ID	task	Deliverable/Milestone	Task Name	1st Quarter Jan	Apr	3rd Quarter Jul	Oct	1st Quarter Jan	Apr	3rd Quarter Jul	Oct	1st Quarter Jan	Apr	3rd Quarter Jul	Oct	1st Quarter Jan	Apr	3rd Quarter Jul	Oct
1	WP20		**Advanced Neutron Tools for Soft- and Bio-Materials**																
2	Task 20.0		JRA management activity																
3	Task 20.1		**A platform for model biological membranes**																
4		M 20.1.1	Optimization of model bilayer systems including natural membrane lipids													◆ 22/01			
5		M 20.1.2	Set up a lipid extraction facility to extract and fractionate membranes from real cells			◆ 12/07													
6		D 20.1.3	Tests and methodologies to perform NR, GISANS and spin echo measurements on membranes																◆
7		D 20.1.4	Protocols for reliable reconstitution of membrane proteins																◆
8		D 20.1.5	D lipids extraction. Modeling of biomembranes																◆

Report of recent ILL activities on WP20 carried out within the Partnership for Soft Condensed Matter initiative

NEUTRONS FOR SCIENCE

PSCM
PARTNERSHIP FOR SOFT CONDENSED MATTER

Giovanna Fragneto, Yuri Gerelli, Irena Kiesel, Robin Delhom, Simon Wulle

| ID	task	Deliverable/ Milestone	Task Name	1st Quarter Jan	Apr	3rd Quarter Jul	Oct	1st Quarter Jan	Apr	3rd Quarter Jul	Oct	1st Quarter Jan	Apr	3rd Quarter Jul	Oct	1st Quarter Jan	Apr	3rd Quarter Jul	Oct
1	WP20		**Advanced Neutron Tools for Soft- and Bio-Materials**																
2	Task 20.0		JRA management activity																
3	Task 20.1		**A platform for model biological membranes**																
4		M 20.1.1	Optimization of model bilayer systems including natural membrane lipids													◆ 22/01			
5		M 20.1.2	Set up a lipid extraction facility to extract and fractionate membranes from real cells							◆ 12/07									
6		D 20.1.3	Tests and methodologies to perform NR, GISANS and spin echo measurements on membranes																◆
7		D 20.1.4	Protocols for reliable reconstitution of membrane proteins																◆
8		D 20.1.5	D lipids extraction. Modeling of biomembranes																◆

A platform for sample preparation and pre-characterization at the service of neutrons and synchrotron radiation

Two wet chemistry labs; a clean room; laboratories for
light scattering, UV & FTIR spectroscopies, rheometry, ellipsometry,
BAM, tensiometer, DSC, z-sizer, microscopes, deuterated Lipid
Extraction/Characterisation, rheometers, meeting space
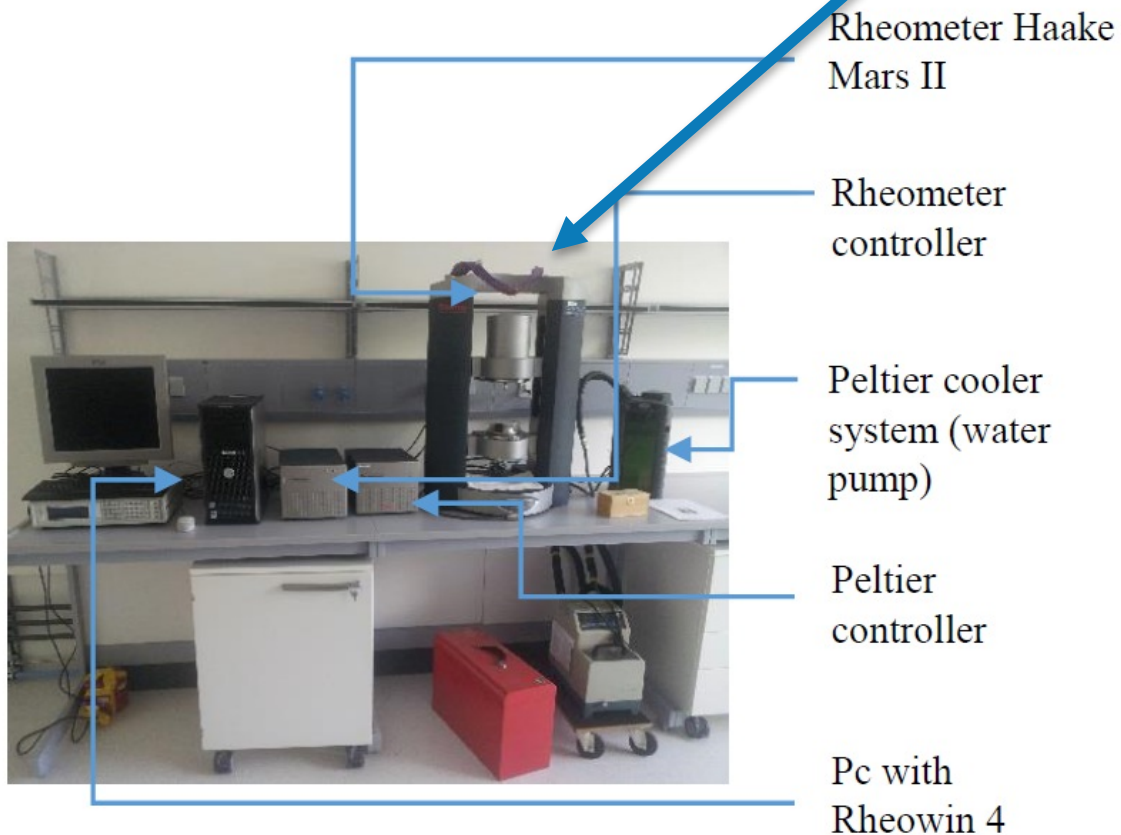AVAILABLE TO ALL ILL USERS

www.epn-campus.eu/users/pscm/
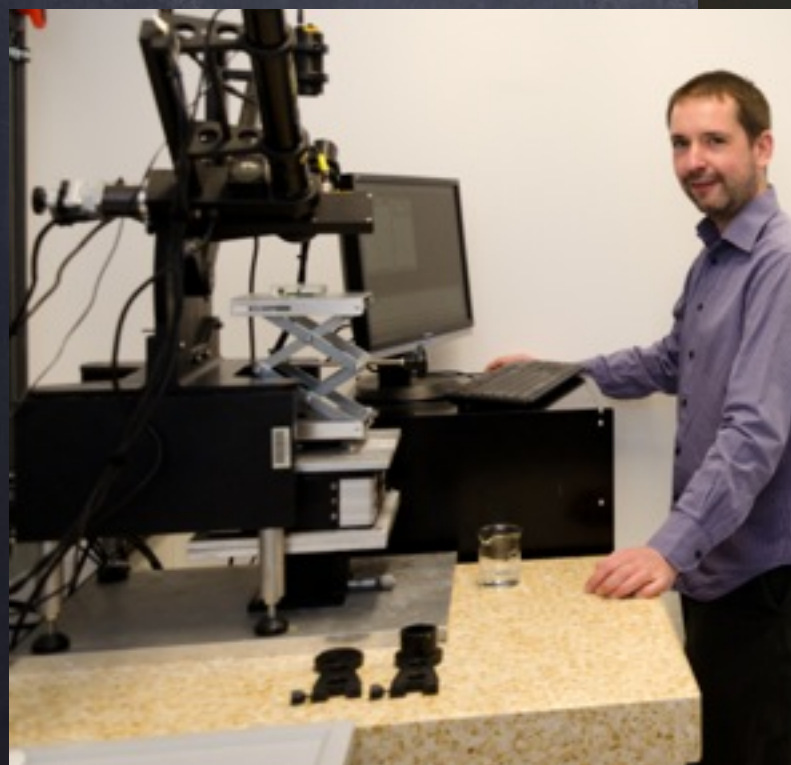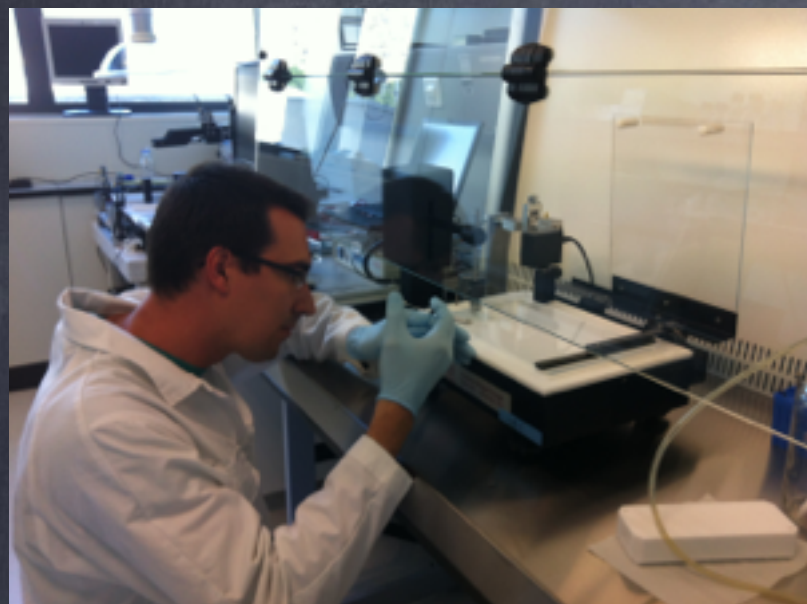
Microscope BX61 @ SB230
(Repaired & upgraded in 2012-2013)

Rheometer @ SB224
(Repaired & upgraded in 2013-2014)

Trough+BAM @ SB230
(Refurbishment planned in 2015)

Rheometer Haake Mars II

Rheometer controller

Peltier cooler system (water pump)

Peltier controller

Pc with Rheowin 4

courtesy D. Pontoni

- Lipid Membranes are known to exhibit thermal fluctuations

- Close to equilibrium conditions are widely investigated
  - GUV's (Video Microscopy)
  - Floating Bilayer (Neutron / Off-specular synchrotron reflectivity)

[1]
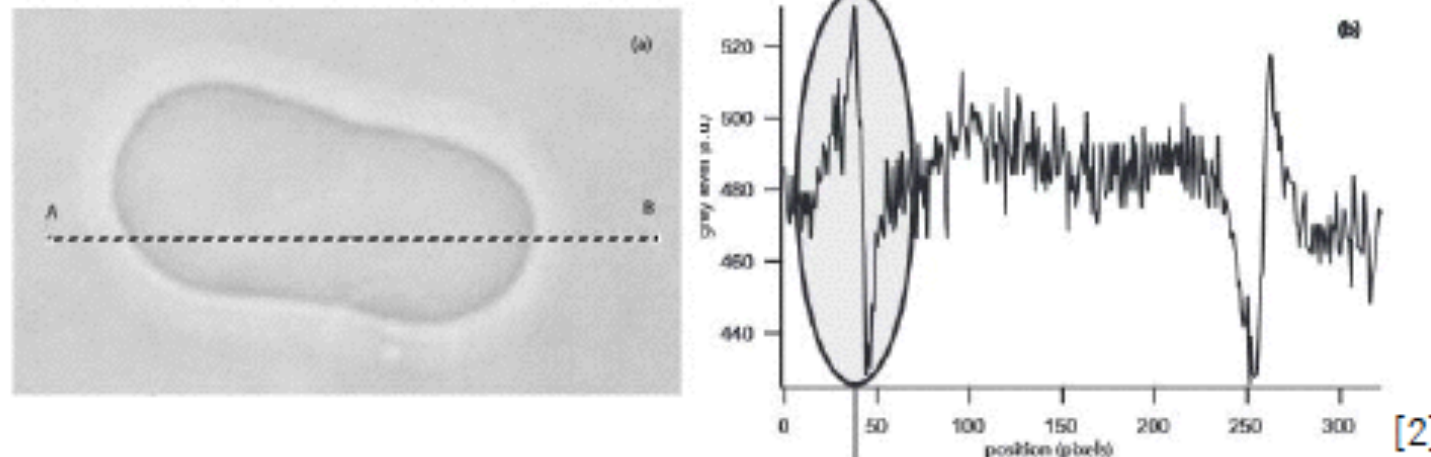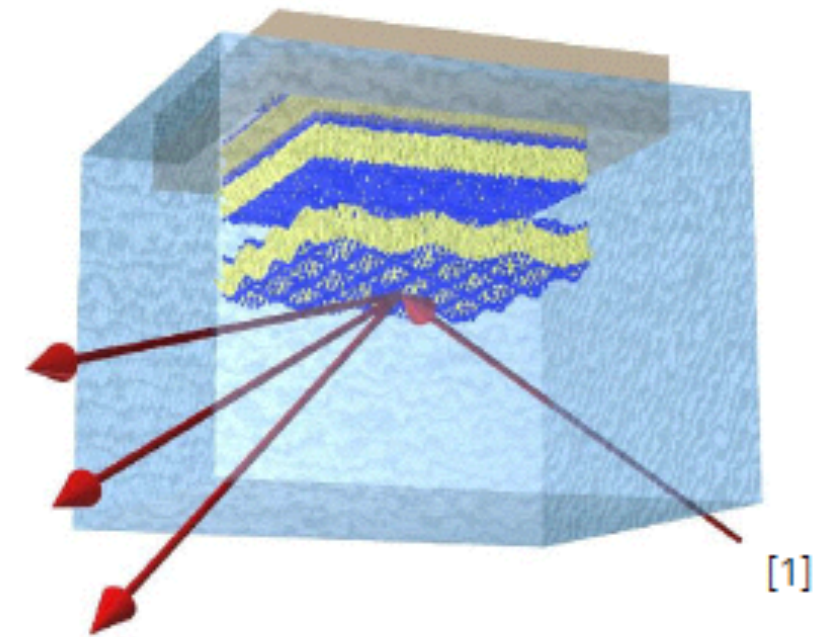
- In Living Systems
  - Protein Activity breaks the fluctuation-dissipation theorem

- <u>Out-of-equilibrium fluctuations</u>

[2]

- Influence of transmembrane Proteins on membranes interesting for advanced drug development

[1] T. Charitat et al., *Eur. Phys. J. B*, 8, 583-593 (1999) [2] J. Pecreaux et al., *Eur. Phys. J. E*, 13, 277-290 (2004)

2

courtesy S. Wulle

- Integrating active Proteins into model membrane
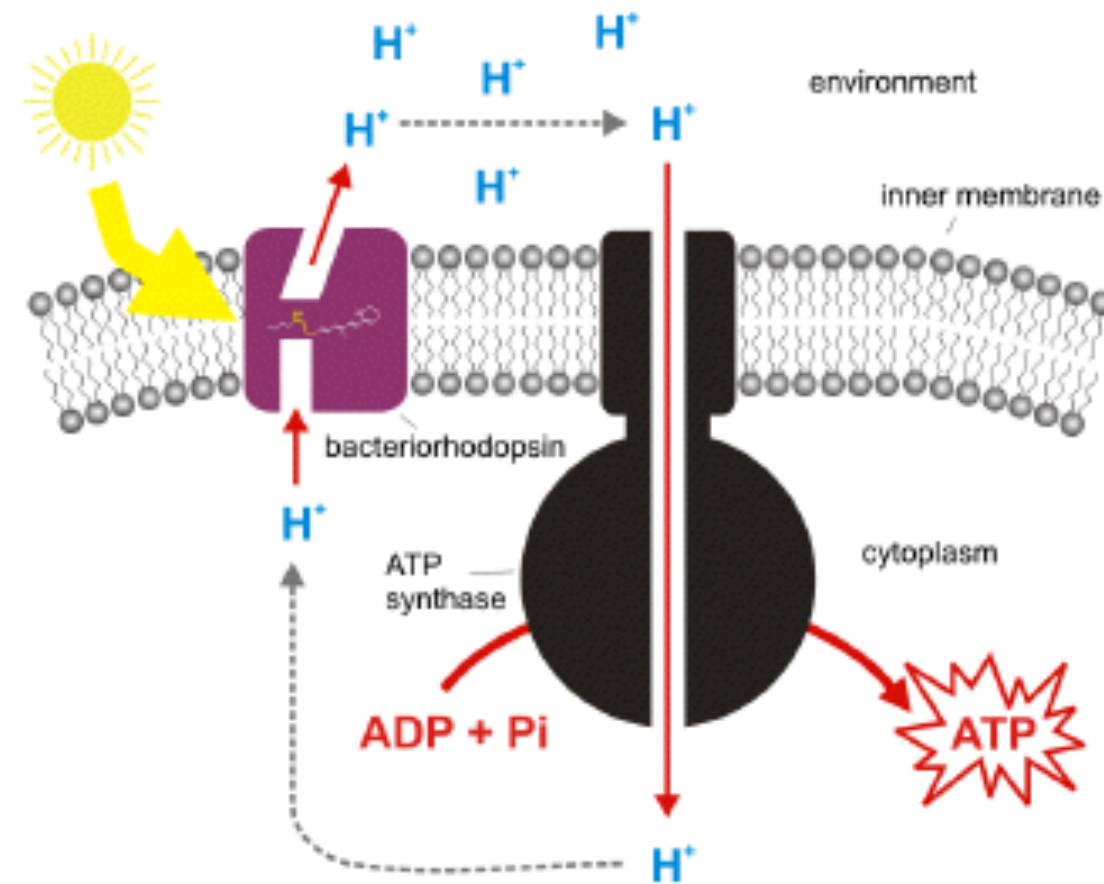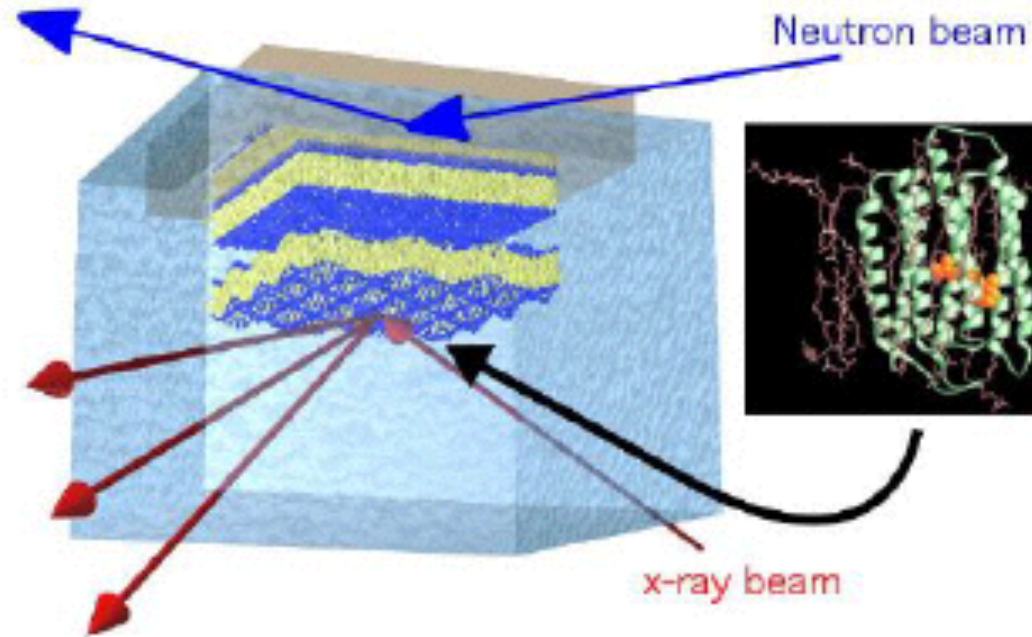
- Bacteriorhodopsin
  - Active Proton Pump
  - Light activated -> Activity easy switchable

- Study the changing fluctuation spectrum using Neutron- and Off-specular X-Ray reflectivity

courtesy S. Wulle

- Integration and purification of TP's into lipid layers is complex and difficult task

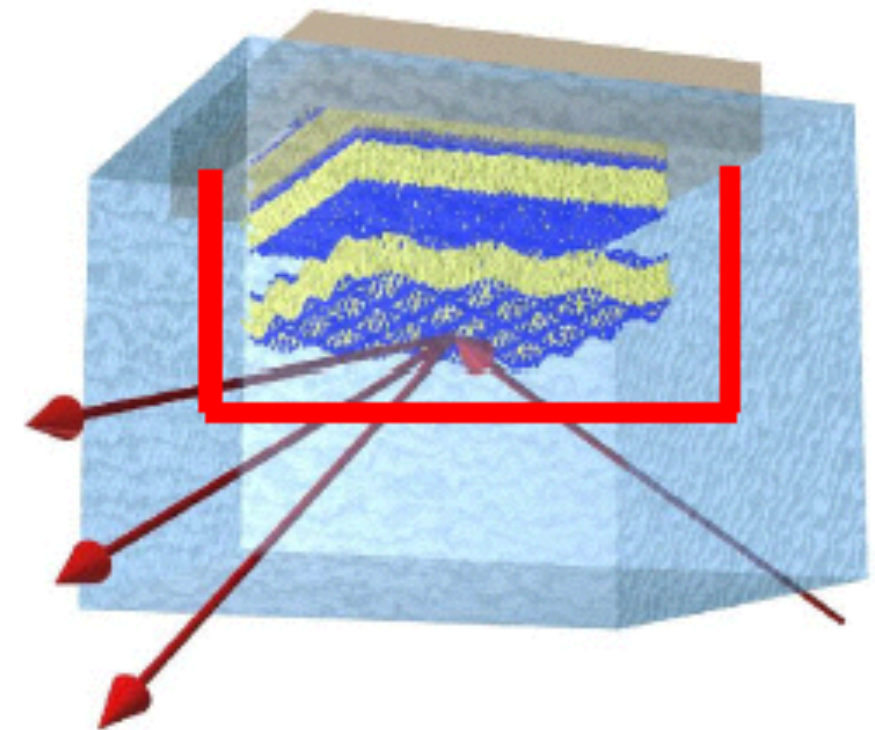- Recently at Institute Curie developed detergent mediated technique is adapted

A

B   t= 0 min a.u.=100   t= 20 min a.u. = 70

[1]

Detergent

Lipid

Purified membrane protein in detergent

[1]
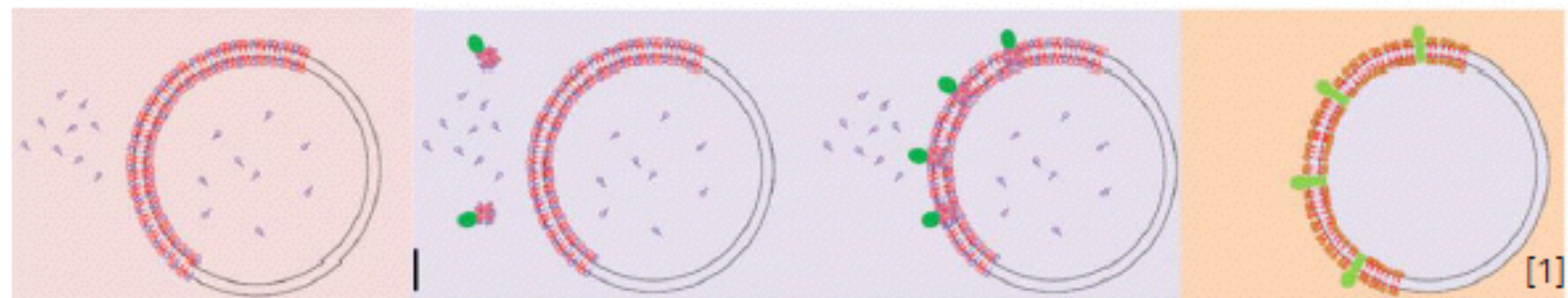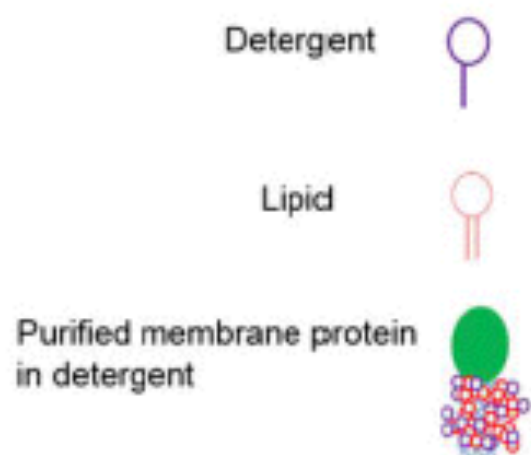
1. Destabilize Membrane using sugar based detergents
2. Deploy puryfied membrane Protein
3. Because of hydrophobic interactions, Proteins will arrange in Bilayer
4. Rinse detergent, Proteins stay in Membrane
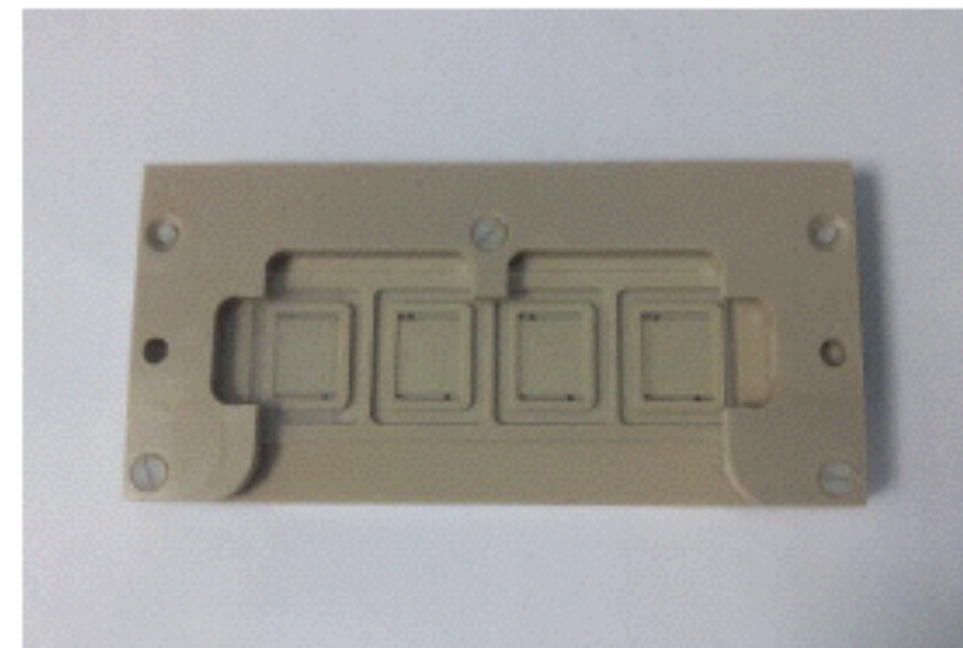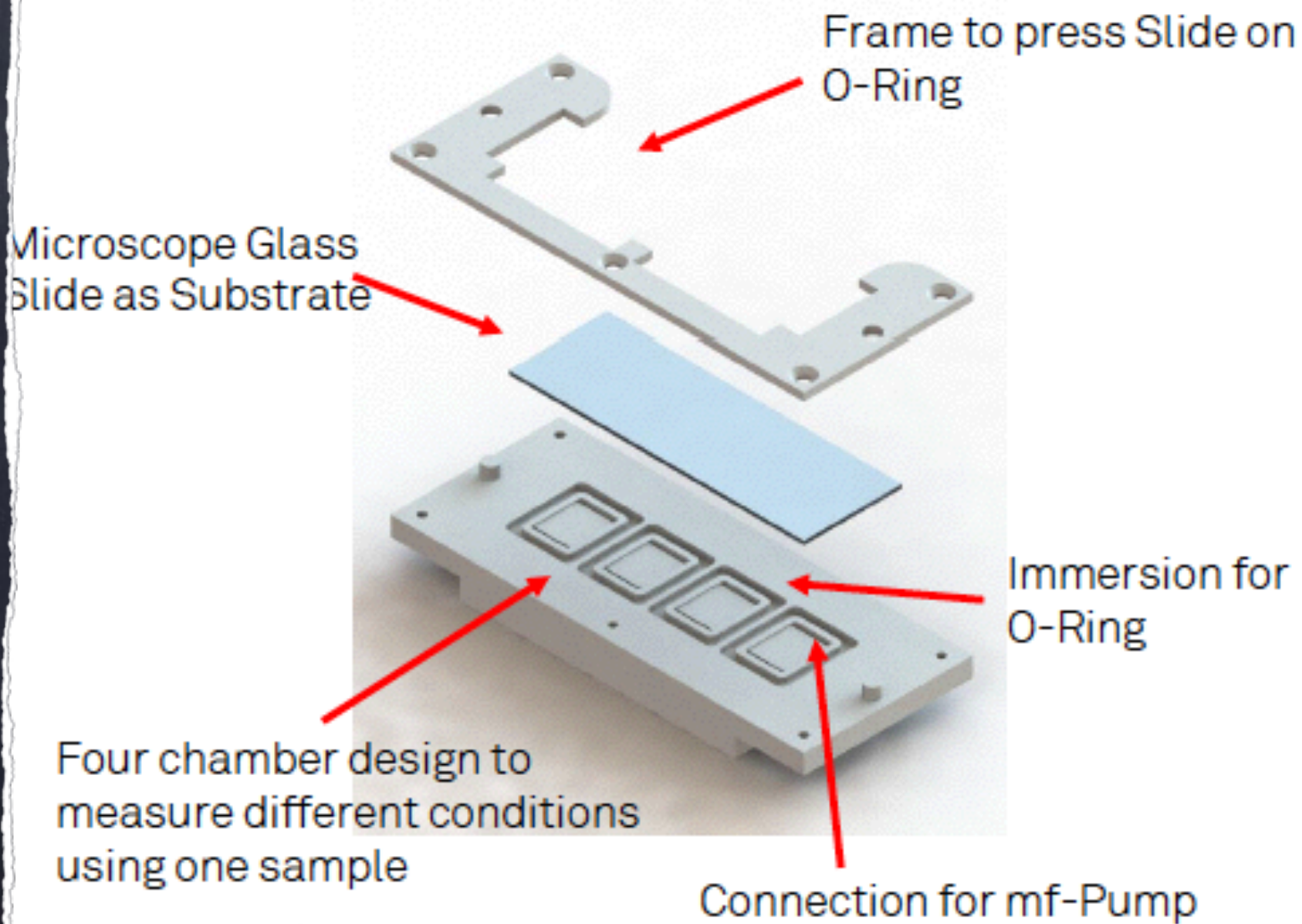
[1] M. Dezi et al. **PNAS**. 18. 7276-7281-593 (2013)
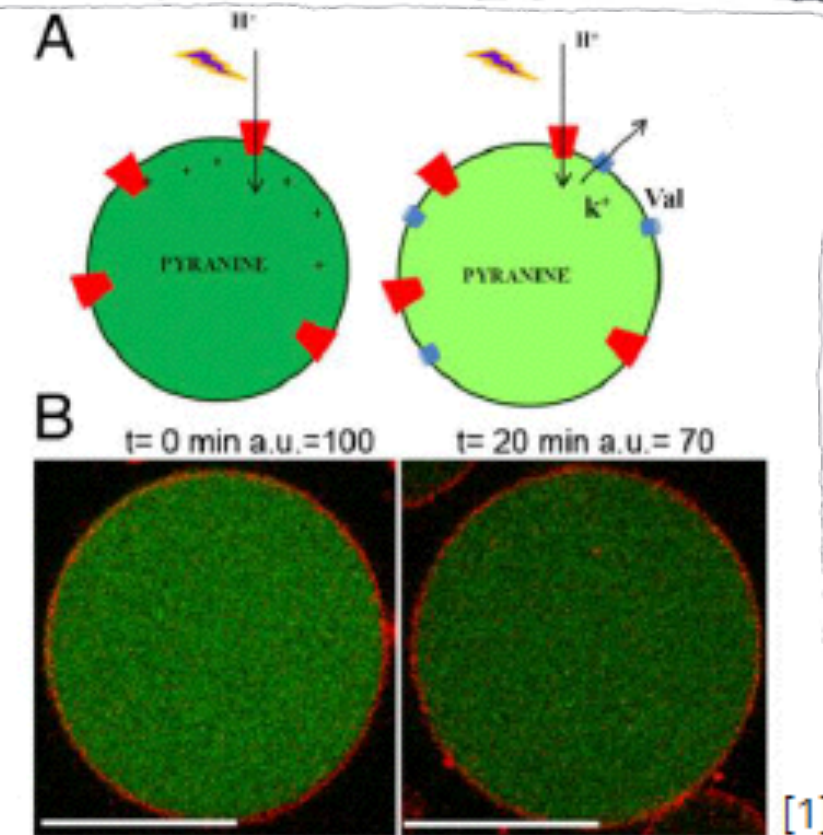
- Develop a Sample Cell for Supported Lipid Bilayers
  - Done in cooperation with the PSCM-ESRF Group

- Requirements
  - Build a completly sealed volume with the substrate
  - Inner water volume exchangeble via microfluidic pump
  - Temperature controlled
  - Limited space available under microscope

courtesy S. Wulle

- **First Design**

Frame to press Slide on O-Ring

Microscope Glass Slide as Substrate

Immersion for O-Ring

Four chamber design to measure different conditions using one sample
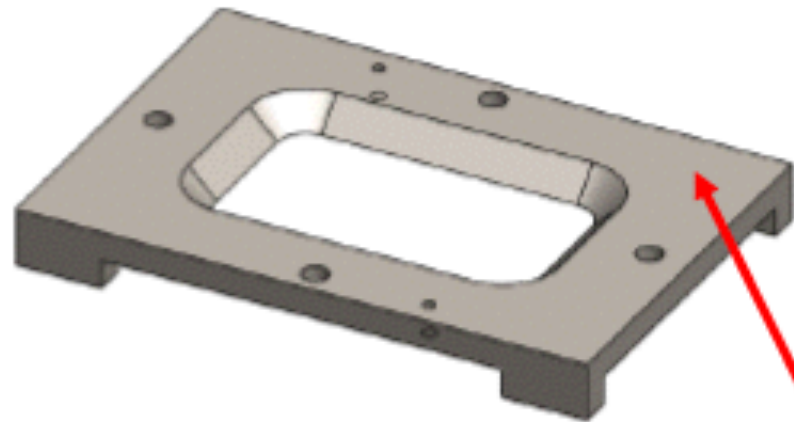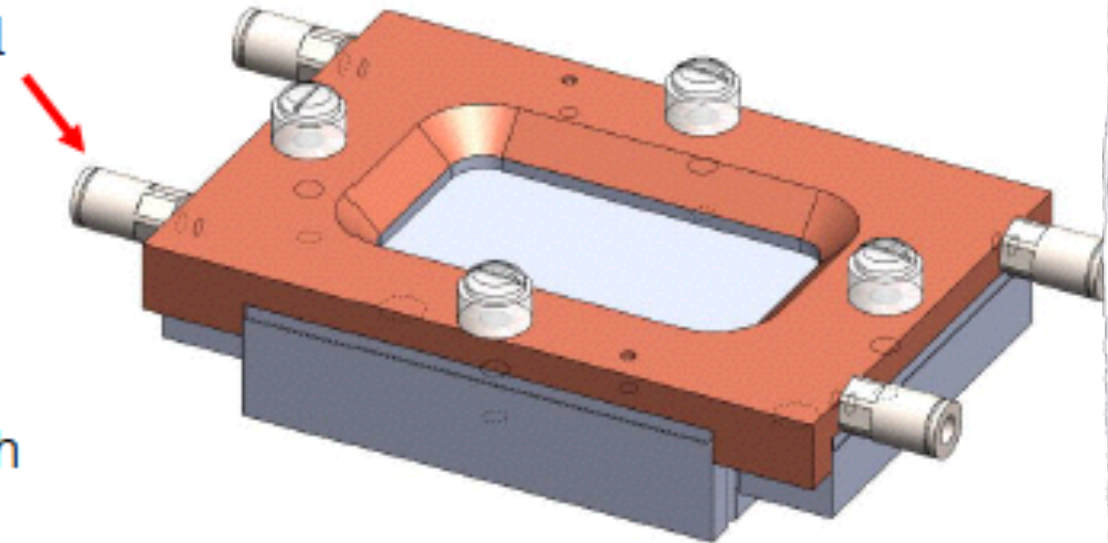
Connection for mf-Pump
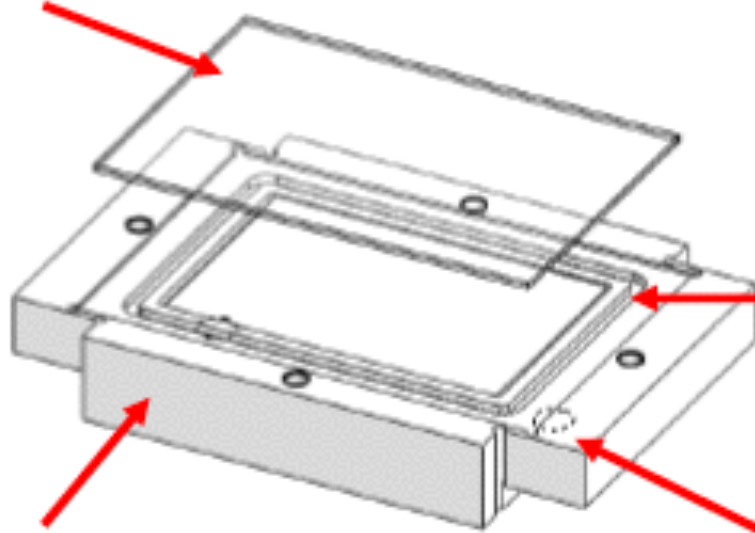
courtesy S. Wulle

EXPERIMENTAL APPROACH

- **Second Design**

Connection for temperature control (Not Build)

Solid metal frame with window for microscope

7x5 Glass Slide

Immersion for O-Ring

One chamber, simple design

Connection for mf-Pump

courtesy S. Wulle

- Problem
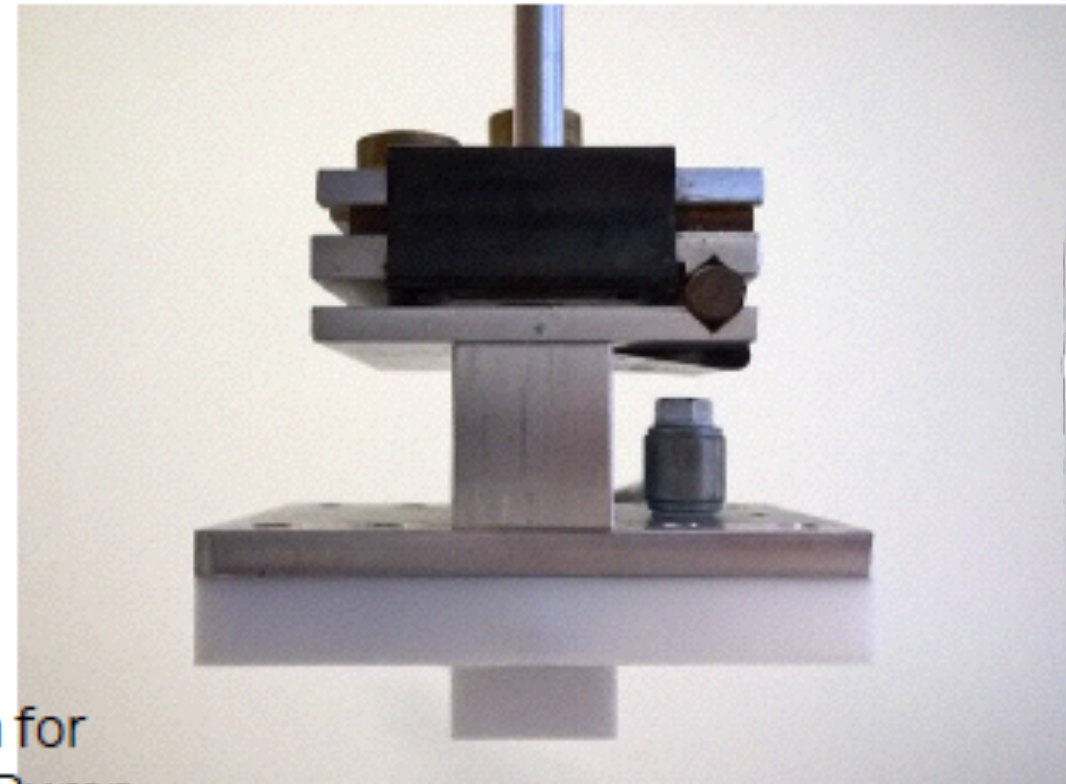 - Substrate for optical microscopy very thin (1 mm)
  - Can't hold it with a „gripper"

- Solution
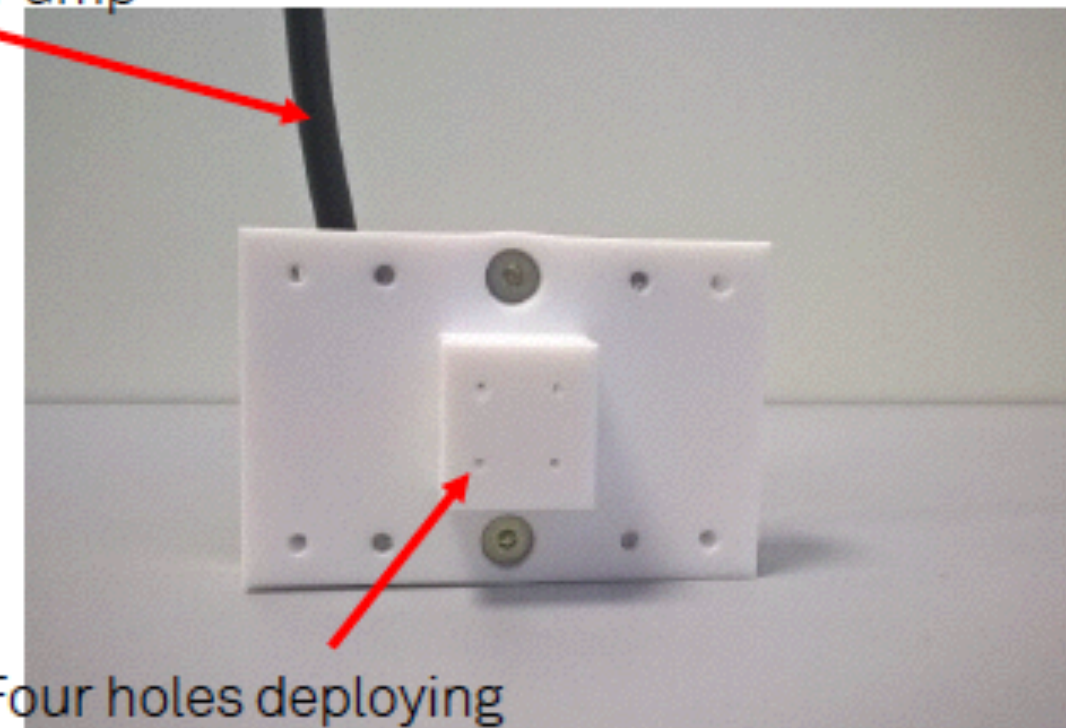 - Development of a vacuum dipper
  - „Sucks" the substrate on via under preassure

- (Surprisingly) Works effortless even with 7x5 silicon blocks
  - Horizontal and vertival geometry!
  - Easier sample preparation also for neutron experiments
  - Available at PSCM

Connection for Waterflow Pump

Four holes deploying underpreassure

courtesy S. Wulle

EXPERIMENTAL APPROACH

courtesy S. Wulle