Plasma membrane structure and dynamics explored via a combined AFM/FCS approach

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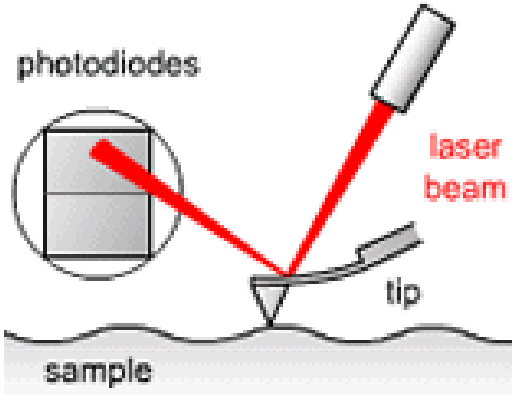
Dresden nanoSeminar, May 2013

Outline

1. Combination of AFM and quantitative fluorescence microscopy
2. Biophysical studies of plasma membrane heterogeneities
3. Advanced models for the plasma membrane: asymmetric bilayers
4. Inter-leaflet coupling in asymmetric membranes
5. Future plans

Experimental approach: Techniques

Atomic force microscopy (AFM)

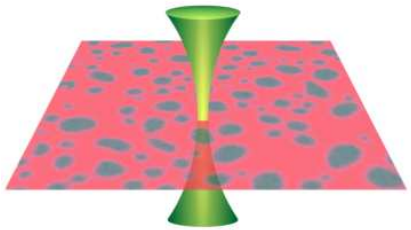


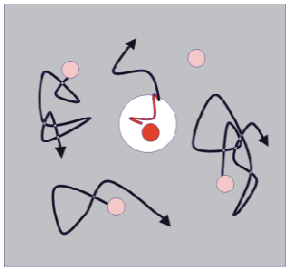
Nm resolution imaging in physiological conditions

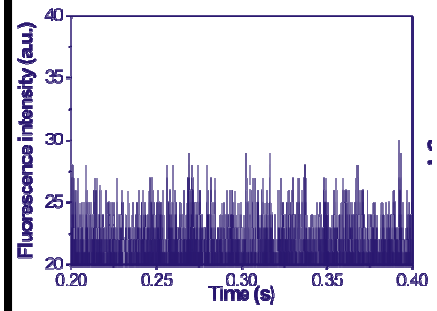
Measuring forces and mechanical properties of sample

Slow

Fluorescence imaging, fluorescence correlation spectroscopy (FCS)







Fluorescence fluctuations (vs. time) to measure dynamics

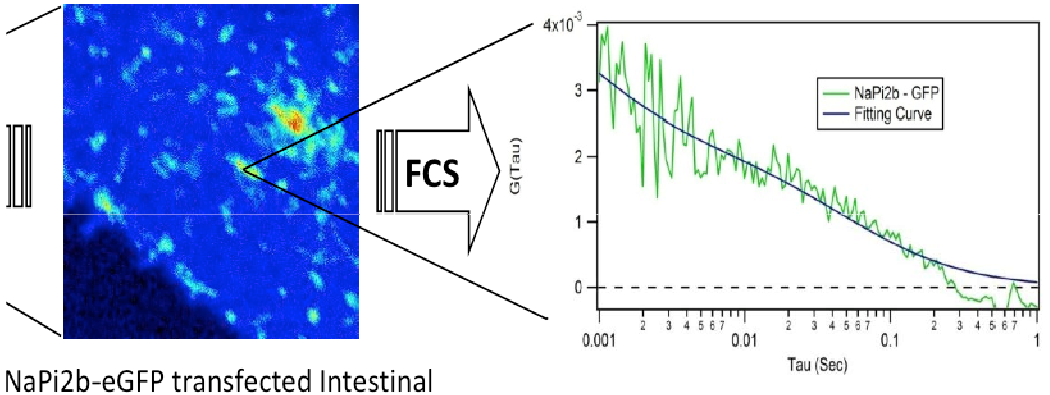
Good temporal resolution (even cLSM)

Single molecule sensitivity

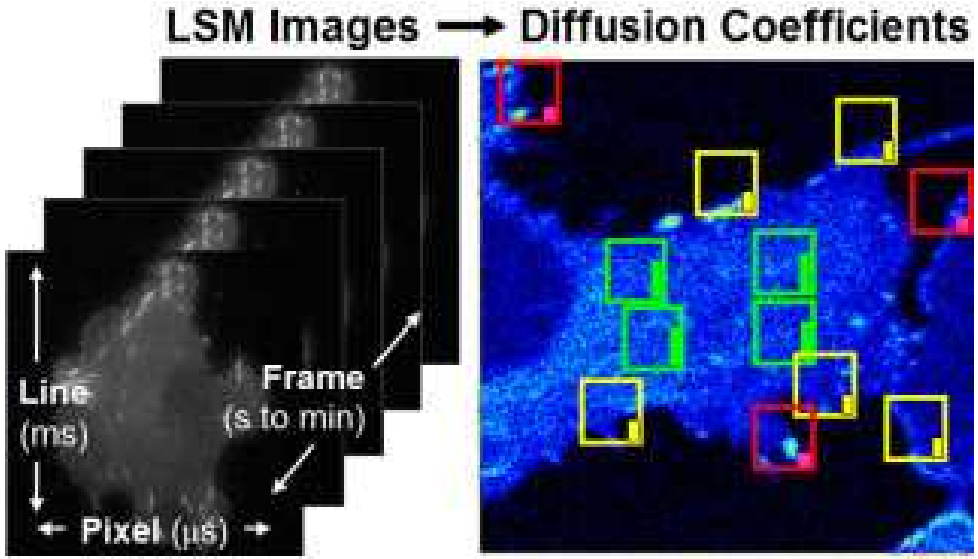
Extension to 1- or 2-dimensional space information (ICS, scanning FCS, RICS)

FCS and other correlation imaging techniques

FCS (Single pixel temporal correlation)



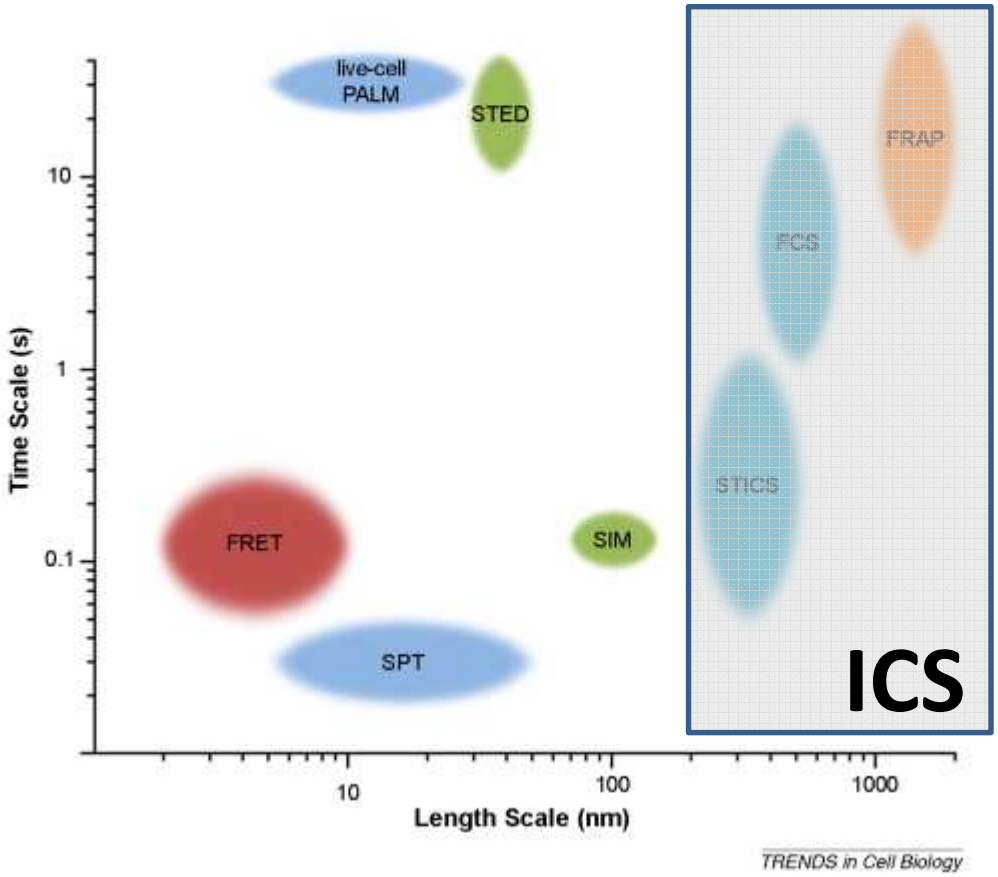
NaPi2b-eGFP transfected Intestinal Epithelial Cells (IEC)



Hidden time structure in scanning process

Other ICS techniques are specific cases:
e.g. Line and pixel times → 0, TICS, STICS
Using also TIRF

Overview of fluorescence-based techniques

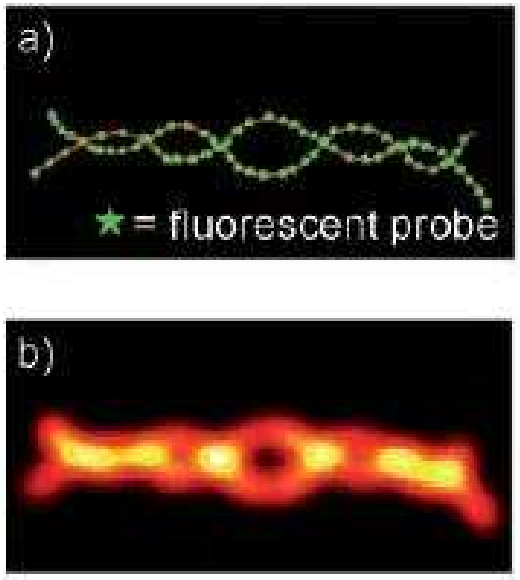


ICS family summary:
Dynamics over large range
Spatial information (e.g. flow)
Concentration
(diffraction limited)

Molecular interactions (CC between different channels)
Aggregation/multimerization state (brightness analysis)

Overview of fluorescence-based techniques

Super-resolution (PALM, STORM):
Effective shrinking of PSF via single-molecule localization precision



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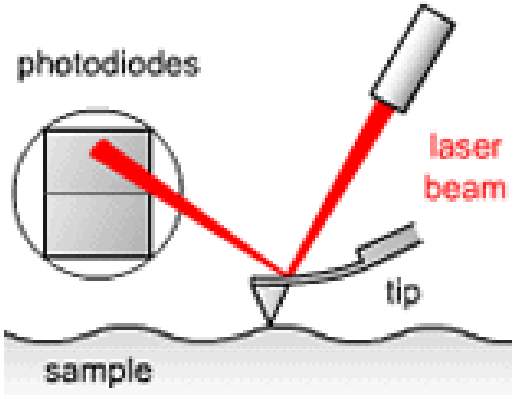

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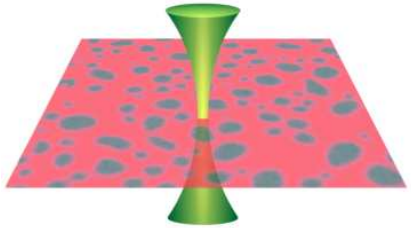

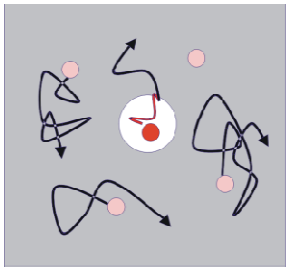

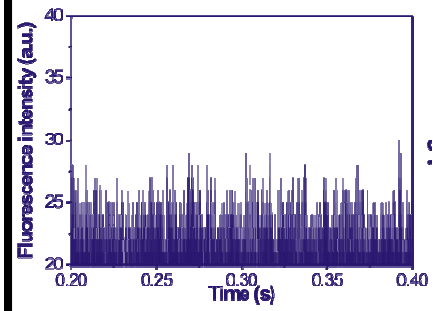

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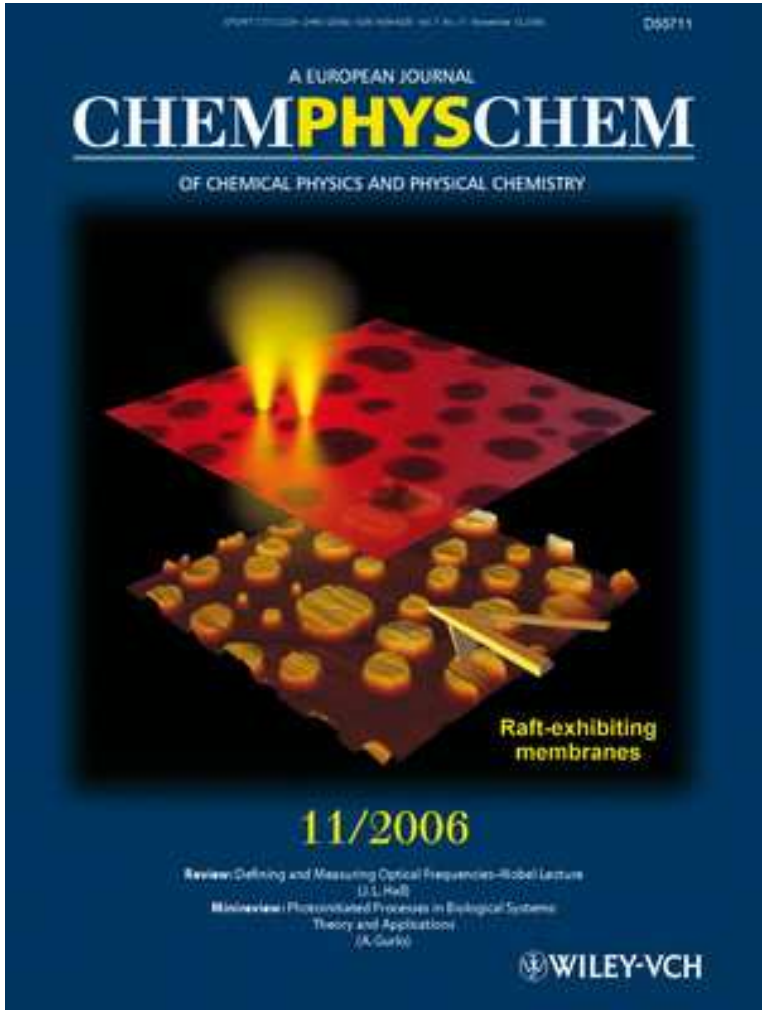
Combination of AFM and ICS/cLSM

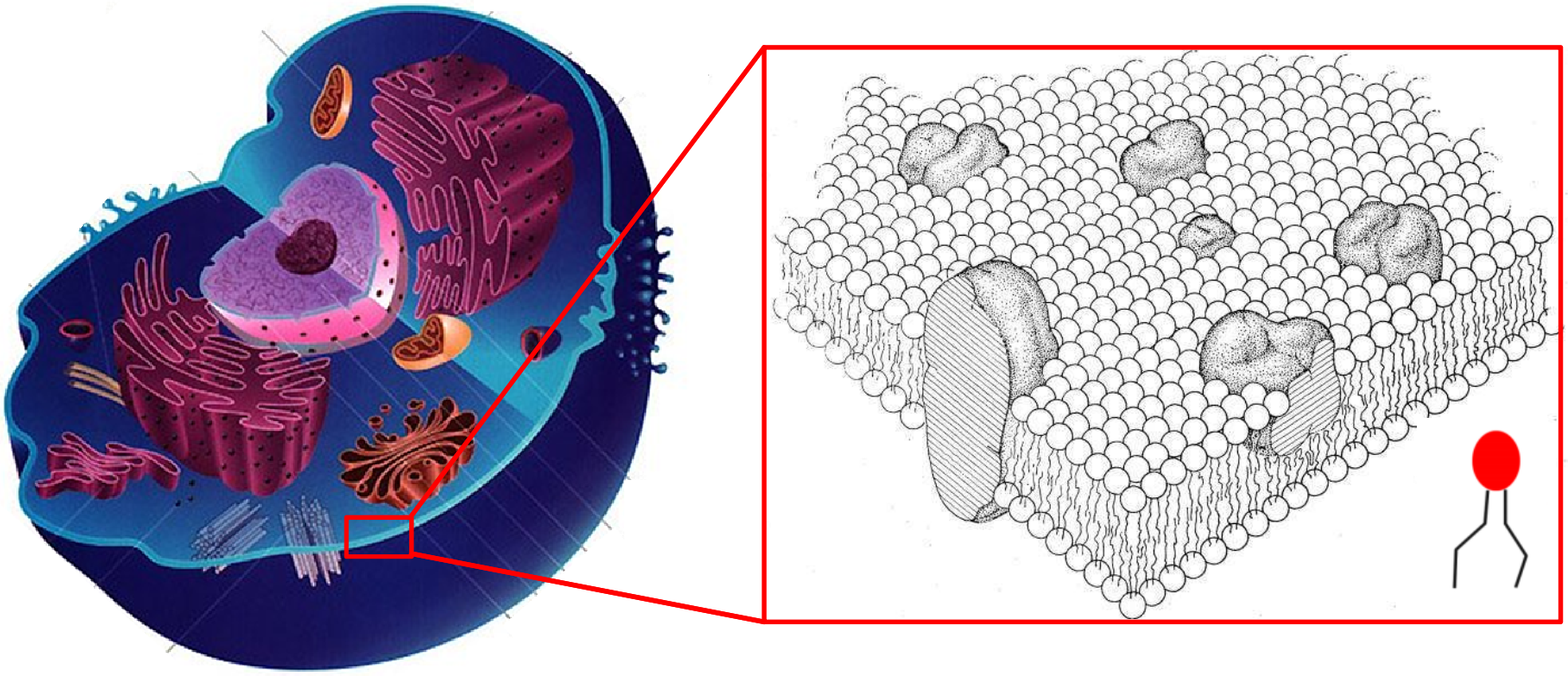


Combination of different experimental approaches on the same sample. Best time and spatial resolution

Complementary information about structure dynamics and inter-molecular forces

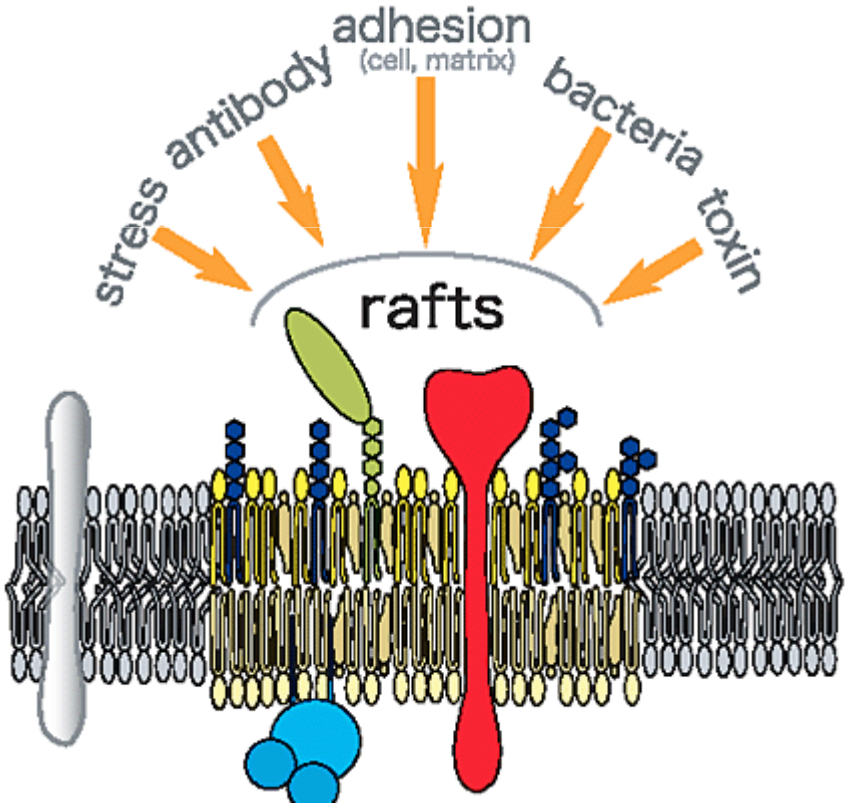
(Chiantia et al. 2006b)

Structure of plasma membrane



Fluid Mosaic Model (Singer and Nicolson, 1972)

Protein-lipid domains (rafts)



-Rich in sphingolipids and cholesterol

-Lipid trafficking

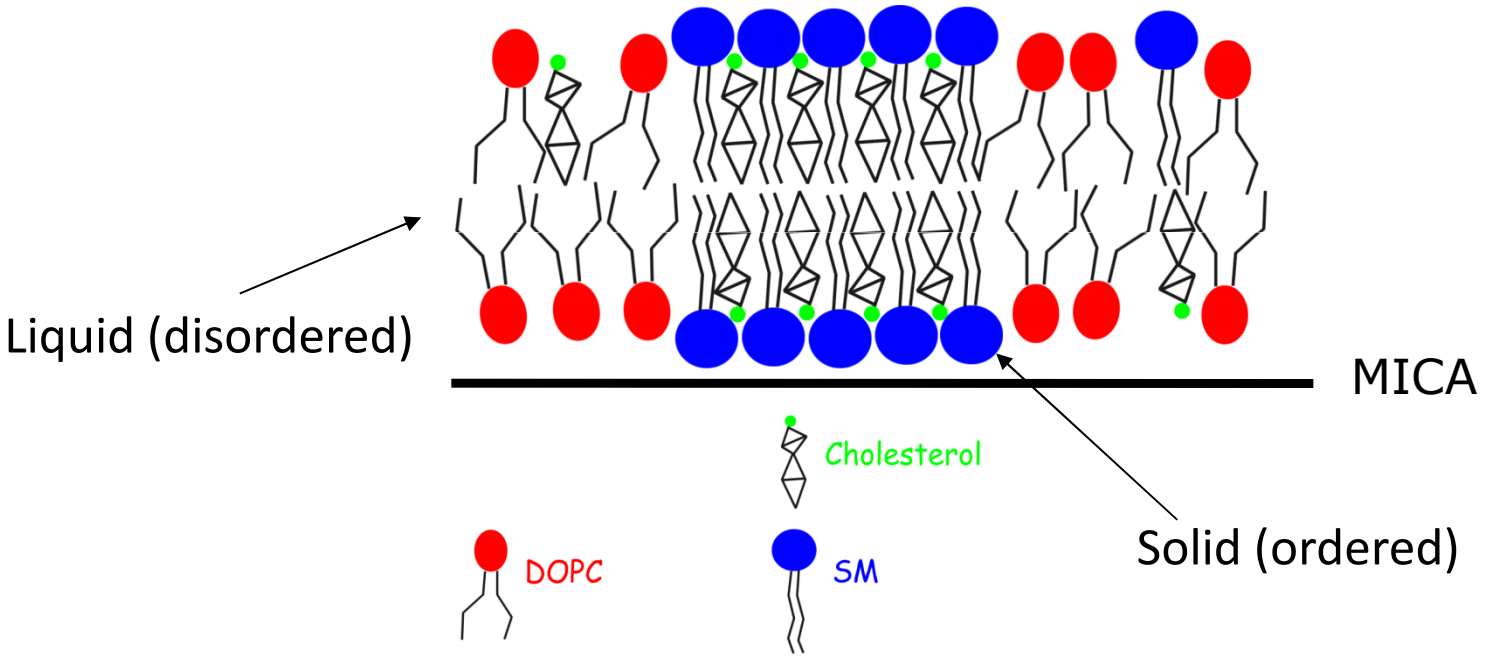
-Protein sorting

-Cell-cell signaling

-Viral infection

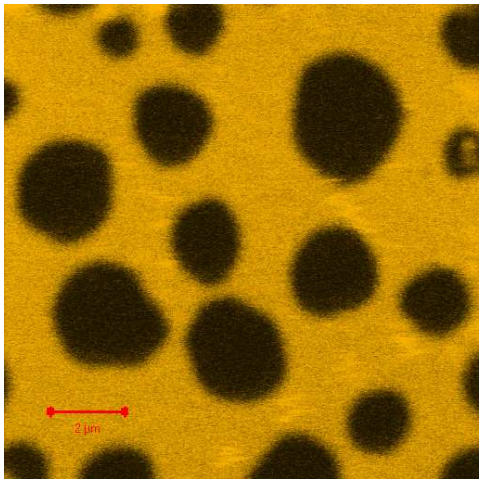
Model for phase-separation in cell membranes

Lipid phase separation



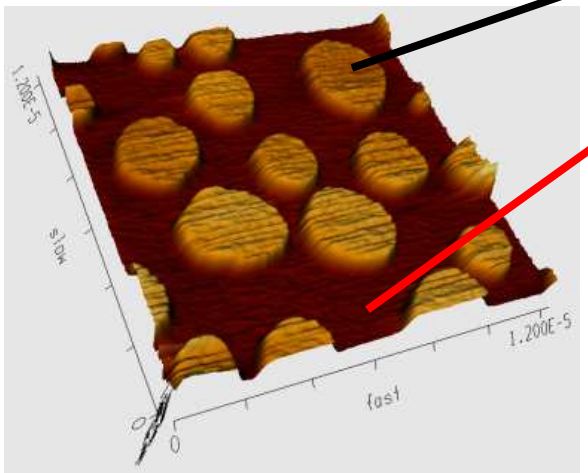
Resistant, stable in time
Can contain proteins (enzymes, receptors…)

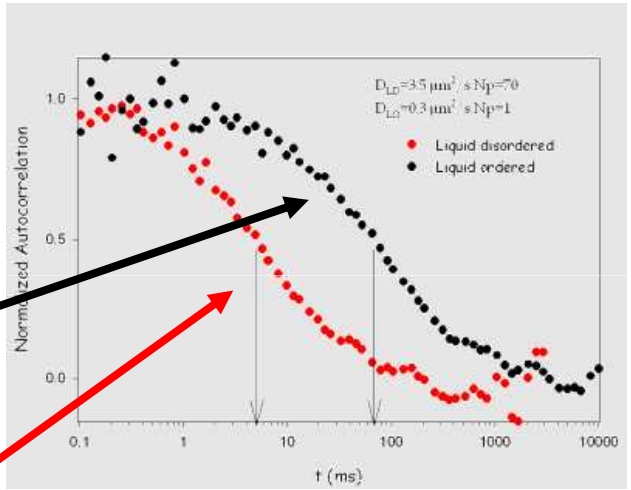
Advantages of the combined AFM-FCS approach



LSM

AFM

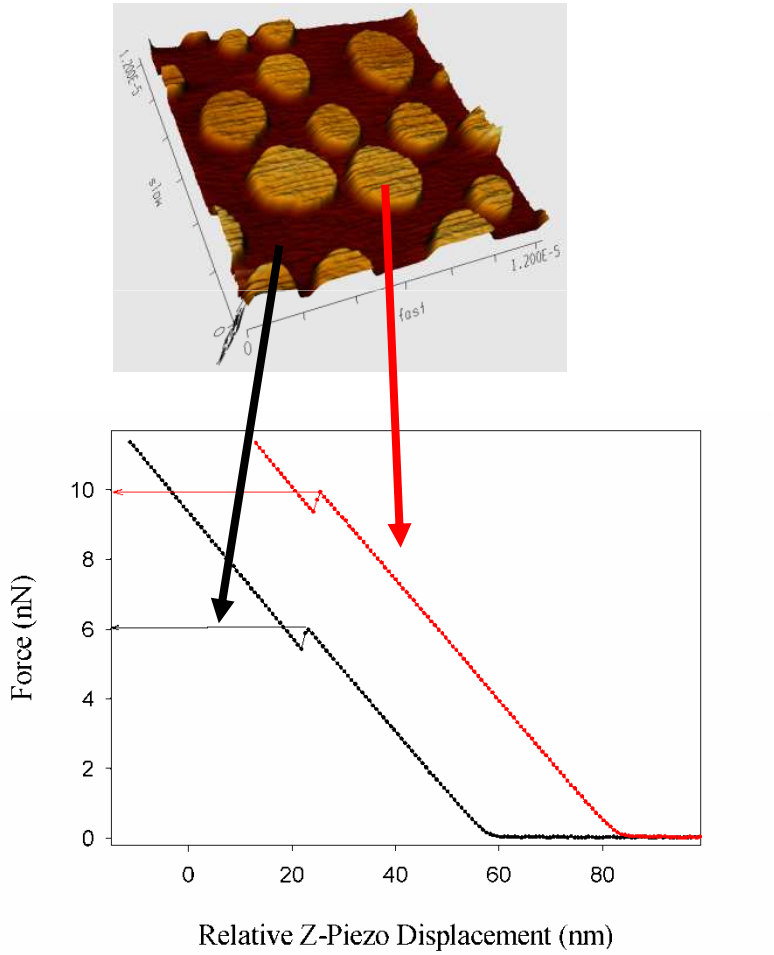


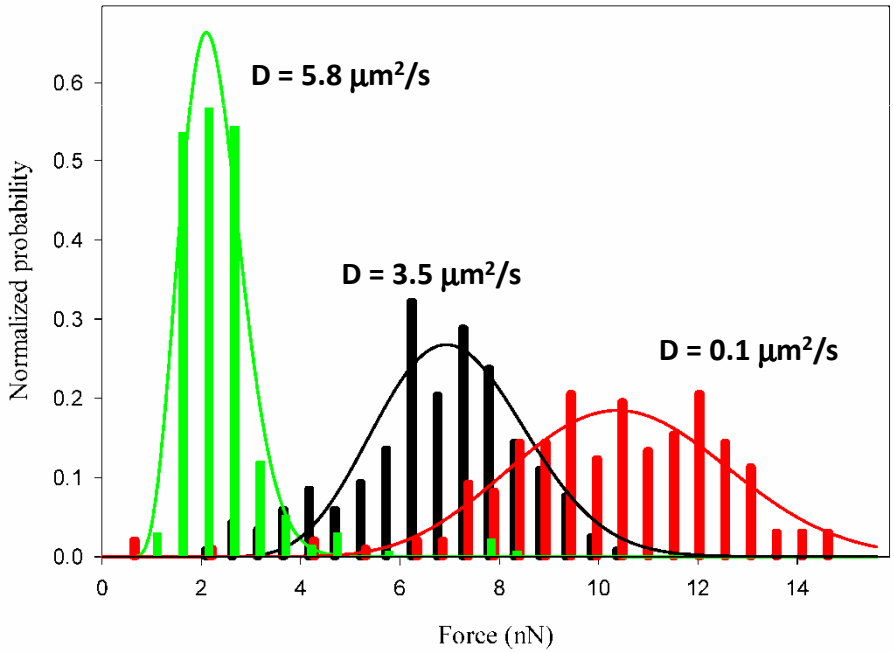


FCS

Advantages of the combined AFM-FCS approach

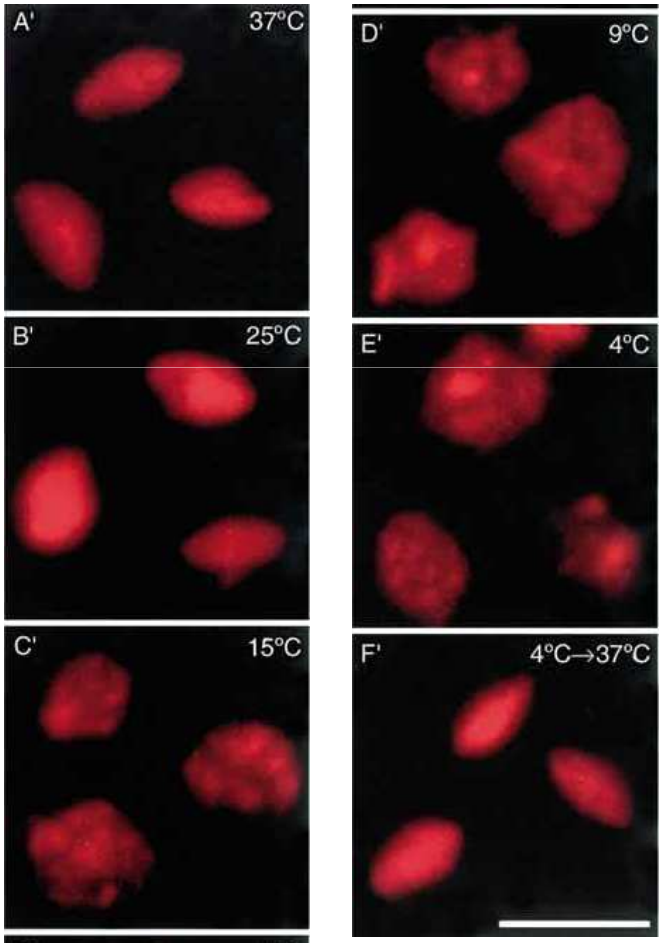
Force measurements





Chiantia et al., ChemPhysChem (2006)

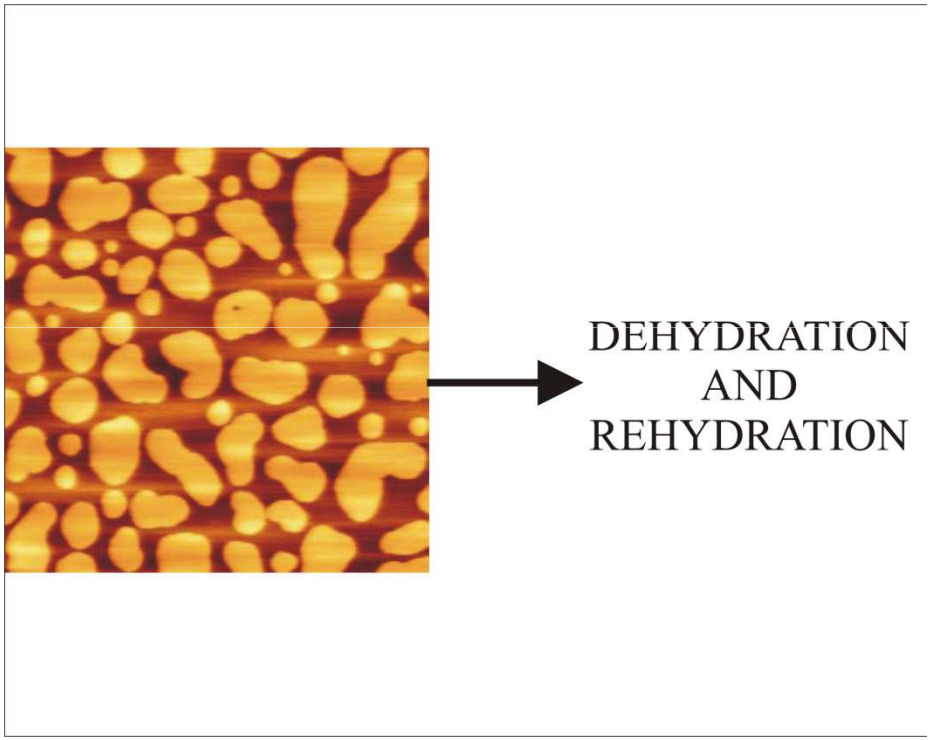
Environmental stress on membranes



Sugars

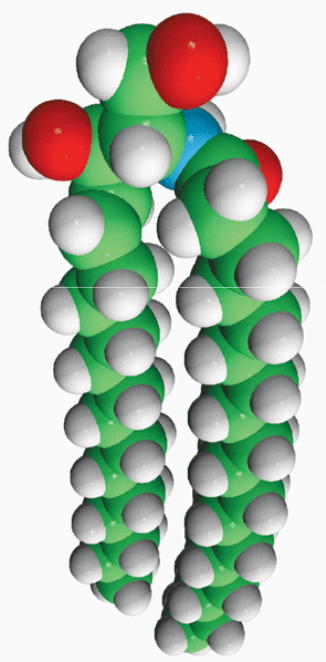
Gousset et al., J. Cell. Phys. (2002)

Environmental stress on membranes



Chiantia et al., Langmuir (2005)

Role of ceramide in plasma membrane organization



Apoptosis, immune response, senescence (Cell growth, cancer therapy)

Stress agents:

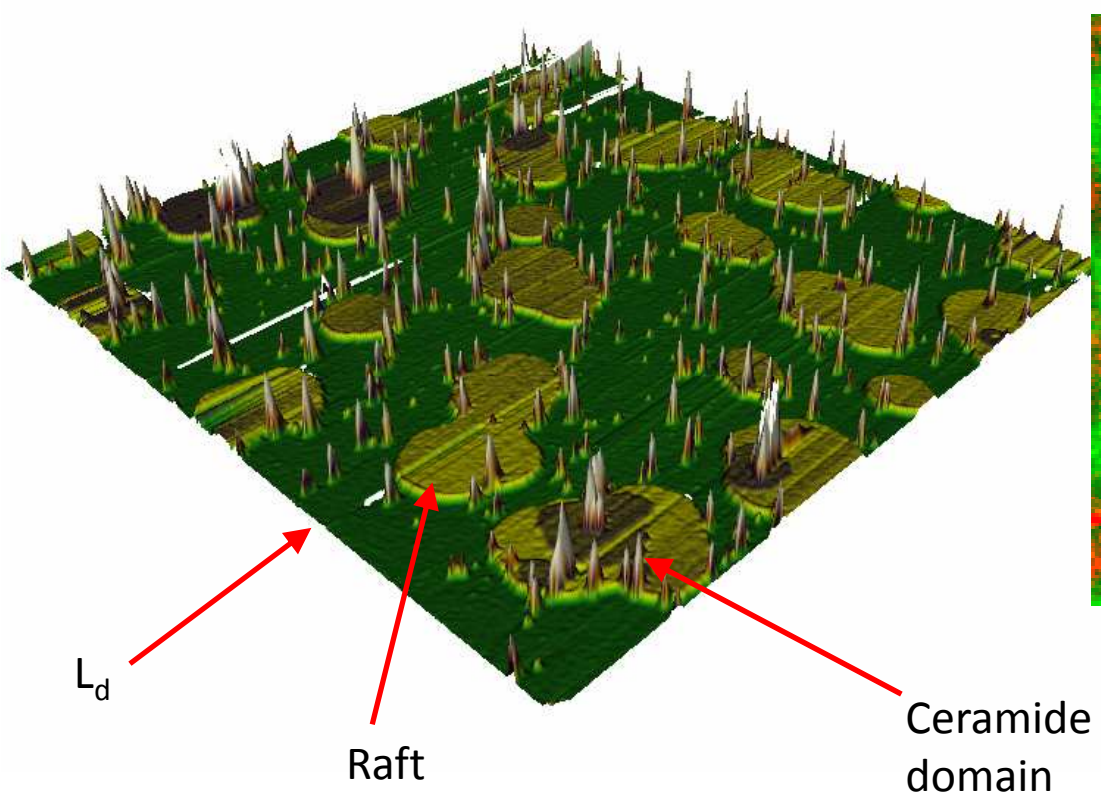
bacterial infections (Neisseria gonorrhoeae, Malaria)

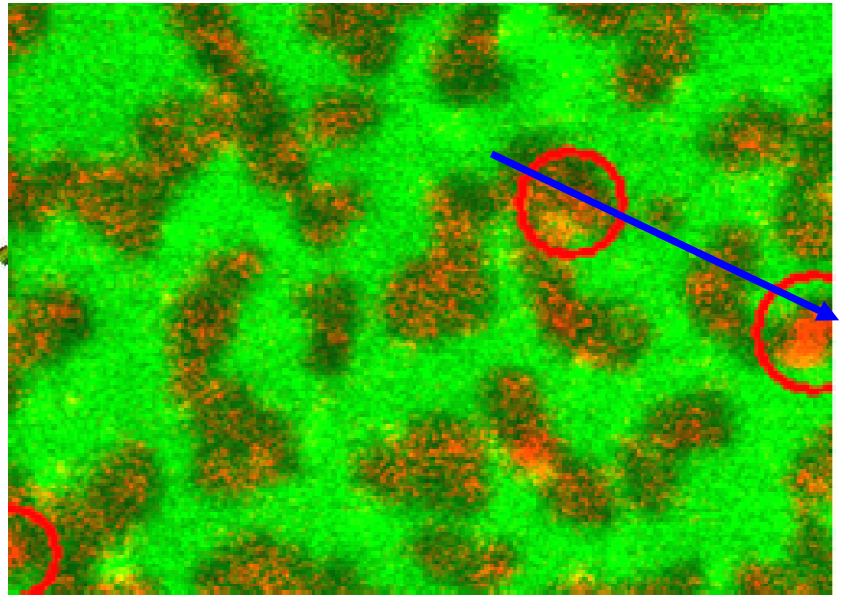
Viral infections (Rhinovirus, Sindbis virus)

UV-light, heat shock → SMase → Ceramide

Lateral organization of plasma membrane, capping

Role of ceramide in plasma membrane organization



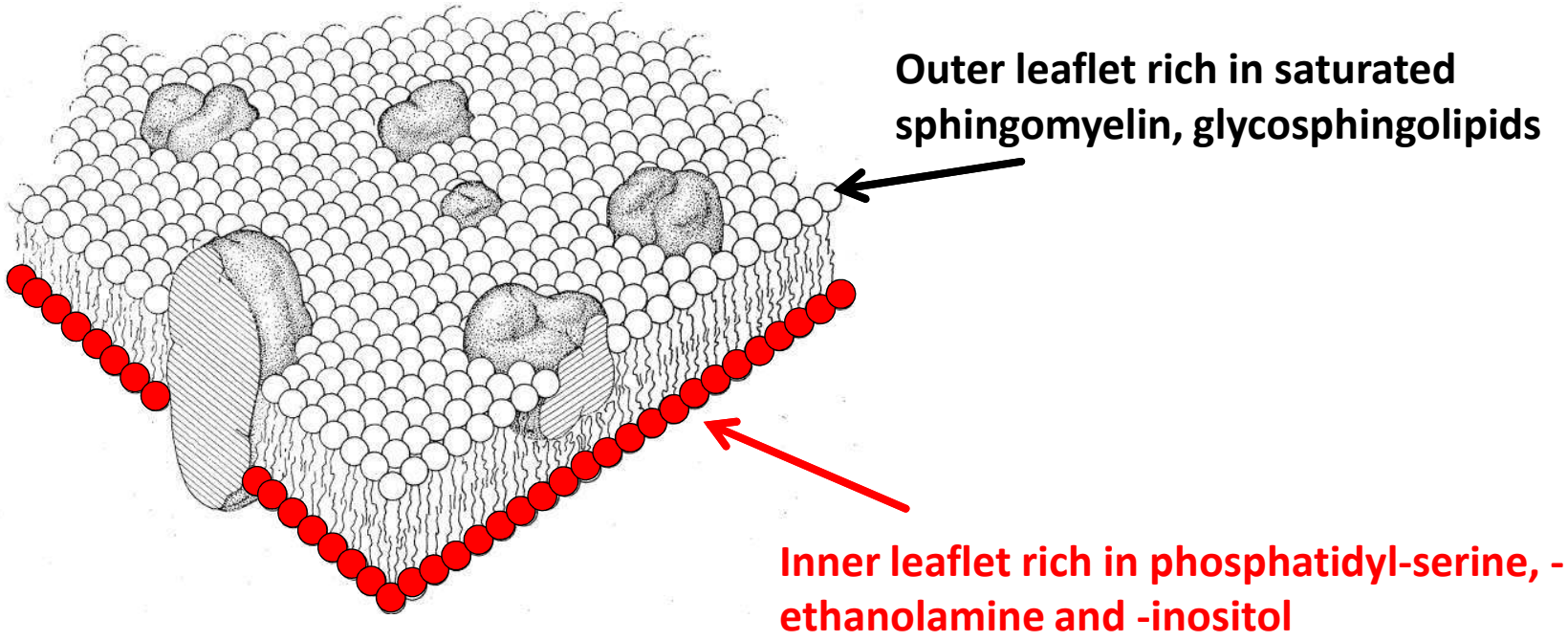


Precise details of membrane topographical structure

Line scanning FCS, protein organization and dynamics

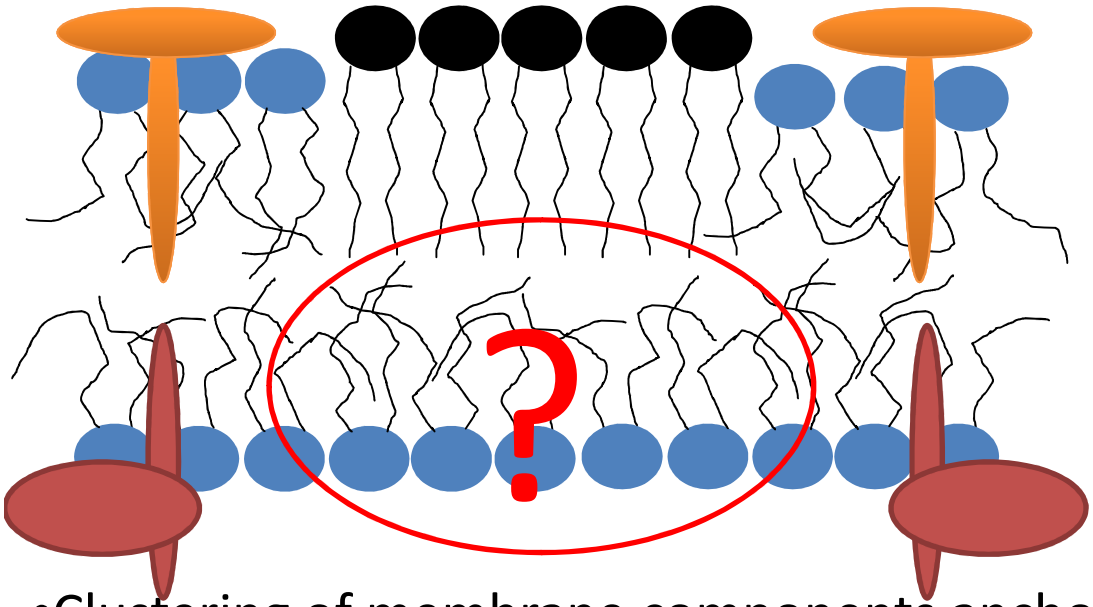
Chiantia et al. 2006a, 2007

Cell membranes are asymmetric



Lipid asymmetry is involved in neuronal development, apoptosis, immune response, platelets activation, tumors, thalassemia and diabetes (Balasubramanian, 2003)

Inter-leaflet coupling



Outer leaflet is rich in saturated sphingolipids→ ordered domains

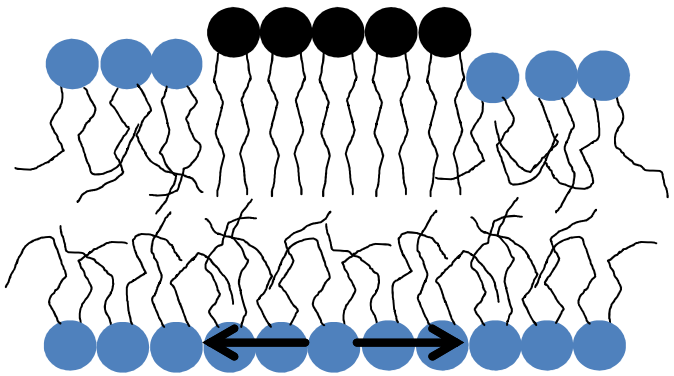
Inner leaflet is rich in unsaturated lipids→ NO domains

- Clustering of membrane components anchored to the outer leaflet triggers formation of domains (e.g. GPI-anchored proteins, PRR in neutrophils) (Chen 2006, 2009, Ewers 2010)
- Proteins associated to the inner inflet are recruited and activated to start signaling (e.g. src-like tyrosine kinases)
- But inner leaflet lipids cannot separate into domains! <u>How is information transmitted across the bilayer? How are the leaflets coupled?</u>
- Model membranes displaying asymmetry did not exist

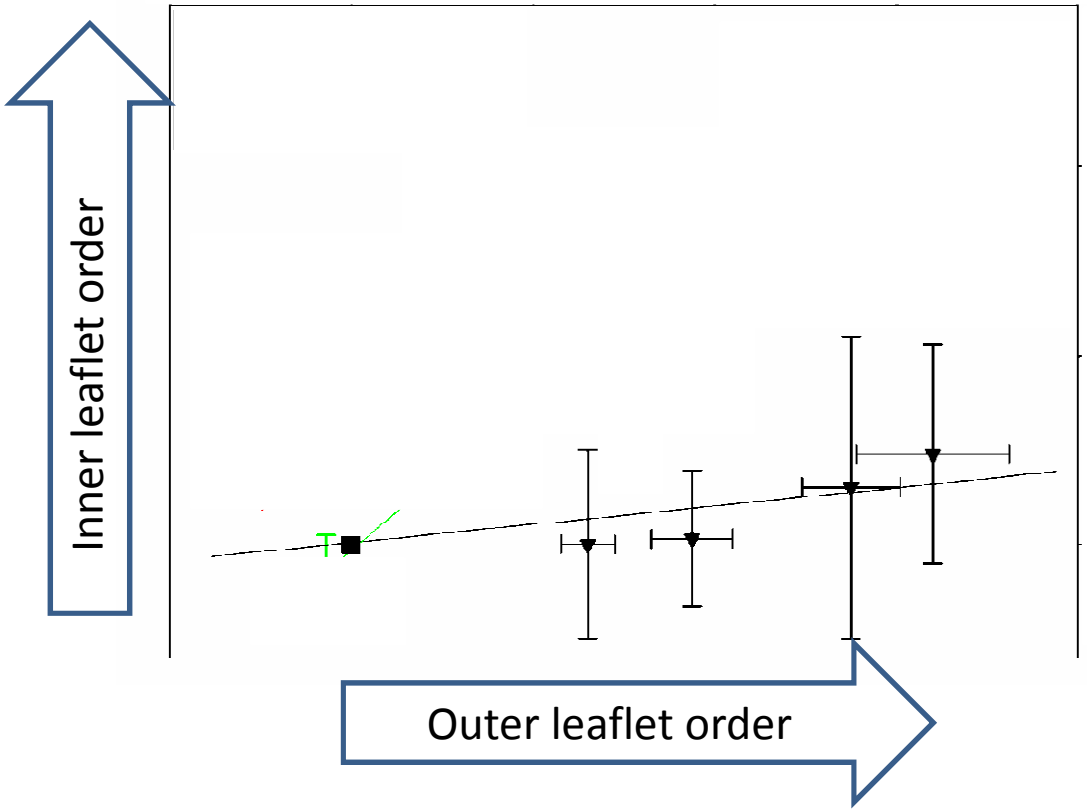
Inter-leaflet coupling: effect of inner leaflet composition

Asymmetric GUVs

SM



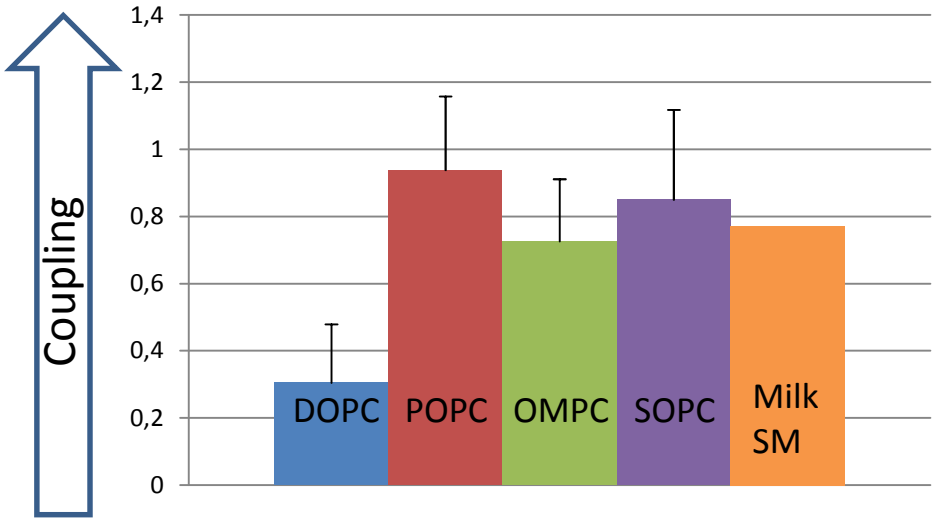
Slow diffusion = high order



Inner leaflet stay disordered when ordered domains are present in the outer leaflet

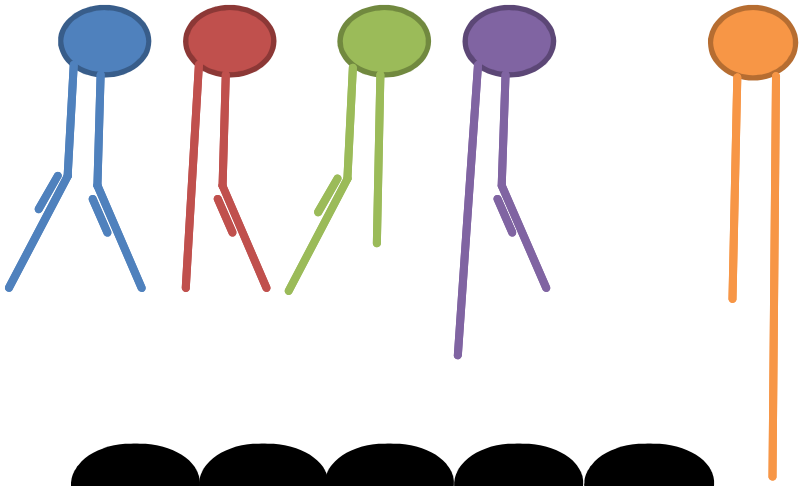
Certain natural lipids mixtures in the inner leaflet increase coupling

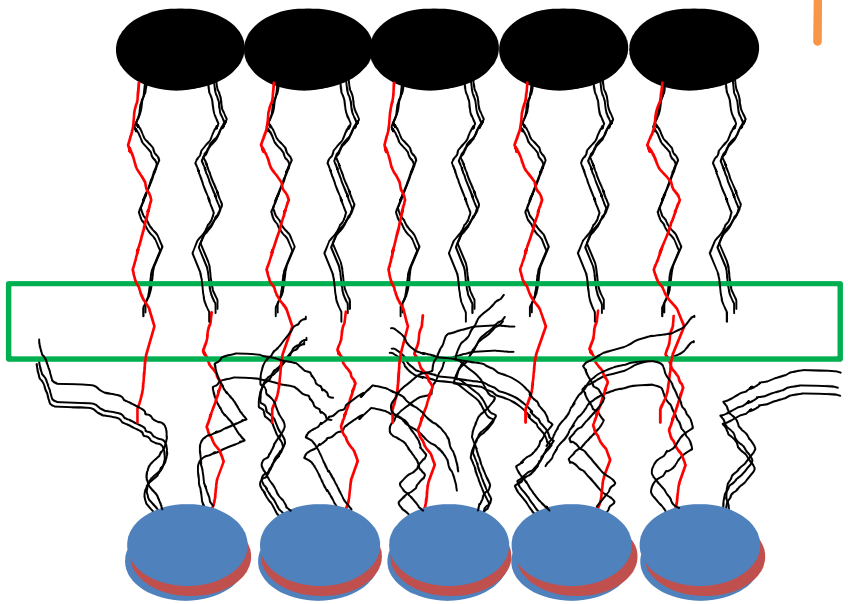
Inter-leaflet coupling: effect of inner leaflet composition



Different PCs in the inner leaflet

Different SMs in the outer leaflet





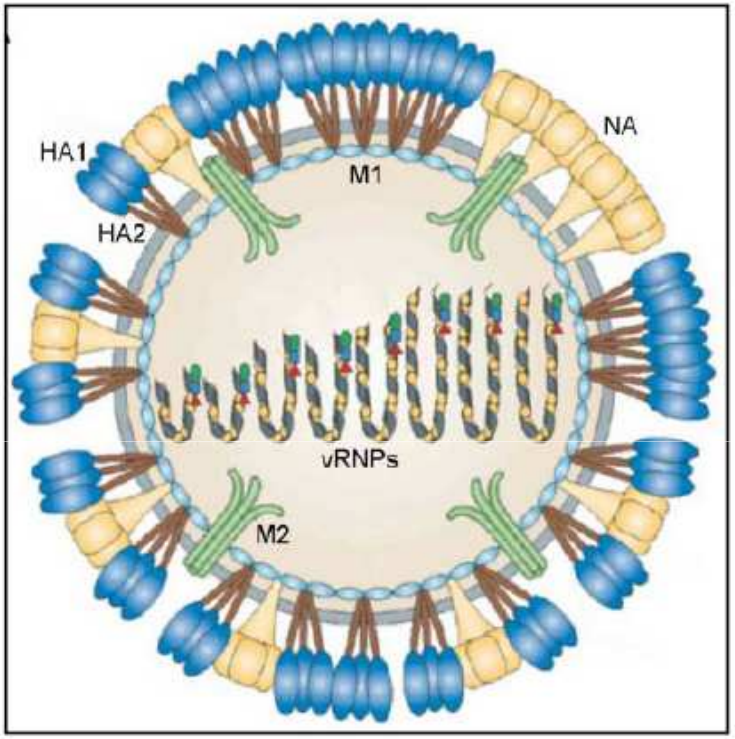
- Coupling depends on the saturation and length of the acyl chains → signaling
- Increased interaction at bilayer midplane for the lipids with higher coupling

Chiantia et al. 2012

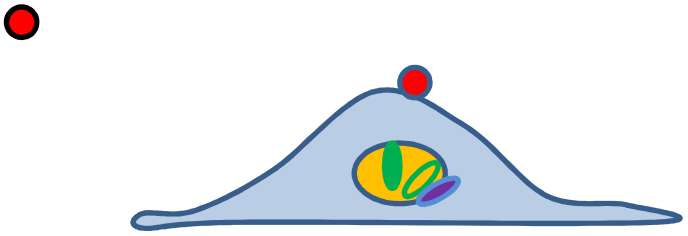
What now?

- Powerful combined approach: AFM and quantitative fluorescence microscopy
- Advanced models for plasma membrane
- We can now study membrane function and structure with unprecedented depth

Influenza: virus structure and life cycle



- 22 Million people infected in 2009
- 20000 influenza-associated deaths in Germany alone
- Virus is often spherical, 100 nm diameter
- Enveloped by a lipid bilayer containing 3 proteins:HA, NA (the spike proteins) and M2
- M1 is the matrix protein

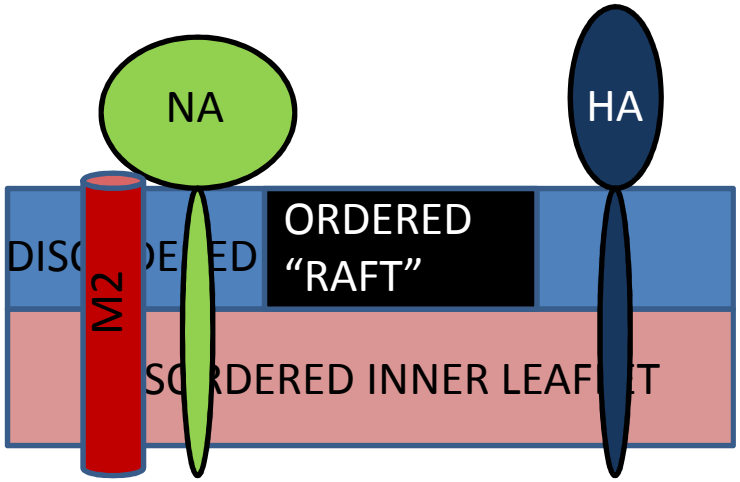


1- Binding and internalization

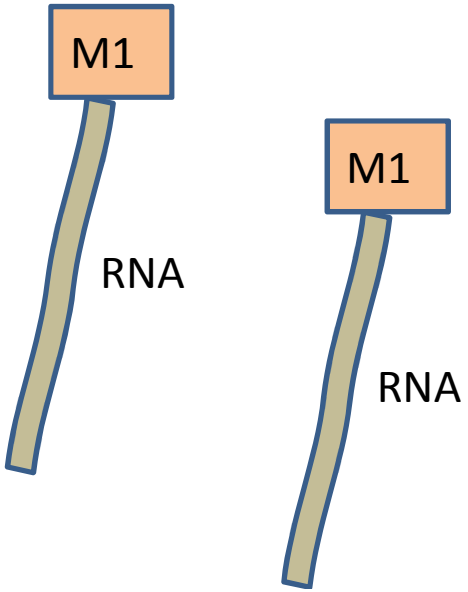
2- Production of viral components

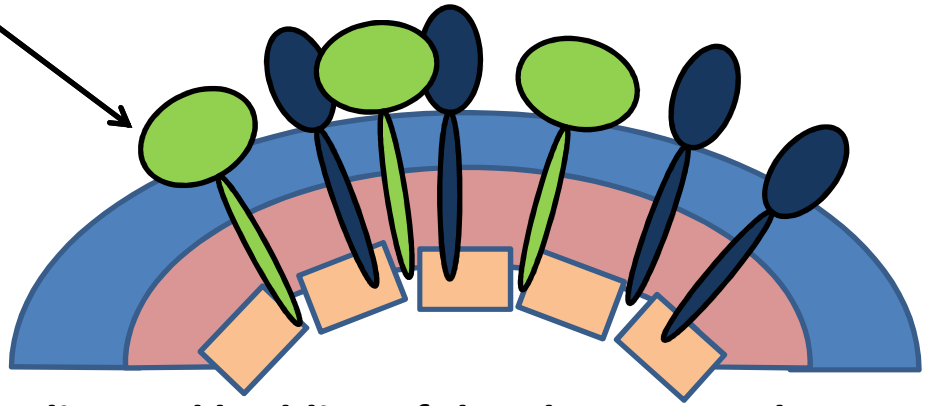
3- Assembly and budding of progeny

Influenza virus assembly at the plasma membrane



Formation of the "budozone"
What is promoting the clustering?



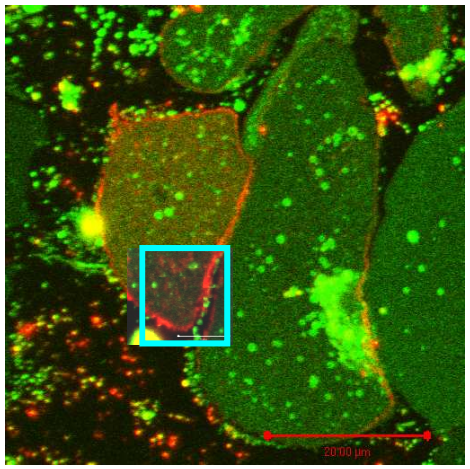


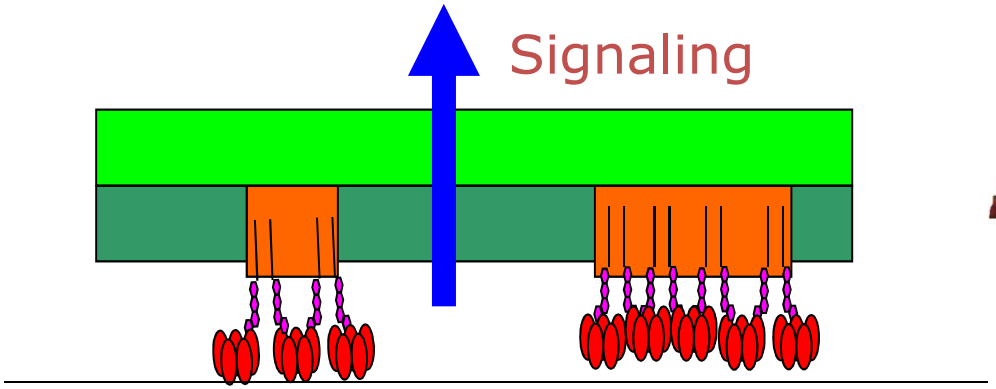
Bending and budding of the plasma membrane

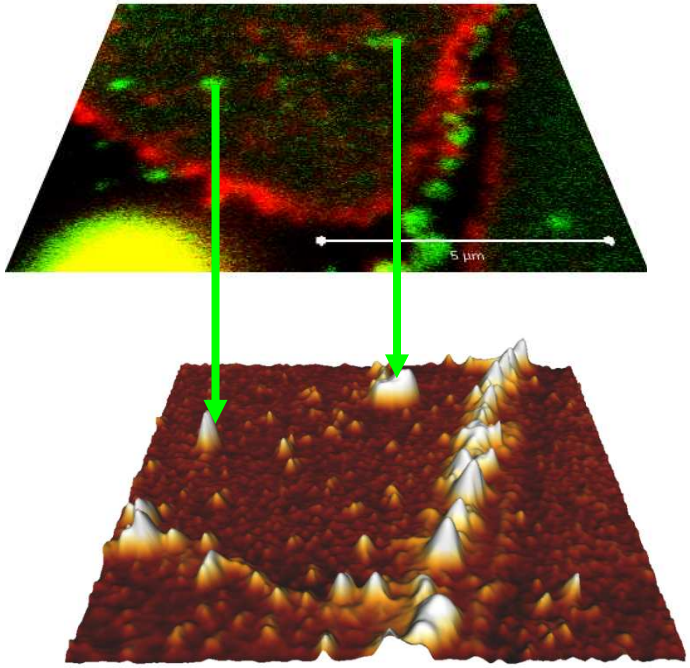
What is bending the membrane?

Trans-membrane signaling in cells

Investigation of inter-leaflet coupling and signalling mechanisms in cellular membranes

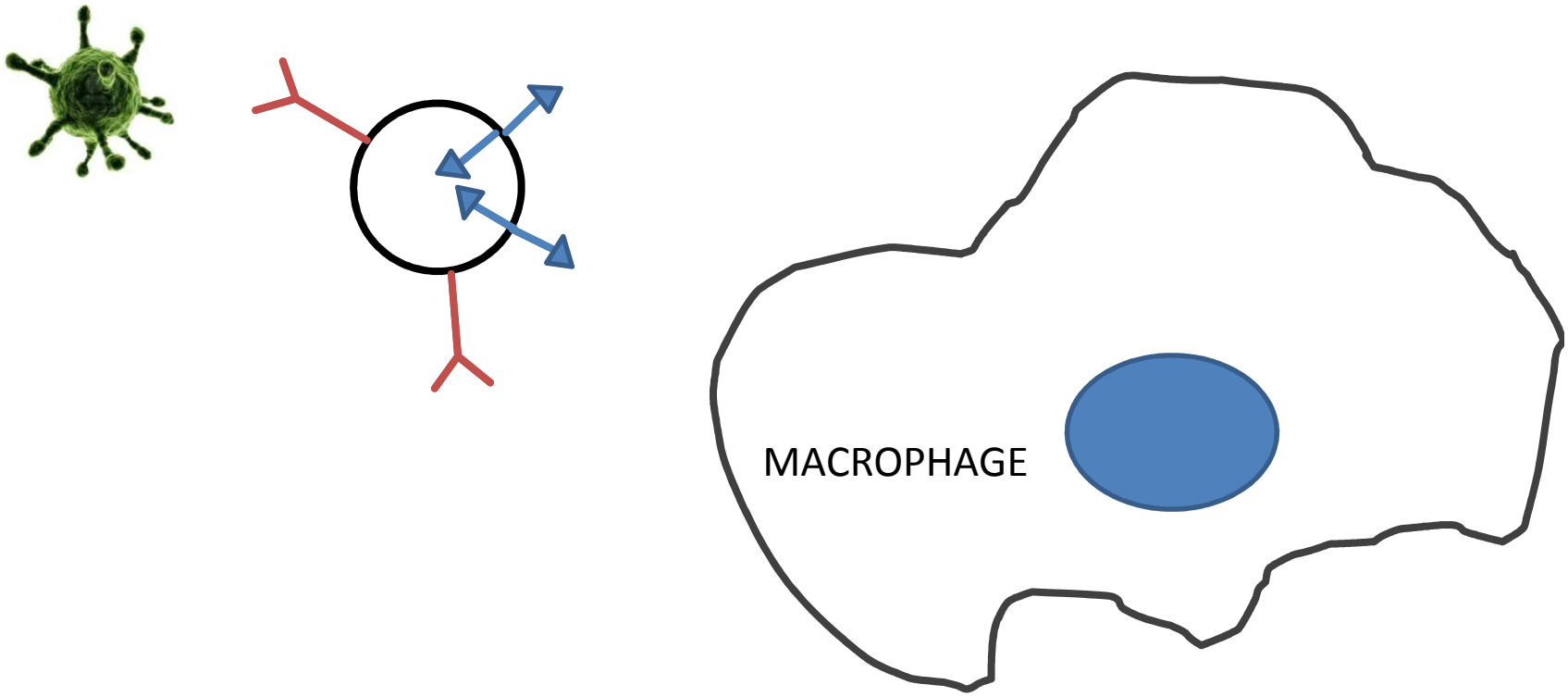






Intelligent drug carriers

Therapeutic nanoparticles (e.g. liposomes), not only for drug delivery

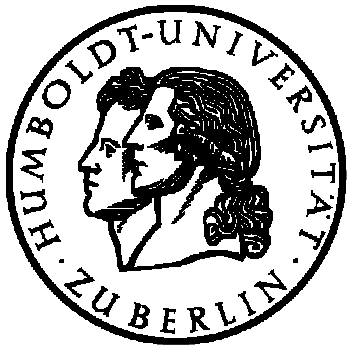


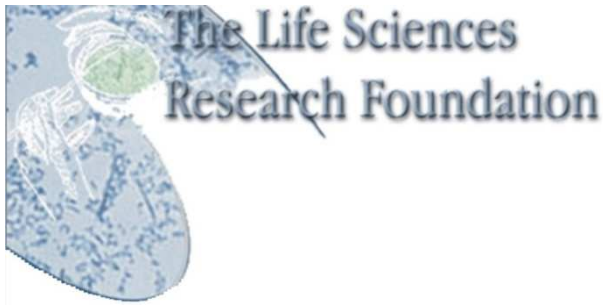
Acknowledgments

- Andreas Herrmann, HU zu Berlin
- Erwin London, State University of NY
- Petra Schwille, MPI Munich

- Funding:

LRSF, HHMI, SFB 740



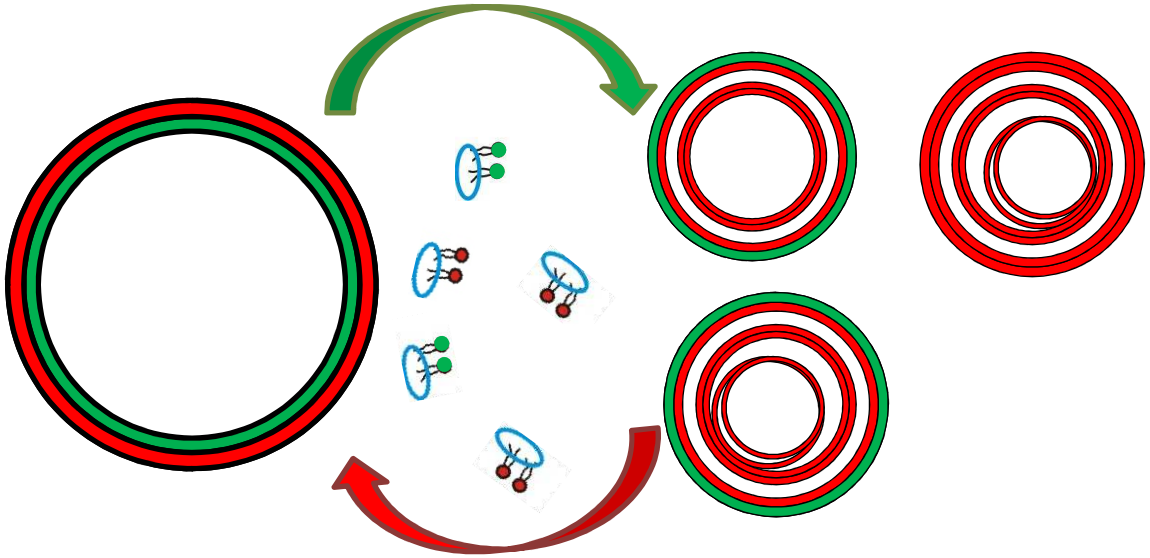


Asymmetric Giant Unilamellar Vesicles (GUV)

- Prepared via mßCD-mediated exchange

Inner leaflet lipids

Outer leaflet lipids



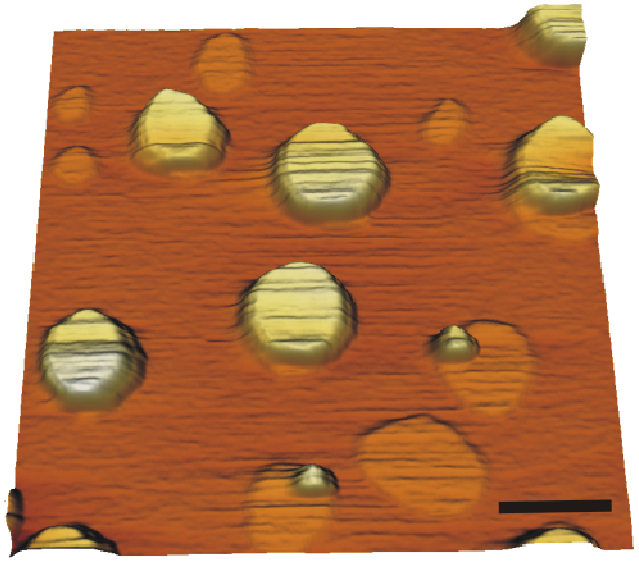
- Compared to other asymmetric model systems: high yield, easy to prepare, trans-membrane protein reconstitution
- GUVs can be used e.g. with optical microscopy and sensitive single molecule techniques
- Can be translated to supported bilayers

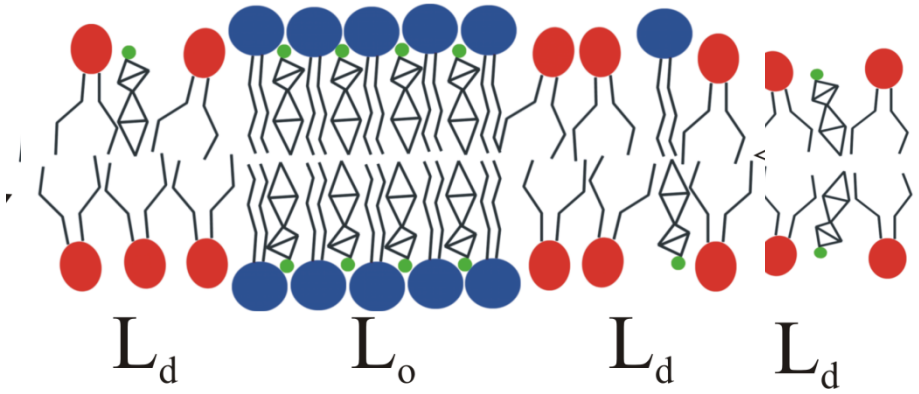
Chiantia et al. 2010

Role of ceramide in plasma membrane organization

Physical properties of ceramide → Effects on membrane organization

Hydrophobic, Donor/Acceptor for H-bonds (Ceramide-enriched domains)





Chiantia et al., Biophysical J. (2006)