Structure of presentations

Introduction : **5-10** min short review of the theme in respect of the historical period

Aim of the study : **2 min**

Methods used : **5-10** min

Results: **15 min**

Conclusion **3 min**

Take home messages **2 min**

Week-1 : In the membrane

A classic review review for background :

Revisiting the fluid mosaic model of membranes, Jacobson et al Science 1995

DOI: 10.1126/science.7770769

Modern reviews :

The mystery of membrane organization: composition, regulation and roles of lipid rafts.

Erdinc Sezgin, Ilya Levental, Satyajit Mayor, Christian Eggeling

Nature Reviews Molecular Cell Biology **volume 18**, pages 361–374 (2017)

https://doi.org/10.1038/nrm.2017.16

NB have a look at the supplements.

Membrane organization and lipid rafts

Kai Simons [1], Julio L Sampaio Cold Spring Harb Perspect Biol

2011 Oct 1;3(10):a004697.

doi: 10.1101/cshperspect.a004697.

Group-1 : In the membrane Presenting on Nov-26

Group 1- Louise DROIN - Romain GUICHONNET - Flavia ZABALA PEREZ Laure CARMINATI

Phospholipids undergo hop diffusion in compartmentalized cell membrane

Takahiro Fujiwara 1 , Ken Ritchie, Hideji Murakoshi, Ken Jacobson, Akihiro Kusumi

Journal of Cell Biology July 2002; 157(6):1071-81. ***DOI:10.1083/jcb.200202050***

PMID: 12058021 PMCID: PMC2174039 **DOI: 10.1083/jcb.200202050**

Abstract

The diffusion rate of lipids in the cell membrane is reduced by a factor of 5-100 from that in artificial bilayers. This slowing mechanism has puzzled cell biologists for the last 25 yr. Here we address this issue by studying the movement of unsaturated phospholipids in rat kidney fibroblasts at the single molecule level at the temporal resolution of 25 micros. The cell membrane was found to be compartmentalized: phospholipids are confined within 230-nm-diameter (phi) compartments for 11 ms on average before hopping to adjacent compartments. These 230-nm compartments exist within greater 750-nm-phi compartments where these phospholipids are confined for 0.33 s on average. The diffusion rate within 230-nm compartments is 5.4 microm2/s, which is nearly as fast as that in large unilamellar vesicles, indicating that the diffusion in the cell membrane is reduced not because diffusion per se is slow, but because the cell membrane is compartmentalized with regard to lateral diffusion of phospholipids. Such compartmentalization depends on the actin-based membrane skeleton, but not on the extracellular matrix, extracellular domains of membrane proteins, or cholesterol-enriched rafts. We propose that various transmembrane proteins anchored to the actin-based membrane skeleton meshwork act as rows of pickets that temporarily confine phospholipids.

Group 2 - Coralie Reuse, Coralie Charpié, Jonas Richard, Vera Dias Gomes.

Sharma, P. et al. Nanoscale organization of multiple GPI-anchored proteins in living cell membranes.

***Cell* 116, 577–589 (2004). The first demonstration of cholesterol-assisted nanoscale clusters in living cells.**

doi: 10.1016/s0092-8674(04)00167-9.

Abstract

Cholesterol and sphingolipid-enriched “rafts” have long been proposed as platforms for the sorting of specific membrane components including glycosyl-phosphatidylinositol-anchored proteins (GPI-APs), however, their existence and physical properties have been controversial. Here, we investigate the size of lipid-dependent organization of GPI-APs in live cells, using homo and hetero-FRET-based experiments, combined with theoretical modeling. These studies reveal an unexpected organization wherein cell surface GPI-APs are present as monomers and a smaller fraction (20%–40%) as nanoscale (<5 nm) cholesterol-sensitive clusters. These clusters are composed of at most four molecules and accommodate diverse GPI-AP species; crosslinking GPI-APs segregates them from preexisting GPI-AP clusters and prevents endocytosis of the crosslinked species via a GPI-AP-selective pinocytic pathway. In conjunction with an analysis of the statistical distribution of the clusters, these observations suggest a mechanism for functional lipid-dependent clustering of GPI-APs.

-

Group 3 - Ami De Carli , Aldric Mercier , Emile Chauvel

2 linked papers

Direct chemical evidence for sphingolipid domains

in the plasma membranes of fibroblasts.

Jessica F. Frisz, Kaiyan Lou, Haley A. Klitzing, +8 , and Mary L. Kraft

Proc Nat Acad Sci USA 110,

https://doi.org/10.1073/pnas.1216585110

Abstract

Sphingolipids play important roles in plasma membrane structure and cell signaling. However, their lateral distribution in the plasma membrane is poorly understood. Here we quantitatively analyzed the sphingolipid organization on the entire dorsal surface of intact cells by mapping the distribution of ^{15}N-enriched ions from metabolically labeled ^{15}N-sphingolipids in the plasma membrane, using high-resolution imaging mass spectrometry. Many types of control experiments (internal, positive, negative, and fixation temperature), along with parallel experiments involving the imaging of fluorescent sphingolipids—both in living cells and during fixation of living cells—exclude potential artifacts. Micrometer-scale sphingolipid patches consisting of numerous ^{15}N-sphingolipid microdomains with mean diameters of ~200 nm are always present in the plasma membrane. Depletion of 30% of the cellular cholesterol did not eliminate the sphingolipid domains, but did reduce their abundance and long-range organization in the plasma membrane. In contrast, disruption of the cytoskeleton eliminated the sphingolipid domains. These results indicate that these sphingolipid assemblages are not lipid rafts and are instead a distinctly different type of sphingolipid-enriched plasma membrane domain that depends upon cortical actin.

Sphingolipid domains in the plasma membranes of fibro blasts are not enriched with cholesterol **-**

Jessica F Frisz [‡], Haley A Klitzing [‡], Kaiyan Lou [§], Ian D Hutcheon [¶], Peter K Weber [¶], Joshua Zimmerberg [‖], Mary L Kraft

J Biol Chem

2013 Apr 22;288(23):16855–16861. **doi: 10.1074/jbc.M113.473207**

Abstract

The plasma membranes of mammalian cells are widely expected to contain domains that are enriched with cholesterol and sphingolipids. In this work, we have used high-resolution secondary ion mass spectrometry to directly map the distributions of isotope-labeled cholesterol and sphingolipids in the plasma membranes of intact fibroblast cells. Although acute cholesterol depletion reduced sphingolipid domain abundance, cholesterol was evenly distributed throughout the plasma membrane and was not enriched within the sphingolipid domains. Thus, we rule out favorable cholesterol-sphingolipid interactions as dictating plasma membrane organization in

fibroblast cells. Because the sphingolipid domains are disrupted by drugs that depolymerize the cells actin cytoskeleton, cholesterol must instead affect the sphingolipid organization via an indirect mechanism that involves the cytoskeleton.

Group 4 Bärlocher Aguilar Cédric Josue,Chopra Shahina, Tergalinsky Arsène Timothée, Van Rossum Marie

A fluorescent membrane tension probe

Nature Chemistry https://doi.org/10.1038/s41557-018-0127-3

Cells and organelles are delimited by lipid bilayers in which high deformability is essential to many cell processes, including motility, endocytosis and cell division. Membrane tension is therefore a major regulator of the cell processes that remodel membranes, albeit one that is very hard to measure in vivo. Here we show that a planarizable push–pull fluorescent probe called **FliptR** (fluorescent lipid tension reporter) can monitor changes in membrane tension by changing its fluorescence life- time as a function of the twist between its fluorescent groups. The fluorescence lifetime depends linearly on membrane tension within cells, enabling an easy quantification of membrane tension by fluorescence lifetime imaging microscopy.

We further show, using model membranes, that this linear dependency between lifetime of the probe and membrane tension relies on a membrane-tension-dependent lipid phase separation. We also provide calibration curves that enable accurate measurement of membrane tension using fluorescence lifetime imaging microscopy.

Nature Protocols protocols https://doi.org/10.1038/s41596-024-01027-6

https://doi.org/10.1016/j.ceb.2023.102294

A supporting review

Mechanotransduction through membrane tension: It's all about propagation?

Author links open overlay panelAndrea Ghisleni, Nils C. Gauthier

Gruop 5 : Rehn Amanda Matilda Olivia, Rossboth Cédric Nathanaël, Rousset Théo, Santos Martins Diogo

Nanodisc and Rhodopsin

Paper: **Transducin activation by nanoscale lipid bilayers containing one and two rhodopsins**

Timothy H Bayburt [1], Andrew J Leitz, Guifu Xie, Daniel D Oprian, Stephen G Sliga

J Biol Chem . 2007 May 18;282(20):14875-81.

doi: 10.1074/jbc.M701433200

Nanodiscs are nanometer scale planar membranes of controlled size that are rendered soluble in aqueous solution via an encircling amphipathic membrane scaffold protein "belt" (Bayburt, T. H., Grinkova, Y. V., and Sligar, S. G. (2002) Nano. Lett. 2, 853-856). Integral membrane proteins can be self-assembled into the Nanodisc bilayer with defined stoichiometry, which allows an unprecedented opportunity to investigate the nature of the oligomerization state of a G-protein-coupled receptor and its coupling to heterotrimeric G-proteins. We generated Nanodiscs having one and two rhodopsins present in the 10-nm-diameter lipid bilayer domain. Efficient transducin activation and isolation of a high affinity transducin-metarhodopsin II complex was demonstrated for a monodisperse and monomeric receptor. A population of Nanodiscs containing two rhodopsins was generated using an increased ratio of receptor to membrane scaffold protein in the self-assembly mixture. The two-rhodopsin population was isolated and purified by density gradient centrifugation. Interestingly, in this case, only one of the two receptors present in the Nanodisc was able to form a stable metarhodopsin II-G-protein complex. Thus there is clear evidence that a monomeric rhodopsin is capable of full coupling to transducin. Importantly, presumably due to steric interactions, it appears that only a single receptor in the Nanodiscs containing two rhodopsins can interact with G-protein. These results have important implications for the stoichiometry of receptor-G-protein coupling and cross talk in signaling pathways

Review: Nanodiscs: A toolkit for membrane protein science

Stephen G. Sligar | Ilia G. Denisov DOI: 10.1002/pro.3994

J Am Chem Soc

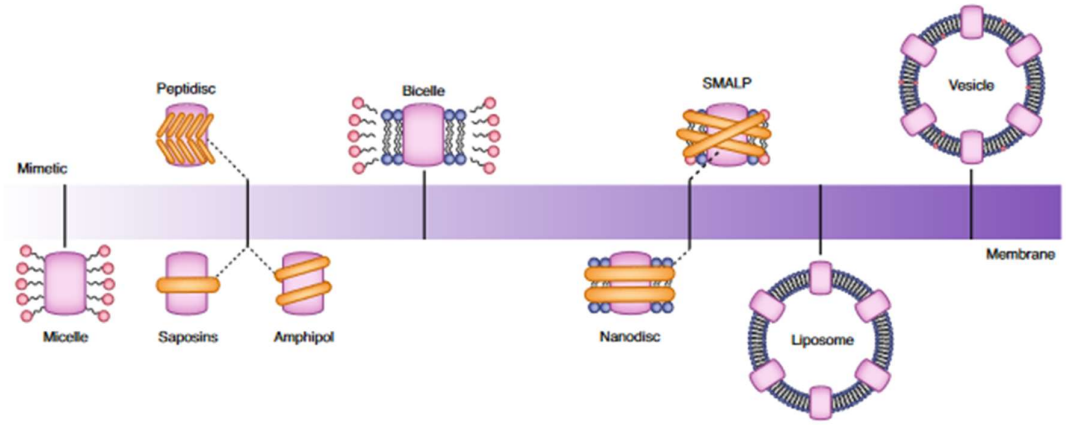

Fig. 2 | From micelles to membranes. The transition from detergent micelles through the various mimetics that have been developed over the last few decades, each with the overall goal of extracting the protein and reconstituting as closely as possible to its original membrane environment. The plethora of membrane protein structures coming to the fore can also be attributed to the new ways of stabilizing these protein complexes in membrane mimetics introduced in recent years. There are now a wide range of such mimetics including nanodiscs, synthetic polymers, amphipols, peptidiscs and saposins complimenting more established approaches of bicelles, liposomes and vesicles. They each have merits and compatibilities with various methods but are rarely universal. For example, bicelles, whereby the membrane protein is encapsulated in lipids flanked by detergent, are compatible with NMR[89]. Nanodiscs are often selected for structure determination via EM[42], and peptidiscs are employed with affinity preparations[55]. Moreover, while all methods are capable of capturing aspects of the membrane environment, inherent differences in their properties lead to more ready application for certain types c protein complexes or applications.

Group 6: Benard Arthur Yves Noël, Bongini Arthur, Cerutti Louma Valentine Jade, De Backer Michiel Hubert L

Paper 1 :- **A Chemogenetic Approach for the Optical Monitoring of Voltage in Neurons**

German Edition: DOI: 10.1002/ange.201812967Imaging Agents

International Edition: DOI: 10.1002/anie.201812967

Mayya Sundukova,* Efthymia Prifti, Annalisa Bucci, Kseniia Kirillova, Joana Serrao, Luc Reymond, Miwa Umebayashi, Ruud Hovius, Howard Riezman, Kai Johnsson, and Paul A. Heppenstall

Abstract: Optical monitoring of neuronal voltage using fluorescent indicators is a powerful approach for the interrogation of the cellular and molecular logic of the nervous system. Herein, a semisynthetic tethered voltage indicator (STeVI1) based upon nile red is described that displays voltage sensitivity when genetically targeted to neuronal membranes. This environmentally sensitive probe allows for wash-free imaging and faithfully detects supra- and sub-threshold activity in neurons

Supporting info online

Filename		Description
anie201812967-sup-0001-misc_information.pdf	923.1 KB	Supplementary
anie201812967-sup-0001-Movie_1.avi	15 MB	Supplementary
anie201812967-sup-0001-Movie_2.avi	526.1 KB	Supplementary
anie201812967-sup-0001-Movie_3.avi	12.8 MB	

Paper 2:

A Genetically Encoded Optical Probe of Membrane Voltage

Author : Micah S Siegel [2] [1], Ehud Y Isacoff [1] ‡

Neuron, Volume 19, Issue 4, October 1997, Pages 735-741

Volume 19, Issue 4, October 1997, Pages 735-741

https://doi.org/10.1016/S0896-6273(00)80955-1

Measuring electrical activity in large numbers of cells with high spatial and temporal resolution is a fundamental problem for the study of neural development and information processing. To address this problem, we have constructed a novel, genetically encoded probe that can be used to measure transmembrane voltage in single cells. We fused a modified green fluorescent protein (GFP) into a voltage-sensitive K^+ channel so that voltage-dependent rearrangements in the K^+ channel would induce changes in the fluorescence of GFP. The probe has a maximal fractional fluorescence change of 5.1%, making it comparable to some of the best organic voltage-sensitive dyes. Moreover, the fluorescent signal is expanded in time in a way that makes the signal 30-fold easier to detect. A voltage sensor encoded into DNA has the advantage that it may be introduced into an organism noninvasively and targeted to specific developmental stages, brain regions, cell types, and subcellular compartments.

Group 7 : Kacem Youssef, Lang Victoire Jacqueline Françoise, Lopez Mejias Dulce Milagro, Mahfouz Maria

Paper

A covalently linked probe to monitor local membrane properties surrounding plasma membrane proteins

Miwa Umebayashi, Satoko Takemoto, Luc Reymond, Mayya Sundukova, , Ruud Hovius, Annalisa Bucci, Paul A. Heppenstall, Hideo Yokota, Kai Johnsson, and Howard Riezman

J Cell Biol (2023) 222 (3): e202206119

https://doi.org/10.1083/jcb.202206119

Abstract

Functional membrane proteins in the plasma membrane are suggested to have specific membrane environments that play important roles to maintain and regulate their function. However, the local membrane environments of membrane proteins remain largely unexplored due to the lack of available techniques. We have developed a method to probe the local membrane environment surrounding membrane proteins in the plasma membrane by covalently tethering a solvatochromic,

environment-sensitive dye, Nile Red, to a GPI-anchored protein and the insulin receptor through a flexible linker. The fluidity of the membrane environment of the GPI-anchored protein depended upon the saturation of the acyl chains of the lipid anchor.

The local environment of the insulin receptor was distinct from the average plasma membrane fluidity and was quite dynamic and heterogeneous. Upon addition of insulin, the local membrane environment surrounding the receptor specifically increased in fluidity in an insulin receptor-kinase dependent manner and on the distance between the dye and the receptor

NB See supplement information

Group 8: Pinsard Bastien, Roudaut Arthur Joël, Santos Martins Diogo, Susanu Octavian

Group 9: Tambey Diane, Verhoeven Anthony David

A fluorogenic probe for SNAP-tagged plasma membrane proteins based on the solvatochromic molecule Nile Red

Efthymia Prifti, Luc Reymond, Miwa Umebayashi, Ruud Hovius, Howard Riezman, Kai Johnsson

ACS chemical biology

https://**doi.org/10.1021/cb400819c**

Published January 14, 2014

Abstract

A fluorogenic probe for plasma membrane proteins based on the dye Nile Red and SNAP-tag is introduced. It takes advantage of Nile Red, a solvatochromic molecule highly fluorescent in an apolar environment, such as cellular membranes, but almost dark in a polar aqueous environment. The probe possesses a tuned affinity for membranes allowing its Nile Red moiety to insert into the lipid bilayer of the plasma membrane, becoming fluorescent, only after its conjugation to a SNAP-tagged plasma membrane protein. The fluorogenic character of the probe was demonstrated for different SNAP-tag fusion proteins, including the human insulin receptor. This work introduces a new approach for generating a powerful turn-on probe for “no-wash” labeling of plasma membrane proteins with numerous applications in bioimaging.

Group 8

Nanodisc and Rhodopsin

Paper: **Transducin activation by nanoscale lipid bilayers containing one and two rhodopsins**

Timothy H Bayburt [1], Andrew J Leitz, Guifu Xie, Daniel D Oprian, Stephen G Sliga

J Biol Chem . 2007 May 18;282(20):14875-81.

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Nanodiscs are nanometer scale planar membranes of controlled size that are rendered soluble in aqueous solution via an encircling amphipathic membrane scaffold protein "belt" (Bayburt, T. H., Grinkova, Y. V., and Sligar, S. G. (2002) Nano. Lett. 2, 853-856). Integral membrane proteins can be self-assembled into the Nanodisc bilayer with defined stoichiometry, which allows an unprecedented opportunity to investigate the nature of the oligomerization state of a G-protein-coupled receptor and its coupling to heterotrimeric G-proteins. We generated Nanodiscs having one and two rhodopsins present in the 10-nm-diameter lipid bilayer domain. Efficient transducin activation and isolation of a high affinity transducin-metarhodopsin II complex was demonstrated for a monodisperse and monomeric receptor. A population of Nanodiscs containing two rhodopsins was generated using an increased ratio of receptor to membrane scaffold protein in the self-assembly mixture. The two-rhodopsin population was isolated and purified by density gradient centrifugation. Interestingly, in this case, only one of the two receptors present in the Nanodisc was able to form a stable metarhodopsin II-G-protein complex. Thus there is clear evidence that a monomeric rhodopsin is capable of full coupling to transducin. Importantly, presumably due to steric interactions, it appears that only a single receptor in the Nanodiscs containing two rhodopsins can interact with G-protein. These results have important implications for the stoichiometry of receptor-G-protein coupling and cross talk in signaling pathways

Review: Nanodiscs: A toolkit for membrane protein science

Stephen G. Sligar | Ilia G. Denisov DOI: 10.1002/pro.3994

J Am Chem Soc

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. 2004 Mar 24;126(11):3477-87.

doi: 10.1021/ja0393574.

Artical

Directed self-assembly of monodisperse phospholipid bilayer Nanodiscs with controlled size

JACS

I G Denisov [1], Y V Grinkova, A A Lazarides, S G Sligar

Journal of the American Chemical Society

Vol 126/Issue 11

- DOI: 10.1021/ja0393574

DOI: 10.1074/jbc.M110.151043

J Biol Chem

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. 2011 Jan 14;286(2):1420-8.

doi: 10.1074/jbc.M110.151043. Epub 2010 Oct 21.

Monomeric rhodopsin is sufficient for normal rhodopsin kinase (GRK1) phosphorylation and arrestin-1 binding