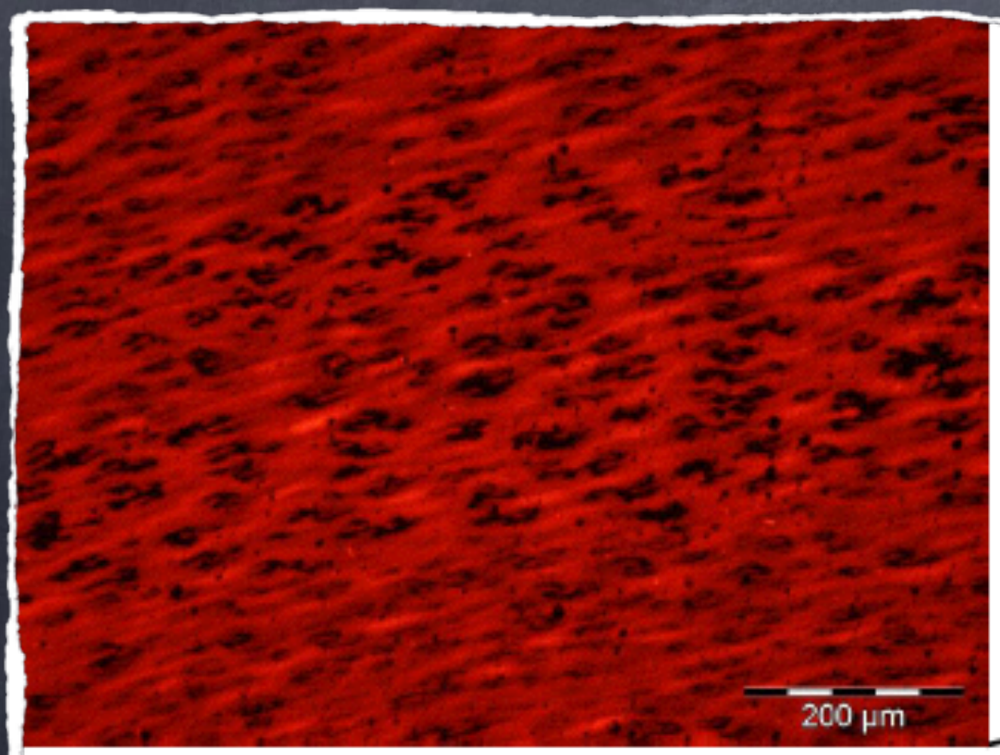
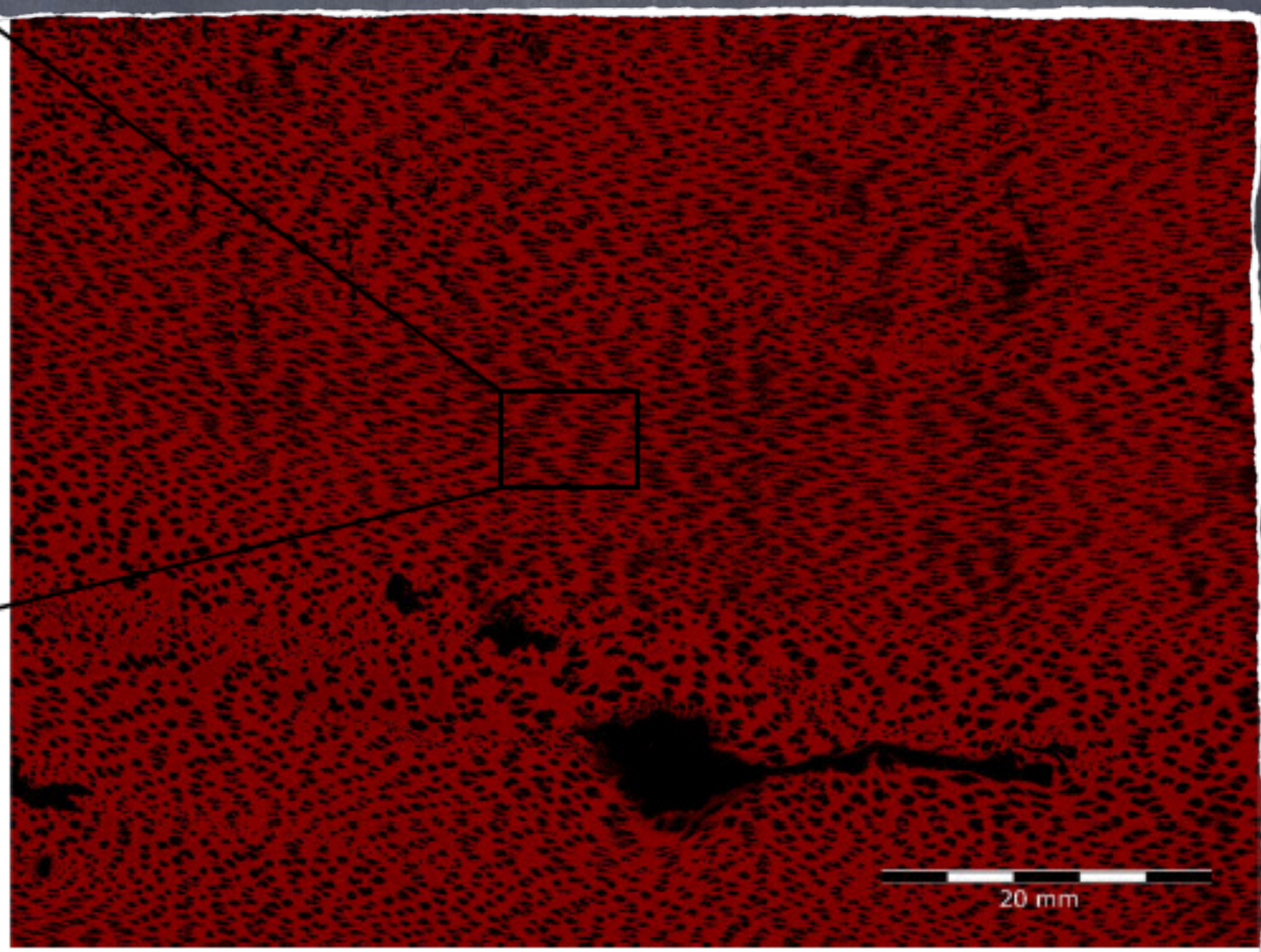
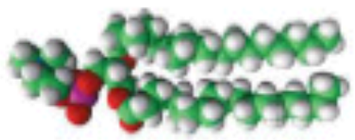
RESULTS



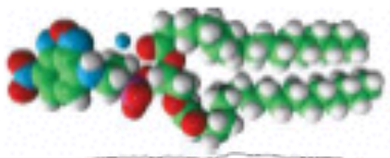


Supported Lipid Bilayer at RT



DSPC (C18)

+



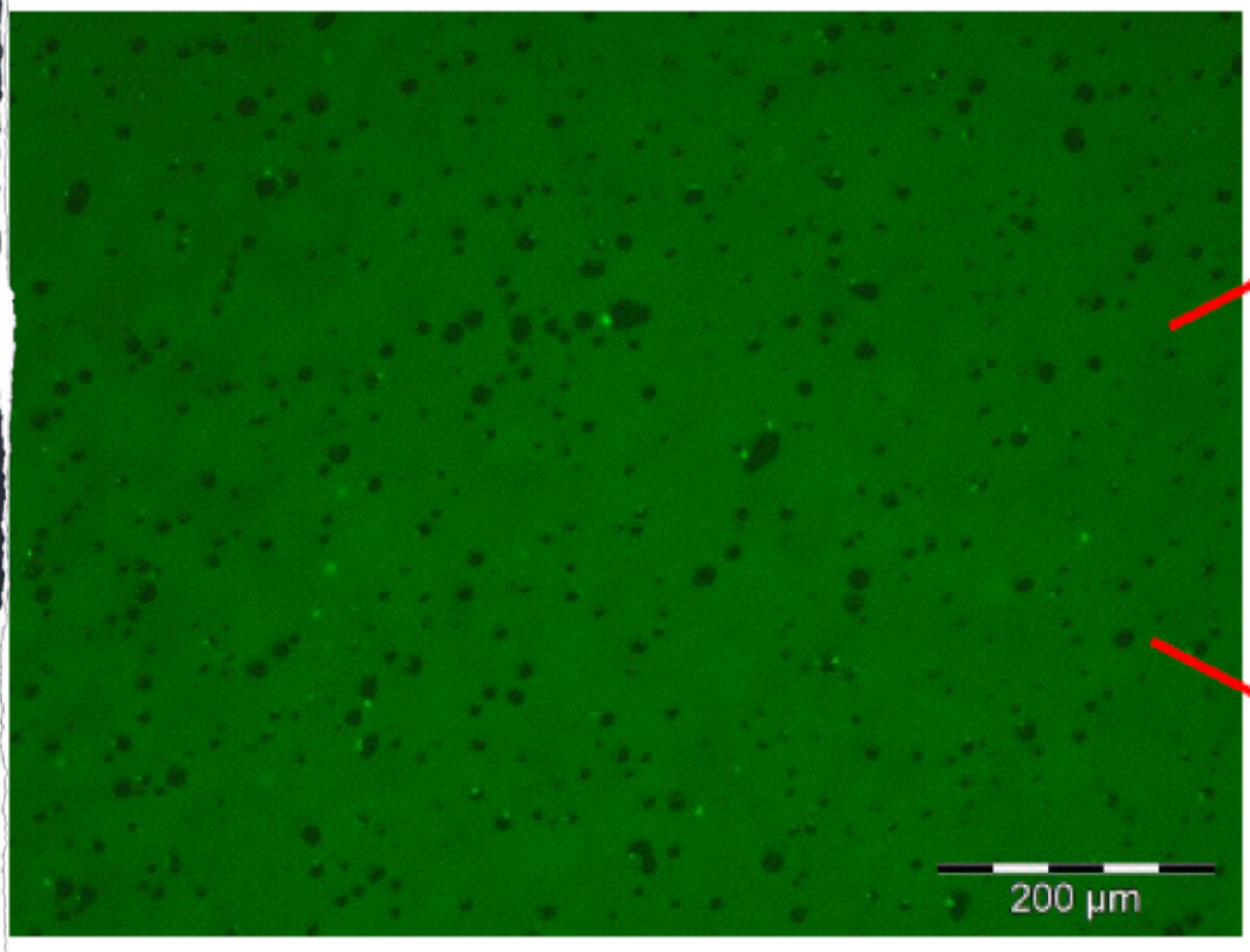
1%
DSPE (C18)
NBD at HG

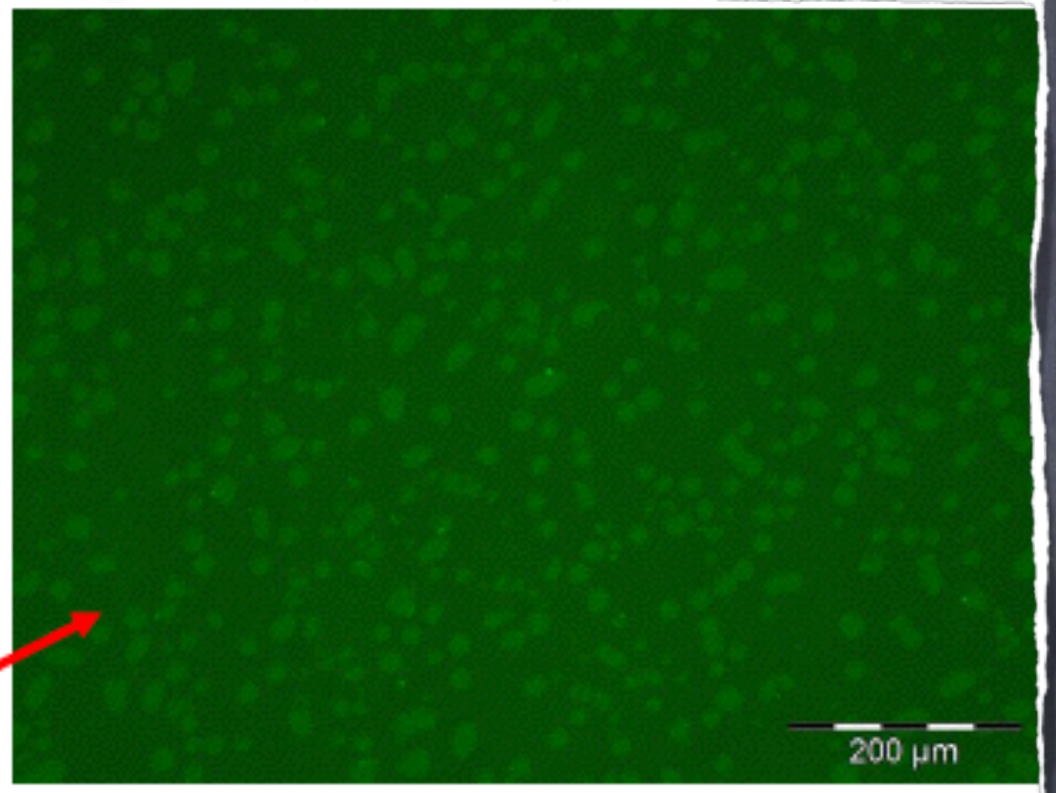
courtesy S. Wulle

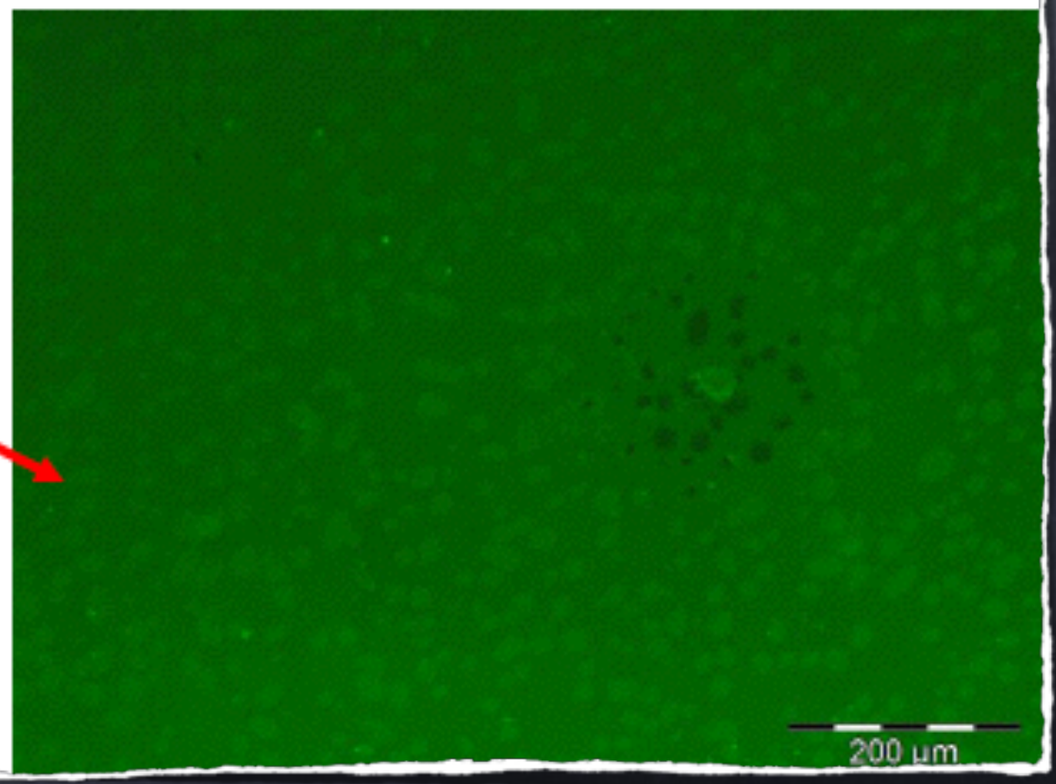
RESULTS

Next Step: Observe the sample in fluid phase

- First Measurement at RT
- Heat Sample for 2h at 57deg
- Go back to RT



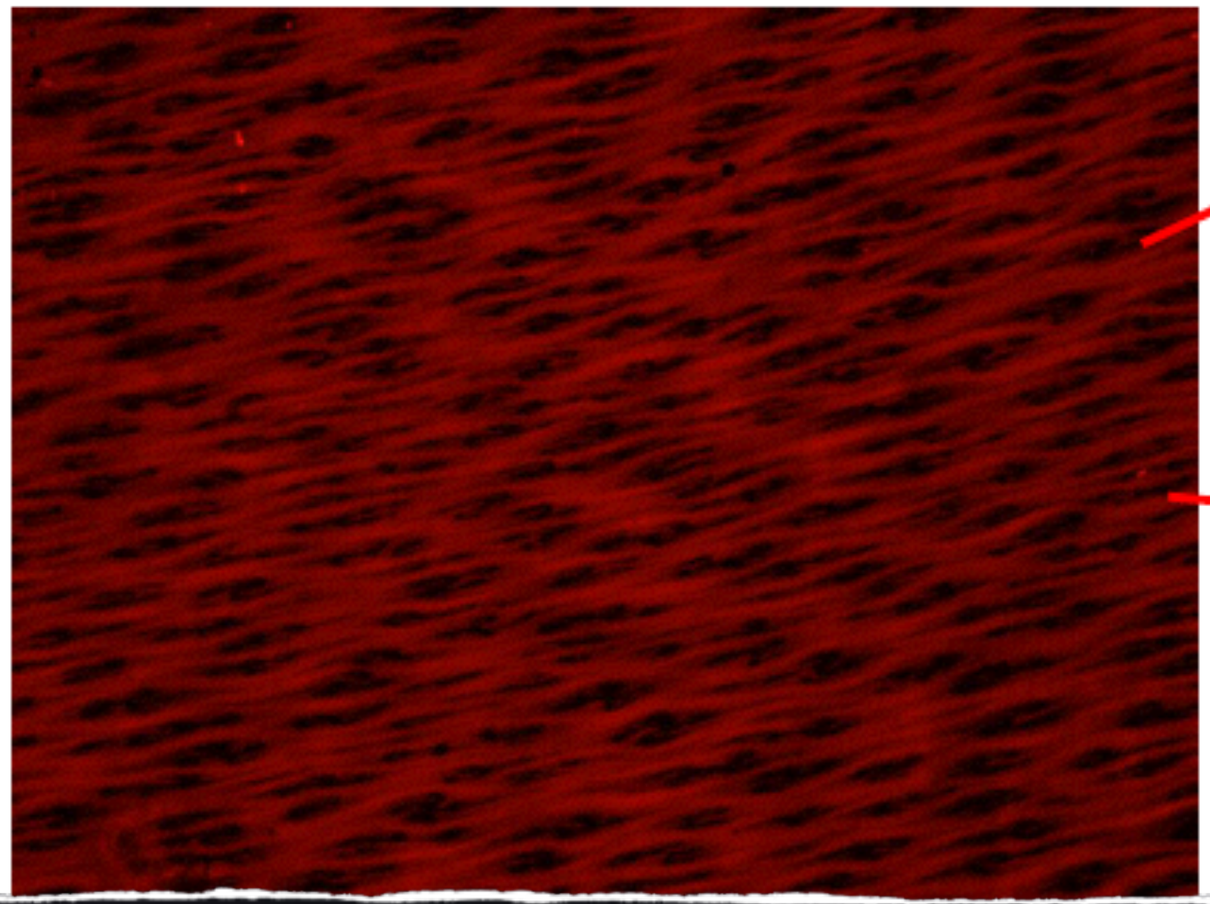


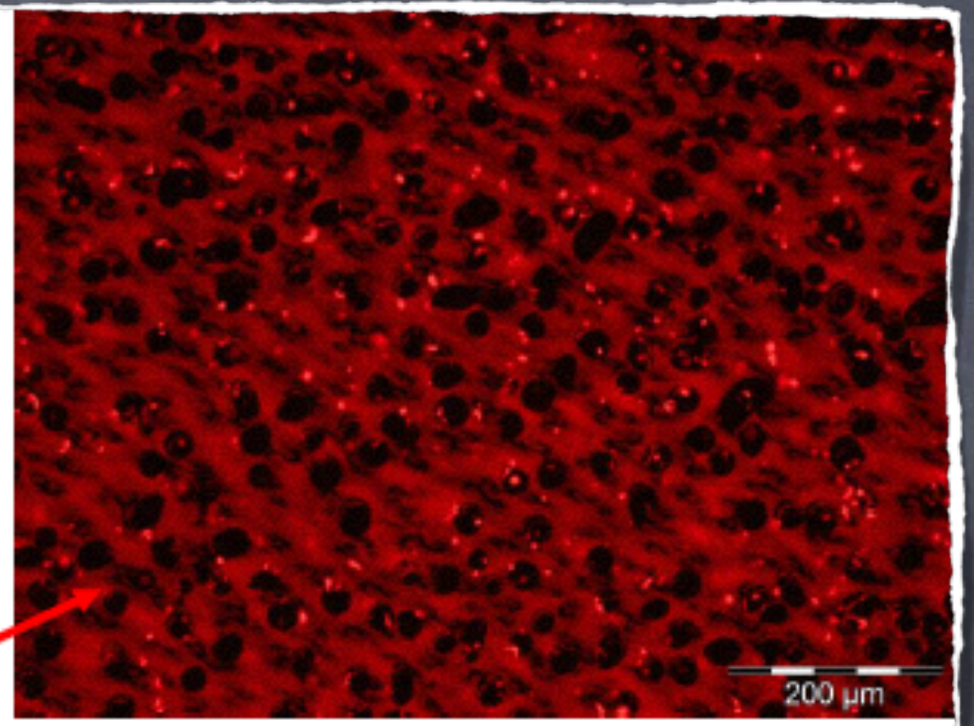


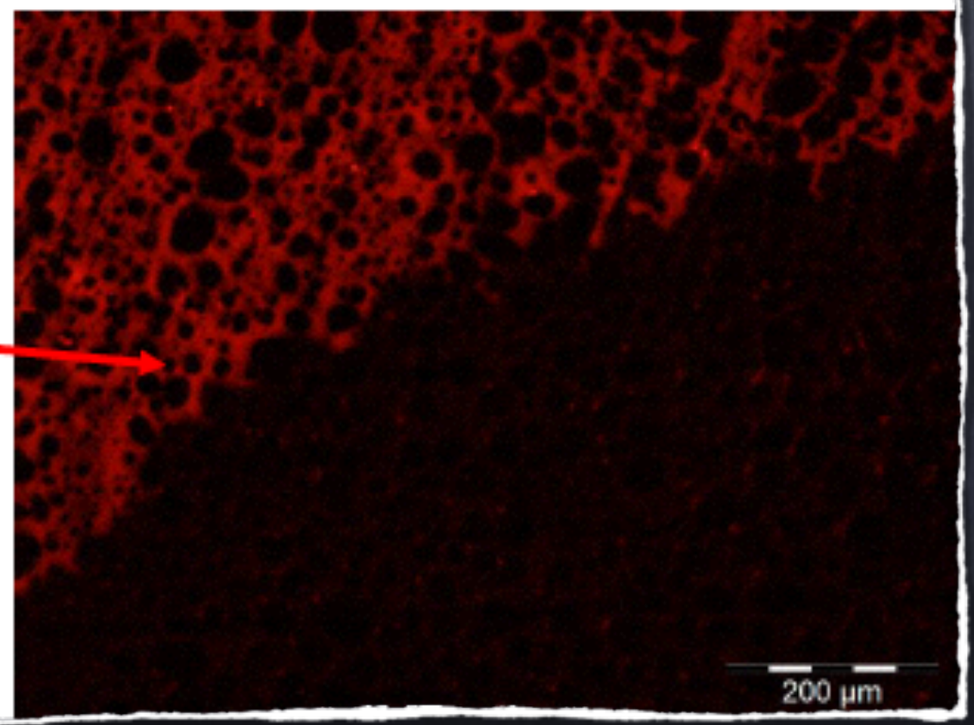
courtesy S. Wulle

RESULTS

- Next Step: Saturate the membrane with detergent
  - First Measurement at RT
  - Insert detergent via mf-pump
  - Heat Sample for 2h at 57deg
  - Rinse detergent
  - Go back to RT



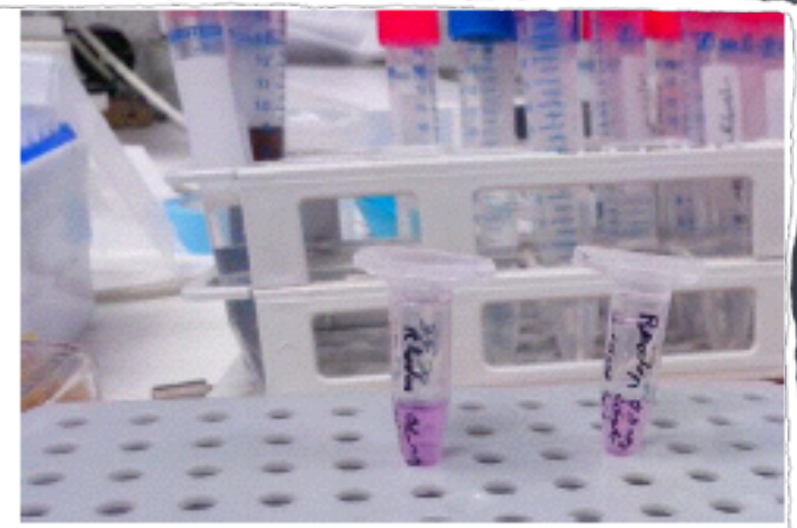


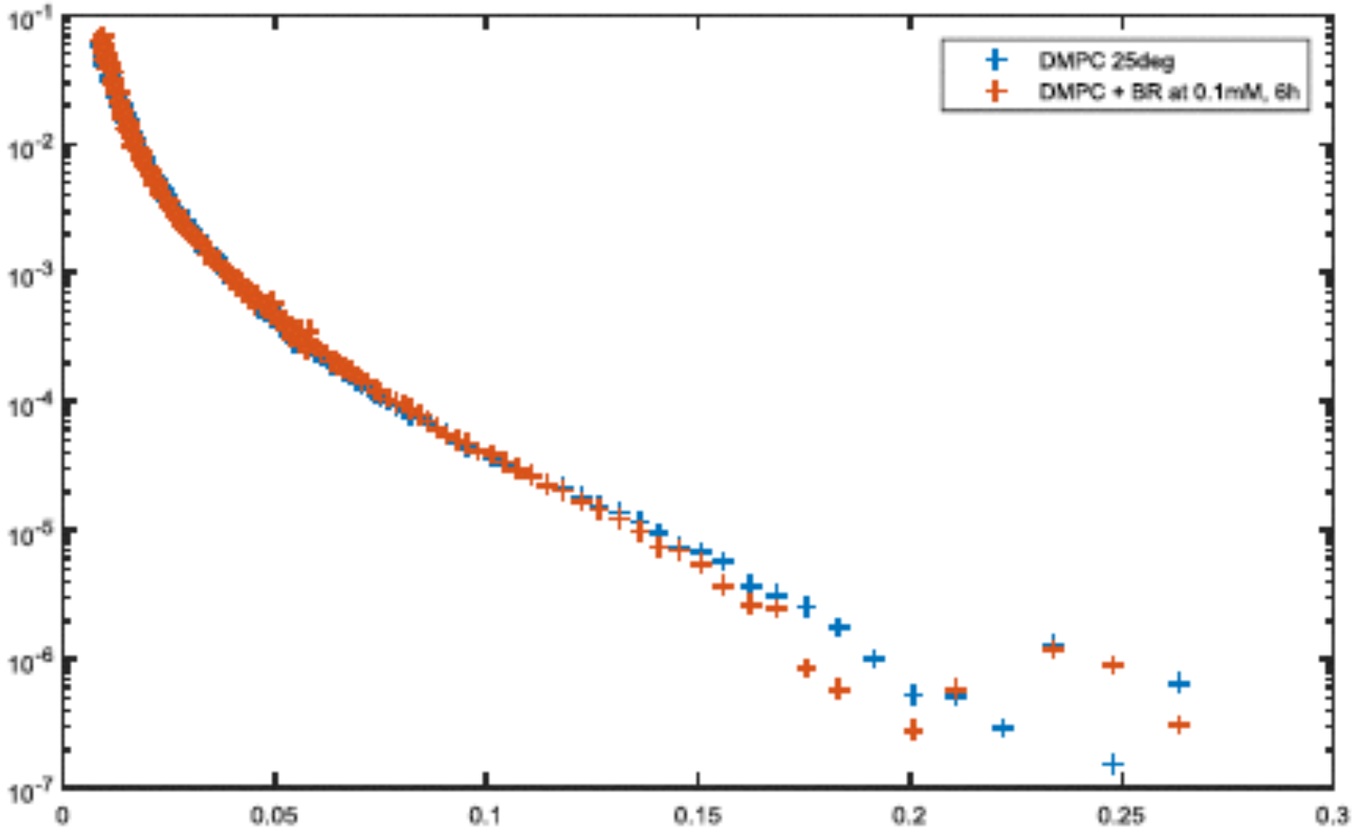


courtesy S. Wulle

RESULTS

- Recently Neutron Reflectivity Experiment at D17
  - Much more stable bilayers on silicon
  - Saturation with detergent and heating much easier
  - Folloews the complete Protein-Insertion procedure
  - Using deuterated DMPC Lipids and undeuterated Proteins
    - -> Insertion should be visible





- DMPC Sample, incubated for 6h with BR
- Indication of protein insertion
  - Changes in the high q
- Data treatment ongoing

courtesy S. Wulle

CONCLUSION OF WORK STILL IN PROGRESS...

- Sample System to observe lipid bilayers with fluorescence microscopy is developed
  - Available at the PSCM!

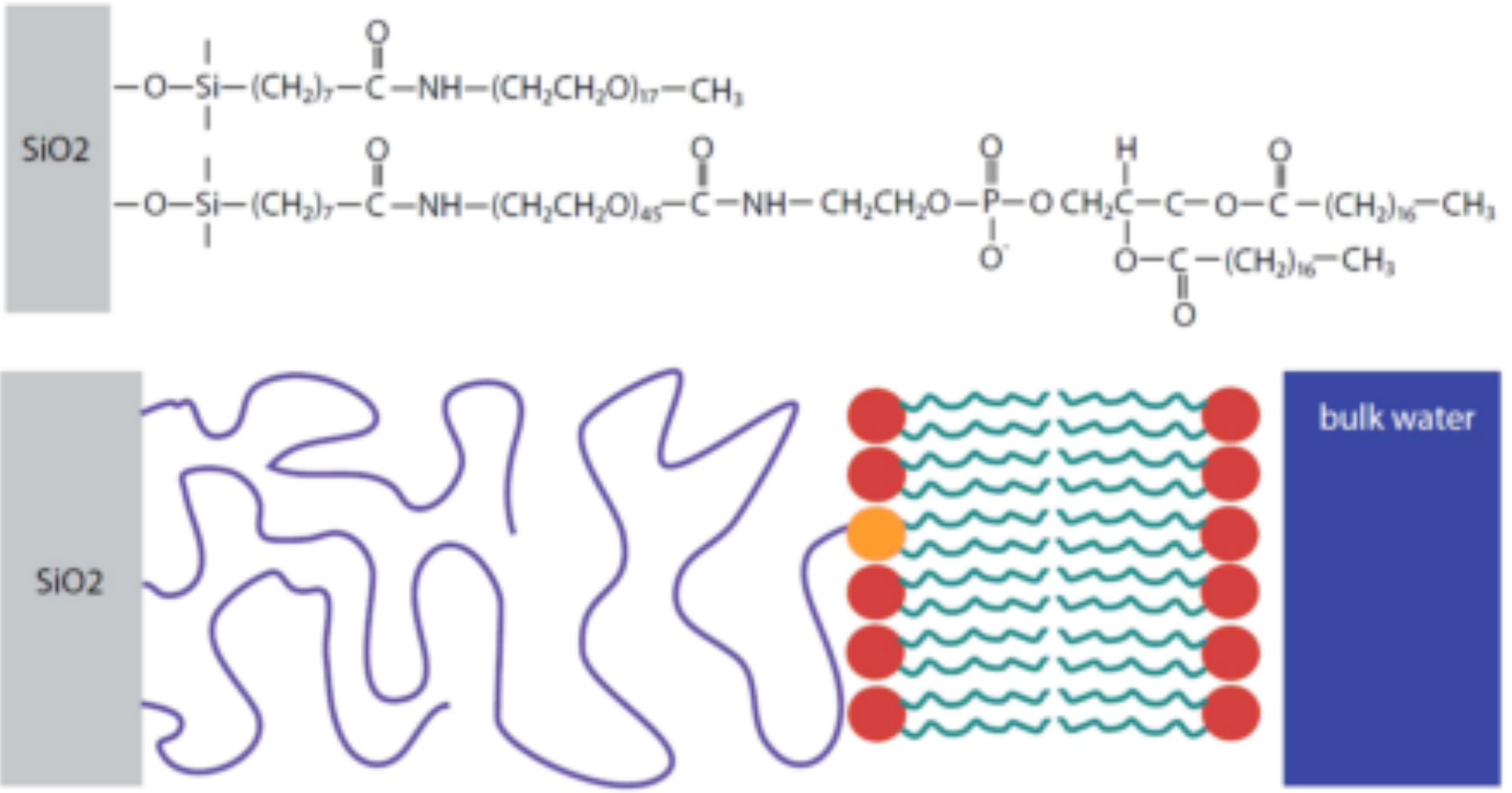
- Bilayer coverage and stability seems to be inferior compared to silicon
  - Accomplishing succesfull protein insertion quite difficult
  - Optimization: Another substrate (polished quartz)

- Neutron reflectivity experiment
  - Indications of succesfull protein insertions could be seen

courtesy S. Wulle

Polymer-tethered membranes

Collaboration with AG Nickel from Munich: Polymer-tehered spin-coated membranes



Hertich et al.,
Langmuir 2014, 30, 9442-944

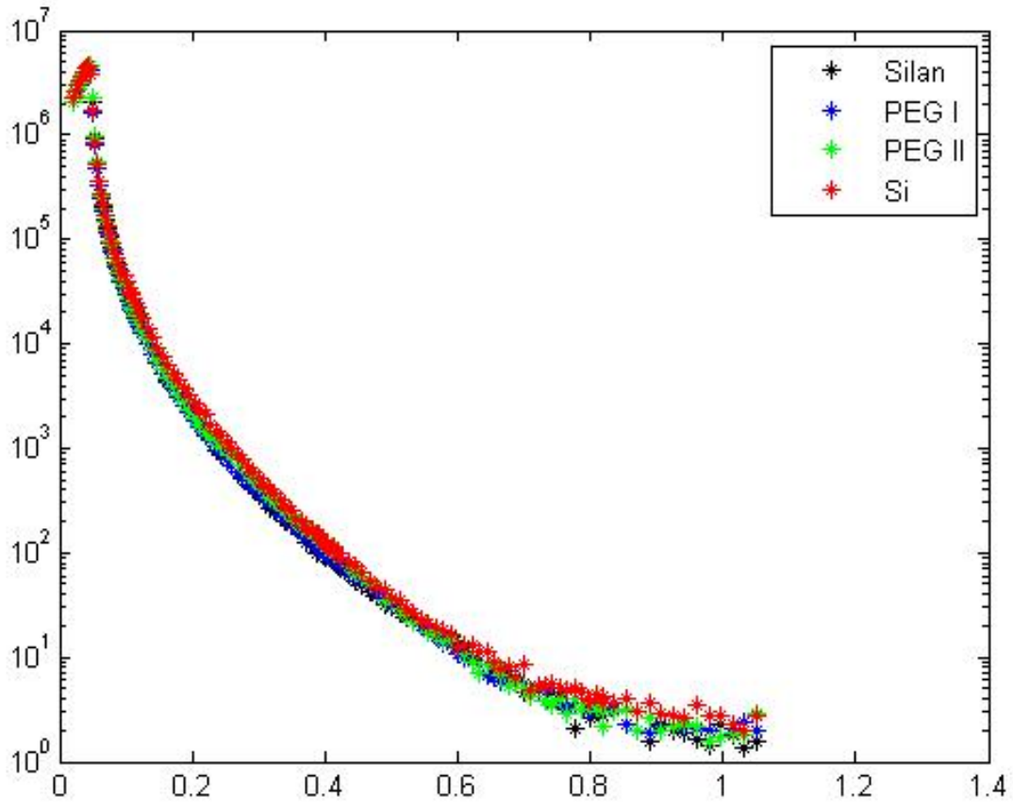
Irena Kiesel

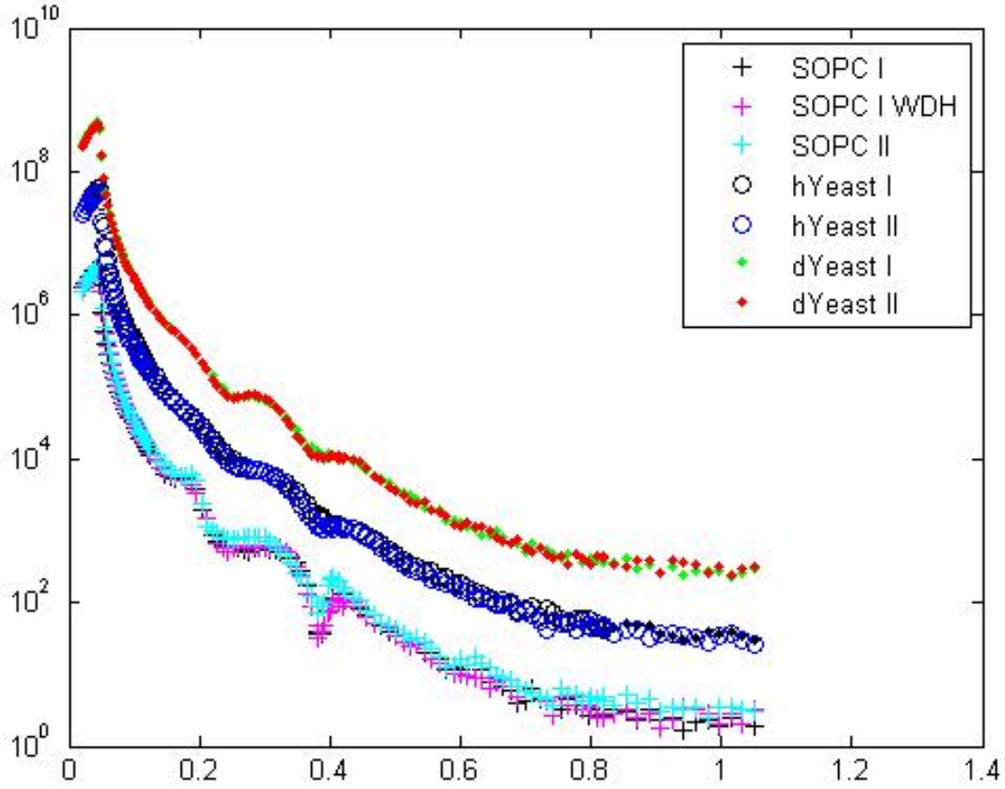
- Short and long polymer brushes (PEG) as tether for membranes, chemically grafted on silicon
- Deuterated and natural lipids extracted from yeast (de Ghellinck, current project PhD Robin Delhom) to mimic natural membranes

Advantage: - Fast easy sample preparation with spin-coating or solvent exchange
- Reusable substrates

courtesy I. Kiesel

Polymer-tethered membranes - XRR





Substrates in water

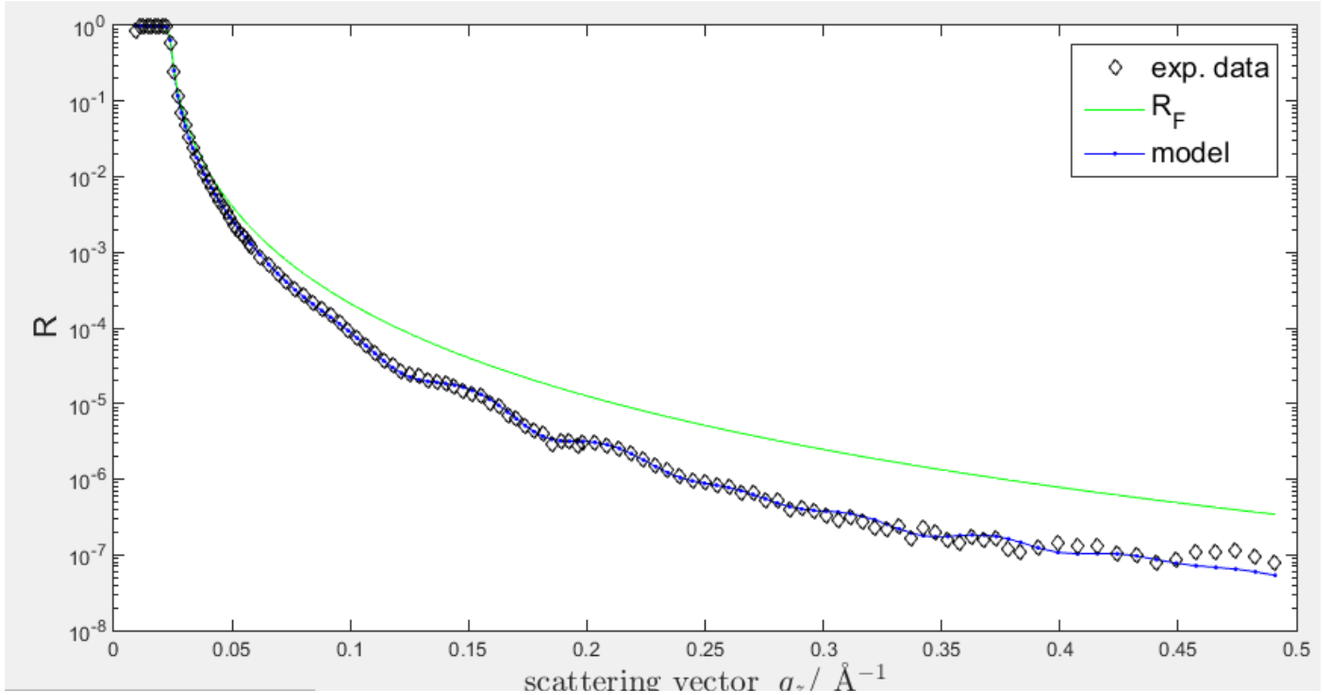
- Clean silicon (red)
- With Octenyltrichlorosilan (black)
  → Nearly not visible in contrast to OTS (hydrophobic gap?)
- Two substrates with PEG brushes (I blue & II green)
  → PEG brushes not visible in water
  → same electron density

Spin-coated lipids on PEG brushes substrate I and II

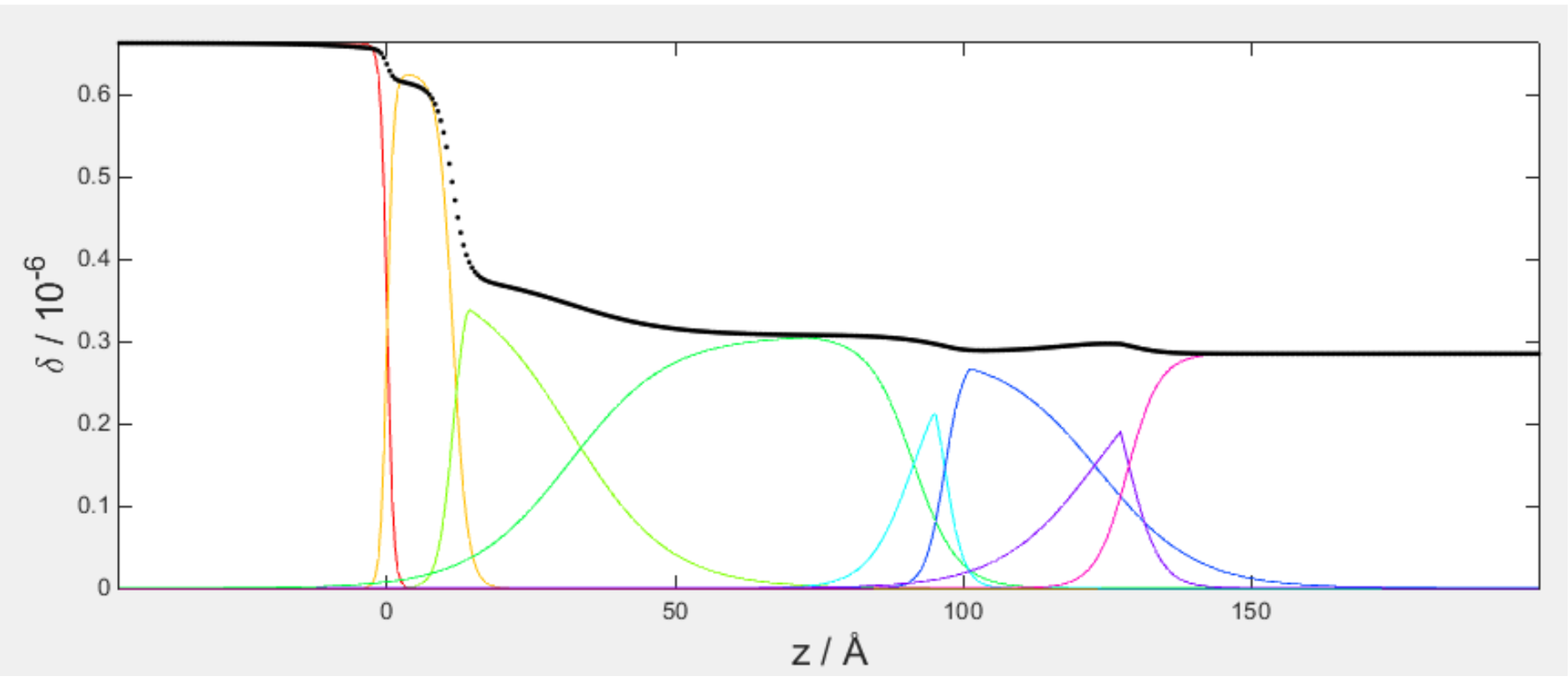
- cleaned with chloroform and methanol in between)
- Reproducible with the same lipid
- With SOPC: stronger features → more ordered (?) than natural mixture of lipids
- H-Yeast and D-Yeast similar

courtesy I. Kiesel

Polymer-tethered membranes - XRR



- H-Yeast on PEG brushes
- PEG brushes denser at the interface
- Low contrast between PEG and lipids
- Interface still a bit too rough to distinguish good between head- and tail-groups

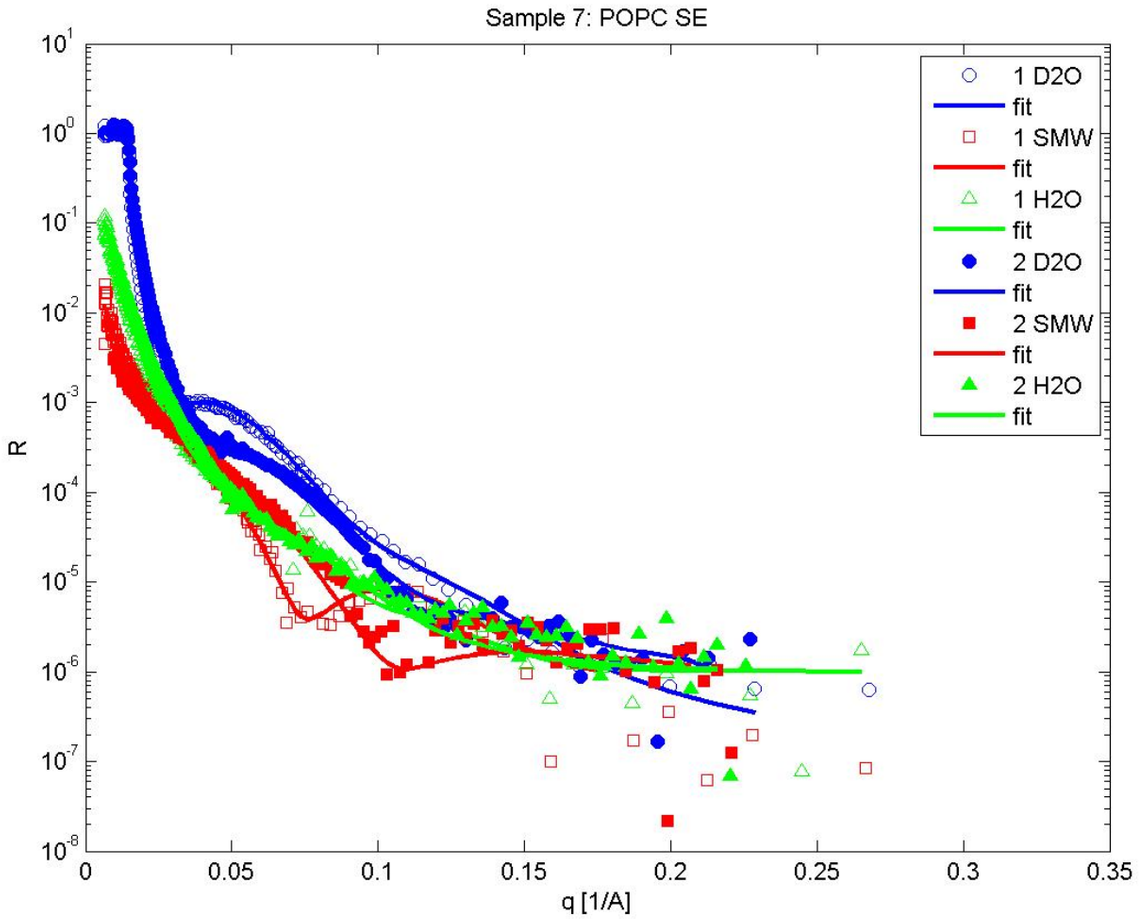


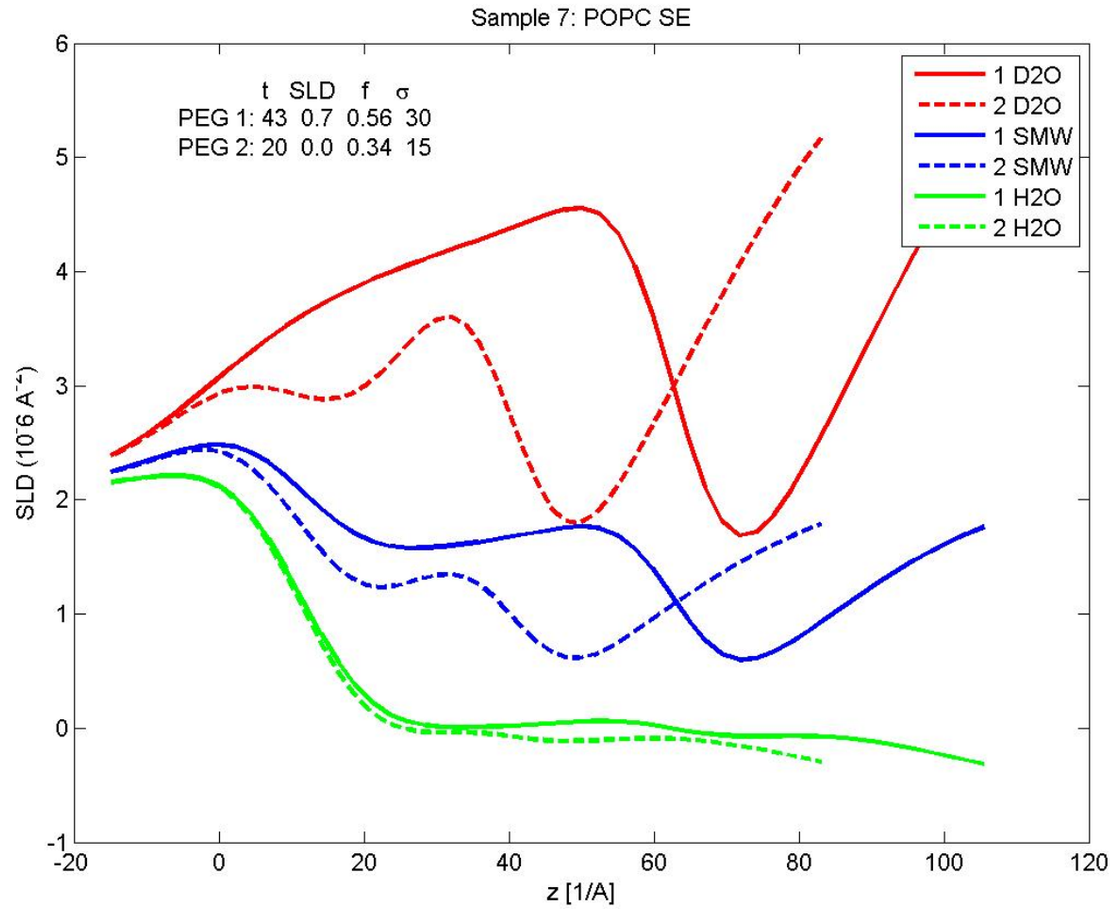
Si SiO2 PEG Lipid-Bilayer Water

courtesy I. Kiesel

Polymer-tethered membranes - NR

Test of the reproducibility with different deposition methods: solvent exchange, spin coating, vesicle fusion, LB-LS





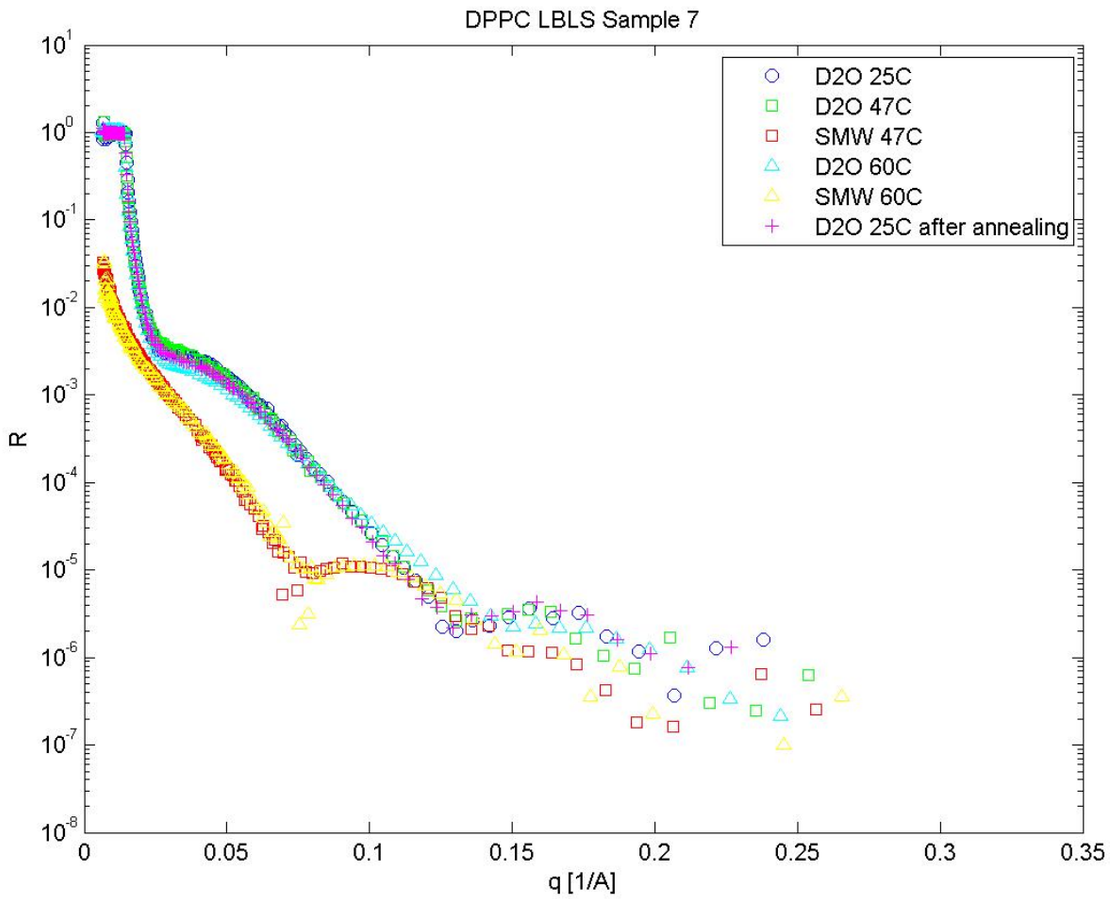
- Same substrates at different experimental times
- Solvent exchange (after cleaning with methanol of a spin-coated lipid layer, measured already a couple of hours in water)
- Reflectivities not similar!
- But: The lipid bilayer is the same, only the PEG layer changes

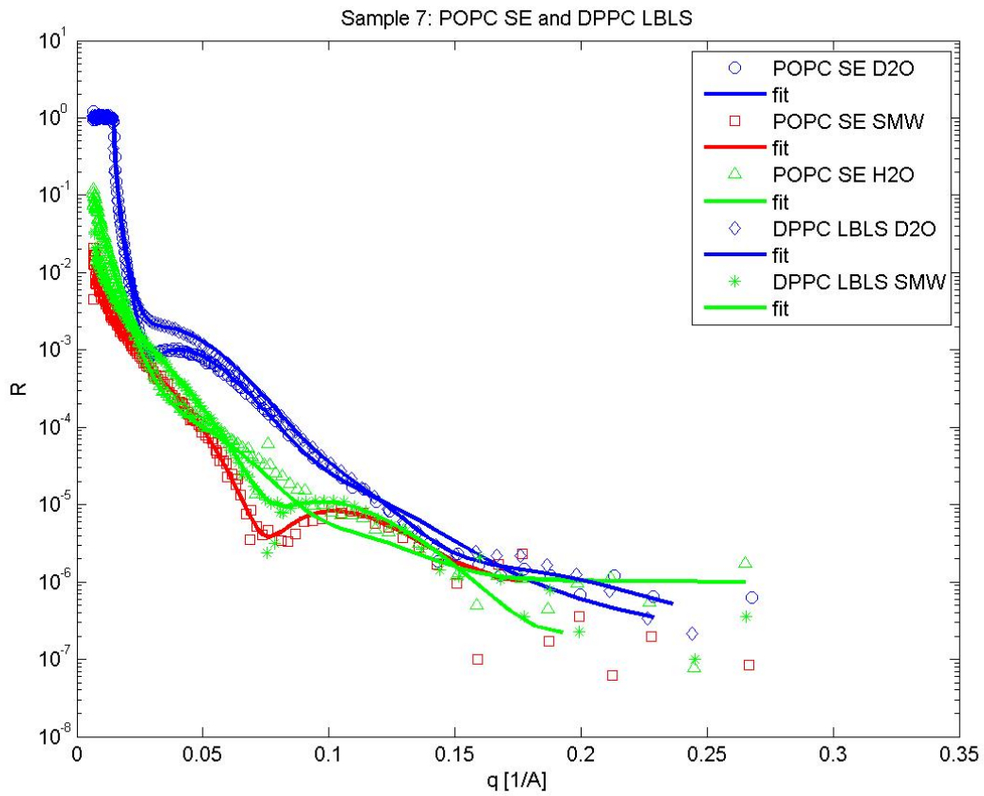
  → Does it need a long time to swell in water?

  → Current tests with ellipsometry and long measurement at FIGARO at the end of this week

courtesy I. Kiesel

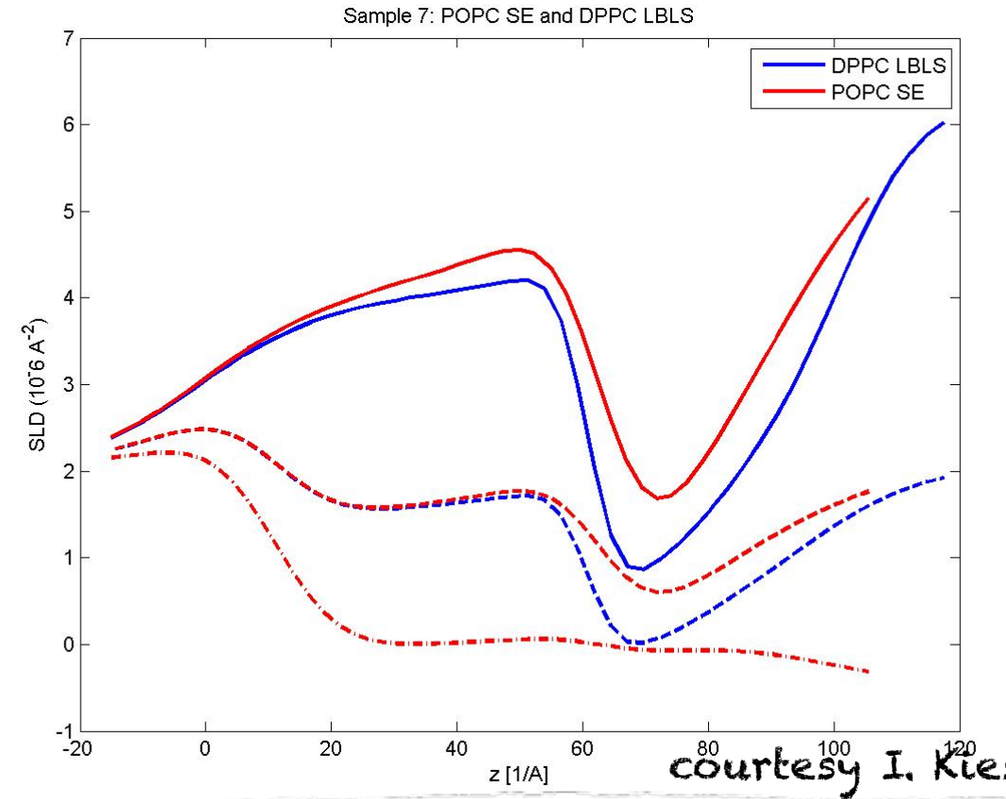
Polymer-tethered membranes - NR





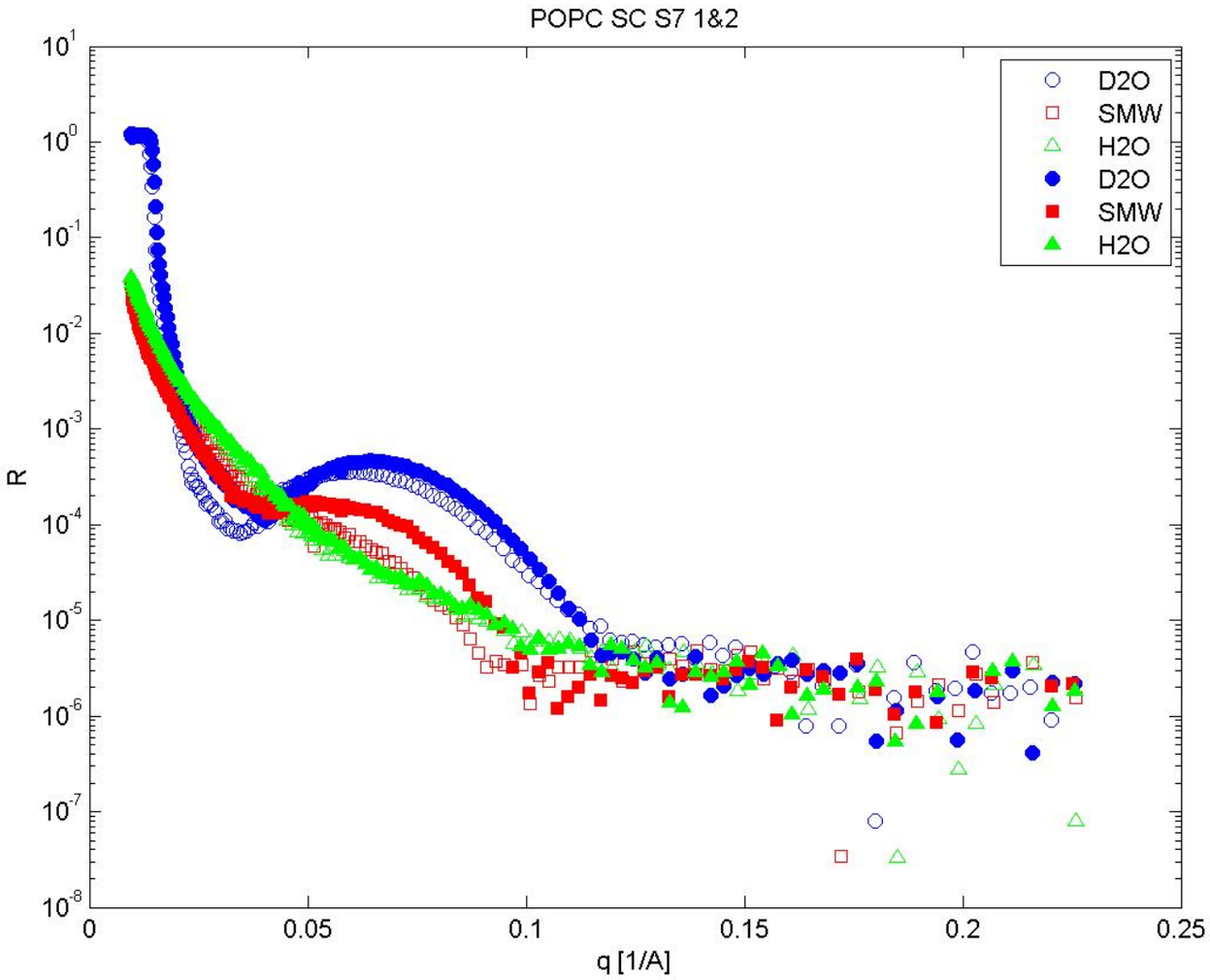
DPPC with LB-LS on same PEG substrate

- Heating to different temperatures for annealing
  → Nearly no change in reflectivity
- Comparison LB-LS/solvent exchange
  → Comparable roughness and thickness, slightly different density (different lipids, coverage?)

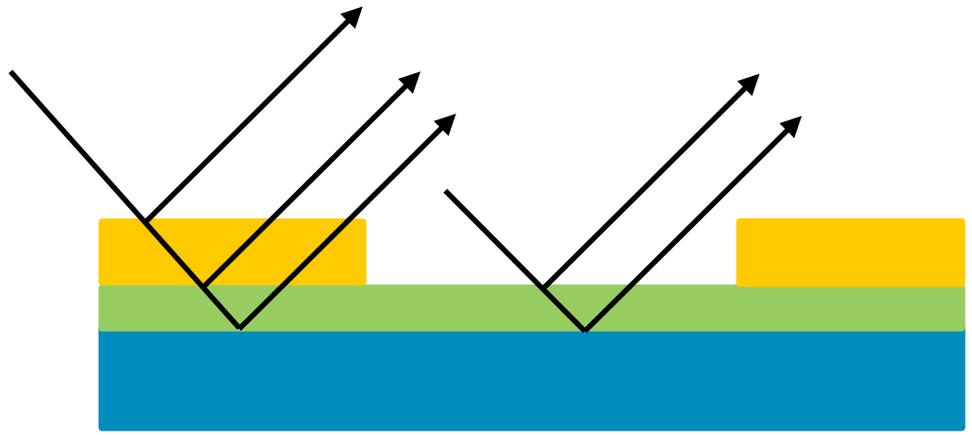


courtesy I. Kiesel

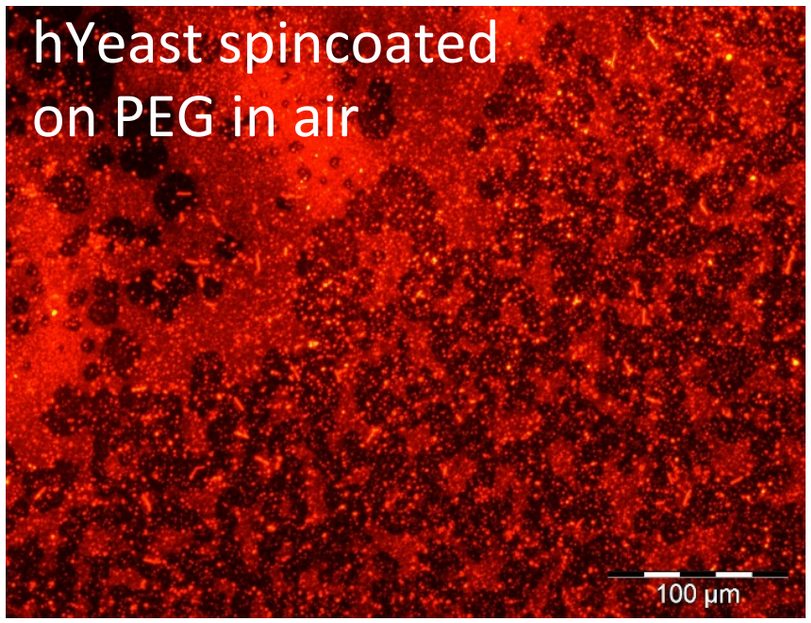
Polymer-tethered membranes - NR

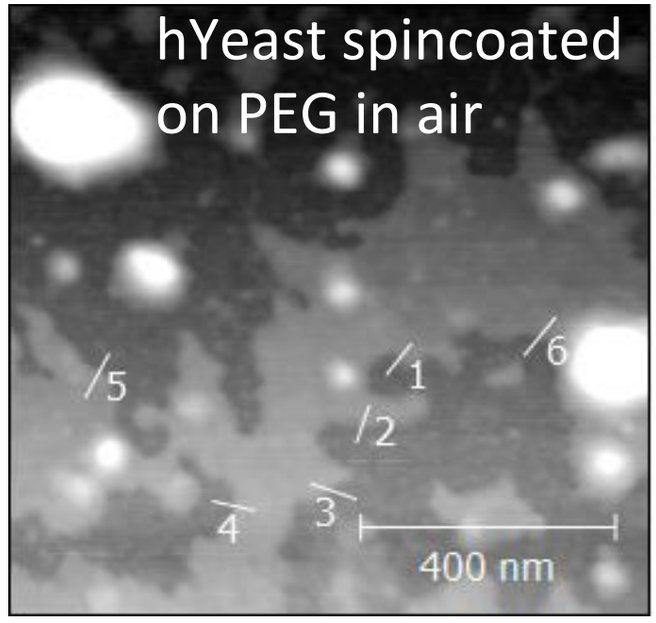


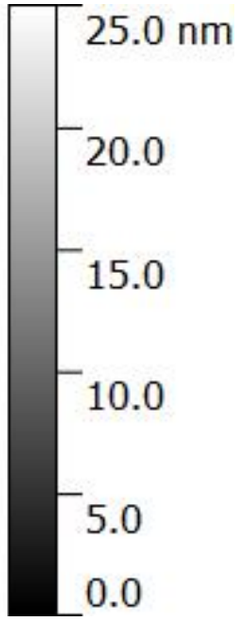
Repeated spin-coated POPC on PEG
Broad minima: Difficult to fit → Patches?

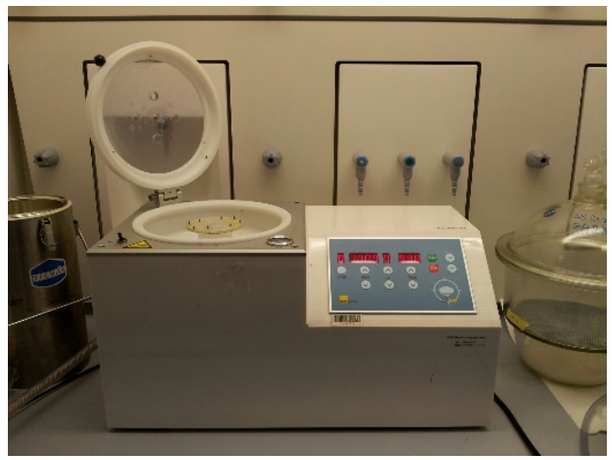


- Check with fluorescence microscopy and AFM
- Optimization of spincoating parameters









Spin Coater Delta6 SUSS MicroTec

courtesy I. Kiesel

Conclusion

- Possibility to create stable lipid bilayer with different techniques on PEG brushes (vesicle, spin-coating, solvent exchange, LB-LS)

- Reproducibility still a problem, maybe due to swelling time after hydration
  - No problem with XRR

  → Smaller samples

  → lower sensitivity?

  → luckily measured at the same time after preparation and hydration?

- Optimizing of spin-coating parameters

- Solvent exchange seems even better than spin-coating

  → cleaning and preparing directly at the instrument possible

  → positively tested with natural lipids as well