dependent endocytosis of the type-I interferon (IFN-α/β) receptor (IFNAR) is required for JAK/STAT signalling. Here we show that the internalized IFNAR1 and IFNAR2 subunits of the IFNAR complex are differentially sorted by the retromer at the early endosome. Binding of the retromer VPS35 subunit to IFNAR2 results in IFNAR2 recycling to the plasma membrane, whereas IFNAR1 is sorted to the lysosome for degradation. Depletion of VPS35 leads to abnormally prolonged residency and association of the IFNAR subunits at the early endosome, resulting in increased activation of STAT1- and IFN-dependent gene transcription. These experimental data establish the retromer complex as a key spatiotemporal regulator of IFNAR endosomal sorting and a new factor in type-I IFN-induced JAK/STAT signalling and gene transcription.

Ludger Johannes, Christian Wunder (2016 Dec 3)
Retromer Sets a Trap for Endosomal Cargo Sorting.
*Cell*: 1452-1454 : [DOI: 10.1016/j.cell.2016.08.032]

Summary

Membrane trafficking from endosomes to the trans-Golgi network or the plasma membrane is driven by the retromer complex. Through structural analysis of the cargo-bound complex, Lucas et al. describe a mechanism by which endosomal membrane recruitment and cargo recognition are integrated through cooperative interactions between retromer subunits.


Enterococcus hirae and Barnesiella intestinihominis Facilitate Cyclophosphamide-Induced Therapeutic Immunomodulatory Effects.

Summary

The efficacy of the anti-cancer immunomodulatory agent cyclophosphamide (CTX) relies on intestinal bacteria. How and which relevant bacterial species are involved in tumor immunosurveillance, and their mechanism of action are unclear. Here, we identified two bacterial species, Enterococcus hirae and Barnesiella intestinihominis that are involved during CTX therapy. Whereas E. hirae translocated from the small intestine to secondary lymphoid organs and increased the intratumoral CD8/Treg ratio, B. intestinihominis accumulated in the colon and promoted the infiltration of IFN-γ-producing γδT cells in cancer lesions. The immune sensor, NOD2, limited CTX-induced cancer immunosurveillance and the bioactivity of these microbes. Finally, E. hirae and B. intestinihominis specific-memory Th1 cell immune responses selectively predicted longer progression-free survival in advanced lung and ovarian cancer patients treated with chemo-immunotherapy. Altogether, E. hirae and B. intestinihominis represent valuable “oncomicrobiotics” ameliorating the efficacy of the most common alkylating immunomodulatory compound.

Inhibitors of retrograde trafficking active against ricin and Shiga toxins also protect cells from several viruses, Chlamydiales and Leishmania.

Chemico-biological interactions: DOI: 10.1009-27971630427-6

Summary

Medical countermeasures to treat biothreat agent infections require broad-spectrum therapeutics that do not induce agent resistance. A cell-based high-throughput screen (HTS) against ricin toxin combined with hit optimization allowed selection of a family of compounds that meet these requirements. The hit compound Retro-2 and its derivatives have been demonstrated to be safe in vivo in mice even at high doses. Moreover, Retro-2 is an inhibitor of retrograde transport that affects syntaxin-5-dependent toxins and pathogens. As a consequence, it has a broad-spectrum activity that has been demonstrated both in vitro and in vivo against ricin, Shiga toxin-producing O104:H4 entero-hemorrhagic E. coli and Leishmania sp. and in vitro against Ebola, Marburg and poxviruses and Chlamydiales. An effect is anticipated on other toxins or pathogens that use retrograde trafficking and syntaxin-5. Since Retro-2 targets cell components of the host and not directly the pathogen, no selection of resistant pathogens is expected. These lead compounds need now to be developed as drugs for human use.


How curvature-generating proteins build scaffolds on membrane nanotubes.
Proceedings of the National Academy of Sciences of the United States of America: 113: DOI: 10.1073/pnas.1606943113

Summary

Bin/Amphiphysin/Rvs (BAR) domain proteins control the curvature of lipid membranes in endocytosis, trafficking, cell motility, the formation of complex subcellular structures, and many other cellular phenomena. They form 3D assemblies that act as molecular scaffolds to reshape the membrane and alter its mechanical properties. It is unknown, however, how a protein scaffold forms and how BAR domains interact in these assemblies at protein densities relevant for a cell. In this work, we use various experimental, theoretical, and simulation approaches to explore how BAR proteins organize to form a scaffold on a membrane nanotube. By combining quantitative microscopy with analytical modeling, we demonstrate that a highly curving BAR protein endophilin nucleates its scaffolds at the ends of a membrane tube, contrary to a weaker curving protein centaurin, which binds evenly along the tube’s length. Our work implies that the nature of local protein-membrane interactions can affect the specific localization of proteins on membrane-remodeling sites. Furthermore, we show that amphipathic helices are dispensable in forming protein scaffolds. Finally, we explore a possible molecular structure of a BAR-domain scaffold using coarse-grained molecular dynamics simulations. Together with fluorescence microscopy, the simulations show that proteins need only to cover 30-40% of a tube’s surface to form a rigid assembly. Our work provides mechanical and structural insights into the way BAR proteins may sculpt
the membrane as a high-order cooperative assembly in important biological processes.


**Quantum dot-loaded monofunctionalized DNA icosahedra for single-particle tracking of endocytic pathways.**

*Nature nanotechnology*: [DOI: 10.1038/nnano.2016.150](http://dx.doi.org/10.1038/nnano.2016.150)

**Summary**

Functionalization of quantum dots (QDs) with a single biomolecular tag using traditional approaches in bulk solution has met with limited success. DNA polyhedra consist of an internal void bounded by a well-defined three-dimensional structured surface. The void can house cargo and the surface can be functionalized with stoichiometric and spatial precision. Here, we show that monofunctionalized QDs can be realized by encapsulating QDs inside DNA icosahedra and functionalizing the DNA shell with an endocytic ligand. We deployed the DNA-encapsulated QDs for real-time imaging of three different endocytic ligands—folic acid, galectin-3 (Gal3) and the Shiga toxin B-subunit (STxB). Single-particle tracking of Gal3- or STxB-functionalized QD-loaded DNA icosahedra allows us to monitor compartmental dynamics along endocytic pathways. These DNA-encapsulated QDs, which bear a unique stoichiometry of endocytic ligands, represent a new class of molecular probes for quantitative imaging of endocytic receptor dynamics.

Laxmidhar Rout, Bibhuti Bhusan Parida, Jean-Claude Florent, Ludger Johannes, Santosh Kumar Choudhury, Ganngam Phaomei, Scalnon Joe, Emmanuel Bertouesque (2016 Aug 10)

**Metal-Free Activation of C(sp)-H Bond of Aromatic Acetylene.**

*Chemistry (Weinheim an der Bergstrasse, Germany)*: [DOI: 10.1002/chem.201603003](http://dx.doi.org/10.1002/chem.201603003)

**Summary**

C(sp)-H bond activation of acetylene molecule still remains a challenge for synthetic organic chemist. In practice, it is activated by strong base and metal atoms. A very first example for activating acetylenic proton under base and metal-free condition has been reported here. It gives a general method for synthesizing propargylic derivatives of cotarine. An array of tetrahydro-isoquinolines alkaloids was synthesized by C(sp)-H bond activation of aromatic acetylenes with cotarine at room temperature. A DFT based mechanism is proposed for following reaction has been revealed.

Cédric M Blouin, Yannick Hamon, Pauline Gonnord, Cédric Boularan, Jérémy Kagan, Christine Viaris de Lesegno, Richard Ruez, Sébastien Mailfert, Nicolas Bertaux, Damarys Loew, Christian Wunder, Ludger Johannes, Guillaume Vogt, Francesc-Xabier Contreras, Didier Marguet, Jean-
Laurent Casanova, Céline Galès, Hai-Tao He, Christophe Lamaze (2016 Aug 9)  
**Glycosylation-Dependent IFN-γR Partitioning in Lipid and Actin Nanodomains Is Critical for JAK Activation.**  
*Cell*: 920-34: DOI: [10.1016/j.cell.2016.07.003](http://dx.doi.org/10.1016/j.cell.2016.07.003)  

**Summary**

Understanding how membrane nanoscale organization controls transmembrane receptors signaling activity remains a challenge. We studied interferon-γ receptor (IFN-γR) signaling in fibroblasts from homozygous patients with a T168N mutation in IFNGR2. By adding a neo-N-glycan on IFN-γR subunit, this mutation blocks IFN-γ activity by unknown mechanisms. We show that the lateral diffusion of IFN-γR2 is confined by sphingolipid/cholesterol nanodomains. In contrast, the IFN-γR2 T168N mutant diffusion is confined by distinct actin nanodomains where conformational changes required for Janus-activated tyrosine kinase/signal transducer and activator of transcription (JAK/STAT) activation by IFN-γ could not occur. Removing IFN-γR2 T168N-bound galectins restored lateral diffusion in lipid nanodomains and JAK/STAT signaling in patient cells, whereas adding galectins impaired these processes in control cells. These experiments prove the critical role of dynamic receptor interactions with actin and lipid nanodomains and reveal a new function for receptor glycosylation and galectins. Our study establishes the physiological relevance of membrane nanodomains in the control of transmembrane receptor signaling in vivo. VIDEO ABSTRACT.

Thi Tran, Mariana De Oliveira Diniz, Estelle Dransart, Alain Gey, Nathalie Merillon, Yu Chun Lone, Sylvie Godefroy, Craig Sibley, Jacques Medioni, Stephane Oudard, Ludger Johannes, Luís Carlos de Souza Ferreira, Eric Tartour (2016 Mar 24)  
**A therapeutic Her2/neu vaccine targeting dendritic cells preferentially inhibits the growth of low Her2/neu-expressing tumor in HLA-A2 transgenic mice.**  
*Clinical cancer research*: an official journal of the American Association for Cancer Research: DOI: [clincanres.0044.2016](http://dx.doi.org/clincanres.0044.2016)  

**Summary**

E75, a peptide derived from the Her2/neu protein, is the most clinically advanced vaccine approach against breast cancer. In this study, we aimed to optimize the E75 vaccine using a delivery vector targeting dendritic cells – the B-subunit of Shiga toxin (STxB) – and to assess the role of various parameters (Her2/neu expression, combination with trastuzumab) in the efficacy of this cancer vaccine in a relevant preclinical model.

Philipp Emanuel Geyer, Matthias Maak, Ulrich Nitsche, Markus Perl, Alexander Novotny, Julia Slotta-Huspenina, Estelle Dransart, Anne Holtorf, Ludger Johannes, Klaus-Peter Janssen (2016 Jan 31)  
**Gastric Adenocarcinomas Express the Glycosphingolipid Gb3/CD77: Targeting of**
**Gastric Cancer Cells with Shiga Toxin B-Subunit.**

*Molecular cancer therapeutics*: 1008-17 : [DOI: 10.1158/1535-7163.MCT-15-0633](https://doi.org/10.1158/1535-7163.MCT-15-0633)

### Summary

The B-subunit of the bacterial Shiga toxin (STxB), which is nontoxic and has low immunogenicity, can be used for tumor targeting of breast, colon, and pancreatic cancer. Here, we tested whether human gastric cancers, which are among the most aggressive tumor entities, express the cellular receptor of Shiga toxin, the glycosphingolipid globotriaosylceramide (Gb3/CD77). The majority of cases showed an extensive staining for Gb3 (36/50 cases, 72%), as evidenced on tissue sections of surgically resected specimen. Gb3 expression was detected independent of type (diffuse/intestinal), and was negatively correlated to increasing tumor-node-metastasis stages (P = 0.0385), as well as with markers for senescence. Gb3 expression in nondiseased gastric mucosa was restricted to chief and parietal cells at the bottom of the gastric glands, and was not elevated in endoscopic samples of gastritis (n = 10). Gb3 expression in established cell lines of gastric carcinoma was heterogeneous, with 6 of 10 lines being positive, evidenced by flow cytometry. STxB was taken up rapidly by live Gb3-positive gastric cancer cells, following the intracellular retrograde transport route, avoiding lysosomes and rapidly reaching the Golgi apparatus and the endoplasmic reticulum. Treatment of the Gb3-expressing gastric carcinoma cell line St3051 with STxB coupled to SN38, the active metabolite of the topoisomerase type I inhibitor irinotecan, resulted in >100-fold increased cytotoxicity, as compared with irinotecan alone. No cytotoxicity was observed on gastric cancer cell lines lacking Gb3 expression, demonstrating receptor specificity of the STxB-SN38 compound. Thus, STxB is a highly specific transport vehicle for cytotoxic agents in gastric carcinoma. Mol Cancer Ther; 15(5): 1008-17. ©2016 AACR.


**Persistent cell migration and adhesion rely on retrograde transport of β(1) integrin**

*Nature Cell Biology*: 18 : 54-64 : [DOI: 10.1038/ncb3287](https://doi.org/10.1038/ncb3287)

### Summary

Integrins have key functions in cell adhesion and migration. How integrins are dynamically relocalized to the leading edge in highly polarized migratory cells has remained unexplored. Here, we demonstrate that β1 integrin (known as PAT-3 in Caenorhabditis elegans), but not β3, is transported from the plasma membrane to the trans-Golgi network, to be resecreted in a polarized manner. This retrograde trafficking is restricted to the non-ligand-bound conformation of β1 integrin. Retrograde trafficking inhibition abrogates several β1-integrin-specific functions such as cell adhesion in early embryonic development of mice, and persistent cell migration in the developing posterior gonad arm of C. elegans. Our results establish a paradigm according to which retrograde trafficking, and not endosomal recycling, is the key driver for β1 integrin function in highly polarized cells. These data more generally suggest that the retrograde route is used to relocalize plasma membrane machinery from...
previous sites of function to the leading edge of migratory cells.