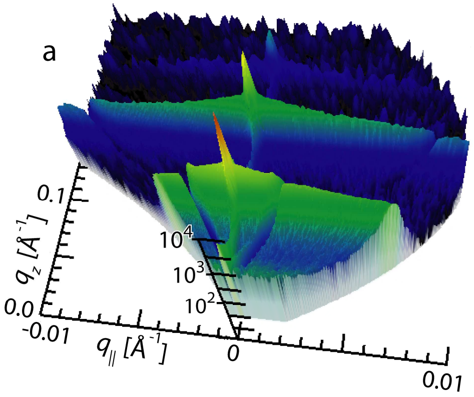
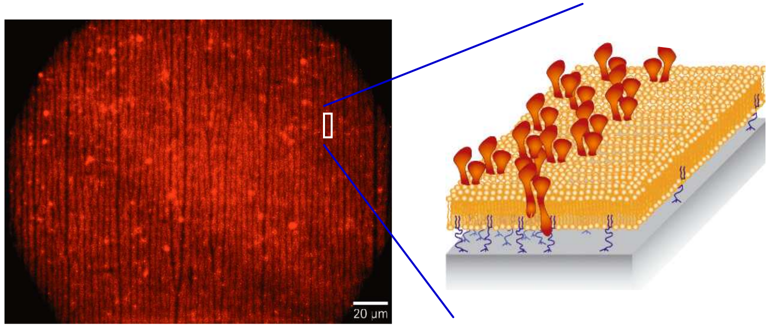
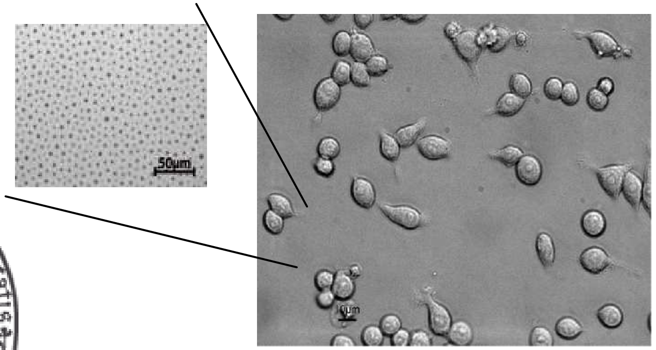
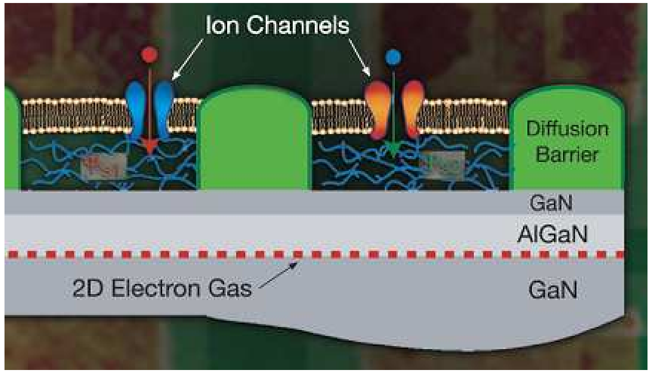
Role of Oligo- and Polysaccharides in Modulation of Biological Interfaces









Motomu Tanaka

Biophysical Chemistry Laboratory II
Institute of Physical Chemistry and
BIOQUANT, University of Heidelberg

EXZELLENZCLUSTER CellNetworks

BioQuant
MODEL base of LIFE

Contents

Motivations, Scientific Background

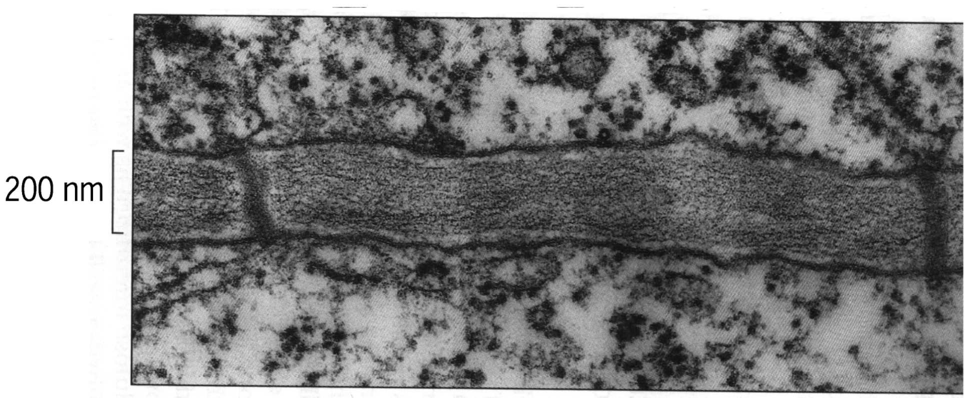
Models of Cell-ECM Contacts: Polymer-Supported Membranes

Interactions Mediated via “Membrane-Bound” Sugars (Glycocalyx)

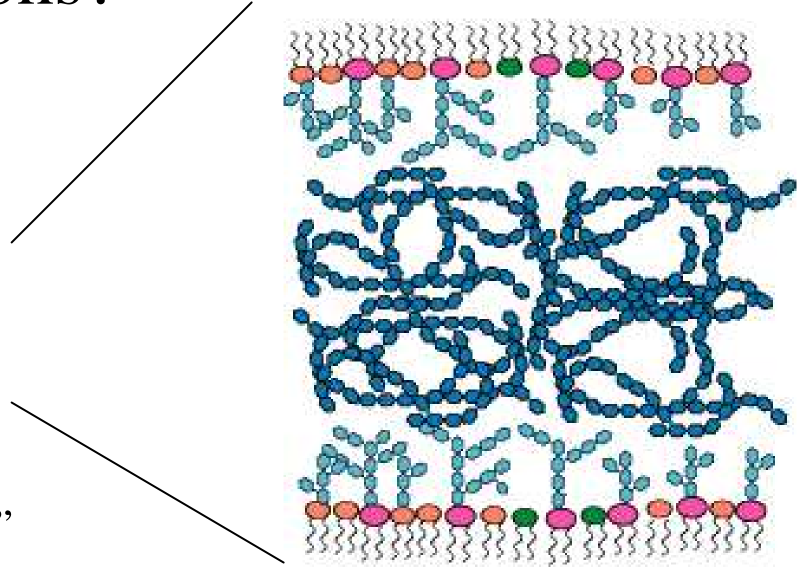
Molecular Mechanism of Bacterial Resistance to CAPs

Fundamental Motivation

How does Nature Regulate Cell-Cell (Cell-Tissue) Interactions?



Alberts “*Molecular Biology of the Cells*”

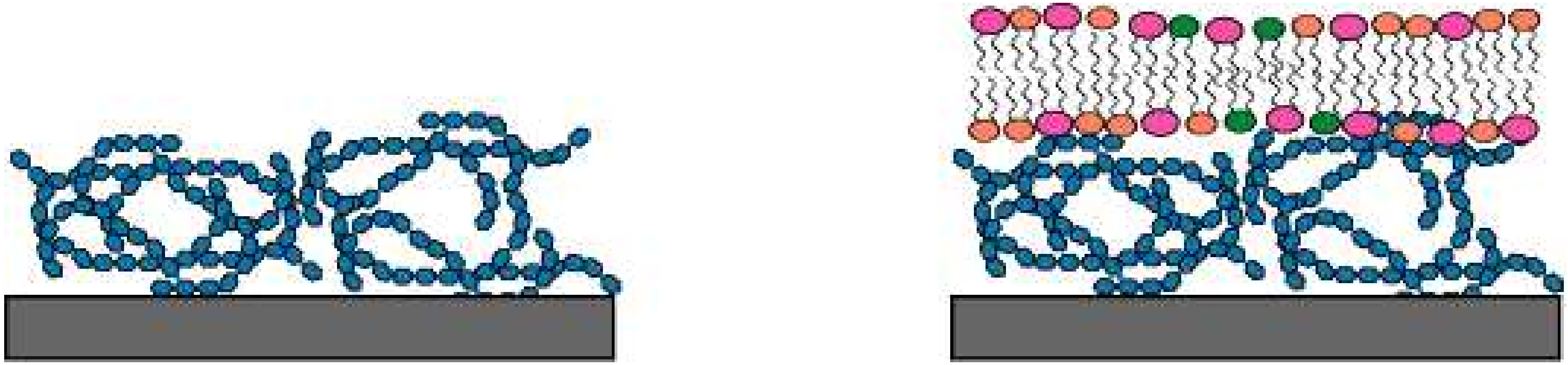


Many contacts between two neighboring cells are mediated via layers of hydrated biopolymers containing saccharide moieties (ECM and glycocalyx).

Design of defined cell membrane models with less complexity can reveal how biopolymers modulate interfacial interactions.

Cell-Cell Contact as “Wetting Problems” (I)

Stratified layers are stable only if “complete wetting” conditions are fulfilled at the interface.



The presence of an “additional” layer (membrane) must result in the gain of the surface free energy.

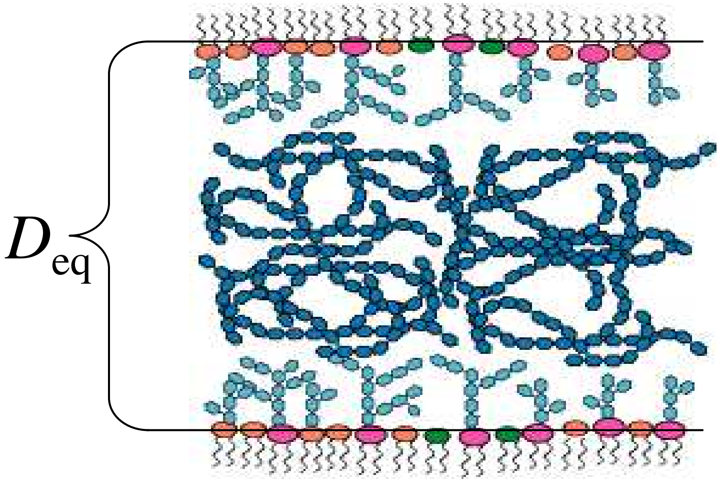
Spreading coefficient: $S = \sigma_{\mathrm{SL}} - (\sigma_{\mathrm{SP}} + \sigma_{\mathrm{PM}} + \sigma_{\mathrm{ML}}) > 0$

Brochard, de Gennes, *Adv. Colloid Interf. Sci*. 1992

Cell-Cell Contact as “Wetting Problems” (II)

To keep a finite intercellular distance of 10 ~ 100 nm, the interaction potential at the interface should be weakly repulsive.

electrostatic
van der Waals
hydration
entropic



Disjoining pressure (net force per unit area)

$$\Pi(D) = -\partial G / \partial D$$

Churaev, Derjaguin, *J. Colloid Interf. Sci.* 1985
Tanaka et al., *J. Phys. Cond. Matter* 2005

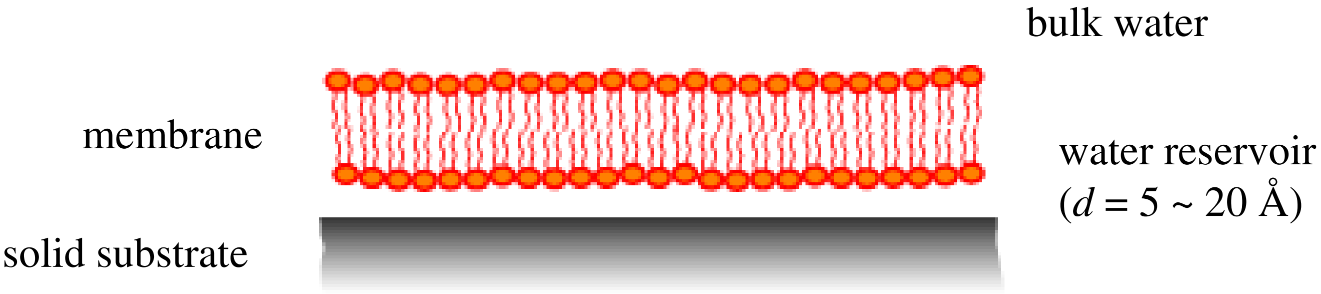
The minimum $\Pi(D_{eq}) = 0$ can be found at $\partial^2 G / \partial D^2 > 0$

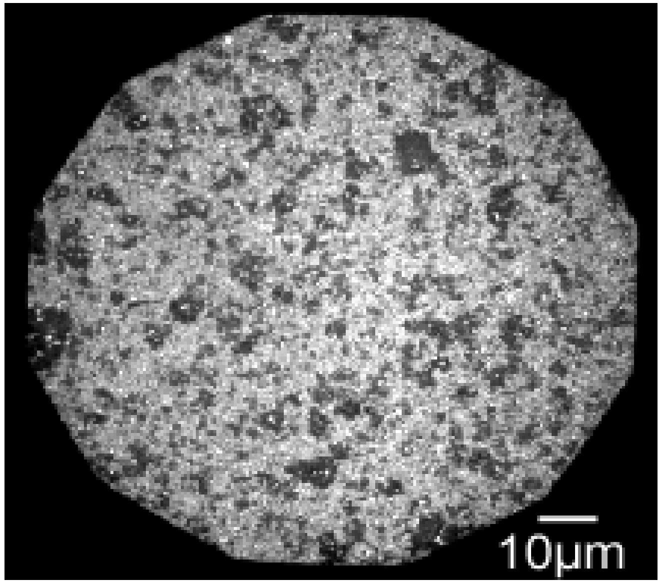
Negative disjoning pressure causes dissipation (de-wetting).

Conventional Strategy

Use of lipid membranes on planar substrates (***solid-supported membranes***) as model biomembranes

A. Brian, H. McConnell *PNAS* 1985, E. Sackmann, *Science* 1996





Human platelet integrin $\alpha_{IIb}\beta_3$ receptors in a solid-supported membrane

Problem: Proteins in the membrane experience direct mechanical contact to hard/solid substrates, resulting in partial/complete denaturing.

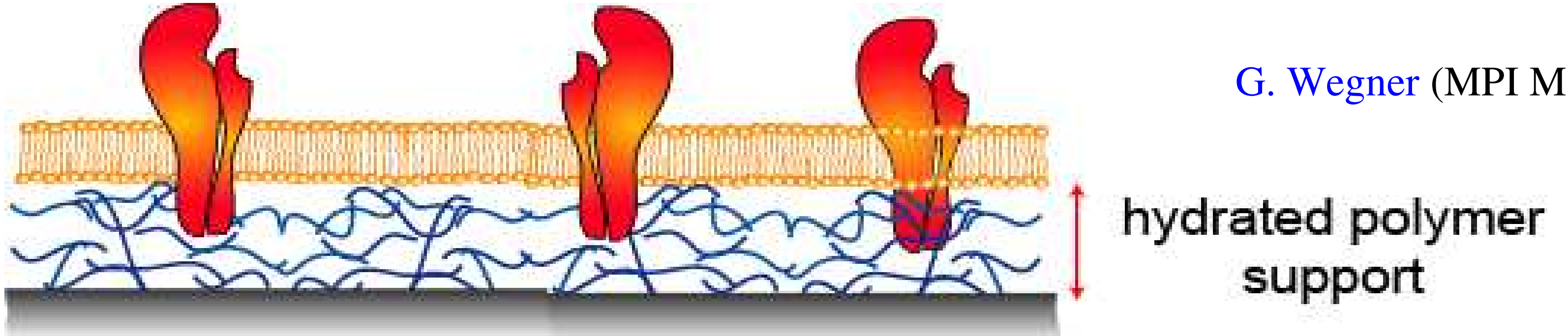
Gönnenwein, Tanaka, Hu, Moroder, Sackmann, *Biophys. J.* 2003

Purrucker, Gönnenwein, Förtig, Rusp, Moroder, Sackmann, Jordan, Tanaka, *SoftMatter* 2007

Our Strategy: “Polymer-Supported Membrane” Concept

Use of hydrated polymer films as biocompatible interlayers that
mimic generic roles of cytoskeleton and extracellular matrix

G. Wegner (MPI Mainz)



regenerated cellulose
thickness: 5 – 50 nm

Tanaka & Sackmann, *Nature*, **437**, 656 (2005)

The presence of ultrathin (d ~ 10 nm) polymer films fascilitates an improved homogeneity and lateral diffusivity of transmembrane cell receptors, retaining their natural functions.

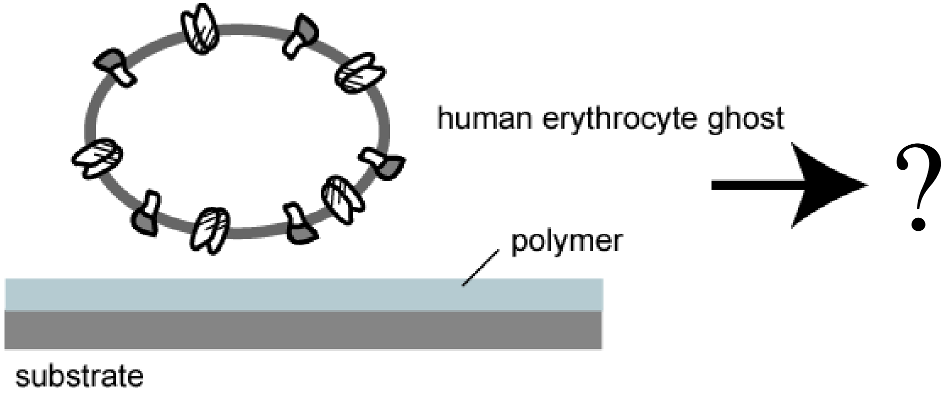
Gönnenwein, Tanaka, Hu, Moroder, Sackmann, *Biophys. J*. (2003)
Purrucker, Gönnenwein, Förtig, Rusp, Moroder, Sackmann, Jordan, Tanaka
ChemPhysChem (2004), *SoftMatter* (2007), *Phys. Rev. Lett.* (2007)

A Breakthrough: Spreading of Native Cell Membranes

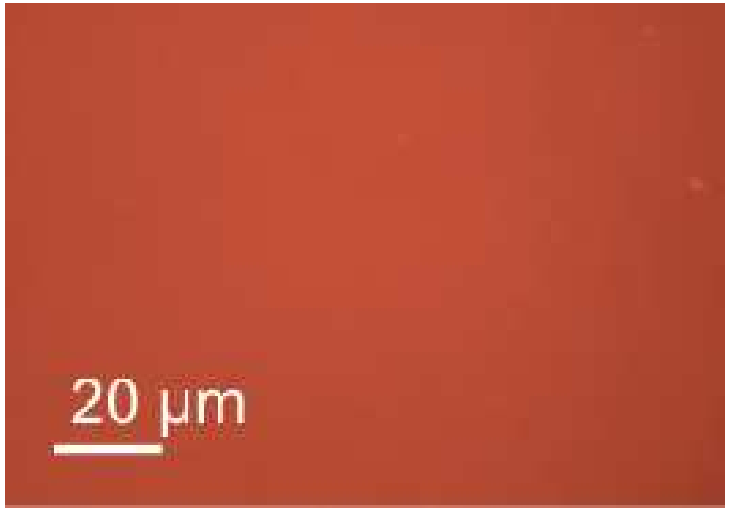
If it works, it would enable to retain the natural composition/density of proteins.

First Attempt: Human Red Blood Cell Membranes (Erythrocytes)

S. Kaufmann



"Inside labeling" on a polymer support



Orientation of cells is identified with antibodies after spreading

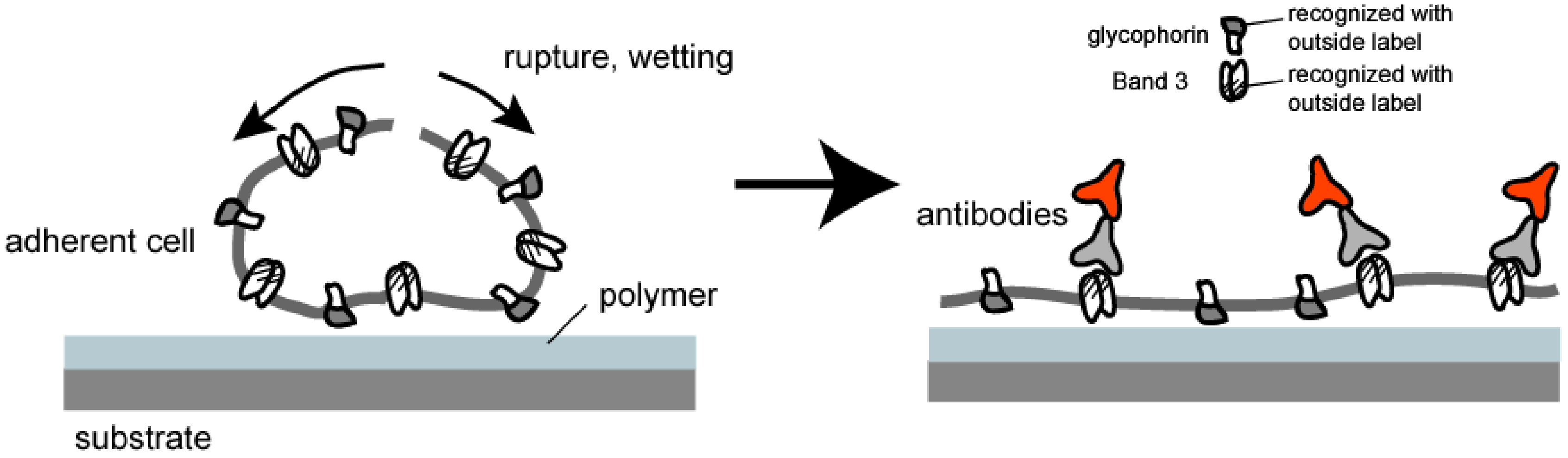
- No adhesion or rupture happens on glass/quartz.

On polymer supports:

- "Inside labeling" results in a homogeneous and continuous signal.
- "Outside labeling" shows no fluorescence.

Complete Wetting of Cell Membranes

After spreading, membranes take "inside-out" orientation.



Transformation of 3D cells into quasi 2D films with the perfectly defined membrane orientation

→ **First Example of "Two-Dimensional Cell Membranes"**

The same principle works for other native membranes (microsomes, sarcoplasmic reticulum, and plasma membrane extracts).

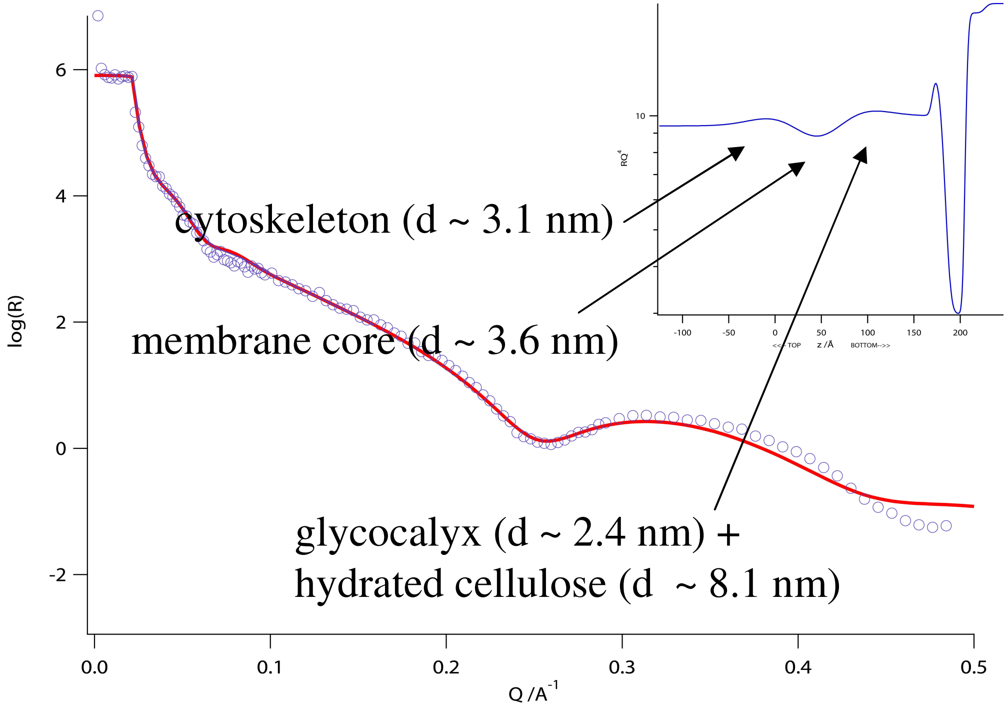
Tanaka, Kaufmann, Nissen, Hochrein, *Phys. Chem. Chem. Phys.* 2002
Tanaka, Wong, Rehfeldt, Tutus, Kaufmann, *J. Am. Chem. Soc.* 2004
Tanaka, Rossetti, Tutus, Schneck, Kaufmann, Weiss *J. Struct. Biol.* 2009.

Structure of Two-Dimensional Cell Membranes

Specular Neutron and X-ray Reflectivity

First X-ray Reflectivity Result of Human Red Blood Cell Membranes on Polymer Support

F. Rossetti (DFG fellow)
E. Schneck, S. Kaufmann



cytoskeleton (d ~ 3.1 nm)

membrane core (d ~ 3.6 nm)

glycocalyx (d ~ 2.4 nm) +
hydrated cellulose (d ~ 8.1 nm)

Measured at ESRF ID10B at 22 keV, where the transmittance of X-ray through 1 mm thick water is > 40%

O. Konovalov (ESRF)

The best fit model indicates the deposition of a uniform "layer" with a clear electron density contrast.

In parallel, we have been carrying out specular neutron reflectivity experiments at ILL to highlight/mask a certain layer by contrast variation.

G. Fragnetto (ILL)

Roles of Biopolymers in Fine-Adjustment of Interfacial Interactions

$\Pi(\mathrm{D}) = 0$ at equilibrium cell-cell (cell-substrate) distance D_{eq}

vdW (asymmetric 5-slab model)

$$\boxed{\Pi_{vdW} = \frac{1}{6\pi}\left[\frac{A_{234}}{D^3} - \frac{\sqrt{A_{121}A_{343}}}{(D+T_2)^3} - \frac{\sqrt{A_{545}A_{323}}}{(D+T_4)^3} + \frac{\sqrt{A_{545}A_{121}}}{(D+T_2+T_4)^3}\right]}$$

Helfrich Repulsion

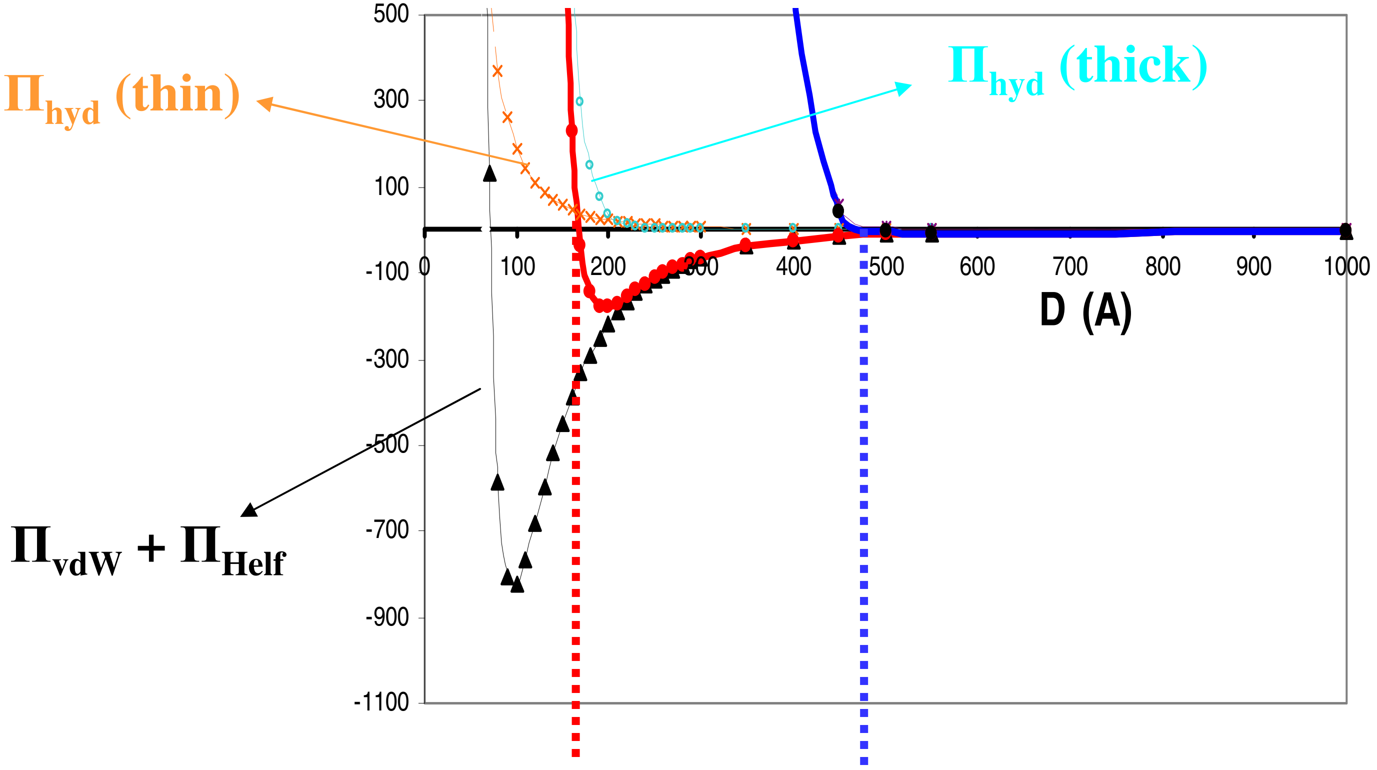
$$\boxed{\Pi_{Helf} = \frac{3\pi^2 (kT)^2}{64\kappa D^3}}$$

Hydration Repulsion

$$\boxed{\Pi_{hyd} = P_0 \exp\left(-\frac{D}{\lambda_0}\right)}$$

All parameters to calculate three major forces can be measured/calculated quantitatively.

Comparison of Theoretically Predicted D_{eq} and D_{exp}



$\Pi(D) = 0$ at $D_{eq} = 170$ Å
$D_{exp} = 200$ Å

$\Pi(D) = 0$ at $D_{eq} = 480$ Å
$D_{exp} = 380$ Å

D_{eq} agrees well with D_{exp}, confirming interplays of interfacial forces.

Rossetti, Schneck, Kaufmann, Fragneto, Konovalov, Tanaka, submitted

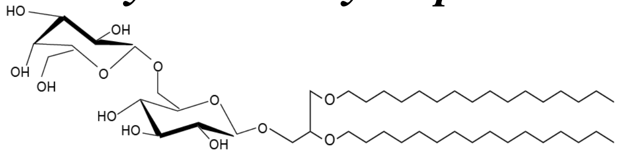
Modeling Interactions Mediated via “Membrane-Bound” Sugars (Glycocalyx)

Planar stacks of synthetic/natural glycolipid membranes hydrated in bulk D_2O or vapor at defined Π_{D2O} and T:

E. Schneck

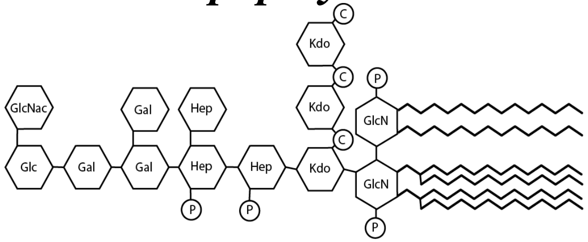
Multiple Polymer-Supported Membranes

Synthetic Glycolipids

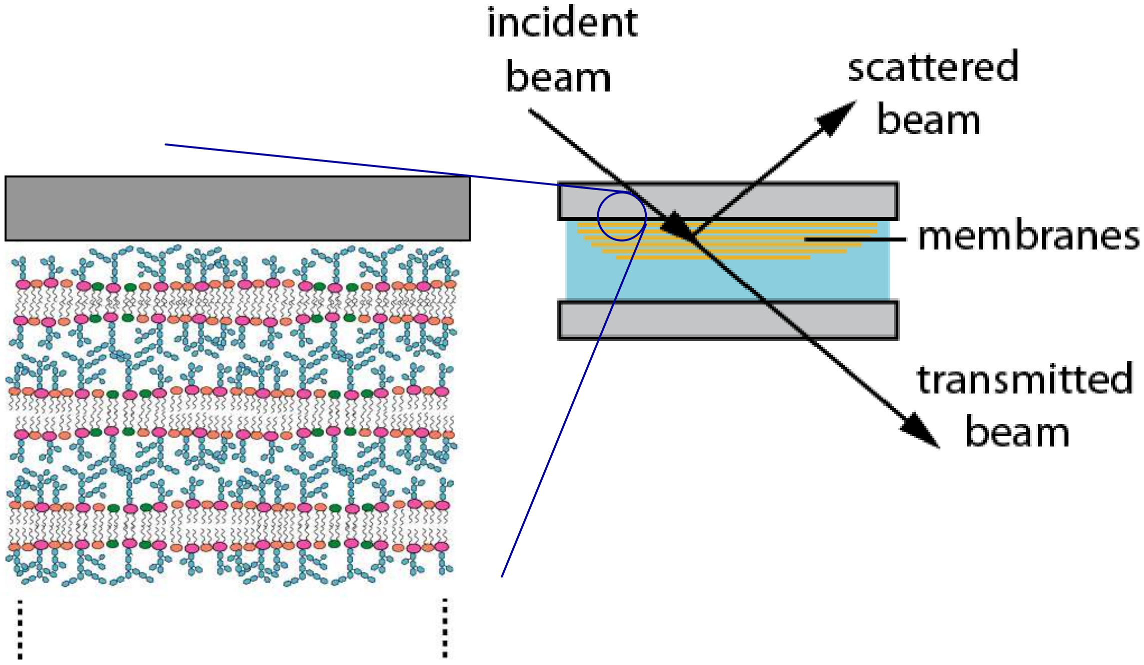


R. Schmidt (Konstanz)

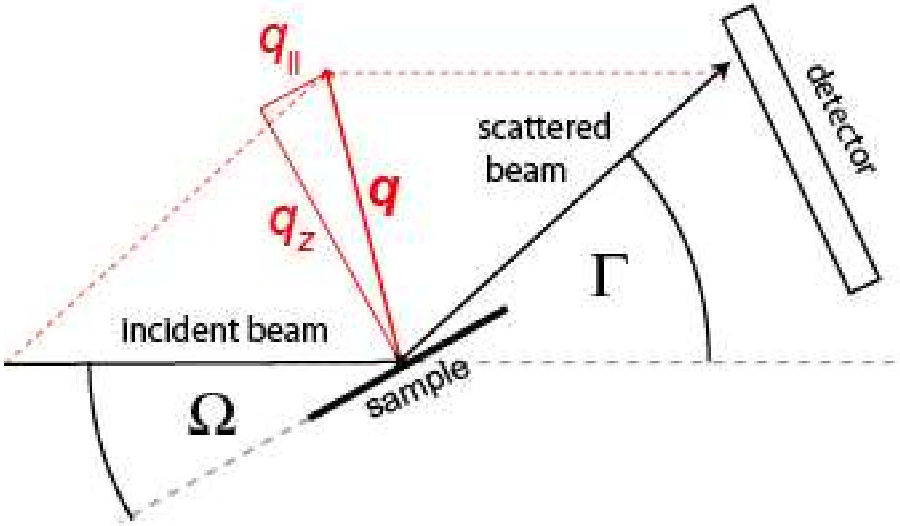
Bacterial Lipopolysaccharides

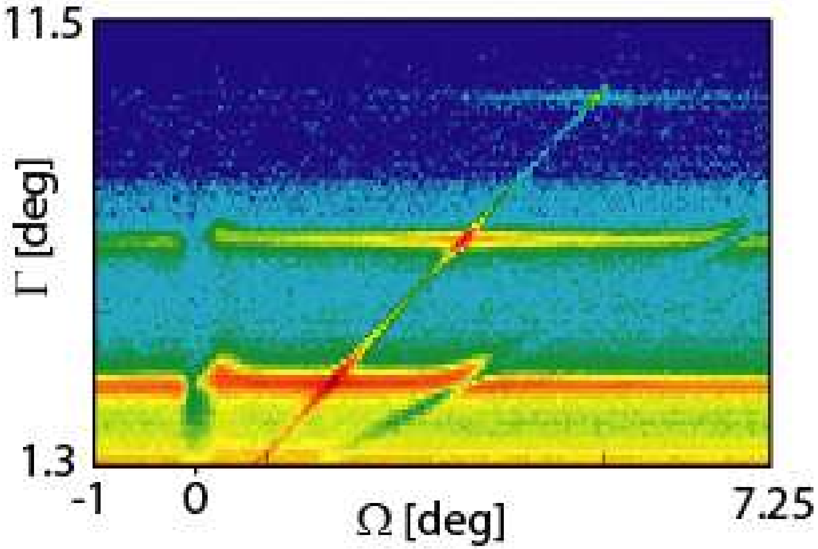


U. Seydel, K. Brandenburg (Borstel), T. Beverage (Guelph)



Specular/Off-Specular Neutron Scattering





Planar Geometry:

E. Schneck

B. Demé (ILL D16)

Identification of vertical and lateral scattering vector components

$$q_z = \frac{2\pi}{\lambda}\left[\sin(\Gamma-\Omega)+\sin(\Omega)\right] \qquad q_{\parallel} = \frac{2\pi}{\lambda}\left[\cos(\Gamma-\Omega)-\cos(\Omega)\right]$$

Safinya, et al. *Phys. Rev. Lett.* (1987), Salditt, *J. Phys. Cond. Matt.* (2005)

Specular Intensity $\Gamma = 2\Omega \quad q_{\parallel} = 0$

Vertical structure, inter-membrane potential

Off-Specular Intensity $\Gamma \neq 2\Omega \quad q_{\parallel} \neq 0$

Lateral structural ordering, membrane mechanics

Simulation of Scattering Signals (1)

Basic Framework: Discrete Smectic Hamiltonian

$$H = \int_A d^2 r \sum_{n=1}^{N-1} \left(\frac{B}{2d}\left(u_{n+1} - u_n\right)^2 + \frac{\kappa}{2}\left(\nabla_{xy}^2 u_n\right)^2 \right)$$

Leibler & Lipowsky, *Phys. Rev. B* (1987)

Two key parameters: compression modulus ***B*** & bending rigidity ***κ***

Scattering from stratified rough interfaces in 1st Born approximation as a function of q_z and $q_\|$

$$S\left(q_z, q_\|\right) \propto \frac{e^{-q_z^2 \sigma^2}}{q_z^2} \left[N + 2\sum_{k=1}^{N} (N-k) \cos\left(k q_z d\right) \int_{-\infty}^{\infty} e^{q_z^2\left(\sigma^2 - g_k(r)/2\right)} e^{-i q_\| r} dr \right]$$

Sinha, *J. Phys. III* (1994)

Displacement correlation function $g_k(r)$ is determined by two mechanical parameters: ***λ* & *η***

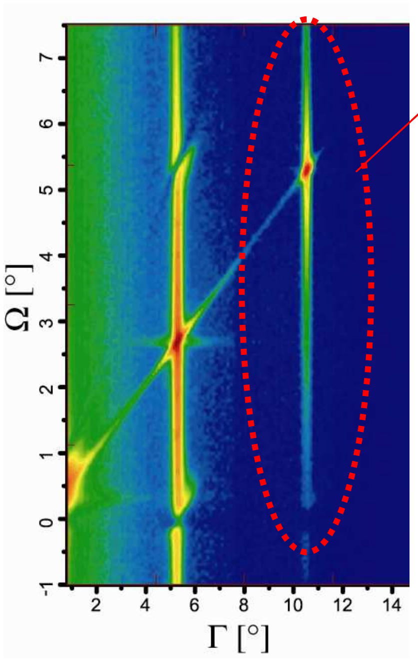
$$g_k(r) = \frac{d^2}{\pi^2} \eta \int_{\boxed{2\pi/R}}^{\infty} \frac{\left[1 - J_o\left(q_\| r\right) \exp\left(-\lambda k q_\|^2 d\right)\right]}{q_\| \sqrt{1 + \frac{\lambda^2 d^2}{4} q_\|^4}} dq_\|$$

Lei, Safinya, Bruinsma, *J. Phys. II* (1995)

$\lambda \propto \sqrt{\frac{\kappa}{B}}$ De Gennes Parameter $\eta \propto \frac{1}{\sqrt{\kappa B}}$ Caillé Parameter

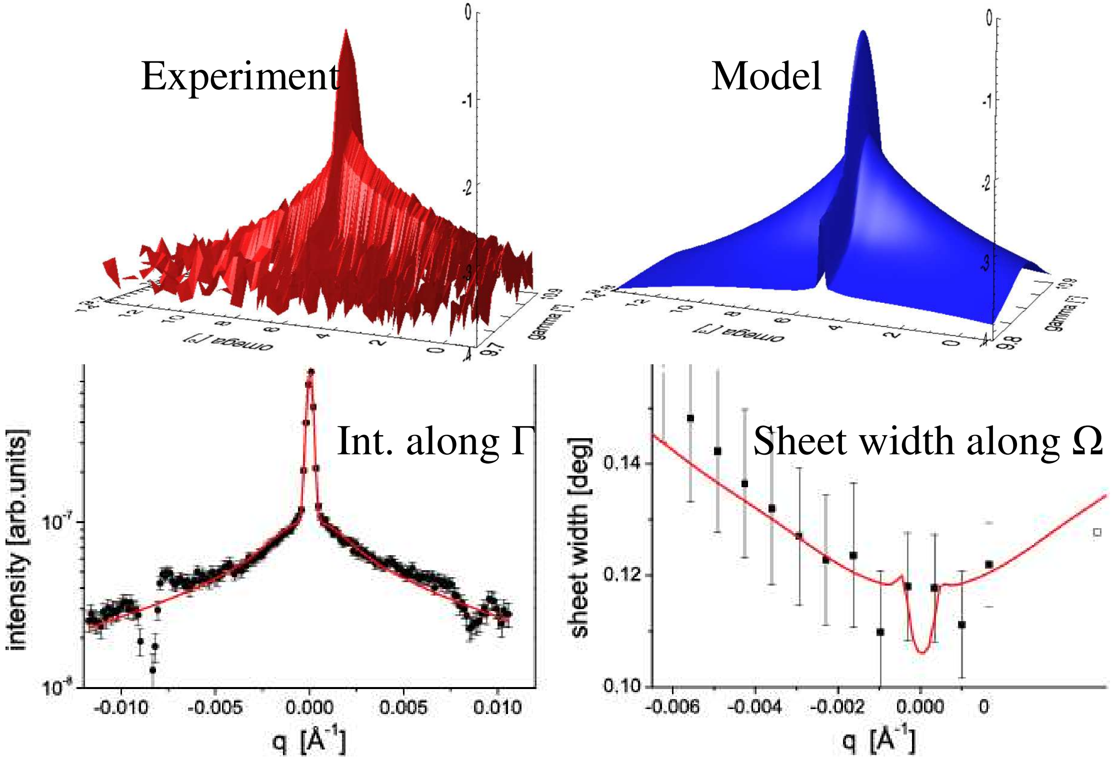
Schneck, Rehfeldt, Oliveira, Gege, Schmidt, Demé, Tanaka, *Phys. Rev. E* (2008)

Simulation of Scattering Signals (2)



Synthetic glycolipids
in bulk D_2O

2nd Bragg Sheet was taken to ensure 1st Born approx.



The strategy is applicable for both synthetic lipids and natural compounds (e.g. lipopolysaccharides from bacteria mutants) to highlight the influence of molecular chemistry and mutation on structural ordering and mechanics of biomembranes, e.g. $\kappa_{(\text{lipid A})} = 0.9$ MPa but $\kappa_{(\text{LPSRa})} = 1.6$ MPa.

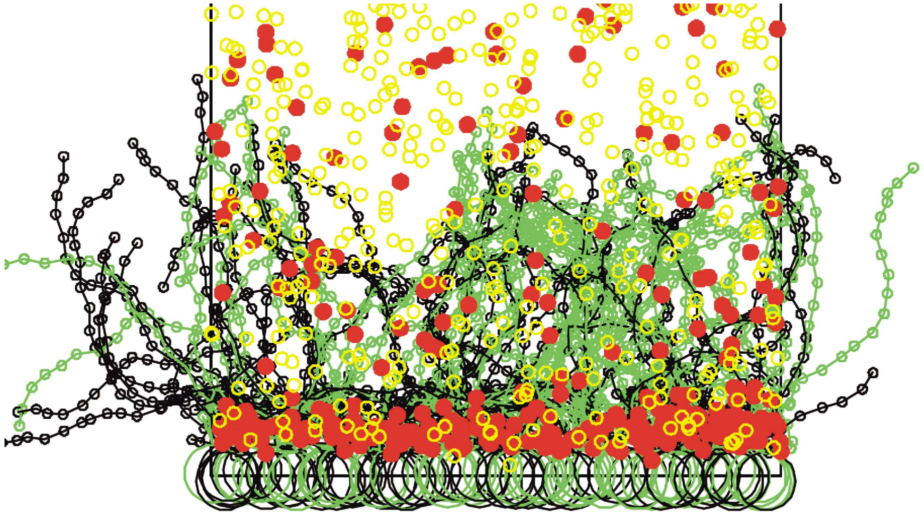
Schneck, et al., *Phys. Rev. E* (2008), *Phys. Rev. E* (2009), Oliveira, et al., *Comptes Rendus Chimie* (2009), Schneck et al., *Roy. Soc. J. Interf.* (2009)

Mechanism of Bacterial Resistance against CAPs

Canadian COE "Advanced Food and Material Network"

In-vivo study (T. Beverage, Univ. Guelph)

Significant (> 5 x) bacterial survival with Ca^{2+}.



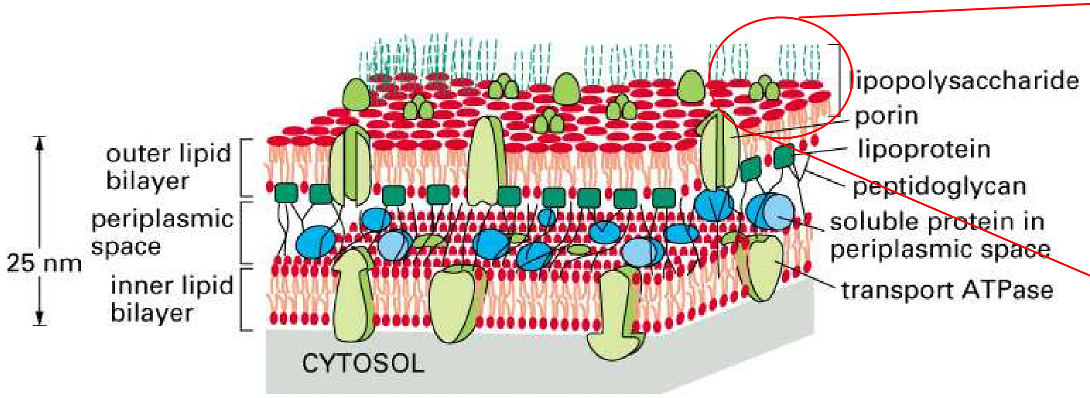
Coarse-Grained MC simulation (D. Pink, Canadian COE)

Sugar chains should "collapse" in the presence of Ca^{2+}.

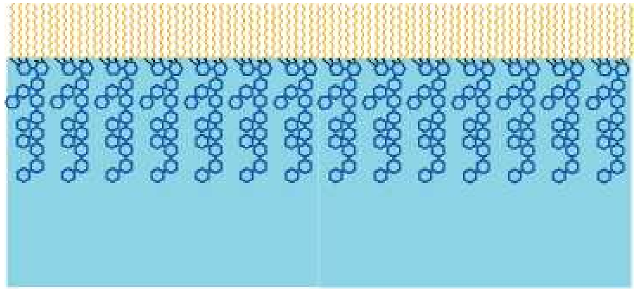
Pink, Truelstrup Hansen, Gill, Quinn, Jericho, Beveradge, *Langmuir* (2003).

No experimental study has revealed the influence of Ca^{2+} on the molecular level.

Our Strategy: Design of Simple Models of the Outer Surface of Bacteria with Well-Defined Building Blocks

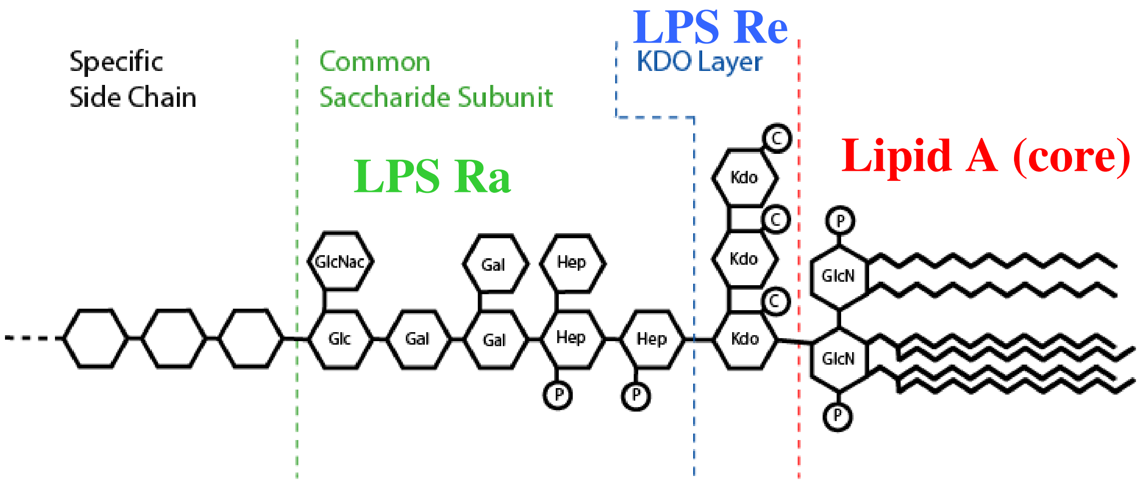


Alberts "*Molecular Biology of the Cells*"



Model: Monolayer of Bacterial LPSs at the air/water interface

Building Blocks: Purified LPSs



PAO1 LPS
Beverage Lab / Guelph

Seydel & Brandenburg Lab / Borstel

Vertical Structures: Grazing Incidence X-ray Scattering Out of Specular Plane (GIXOS) at the Air/Water Interface

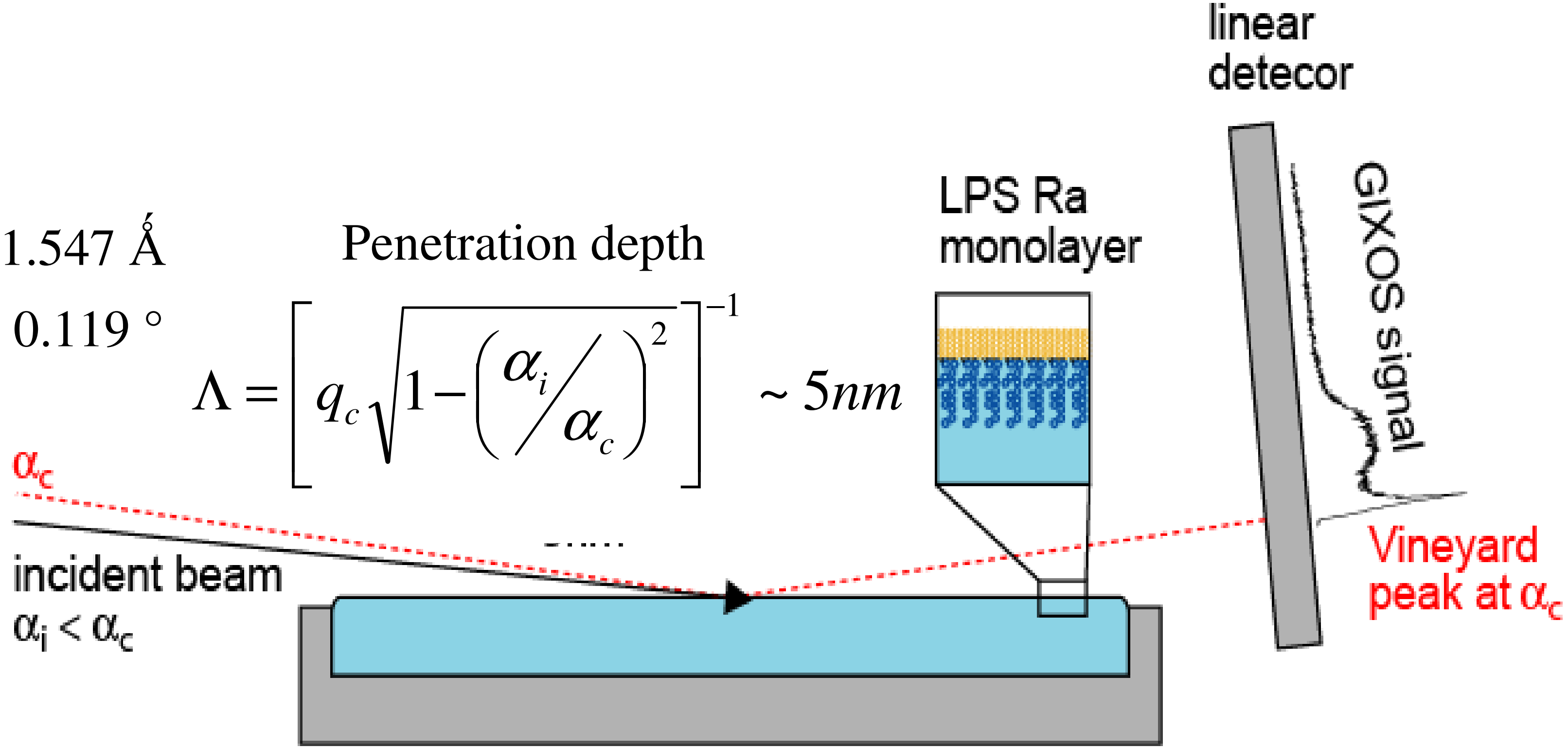
ESRF ID10B beam line (O. Konovalov)

$\lambda = 1.547$ Å

$\alpha_i = 0.119\ °$

Penetration depth

$$\Lambda = \left[q_c \sqrt{1-\left(\alpha_i / \alpha_c\right)^2}\,\right]^{-1} \sim 5nm$$



Detection with a linear PSD at $q_{\|} \sim 0.029$ Å^{-1}

Oliveira, Schneck, Konovalov, Brandenburg, Seydel, Quinn, Pink, Tanaka
Comptes Rendus,(2009), *Phys. Rev. E* (2009)

GIXOS: Principles

For conformal layers, $I(q_z)$ at $q\| \sim 0$ is connected to the corresponding reflectivity:

$$I(q_z) \propto \left|T(k_{out})\right|^2 \frac{R(q_z)}{R_F(q_z)}$$

Vineyard Function

Mora et al., *Europhys. Lett*. (2004)

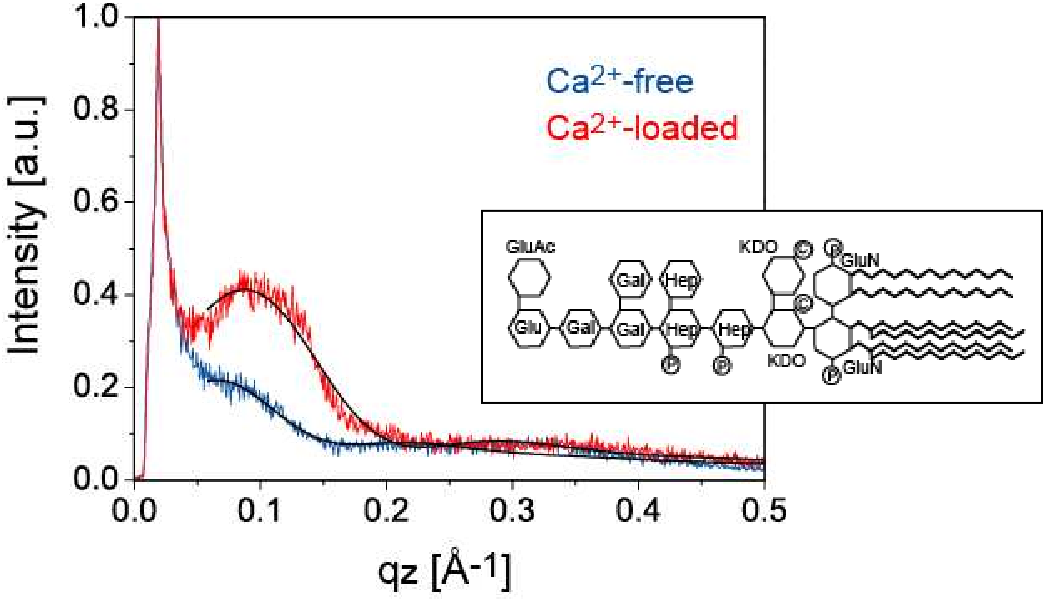
To obtain a least square fit to each result, we developed a new fitting routine based on the Master Formula for specular reflectivity:

$$R(q_z) = R_F(q_z)\left|\frac{1}{\rho}\int \frac{d\rho(z)}{dz}\exp(iq_z z)dz\right|^2$$

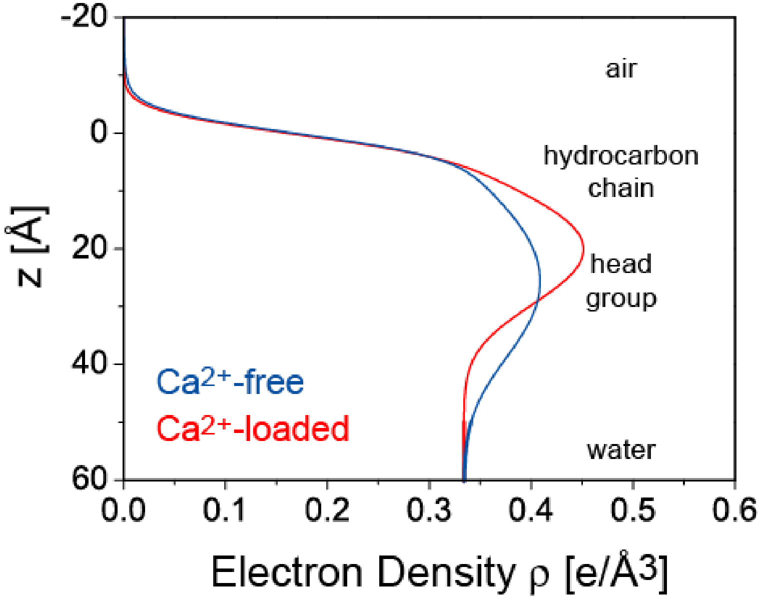
Compared to specular reflectivity (θ-2θ scan), GIXOS can minimize the radiation time (< 100 times) that is advantageous for biological samples.

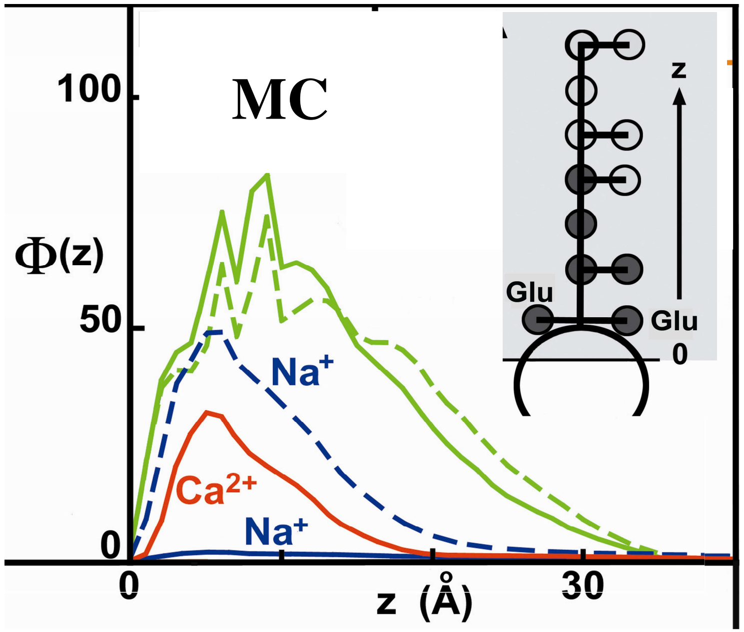
Influence of Ca^{2+} on LPSRa

GIXOS at A = 180 Å²



Electron density profiles





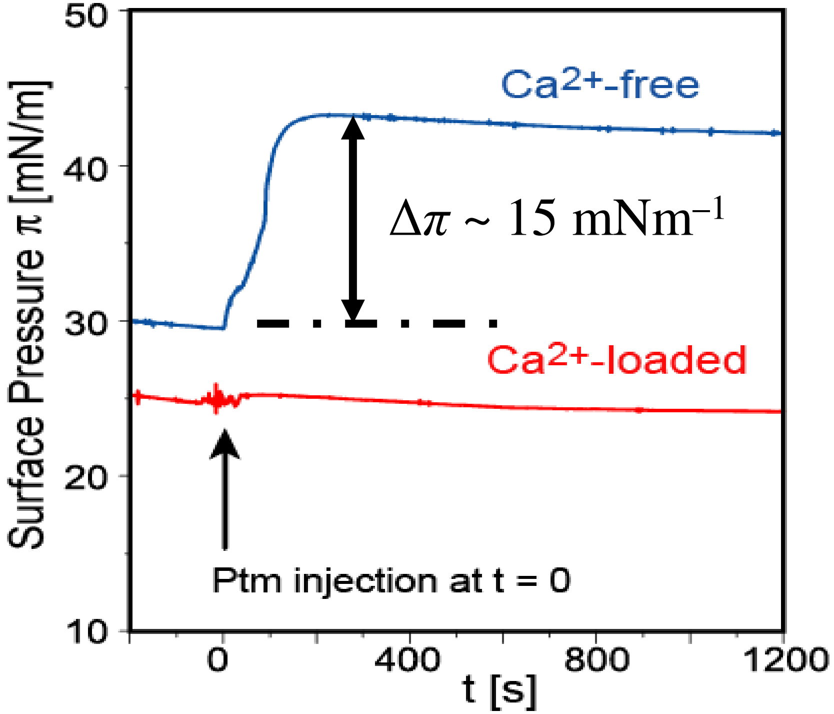
Collapse of sugar chains in the presence of Ca^{2+} could be detected. Coarse-grained MC simulations based on linearlized PB theorem fully supported our experimental findings.

Oliveira, et al. *Comptes Rendus* (2009)

Impact of Herring Protamine (Ptm)

Canadian Inst. Fishery

π vs. time at A = 180 Å^2

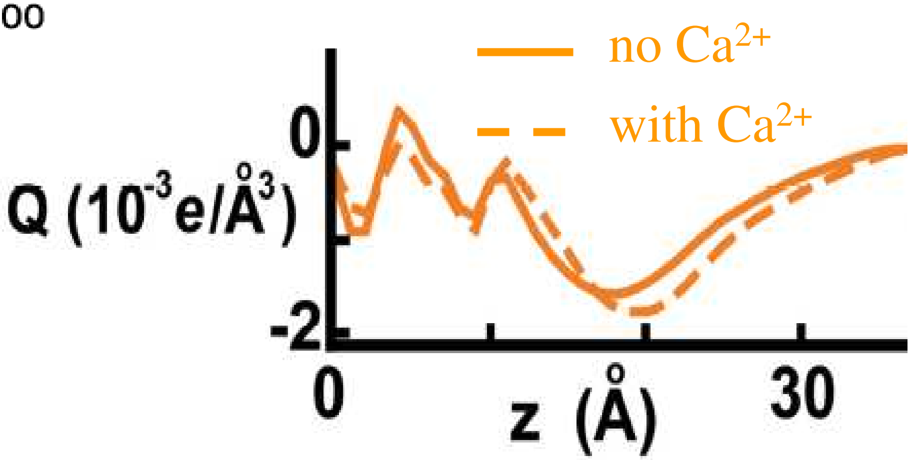


Ca^{2+}-free:

Destruction of layered structure by Ptm intrusion

Ca^{2+}-loaded:

Membrane remains intact!



MC simulation predicts the creation of an electrostatic energy barrier (at around z = 20 Å) repelling positively charged protamine.

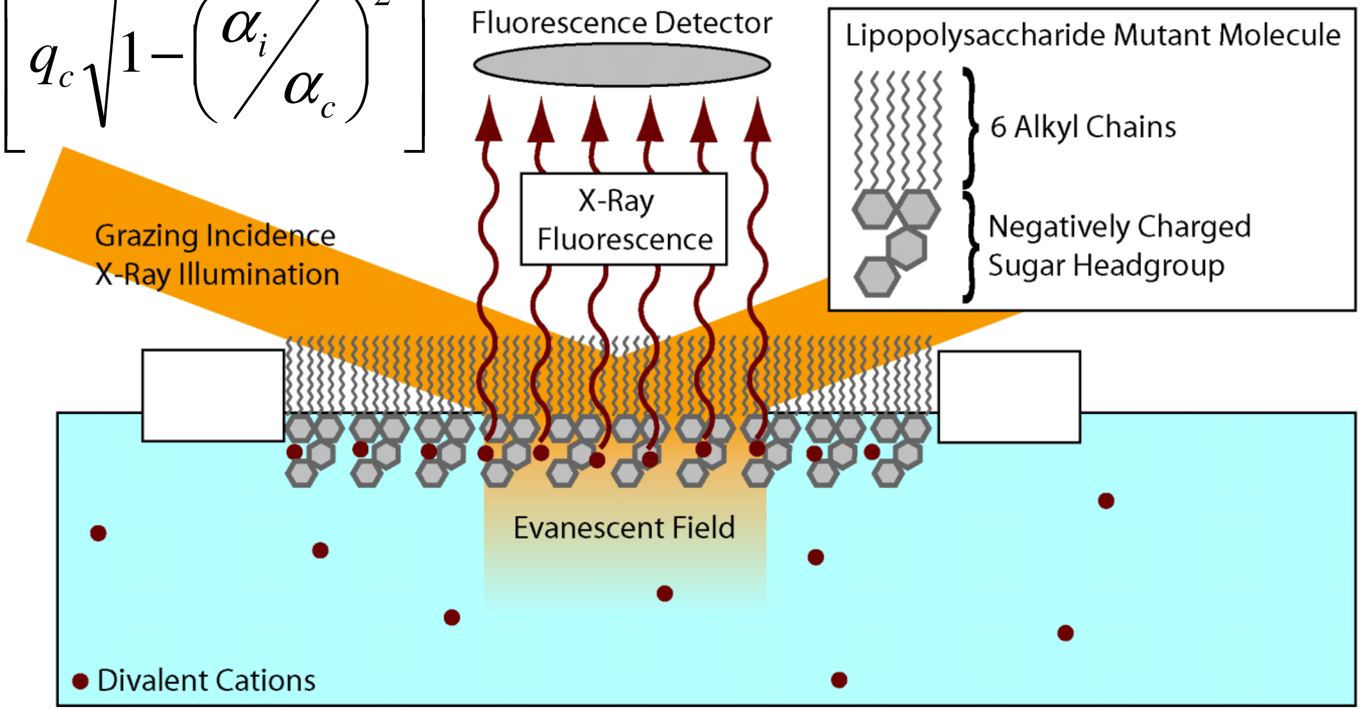
Oliveira, Schneck, Konovalov, Brandenburg, Seydel, Quinn, Pink, Tanaka, *Compt. Rendus* (2009)

Next Step: Determination of Ion Specific Density Profiles

Grazing Incidence X-ray Fluorescence

Variation of α_i (and thus Λ)

$$\Lambda = \left[q_c \sqrt{1 - \left(\alpha_i / \alpha_c \right)^2} \right]^{-1}$$



Fluorescence from the target elements (K: 3.31 keV, Ca: 3.69 keV) measured at various α_i enables us to calculate the **depth profiles/amounts of ions** instead of the whole electron density profiles.

Schneck, Schubert, Brandenburg, Konovalov, Quinn, Pink, Tanaka, submitted.

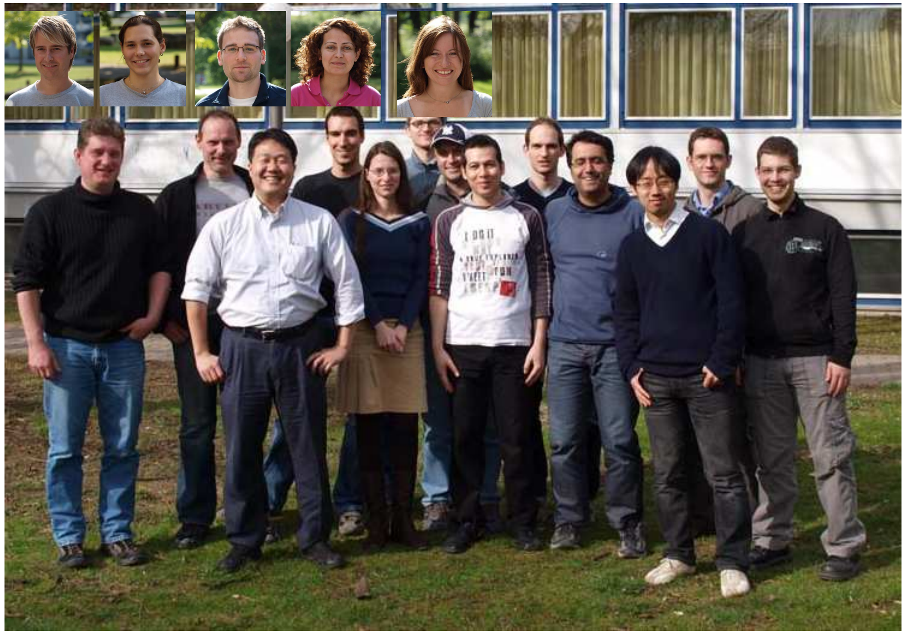
Conclusions

- Specular reflectivity at solid/liquid interface of well-defined polymer-supported membranes enable us to quantify the physical roles biopolymers in modulating bioloical interfaces.

- The impact of membrane-bound sugars on the cell/cell contacts can be evaluated with the vertical compressibility and bending rigidity measured by specular/off-specular scattering of planar membrane stacks.

- Crucial roles of Ca^{2+} on the bacterial resistance against intruders (CAPs) can be revealed by grazing-incidence scattering at the air/water interface.

Thanks!



Current Members

S. Kaufmann, F. Rossetti , H. Yoshikawa, M. Kazanci, F. Al-Ali, J. Oelke, E. Schneck, M. Tutus, T. Schubert, P. Seitz, W. Abuillan, T. Kaindl, D. Gassull, H. Rieger, K. Hock, M. Hermann, J. Oswald, A. Eichmann, M. Pascuci, L. Wolber, C. Harasim, …

R. Jordan, M. Stutzmann, M. Eickhoff, G. Abstreiter, M. Tornow, E. Sackmann (München), R. Schmidt (Konstanz), G. Wegner (MPI Mainz), S. Armes (Sheffield), M. Lanzer, T. Holstein, A. Ho (Heidelberg), A. Gast, S. Boxer (Stanford), U. Seydel (FZ Borstel), S. Funari, (HASYLAB), O. Konovalov (ESRF), B. Demé, G. Fragnetto (ILL), Bayer (Leverkusen), General Mills (Conneticut), Advalytix (München)…

German Science Foundation (DFG), German Excellence Initiatives, Ministry of Research and Education (BMBF), Helmholtz Society, State Baden-Württenberg, Heidelberg Academy of Science, Humboldt Foundation, DAAD, EU FP6/7, VDI, Canadian COE etc.

EXZELLENZCLUSTER CellNetworks

BioQuant MODEL base of LIFE

afmnet
Réseau des aliments et des matériaux d'avant-garde
ADVANCED FOODS & MATERIALS NETWORK
Inspiration from the ground up | Aux racines de l'inspiration