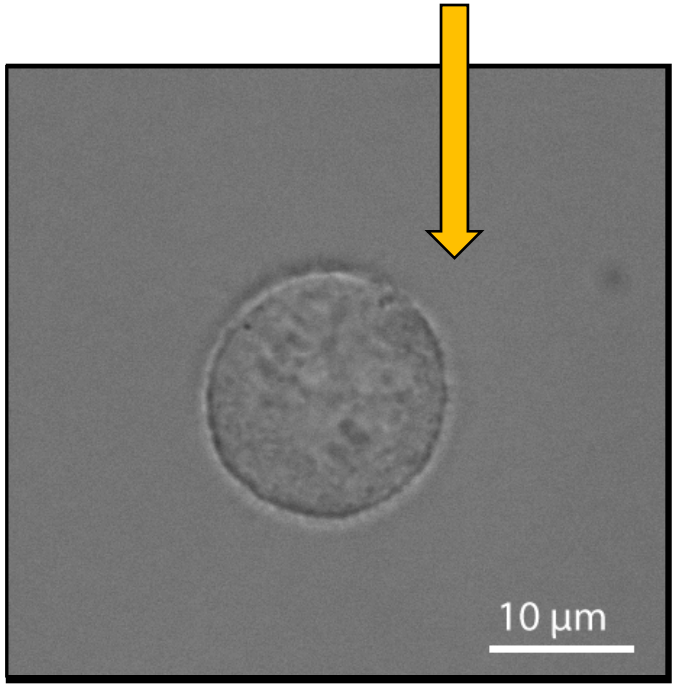
The Pericellular Coat…

…or getting a grip on strongly hydrated biomolecular films



by Ralf Richter

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The Pericellular Coat…

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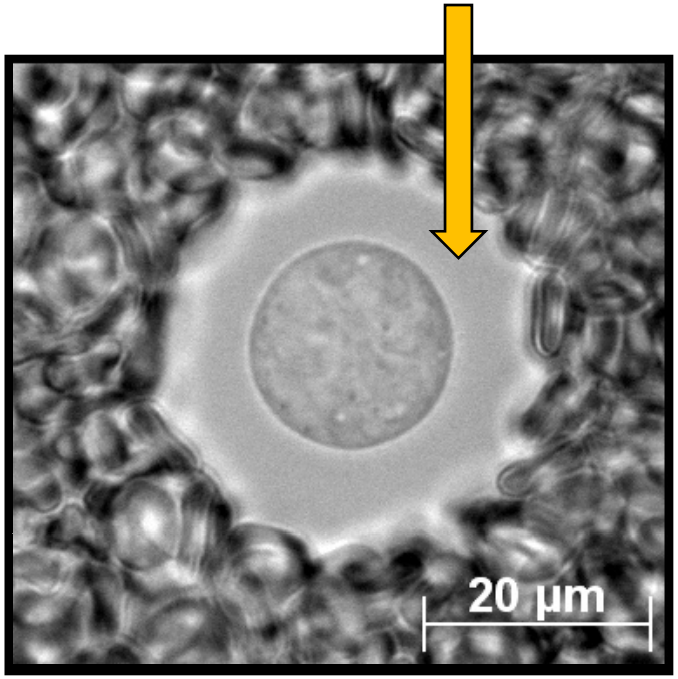
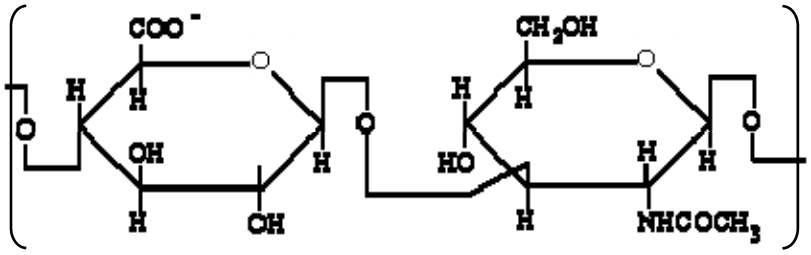


movie by Heike Boehm (MPI Stuttgart)

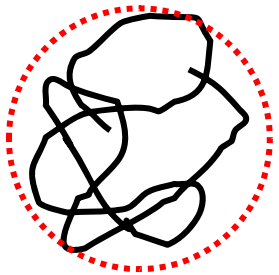
How is this coat made and how does it function?

The Protagonist: Hyaluronan



glucuronic acid N-acetylglucosamine

- linear polymer of a repeating disaccharide
- this simple structure is highly preserved during evolution
- persistence length: ~4 nm
- contour length: from nanometers to several micrometers



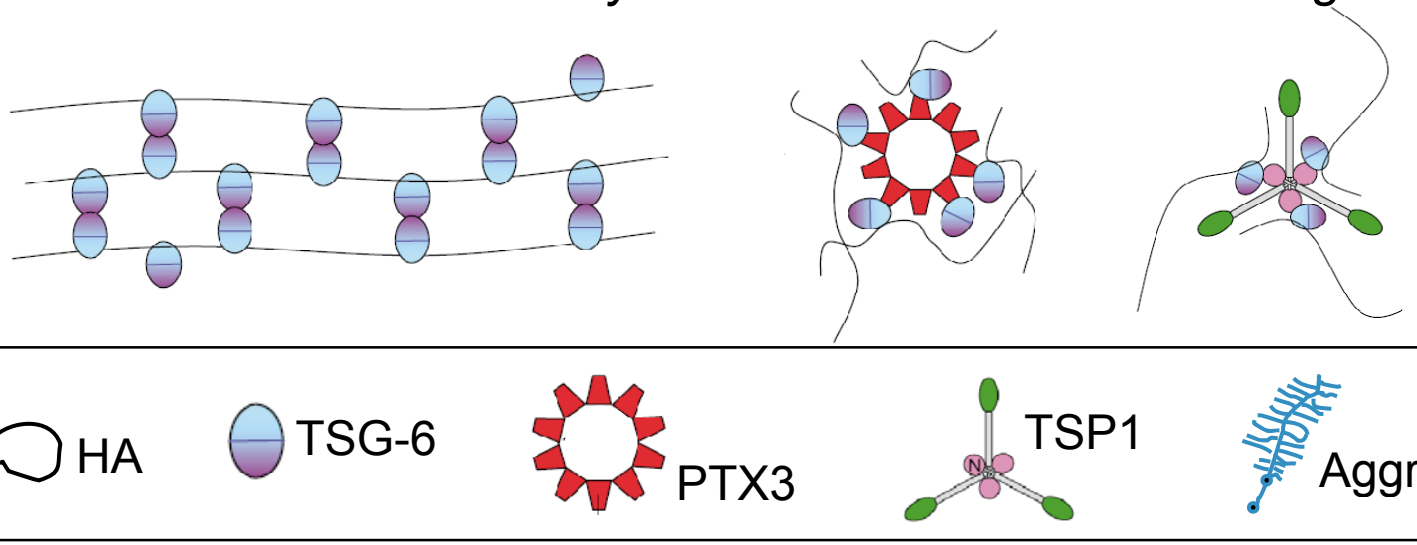
contains
99.95% water

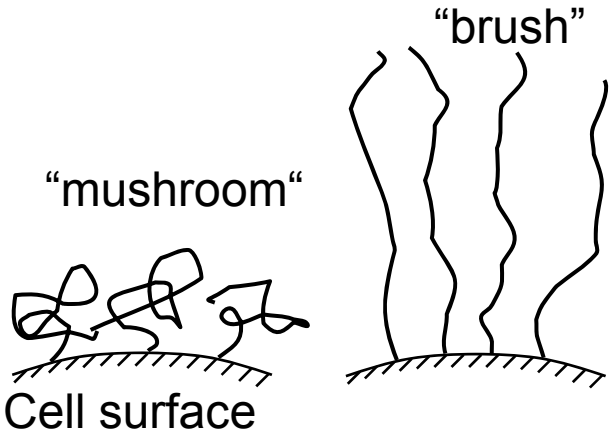
➢ *From the point of view of polymer physics, hyaluronan is well-described as a polyelectrolyte.*

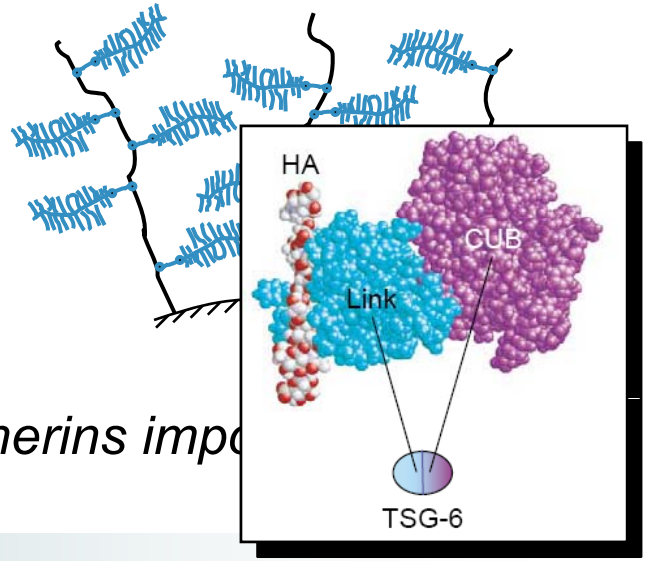
➢ *A single hyaluronan molecule can have hundreds of sites for specific binding of certain proteins (hyaladherins).*

Putative Structures of Hyaluronan-Complexes

Interaction with soluble hyaladherins creates networks/gels



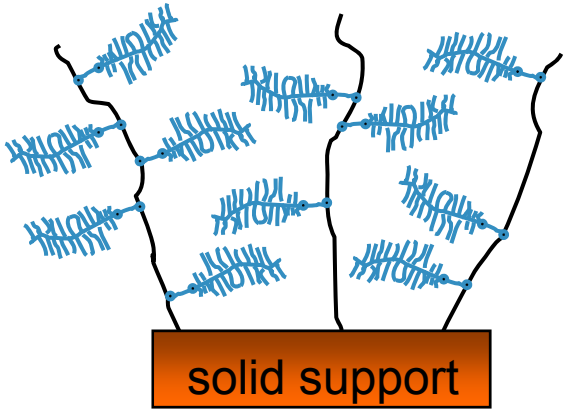




Interaction with membrane-bound hyaladherins impo
confinement in 2 dimensions

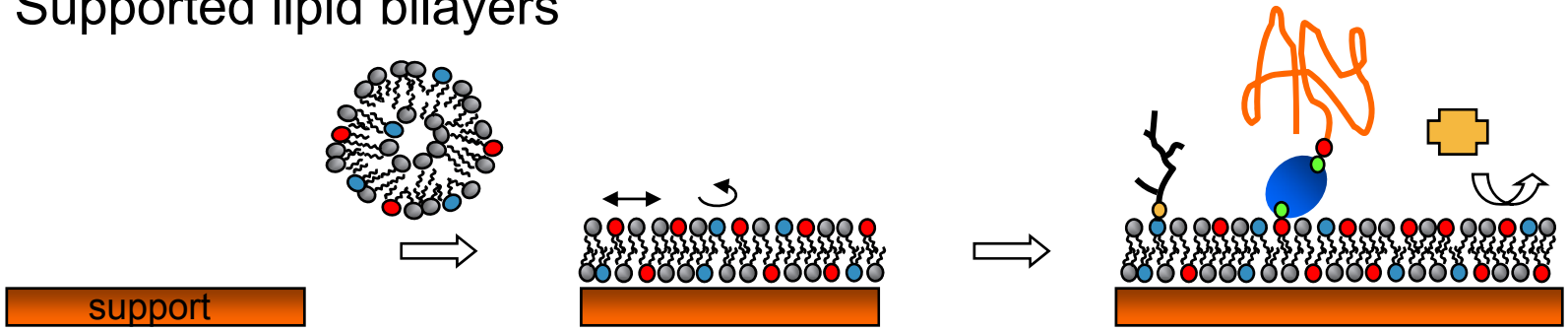
Our Objectives

- Create well-defined model systems of pericellular coats
- Characterize the properties of such artificial coats
- Understand, how the supramolecular structure relates to collective properties of the coats (and their biological function)



Biofunctionalization Strategies

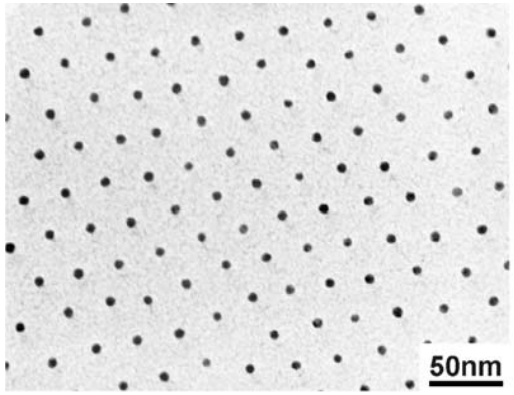
- Supported lipid bilayers



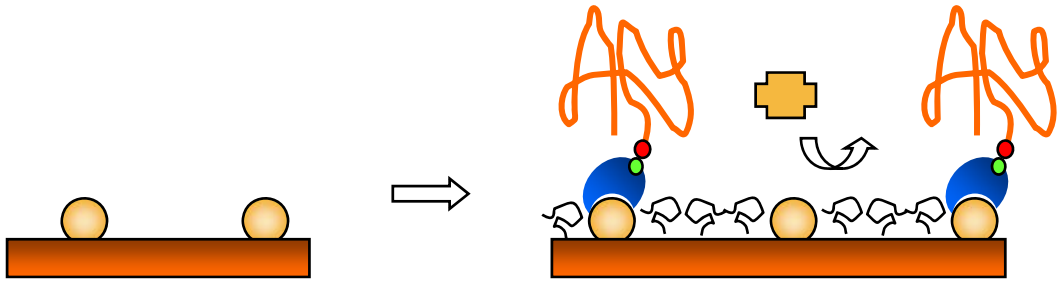
➔ *Density & 2D mobility of individual ligands controlled*

➔ *Several different ligands can be attached on the same support*

- Nanostructuration



Glass et al. (2003) *Adv. Funct. Mat.* 13:571



Arnold et al. (2004) *ChemPhysChem* 3:383

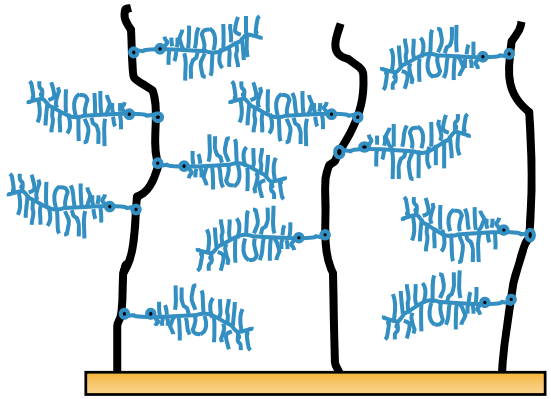
➔ *Density & distances of individual ligands controlled*

Characterization of our model systems

Molecular Composition

Characteristic forces

Water content



Viscoelastic Properties

Permeability

Reaction to external cues

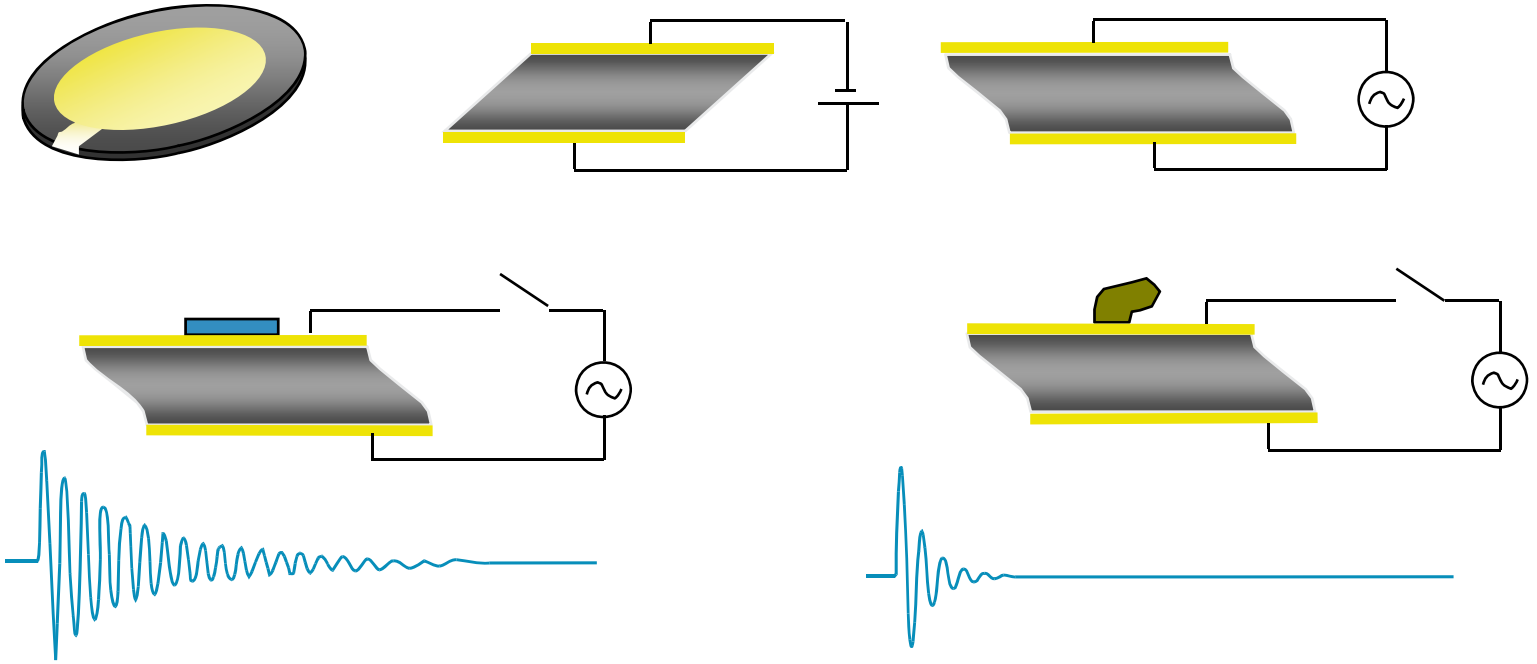
Thickness

Self-Assembly Dynamics

A toolbox of surface-sensitive methods can provide the answers...

QCM-D

Quartz crystal microbalance with dissipation monitoring



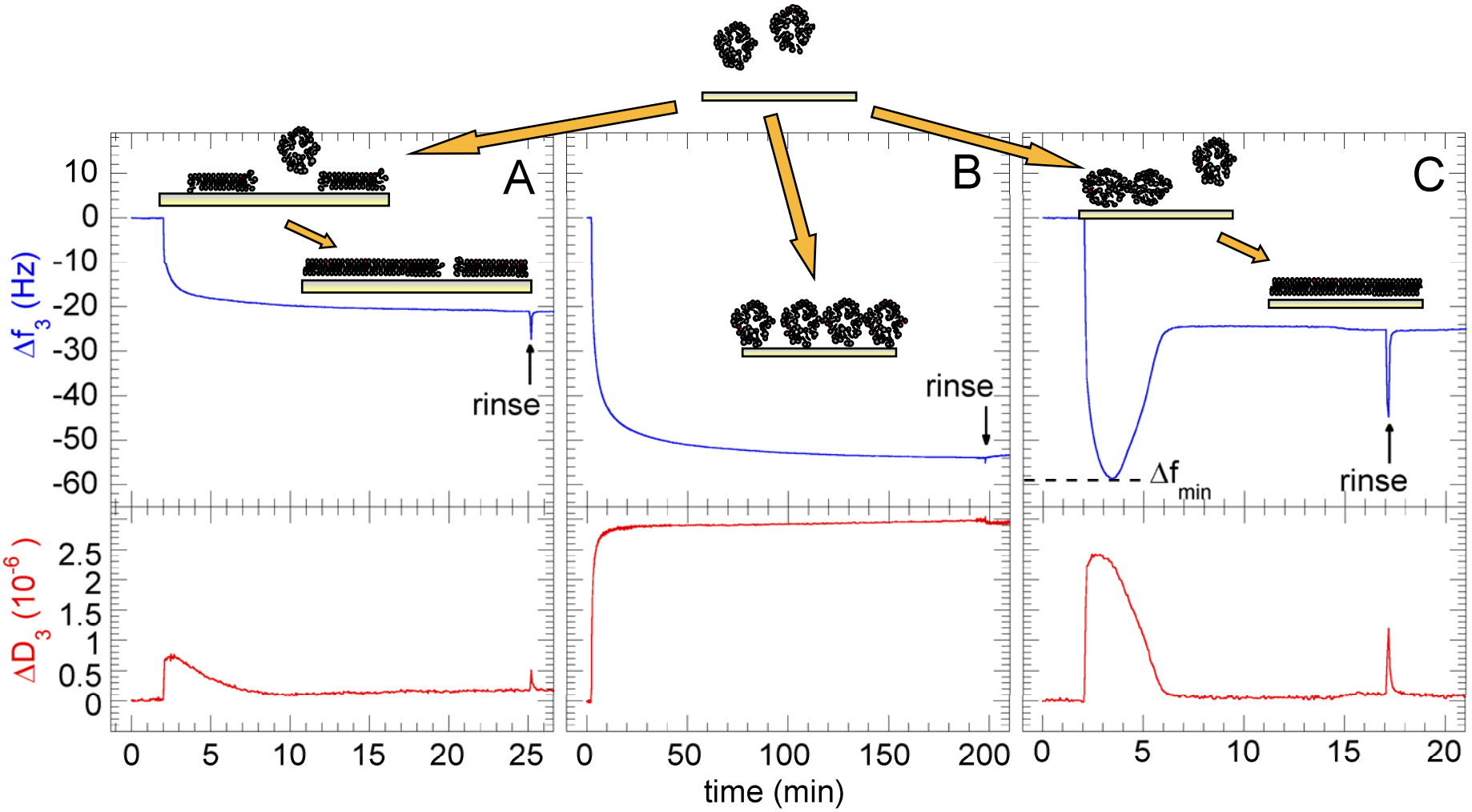
Δf is related to the mass of the attached film

ΔD is related to its viscoelasticity

- ⇨ *« Feel for hydration »*
- ⇨ *Information about mass and structure of the ensemble*
- ⇨ *Good time resolution (seconds)*

Lipid deposition on silica

SUVs in 2mM EDTA



DOTAP (++) ➔ SLB-formation (II)

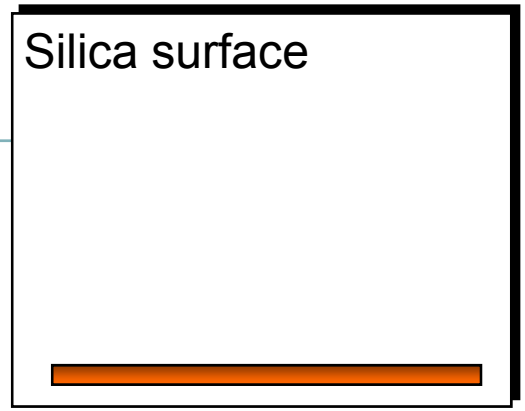
DOPC/DOPS (1:1) (--) ➔ SVL-formation

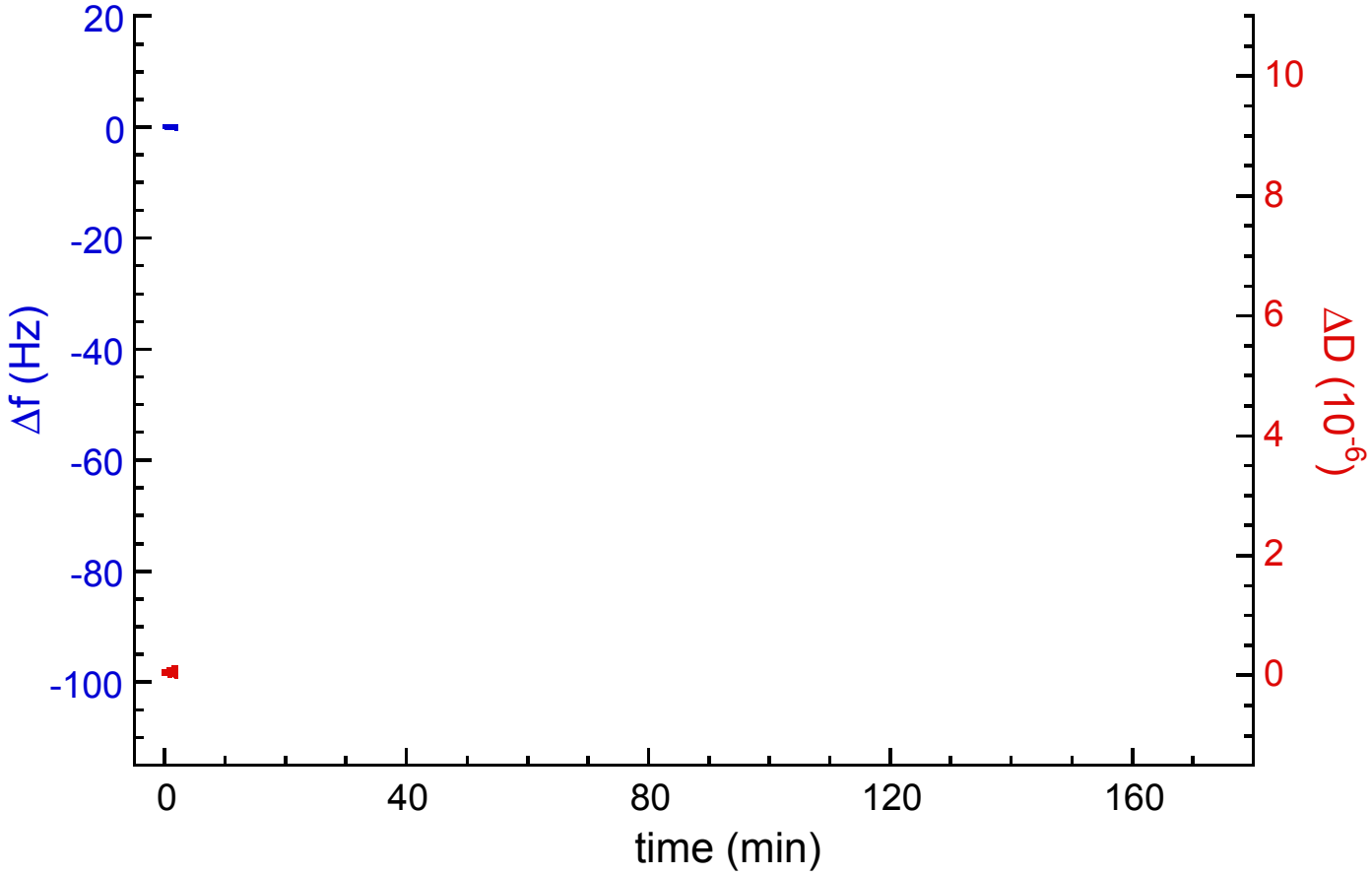
DOPC/DOPS (4:1) (-) ➔ SLB-formation (I)

Keller & Kasemo 1998 *Biophys. J.* 75:1897, Richter et al. 2003 *Biophys. J.* 85:3035 & 2006 *Langmuir* 22:3497

Creating a well-defined HA-Film

„Hyaluronan-Lego“
monitored by QCM-D

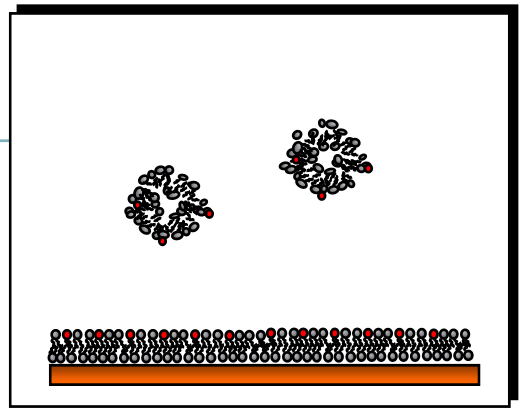




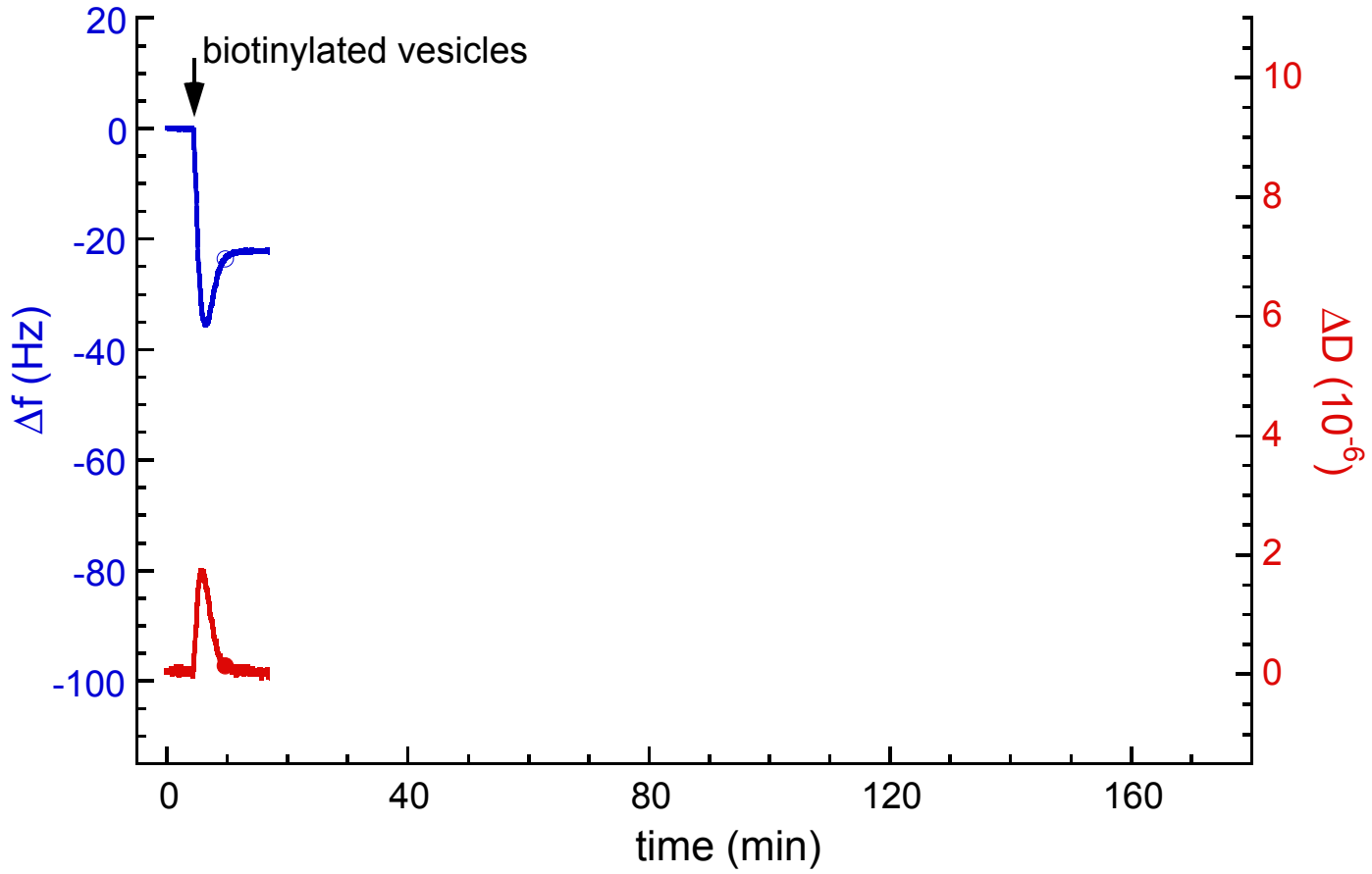
Richter et al. (2007) *JACS* 127:5306

Creating a well-defined HA-Film

„Hyaluronan-Lego“



Step 1: Formation of a supported lipid bilayer



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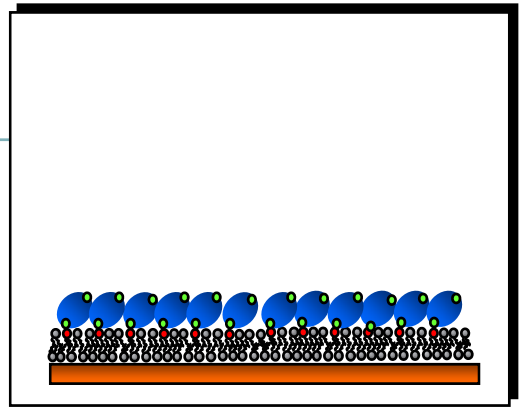
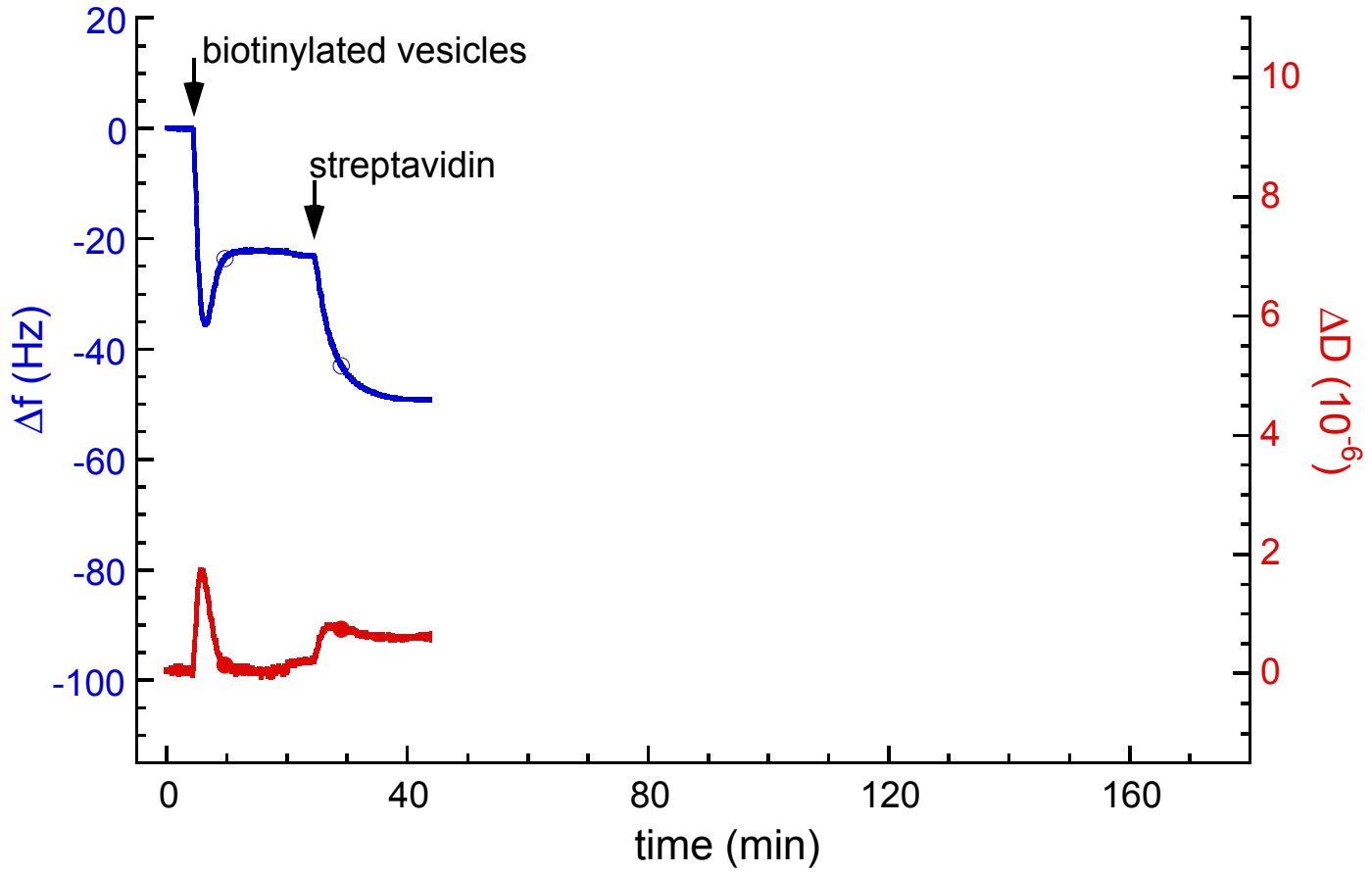


Step 2: Deposition of a streptavidin monolayer



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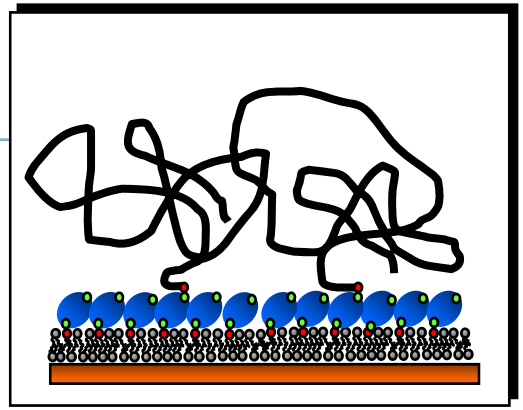
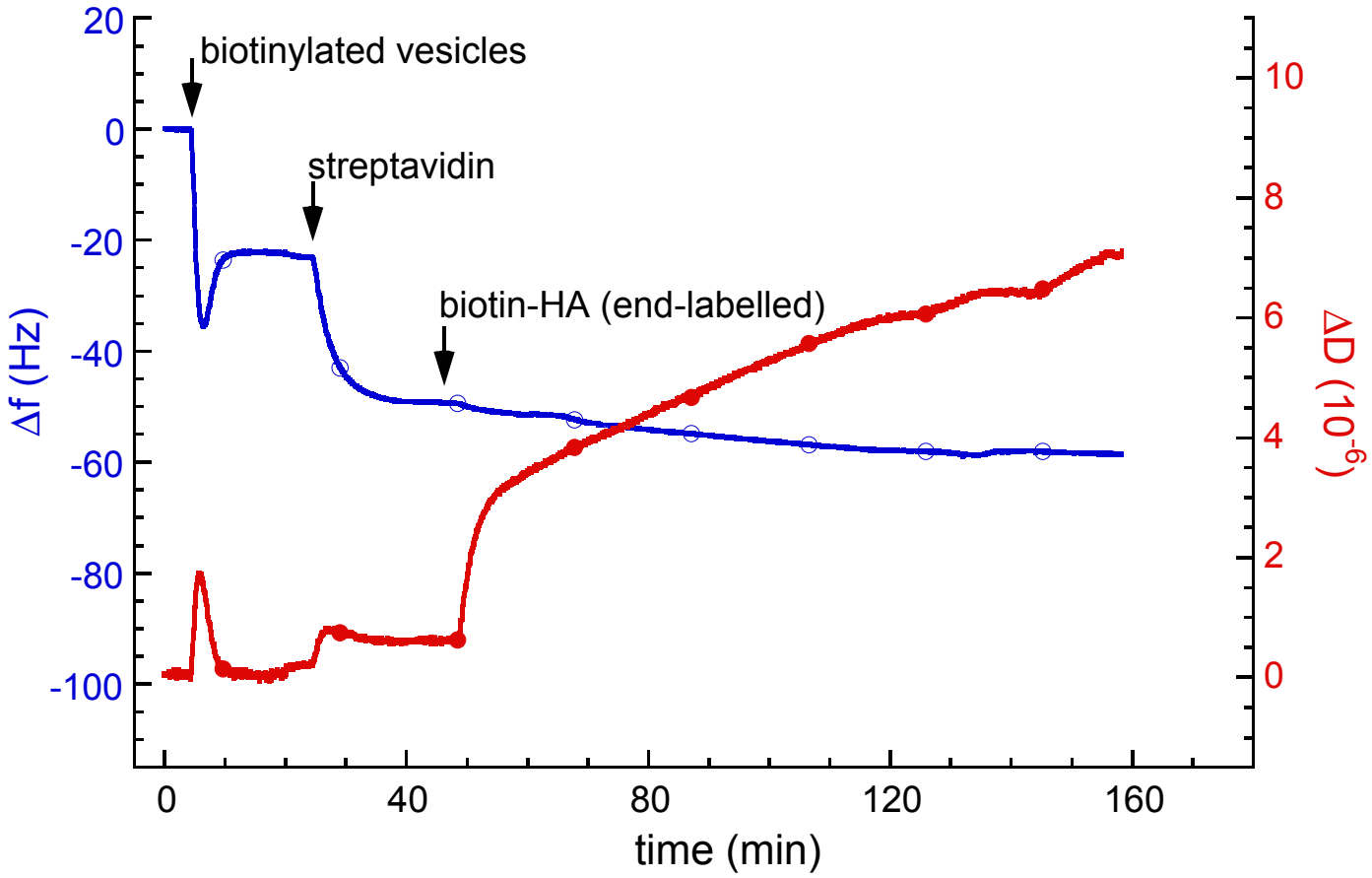


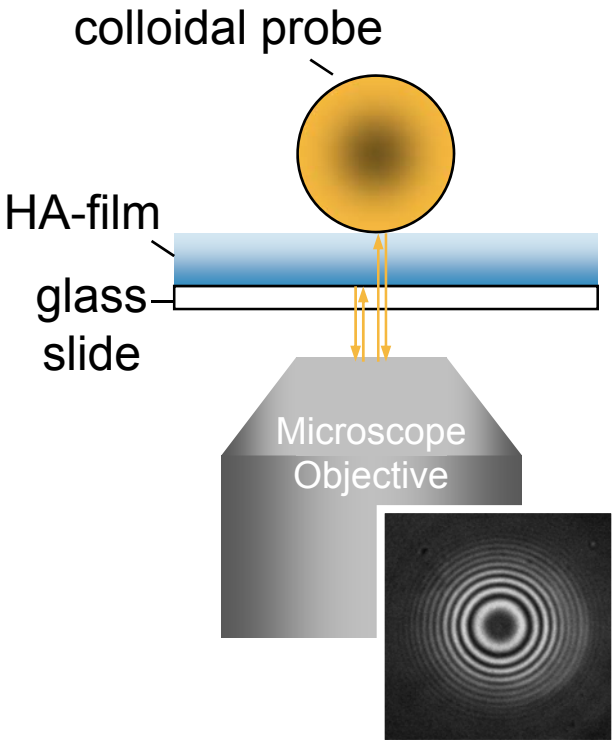
Step 3: “End-on” attachment of HA (3µm length)

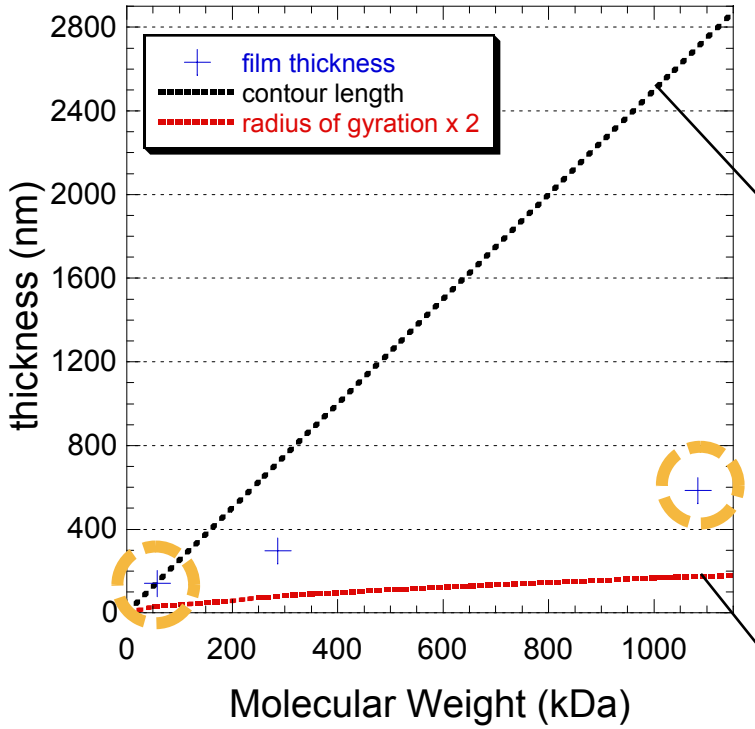


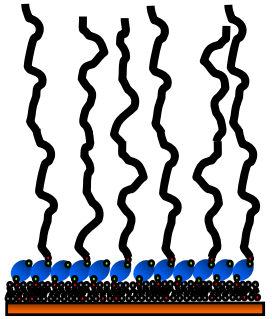
Richter et al. (2007) *JACS* 127:5306

How Thick Is the Film?

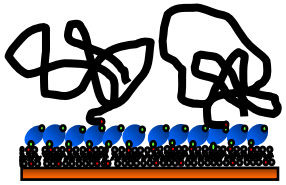
by reflection interference contrast microscopy (RICM)







expected for full stretching



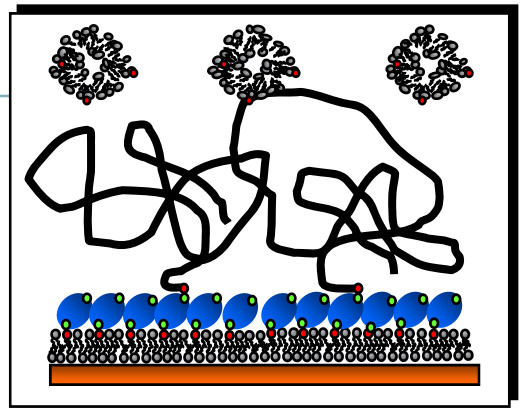
expected for a mushroom layer

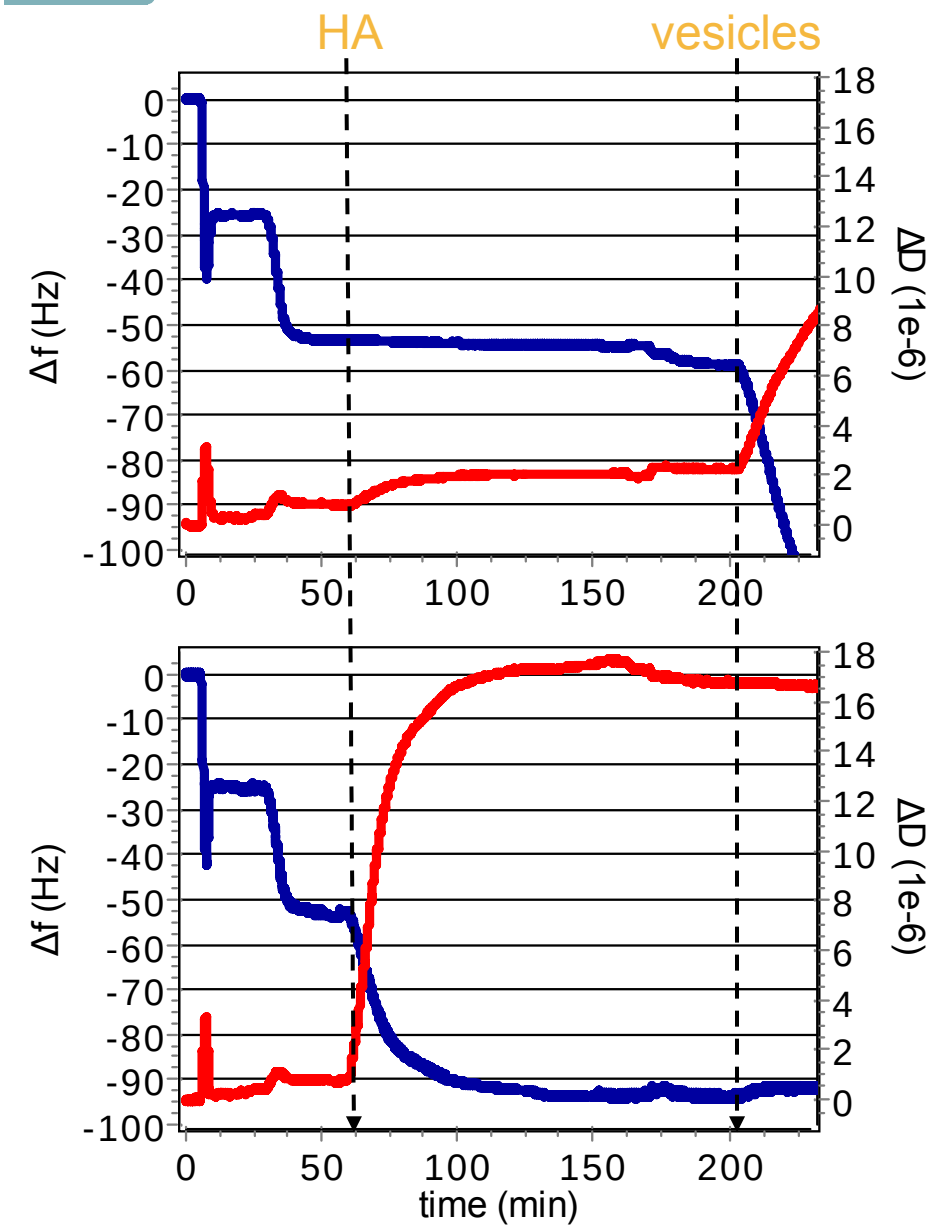
➢ *even the longest HA-chains stretch into the brush regime*

Richter et al. (2007) *JACS* 127:5306

Film Permeability

by QCM-D





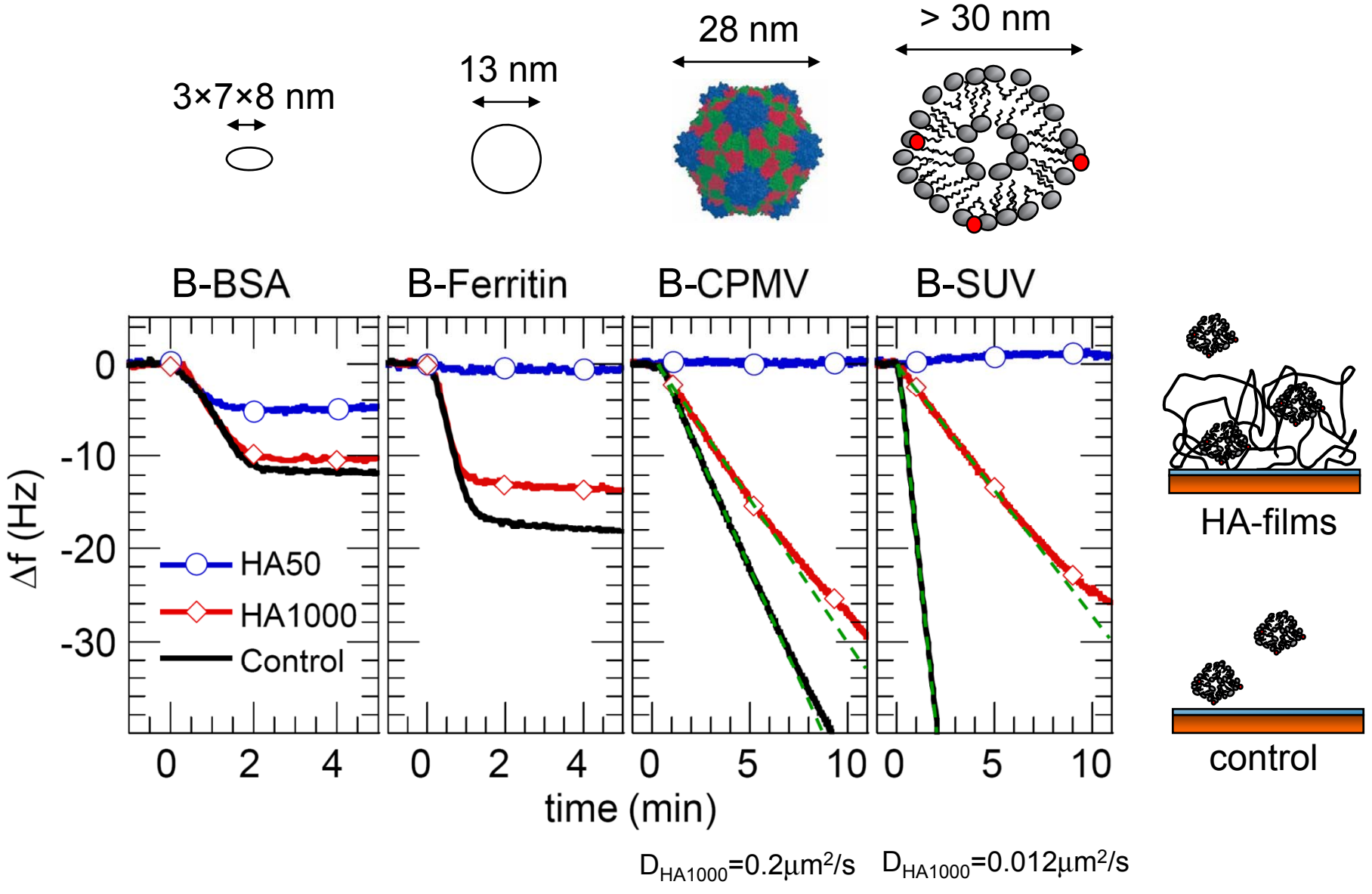
HA1000

L_c=2900nm

HA50

L_c=150nm

Probing Permeability



D_{HA1000}=0.2μm²/s D_{HA1000}=0.012μm²/s

Richter et al. (2007) *JACS* 127:5306

Conclusions

- We can create well-defined model systems of HA-rich cellular coats, based on supported lipid membranes.
- Surface biofunctionalization techniques allow for the controlled build-up and tuning of the model systems.
- A toolbox of surface-sensitive techniques provides detailed characterization.

Thanks to...

Collaborations

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Joachim Spatz (Heidelberg & Stuttgart, D)

Work in the Lab

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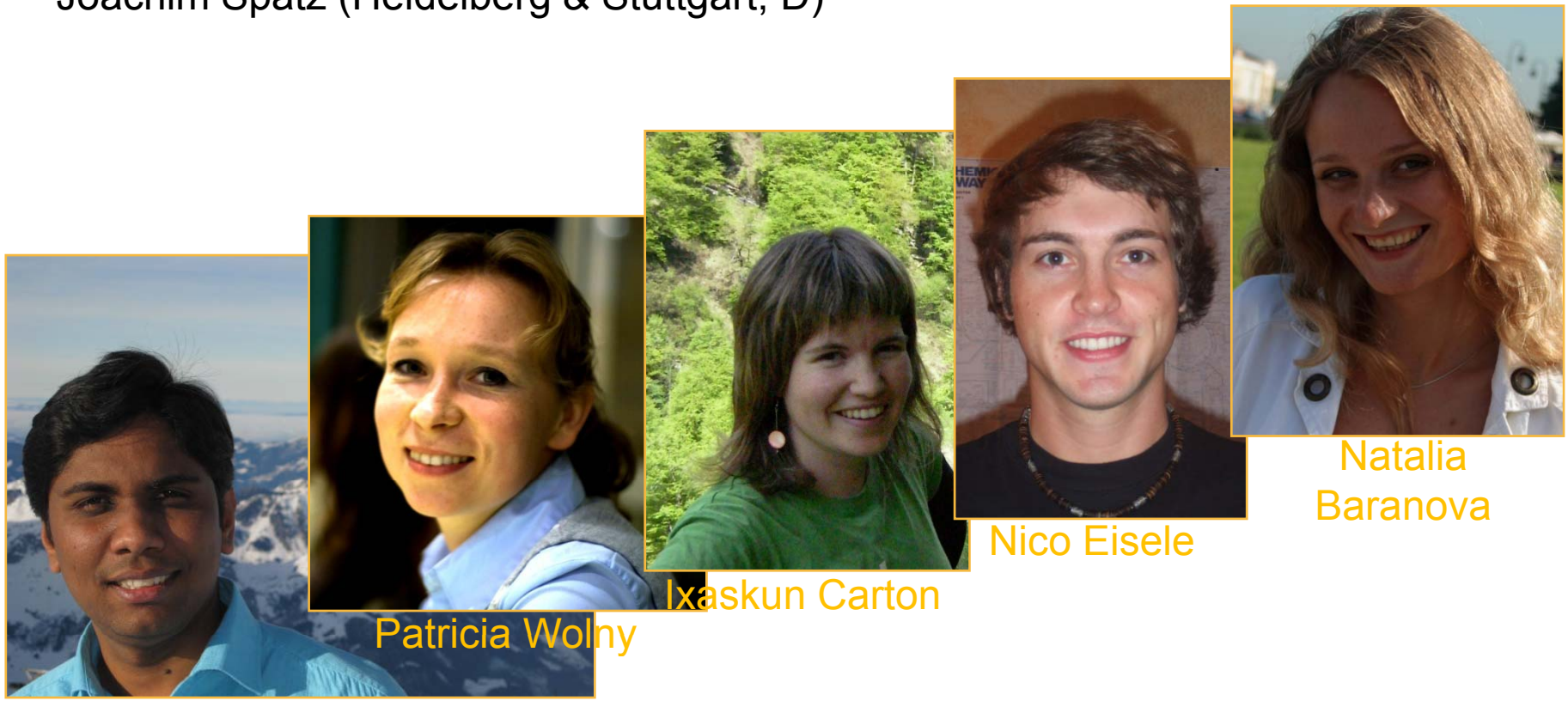
Nico Eisele
Jeffrey Burkhartsmeyer
Heike Böhm



Maybe, you are still wondering, what is the...

CIC = Cooperative Research Centre

¿ CIC biomaGUNE ?

bioma = Biomaterials

GUNE = Place (in Basque language)

We are :

- A newly established research institute in San Sebastian (Spain)
- Part of the BioBasque initiative to develop Biosciences in the Basque Country.
- Home to some 50 young and established researchers, students and postdocs working in bio- and nano-materials research.

Thanks to the generous funding by the local government, the institute boasts some of the best research facilities currently available in Europe.