SCHOOL ON SYNCHROTRON RADIATION AND APPLICATIONS
In memory of J.C. Fuggle & L. Fonda

19 April - 21 May 2004

Miramare - Trieste, Italy

1561/25
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SAXS under extreme conditions

H. Amenitsch

SAXS Under Extreme Conditions

H.Amenitsch

Outline:

-Why? What is extreme?
Scientific case
How it all began?

-How to trigger transitions?
Jump-relaxation methods
Other (oscillatory) methods

-Applications

Biology and Biomedicine
Physical Chemistry
Material Science

-Outlook

AUSTRIAN SAXS - BEAMLINE AT ELETTRA
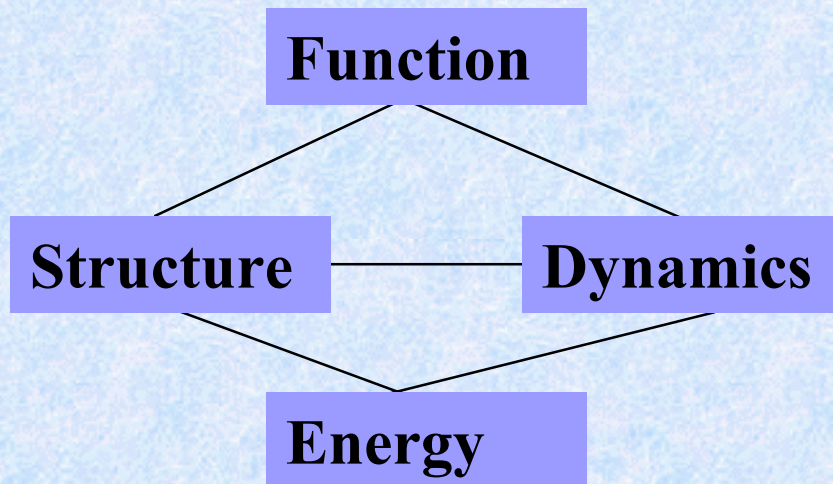H. Amenitsch, S. Bernstorff, P. Dubcek, M. Rappolt & P. Laggner

Why?

What is extreme?
- Temperature: mK, 10^3 K, 10^6 K
- Time scales: years, s, ms, μs
- Pressure: MPa, GPa
- Chemical potential
- Non equilibrium states => Transitions

Scientific Case:

Function

Structure — Dynamics

Energy

Biology and Biomedicine:
- understand molecular and cellular function
- find ways to cure diseases

Material Science:
- understand macro- and supramolecular assembly
- find new, purpose-designed materials

AUSTRIAN SAXS - BEAMLINE AT ELETTRA
H. Amenitsch, S. Bernstorff, P. Dubcek, M. Rappolt & P. Laggner

Why? - How it all began - Muscle Contraction

September 1970: DESY
Rosenbaum, Holmes & Witz, Nature (1971), **230**,434
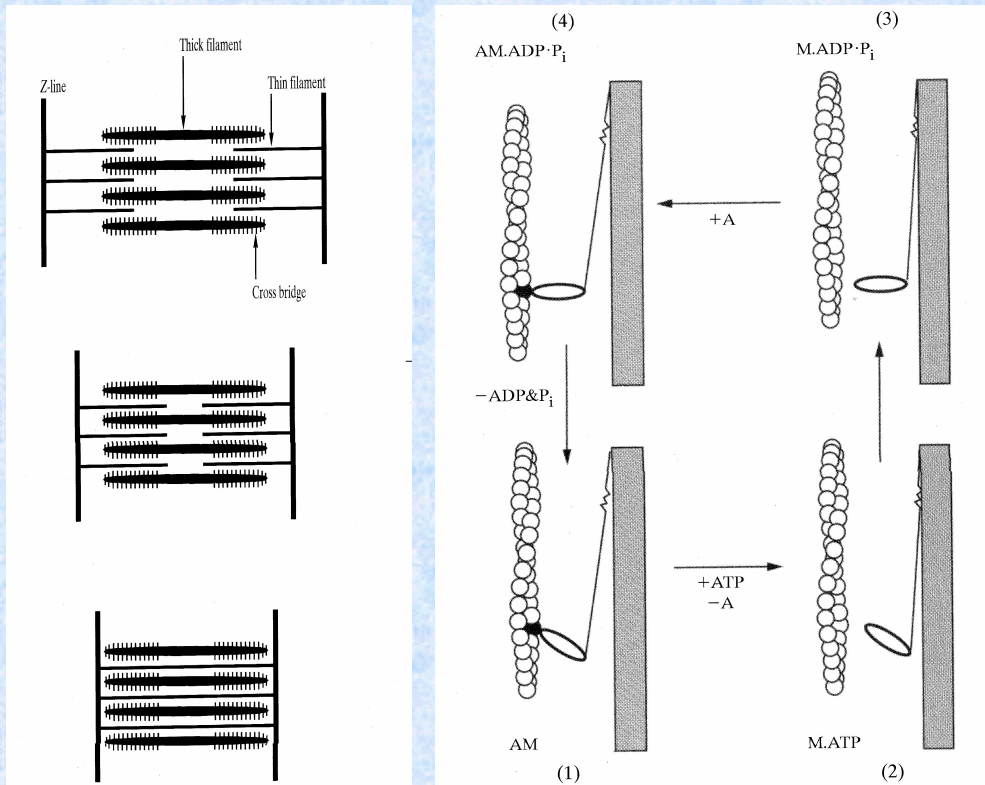
Fig. Lymn-Taylor cycle. (Lymn, Taylor Biochemistry, (1971)10, 4617 Mysoin-cross-bridge is bound in rigor (1) ATP binds->quick dissociation (2) ATP->ADP + P (hydrolysis) binding of myosin to actin 90 up (3) release of components, rowing to (1)
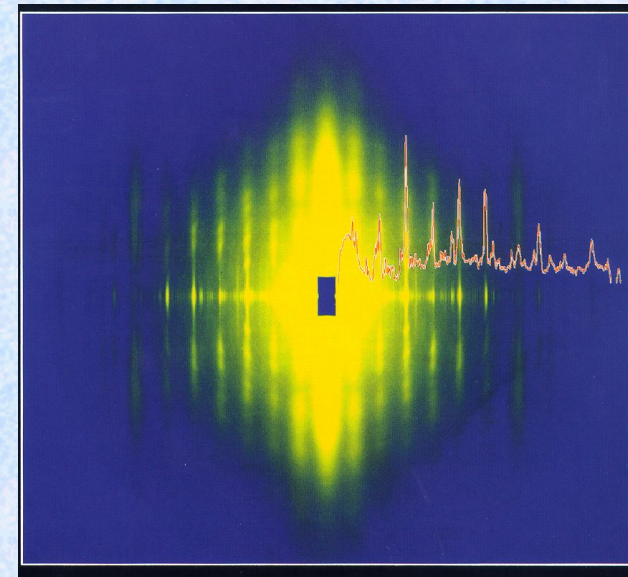
Fig. Muscle Contraction thick (myosin)-, thin-(actin) fibers are interdigitating
K.C Holms Acta Cryst. **A54**, (1997), 789

Fig. Diffraction pattern of life skeletal frog muscle Cover page: Yagi, et.al. J.Synchrotron. Rad (1996), 3,247

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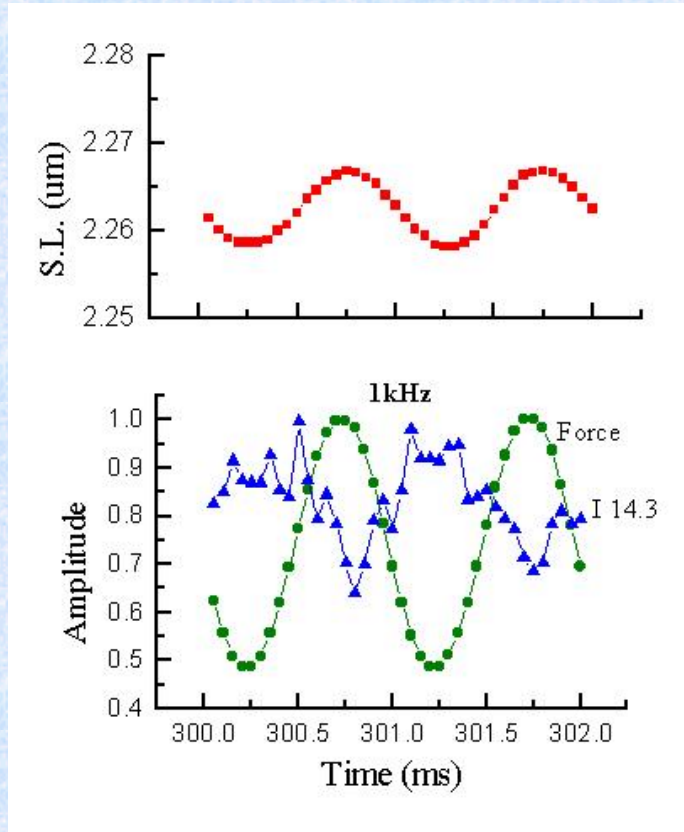# Why? - How it all began - Muscle Contraction

Fig.: Sarcomere length (S.L., filled squares), force (filled circles) and IM3 (I14.3, filled triangles) for a single fibre undergoing 1 kHz sinusoidal length oscillations
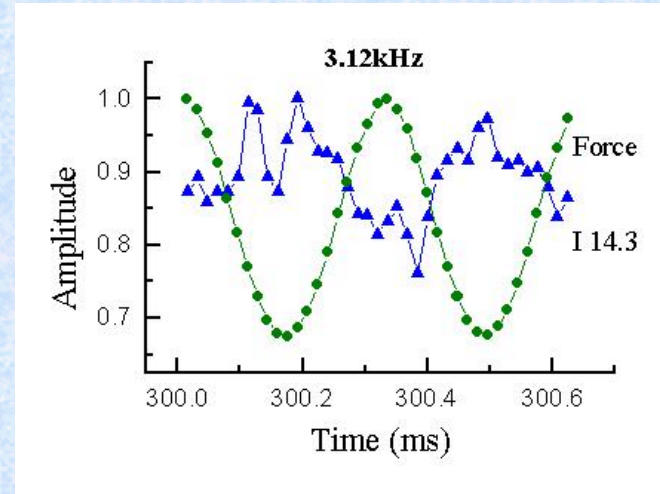
Fig.: IM3 (I14.3, filled triangles) and force (filled circles) for a two fibre bundle undergoing 3.12 kHz sinusoidal length oscillations. Sampling time 16 micro-seconds.

H. Amenitsch, C.C. Ashley, M.A. Bagni, S. Bernstorff, G. Cecchi, B. Colombini and P.J. Griffiths, Elettra News Letter, Number 26 (1), August 31, 1998

Literature:

Bagni MA, et.al., BIOPHYSICAL

JOURNAL **80**, 2809, (2001)

Piazzesi G, et.al. NATURE **415**, 659, (2002)

How to trigger transitions?

-T-jump (heating): Erbium Glass Laser
                                "heat exchanger"
-T-cool jump: "heat exchanger"
-p scans: High pressure cells
                                hydrostatic pressure
                                diamond anvil cells

-p-jumps
-Stopped-flow cells: -M.C.Ramachandra et.al. Biophysical Journal **74**, (1998), 2714
-Segel DJ, Bachmann A, Hofrichter J, Hodgson KO, Doniach S, Kiefhaber T, JOURNAL OF MOLECULAR
          BIOLOGY, 288, 489, (1999)
-Pollack L, Tate MW, Darnton NC, Knight JB, Gruner SM, Eaton WA, Austin RH, PNAS, 96,10115, (1999)
-Batch reactor
-Magnetic field
-Shear experiments

SAXS - Applications: T-jump Device

Detector

Erbium
IR-Laser

Thermoelectric
Sample Cuvette
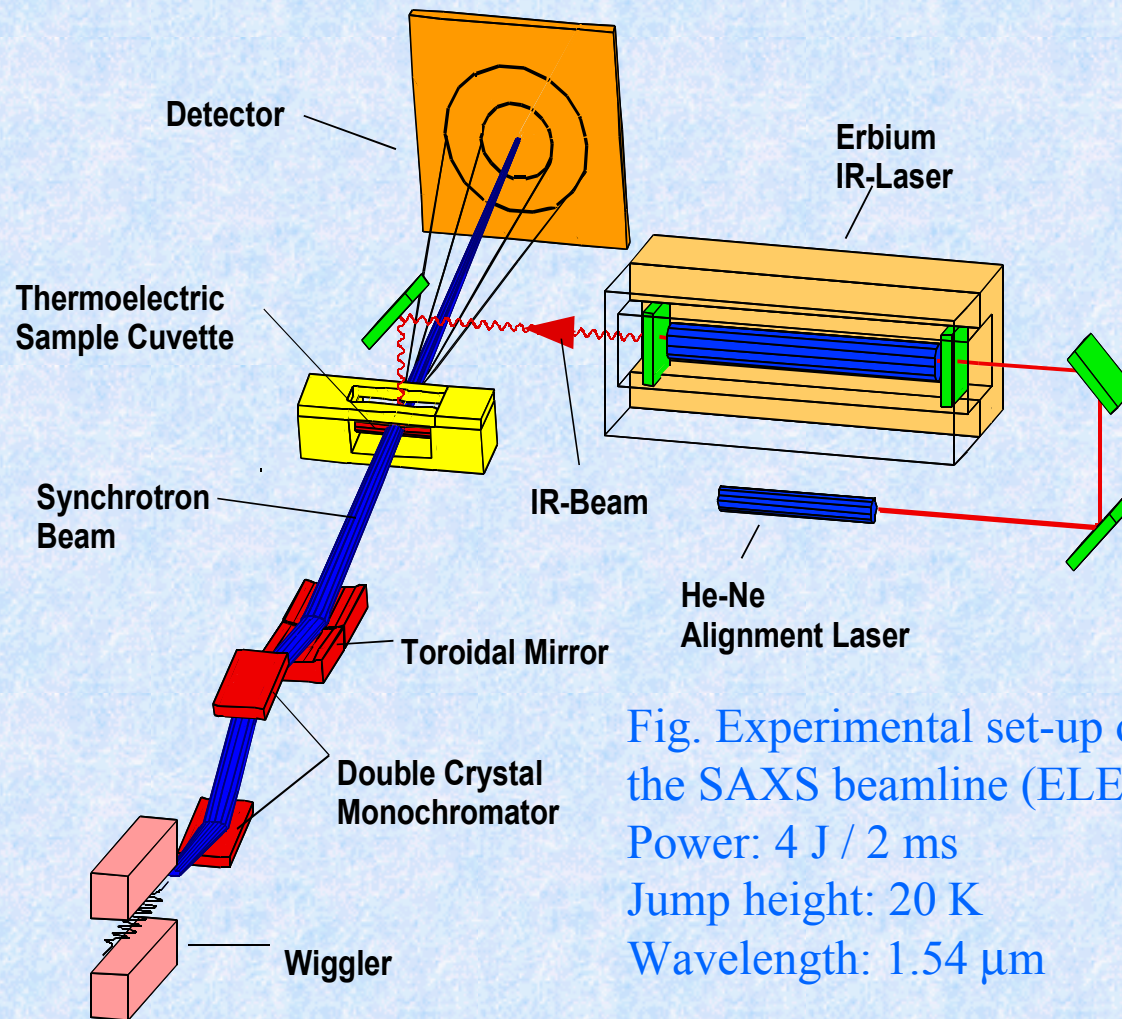
IR-Beam

Synchrotron
Beam

He-Ne
Alignment Laser

Toroidal Mirror

Fig. Experimental set-up of the T-jump device at
the SAXS beamline (ELETTRA).
Power: 4 J / 2 ms
Jump height: 20 K
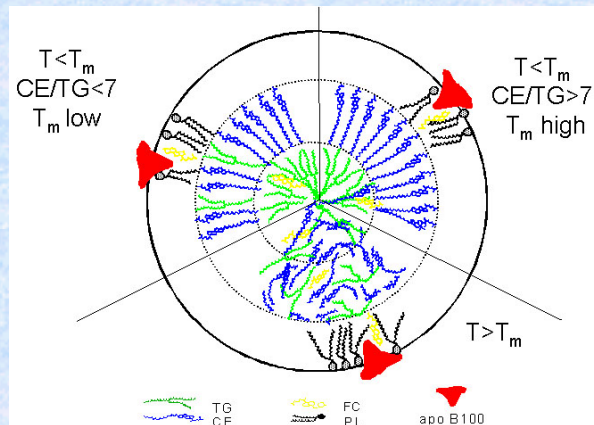Wavelength: 1.54 μm

Double Crystal
Monochromator

Wiggler

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T-jump on Low Density Lipoprotein

10 ms time-resolved x-ray diffraction of the core lipid transition of

human Low Density Lipoproteins

Sketch of the LDL lipo-protein in the 3 different states:
-core liquid crystalline state
-core isotropic state TG, CE, FC, PL denotes triglycerides, esterified cholesterol, unesterified cholesterol, phospholipids.
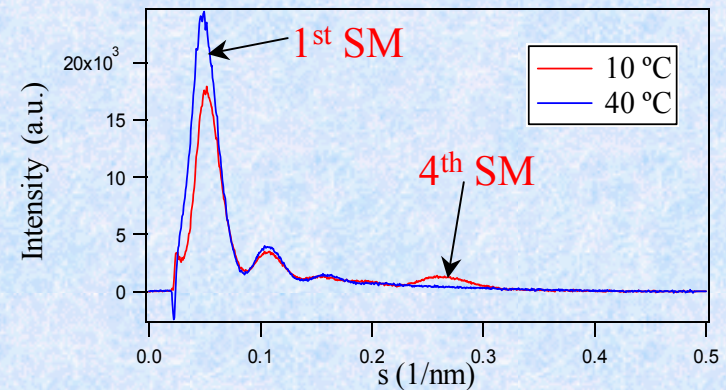

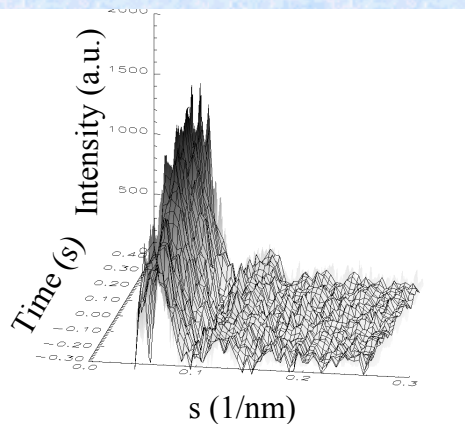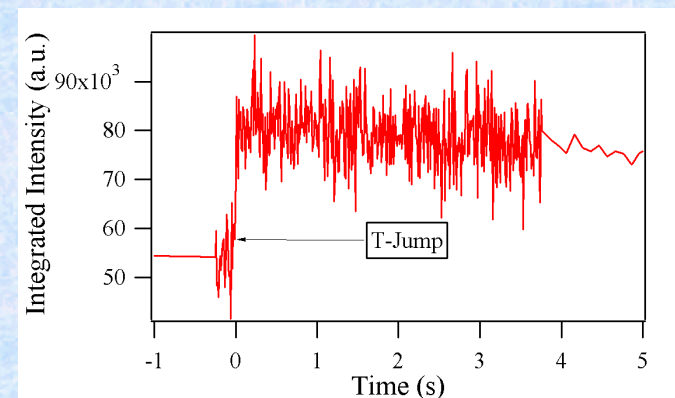Fig. Static diffraction pattern at different temperatures

Fig. Time-resolved diffraction pattern during the T-jump left pattern – right integrated intensity 1st side maximum

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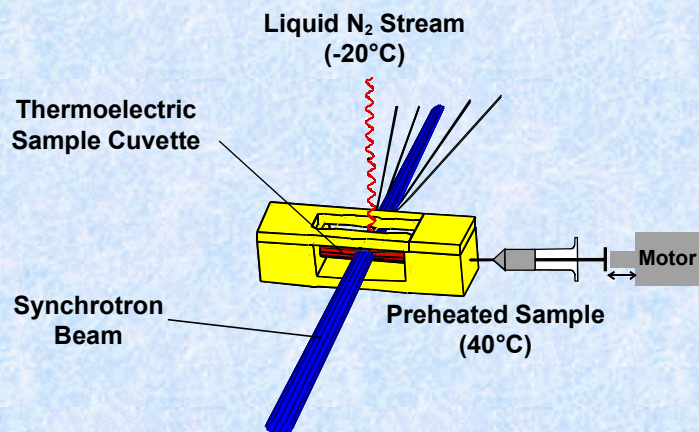
Cool-jump on Low Density Lipoprotein
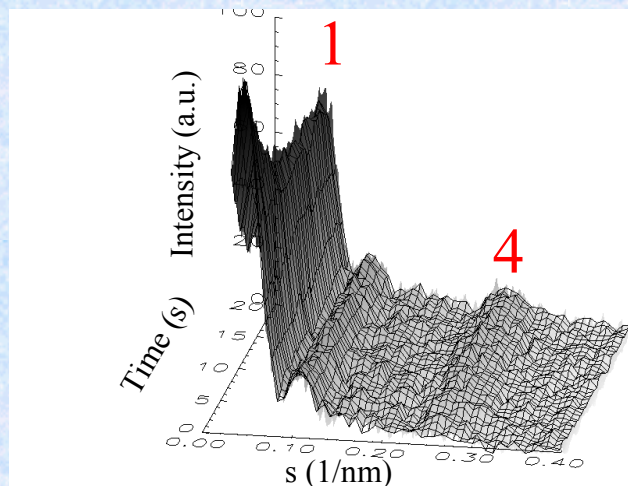

Fig. Sketch of cool-jump set-up

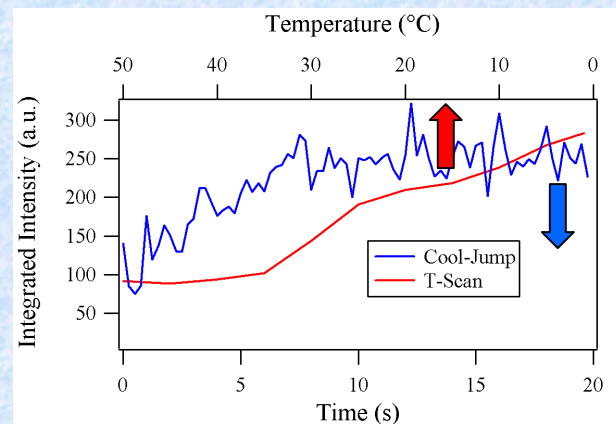Fig. Time-resolved diffraction pattern during the cool-jump
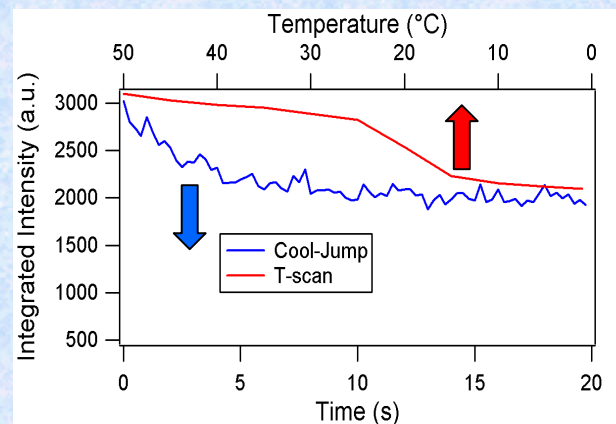

Fig: Integrated intensity 4th side maximum

Fig: Integrated intensity 1st side maximum
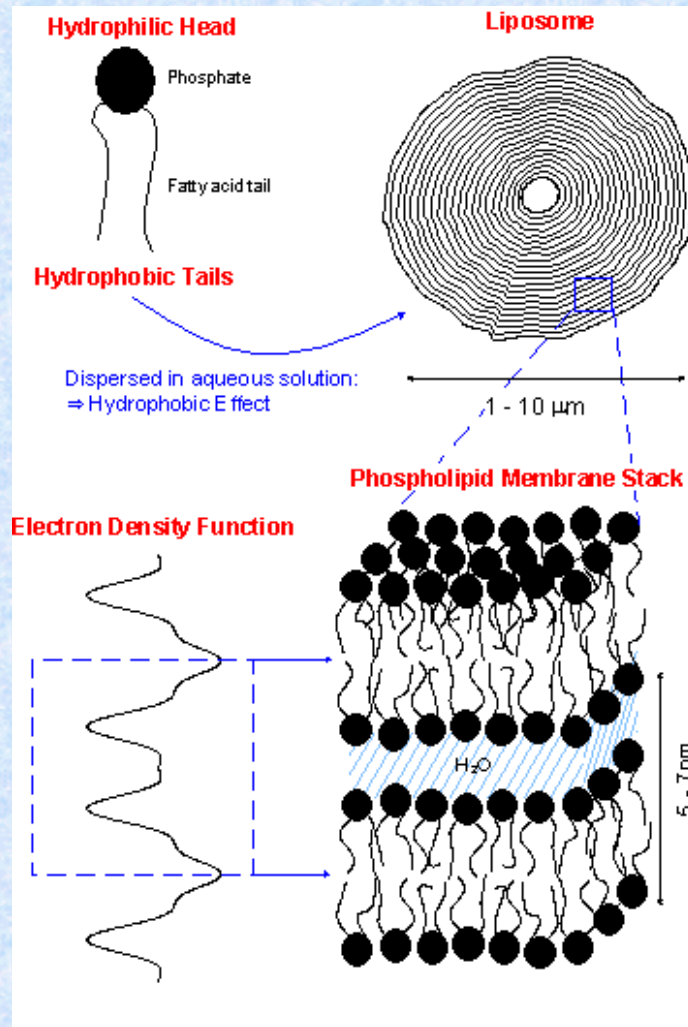
AUSTRIAN SAXS - BEAMLINE AT ELETTRA
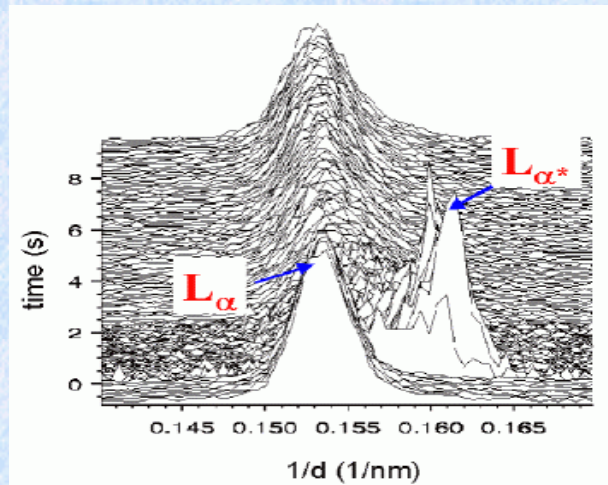H. Amenitsch, S. Bernstorff, P. Dubcek, M. Rappolt & P. Laggner
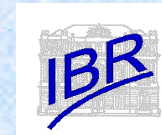
SAXS - Applications: T-jump Device

The formation of a phospholipid membrane. Phospholipids aggregate spontaneously into ordered supra-molecular structures in the presence of water. This can be explained in simple terms by the fact that phospholipids feature a hydrophilic headgroup (attracting water) and hydrophobic hydrocarbon-chains. The average 1- dimensional repeat distance d, i.e., bilayer plus waterlayer of the depicted liquid crystalline phase (La) is in the range of 5-7 nm. The electron density distribution of a bilayer (bottom left corner) has maxima in the headgroup regions and a minimum at the methyl terminus of the hydrocarbon-chains. The dashed rectangle marks the part of the electron density distribution shown in the fig below.

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SAXS - Applications: T-jump Device

The first order diffraction peaks of a phospholipid sample during a T-jump experiment (time resolution = 5 ms). The IR-laser was triggered at time zero.
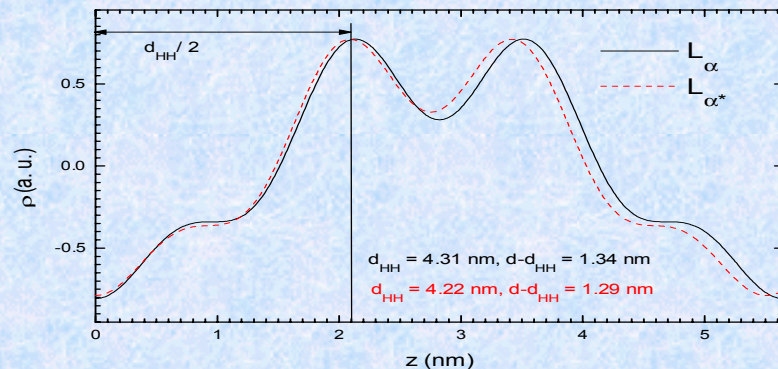
Superimposed electron density distributions of the original L_α-phase (straight line) and of the intermediate phase $L_{\alpha*}$ (dashed line) immediately after the laser flash

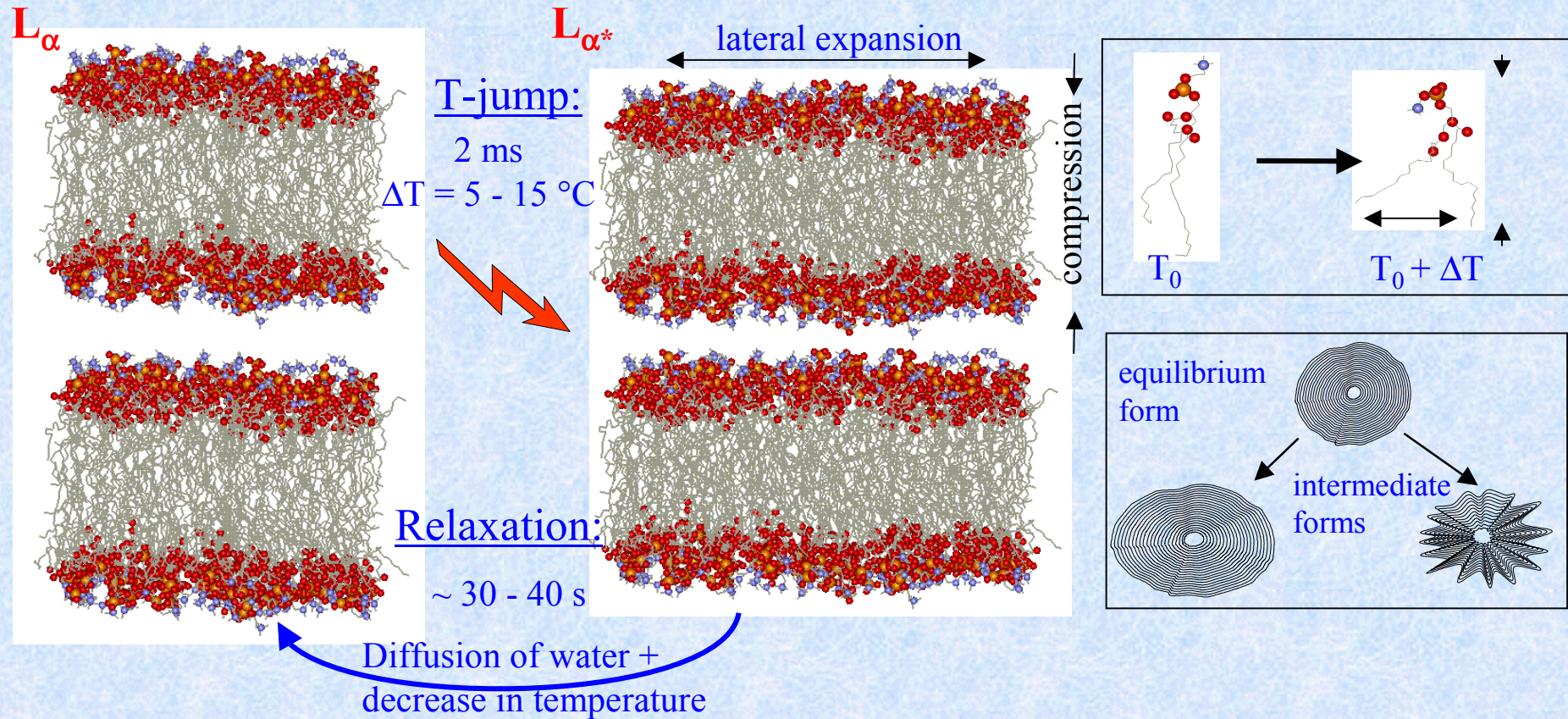**G. Pabst, M. Rappolt, H. Amenitsch, S. Bernstorff & P. Laggner (1998)**

AUSTRIAN SAXS - BEAMLINE AT ELETTRA
H. Amenitsch, S. Bernstorff, P. Dubcek, M. Rappolt & P. Laggner

T-jumps: Phospholipid Phase Transition

L_α $L_{\alpha*}$ lateral expansion

T-jump:

2 ms

$\Delta T = 5 - 15\ ^\circ C$

compression

T_0 $T_0 + \Delta T$

equilibrium form

intermediate forms

Relaxation:

~ 30 - 40 s

Diffusion of water +
decrease in temperature

**G. Pabst, M. Rappolt, H. Amenitsch, S. Bernstorff & P. Laggner,
Biophys. J., (2000)**

AUSTRIAN SAXS - BEAMLINE AT ELETTRA
H. Amenitsch, S. Bernstorff, P. Dubcek, M. Rappolt & P. Laggner

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High Pressure Cell

Details of the inside of the cell (B) with Be-windows
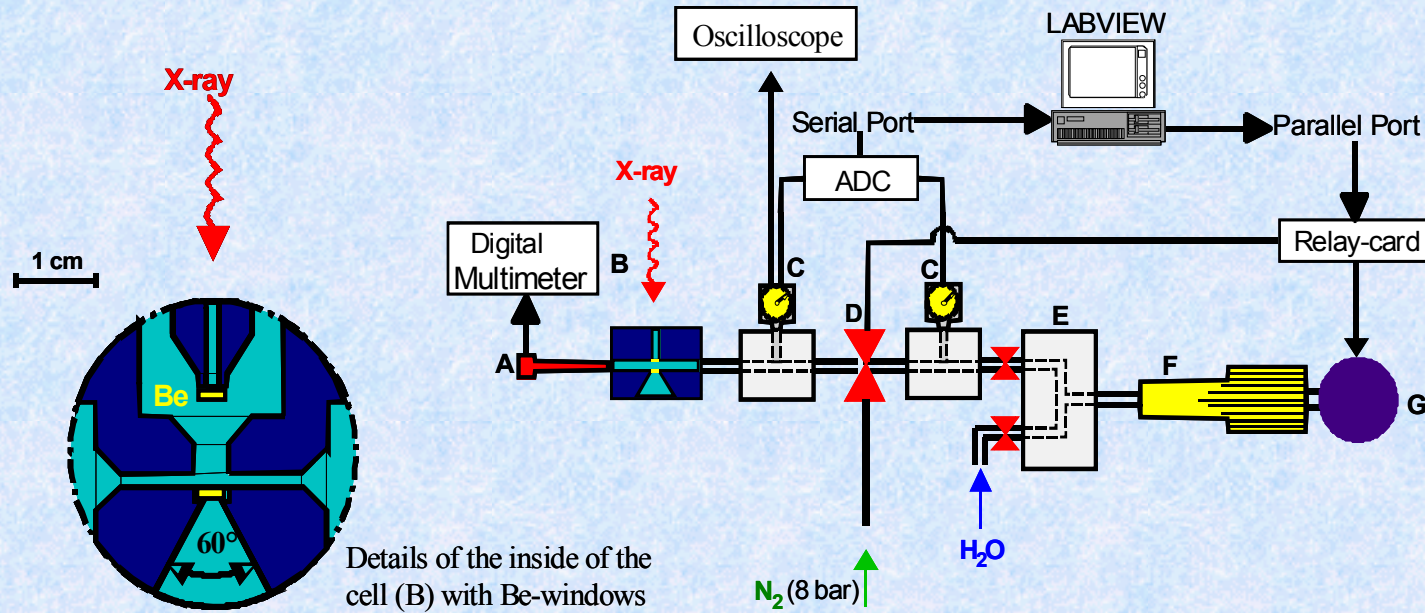
M.Steinhart, M.Kriechbaum, K.Pressl, H.Amenitsch, P.Laggner and S.Bernstorff, Rev.Sci.Instrum. 70, 1540-1545 (1999).
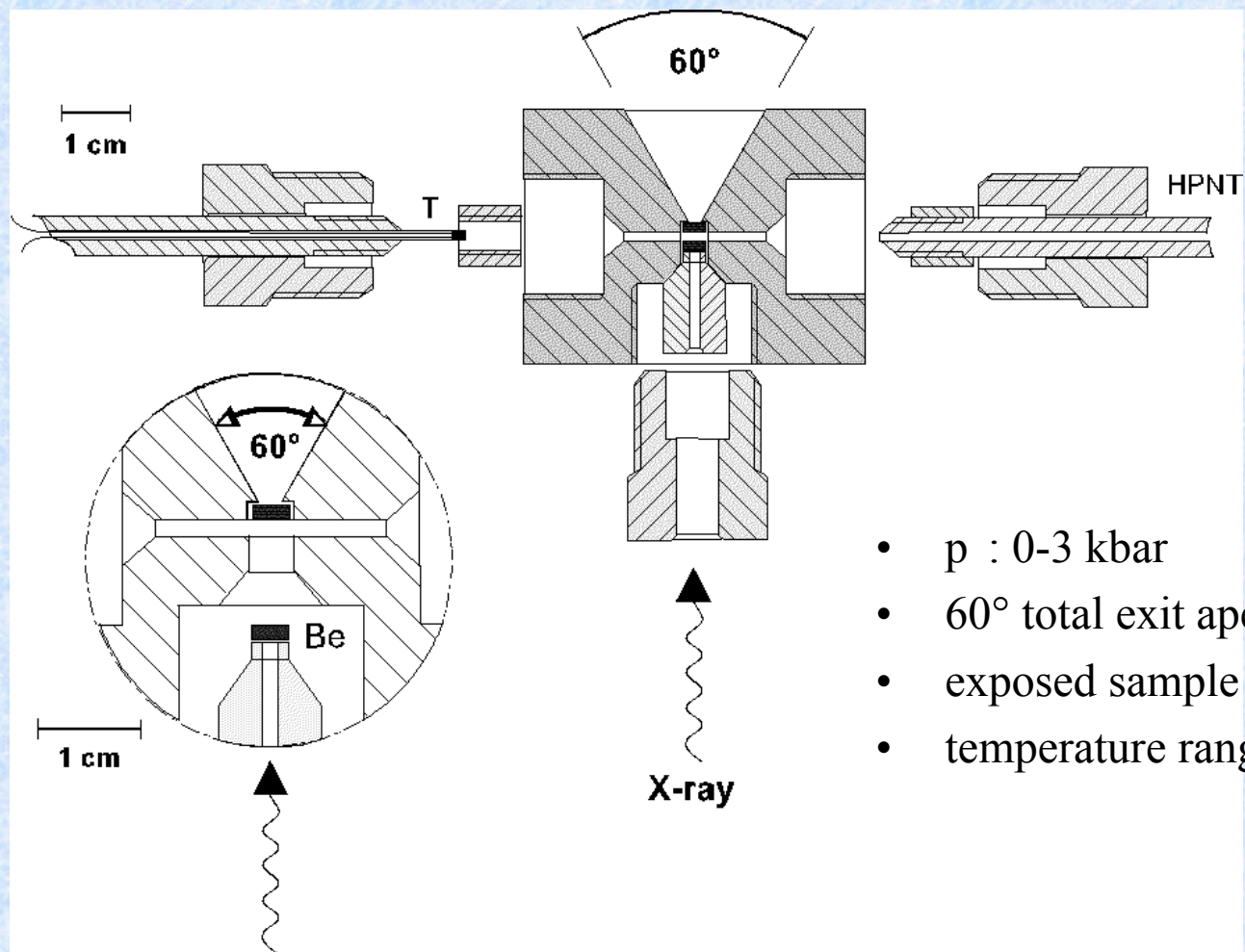
AUSTRIAN SAXS - BEAMLINE AT ELETTRA
H. Amenitsch, S. Bernstorff, P. Dubcek, M. Rappolt & P. Laggner

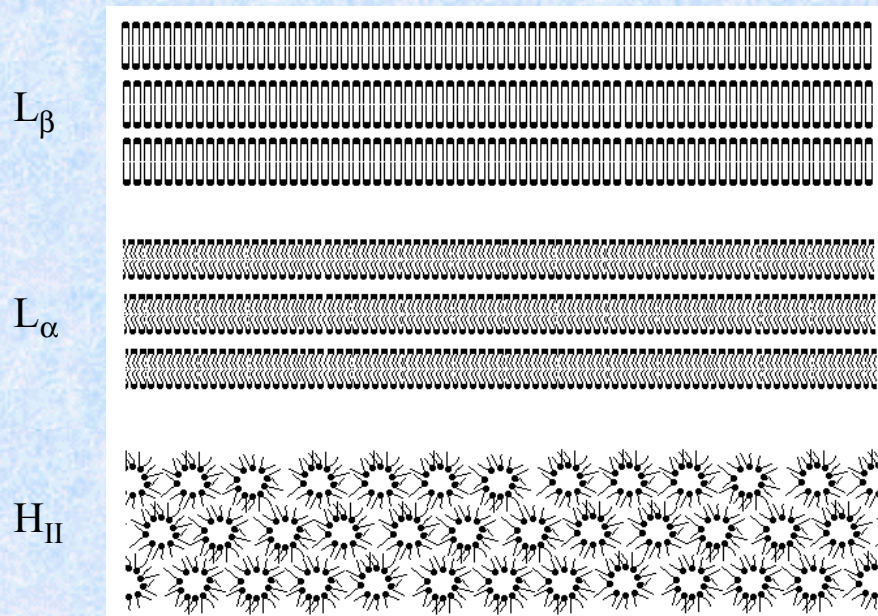# SAXS - APPLICATIONS high pressure cell

- p : 0-3 kbar
- 60° total exit aperture
- exposed sample volume: 1 ml
- temperature range: 0-80 °C
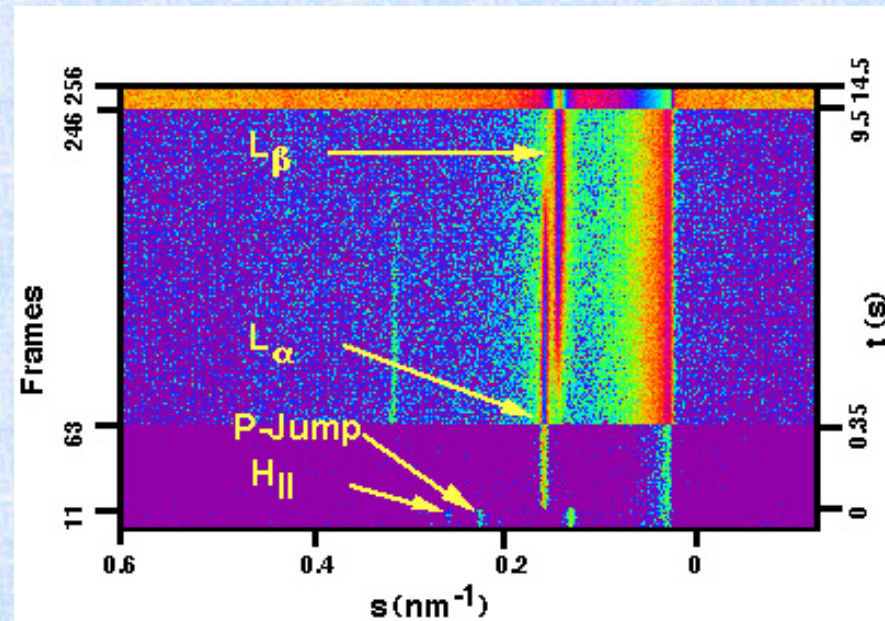
SAXS - APPLICATIONS high pressure cell

EXAMPLE: p-jump on DOPE (Dioleoylphosphatidylethanolamine)
from *150 bar* to *2.3 kbar* at *20° C*. (A) Phases and (B) SAXS-pattern.
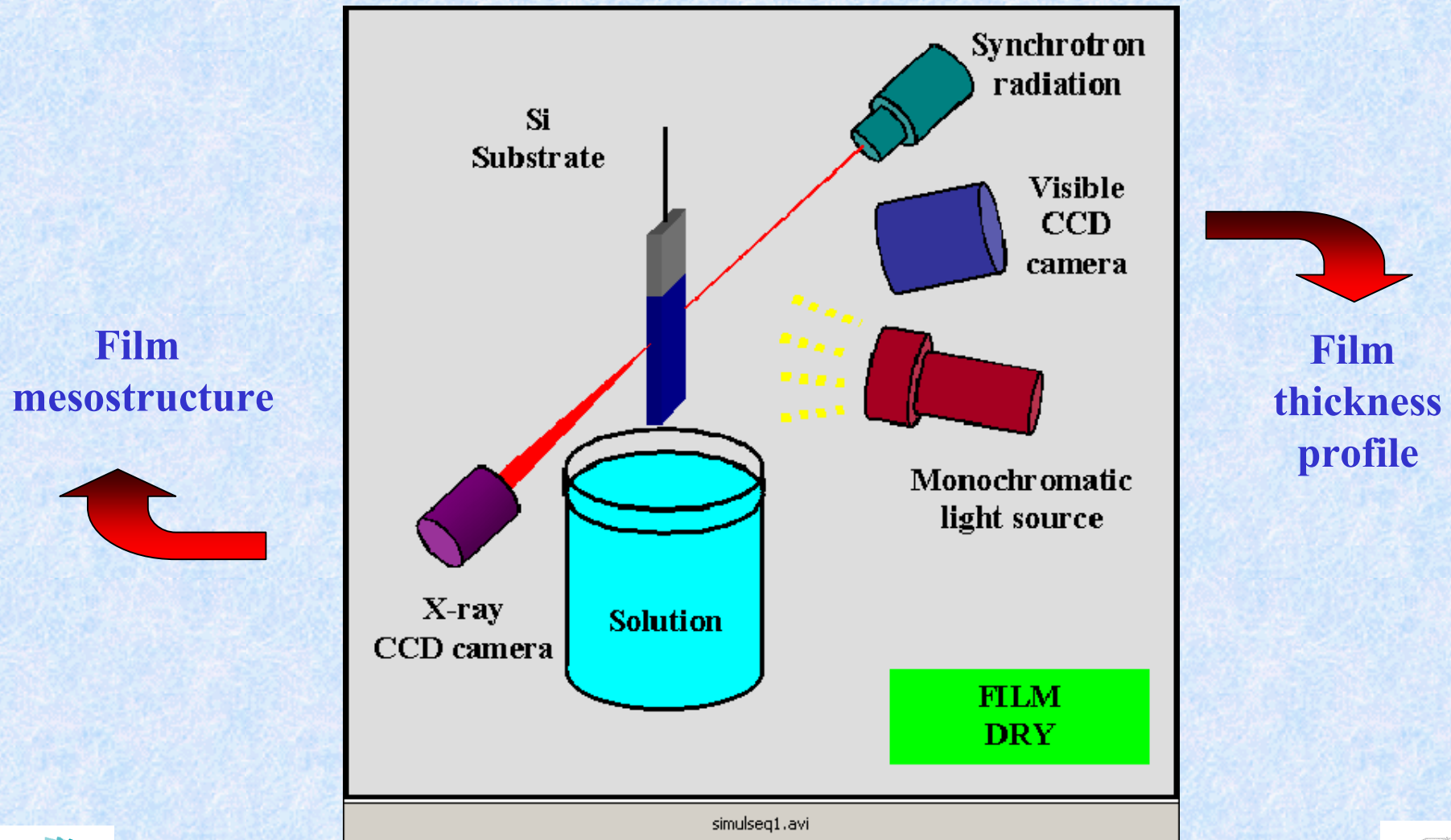*M. Kriechbaum, M. Steinhart, P. Laggner, H. Amenitsch and S. Bernstorff*

A

B

The Self-Assembly of thin films as seen by In-Situ SAXS and interferometry

Film mesostructure

Film thickness profile

Synchrotron radiation

Si Substrate

Visible CCD camera

Monochromatic light source

X-ray CCD camera

Solution

FILM DRY

simulseq1.avi
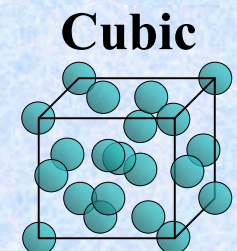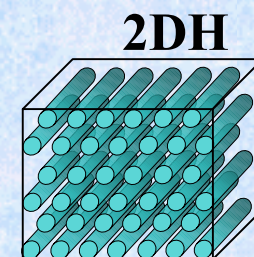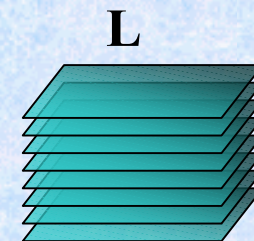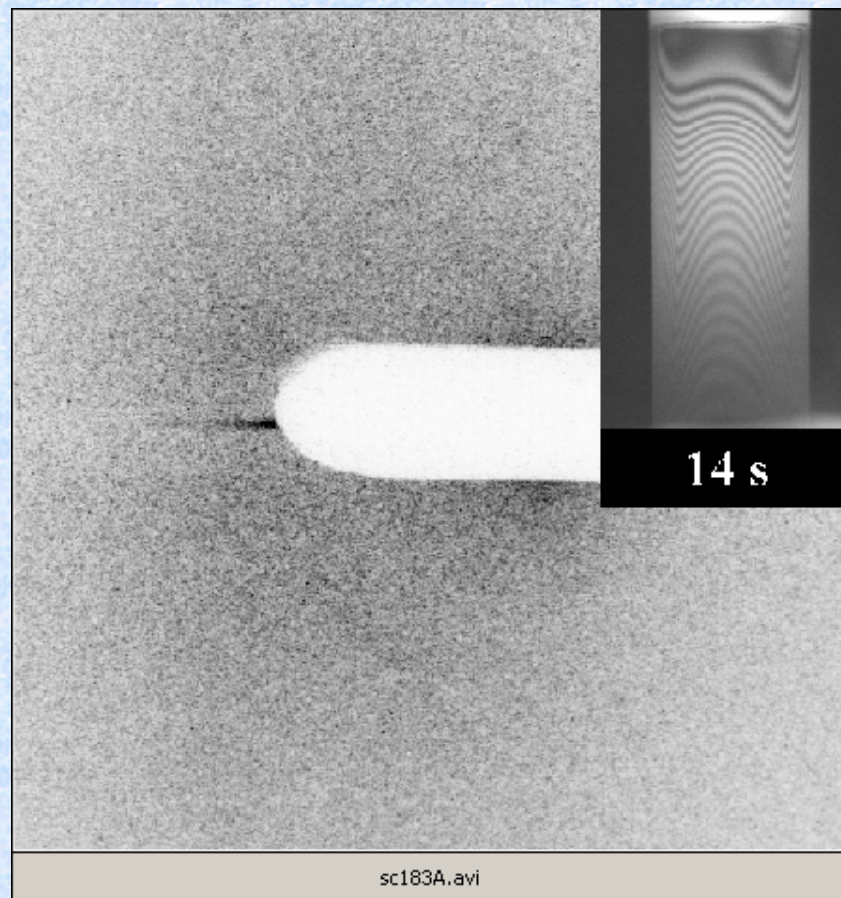
AUSTRIAN SAXS - BEAMLINE AT ELETTRA

H. Amenitsch, S. Bernstorff, P. Dubcek, M. Rappolt & P. Laggner

Surface diffraction: Formation of aligned mesoporous thin films

CTAB / Si = **0,18**
H_2O / Si = 5
HCl / Si = 0.15
Ageing time
Relative Humidity

14 s

sc183A.avi

L

2DH

Cubic

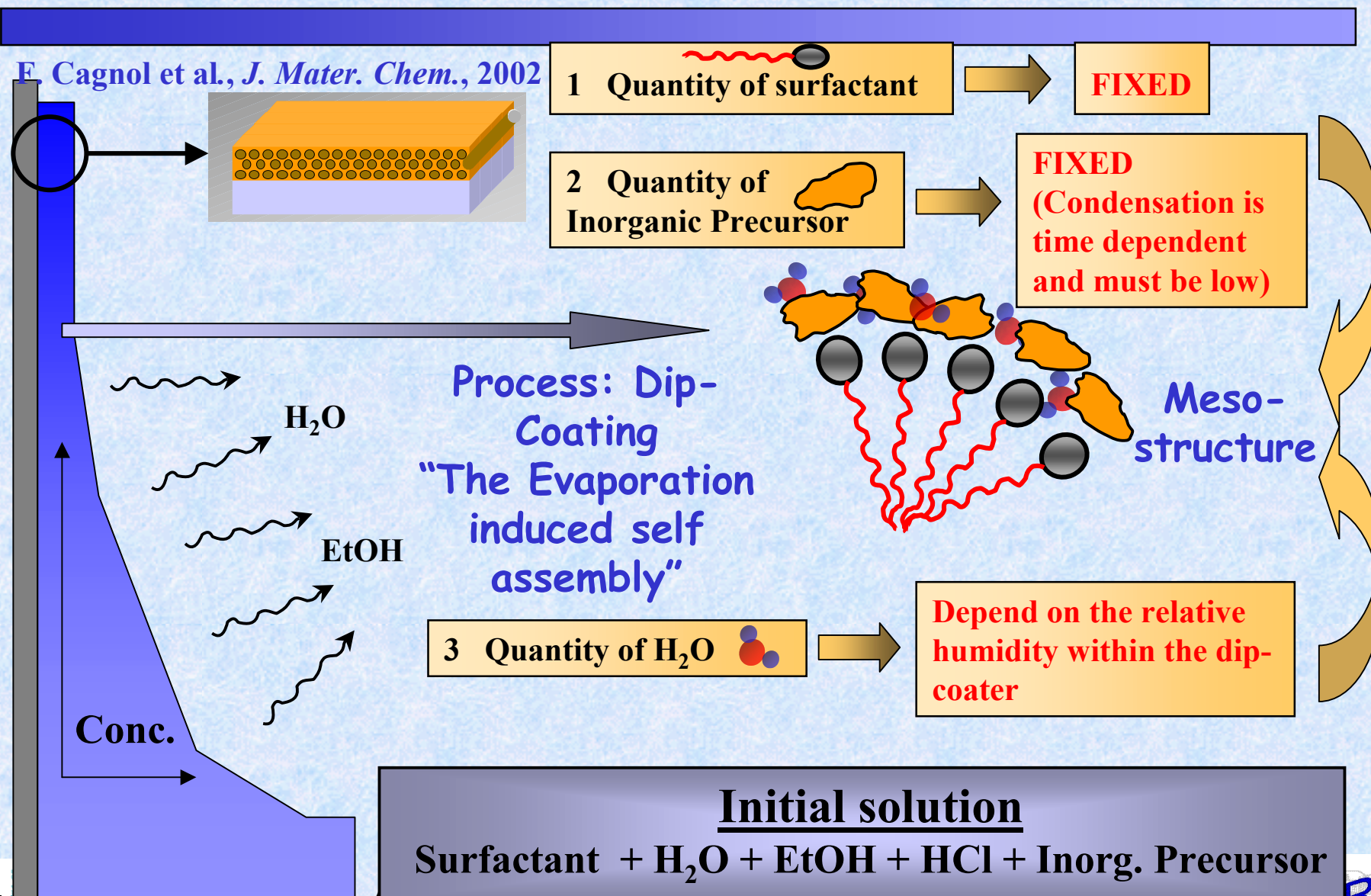**Grosso D, et.al., CHEMISTRY OF MATERIALS *14*, 931,(2002)**

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The Modulable Steady State

1 Quantity of surfactant → **FIXED**

2 Quantity of Inorganic Precursor → **FIXED (Condensation is time dependent and must be low)**

Process: Dip-Coating "The Evaporation induced self assembly"

H_2O

EtOH

Conc.

Meso-structure

3 Quantity of H_2O → **Depend on the relative humidity within the dip-coater**

Initial solution

Surfactant + H_2O + EtOH + HCl + Inorg. Precursor

AUSTRIAN SAXS - BEAMLINE AT ELETTRA

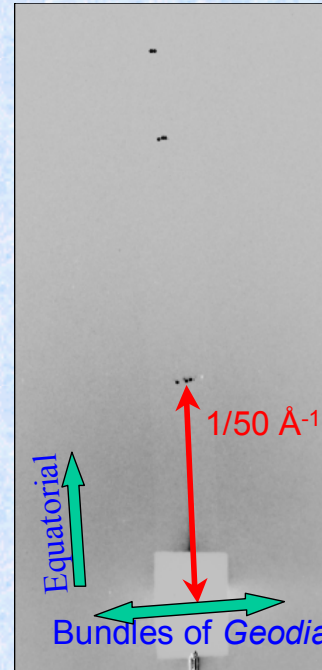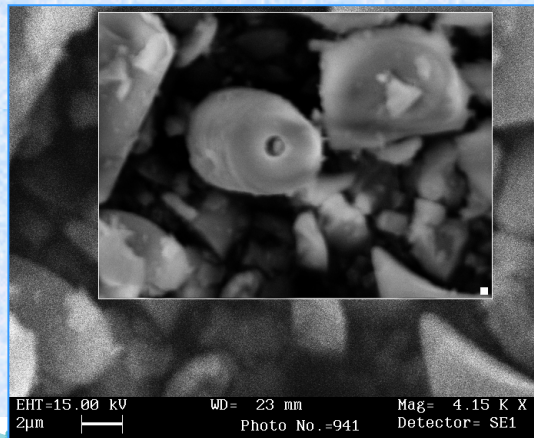H. Amenitsch, S. Bernstorff, P. Dubcek, M. Rappolt & P. Laggner

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SAXS STUDY OF SPICULES FROM MARINE SPONGES

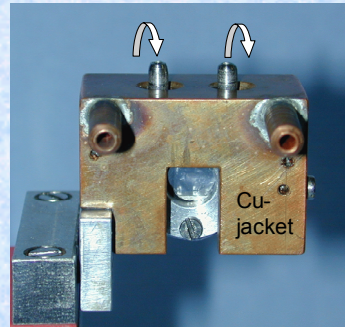➤ The scientific name is "Porifera" which translates into "pore-bearing"

Biomineralisation

Equatorial

1/50 Å⁻¹

Equatorial

Bundles of *Geodia*

Equatorial

73 Å

Meridional
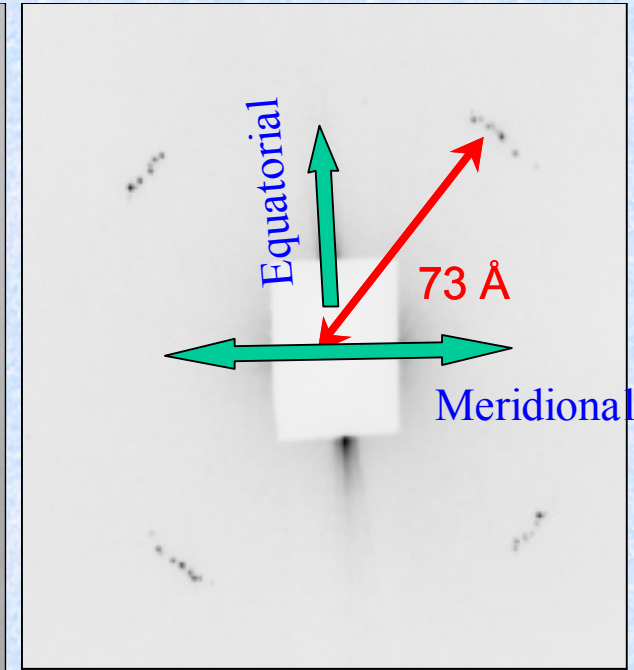
Geodia   Scolimastra

Croce,G. et al., 2004. *Biophysical Journal* 86:526-534.
Croce,G., et al.,2003. *Microscopy Research and Technique* 62:378-381

EHT=15.00 kV   WD= 23 mm   Mag= 4.15 K X
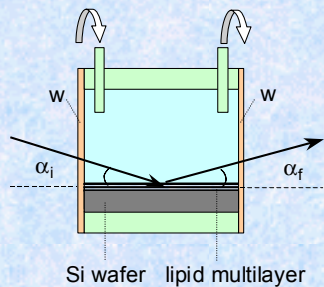2µm   Photo No.=941   Detector= SE1

AUSTRIAN SAXS - BEAMLINE AT ELETTRA
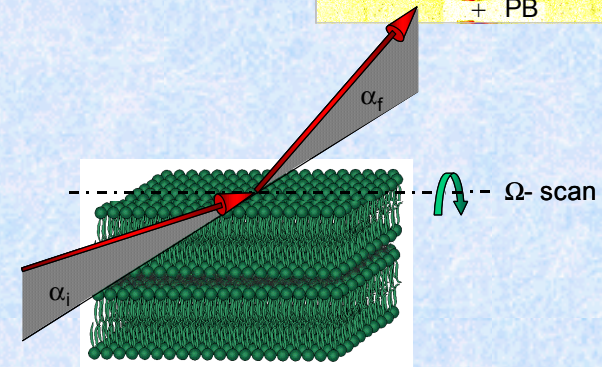H. Amenitsch, S. Bernstorff, P. Dubcek, M. Rappolt & P. Laggner

Surface Diffraction Lipids – Surface Chemistry

w   w

α_i α_f

Si wafer   lipid multilayer

Cu-jacket

Sketch of the exp. set-up and 2D diffraction pattern of POPC and 0.5 M LiCl

2. BP

LS

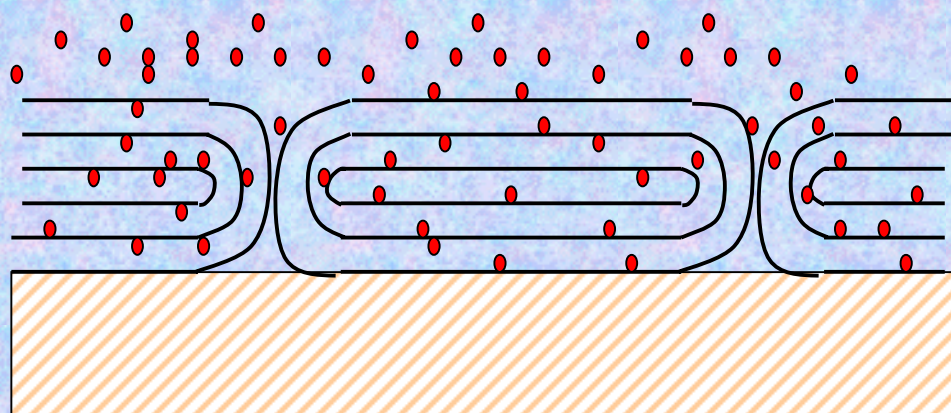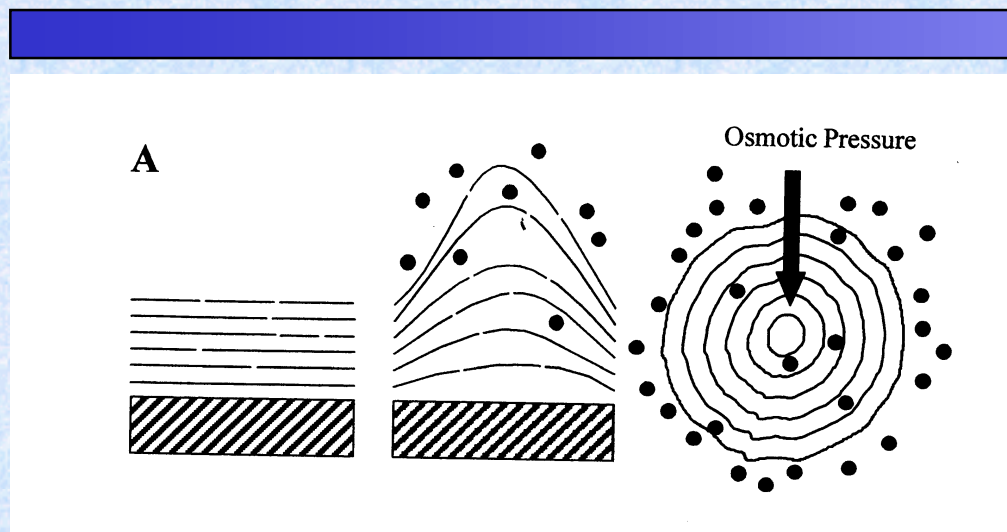1. BP

SB

+ PB

α_f

Ω- scan

α_i

Sketch and photograph of the sample cell in transmission geometry for GISAXS.
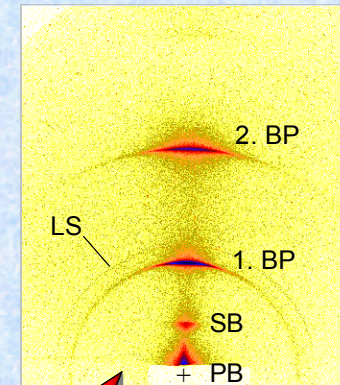
Surface Diffraction Lipids – Surface Chemistry
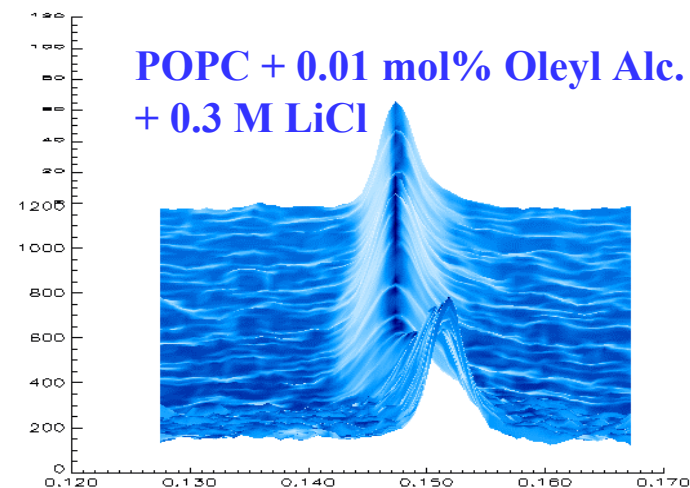

A

Osmotic Pressure

Amenisch, H., et al., (2004) Langmuir
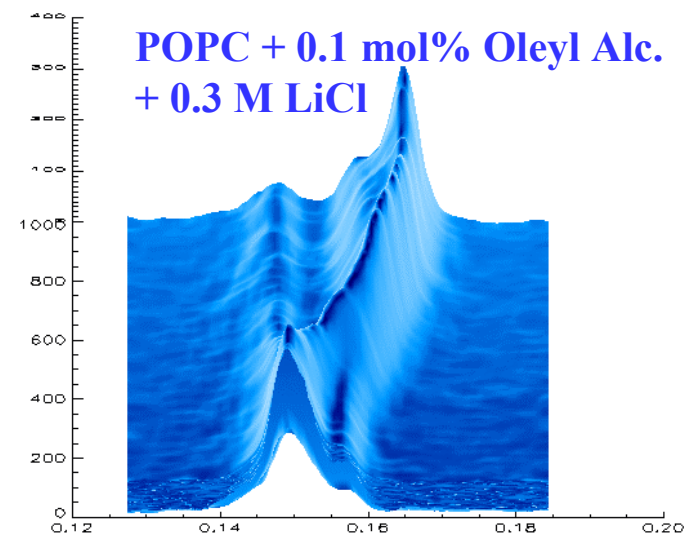
POPC + 0.01 mol% Oleyl Alc. + 0.3 M LiCl

POPC + 0.1 mol% Oleyl Alc. + 0.3 M LiCl
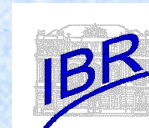
Summary - Outlook

-Why?   Extreme?
-How to trigger transitions?
-Applications

                    Biology and Biomedicine
                    Physical Chemistry
                    Material Science

"Frontiers in Material Science", Science, 277, (1997), 1213-1253

-Outlook: USE of NEW DETECTORS!
                    Use of coherence in SAXS!
                    (photon correlation spectroscopy)
                    Use of new sources FEL's!

         Think for yourself of new ways
         to use SAXS and SR

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