The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
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IAEA
International Atomic Energy Agency

1936-40

Advanced School on Synchrotron and Free Electron Laser Sources and their Multidisciplinary Applications

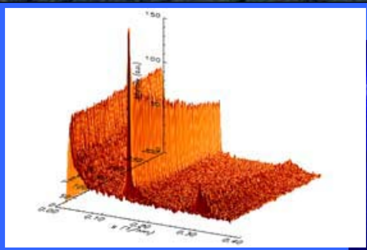
7 - 25 April 2008

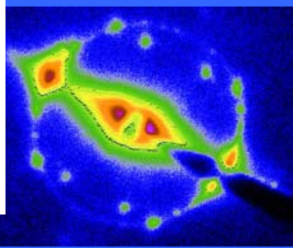
SAXS under extreme conditions: Nanomaterials and proteins.

A. Amentisch
Austrian Academy of Sciences.

SAXS under extreme conditions: Nanomaterials and proteins

By H.Amenitsch (amenitsch@elettra.trieste.it)





Sub-millisecond Exposures for Time-Resolved Structure Analysis on Synthetic and Biopolymers, Colloids, Ceramics, Glasses and Alloys

Layout of Presentation

Time-Resolved Measurements:
- -How to Trigger the Reactions
- -Biological Samples
  - Muscle, Proteins, Phospholipids
- -Material Science
  - Nanoparticles
  - Mesoporous Materials

Grazing Incidence Small Angle Scattering (GISAXS/GISAD):
- -Surface Diffraction Lipids
- -Mesoporous Materials
- -Nanoparticles/Nanocrystals

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

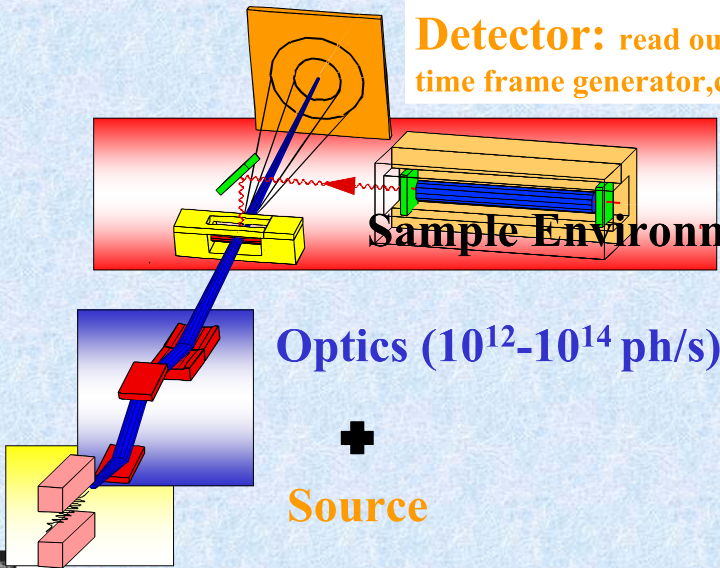
Structure — **Dynamics**

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

What do you need?



Detector: read out times, time frame generator,cps, efficiency

Sample Environment!!!

Optics (10^{12}-10^{14} ph/s)

+

Source

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How to trigger transitions?

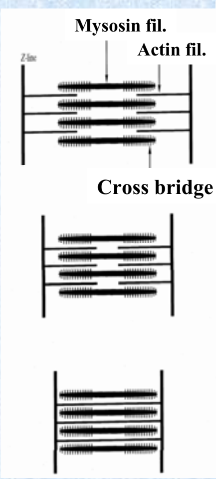
-T-jump (heating): Erbium Glass Laser (ms)
"heat exchanger"
-T-cool jump: "heat exchanger" (s)
-p scans (jumps): High pressure cells (ms)
-hydrostatic pressure
-diamond anvil cells
-Stopped-flow cells, jet-cells (µs-ms)

-M.C.Ramachandra et.al. Biophysical Journal 74, (1998), 2714
-Segel DJ et.al. , JOURNAL OF MOLECULAR BIOLOGY, 288, 489, (1999)
-Pollack L et.al., PNAS, 96,10115, (1999)
-Akiyama S et.al., PNAS, 99,1329, (2002)
-Rössle M et.al. *Biomacromolecules*, *4*, 981-986, (2003)

-Batch reactor(s)
-Magnetic field
-Shear experiments
-Mechanical stretch (µs-ms-s)

How it all began - Muscle Contraction

September 1970: DESY
Rosenbaum,Holmes & Witz, Nature (1971), **230**,434



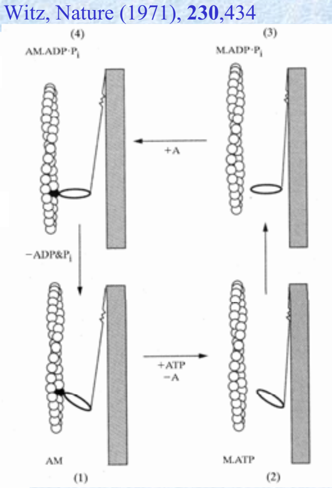


Fig. Muscle Contraction thick (myosin)-, thin-(actin) fibers are interdigitating K.C Holms Acta Cryst. **A54**, (1997), 789

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)

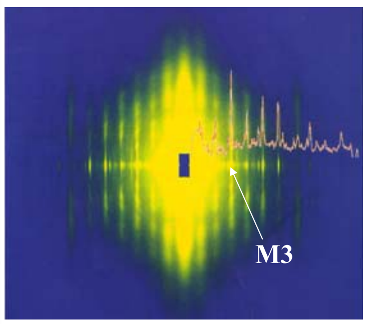
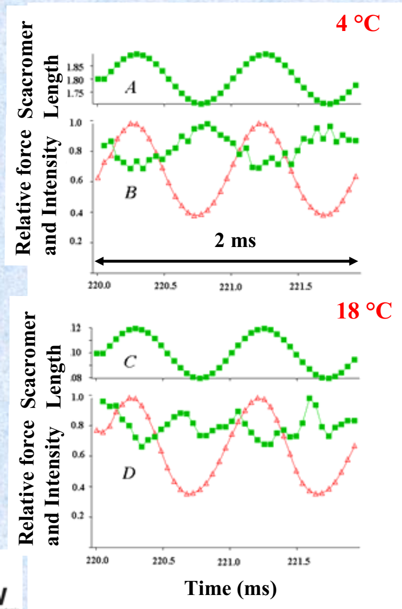


Fig. Diffraction pattern of life skeletal frog muscle Cover page: Yagi, et.al. J.Synchrotron. Rad (1996), 3,247

How it all began - Muscle Contraction



***Rana temporaria* (tibialis anterior fibers)**
Frequency: 1 kHz
Time resolution: 50 µs

Frequency: 3 kHz
Time resolution: 16 µs

Literature:

Bagni MA, et.al., BIOPHYSICAL JOURNAL **80**, 2809, (2001)

Griffiths P J, et.al. PNAS, **99**, 5384, (2002)

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

Reconditi M, et.al. NATURE **428**, 587, (2004)

Biomechanics on Human Aorta: Motivation

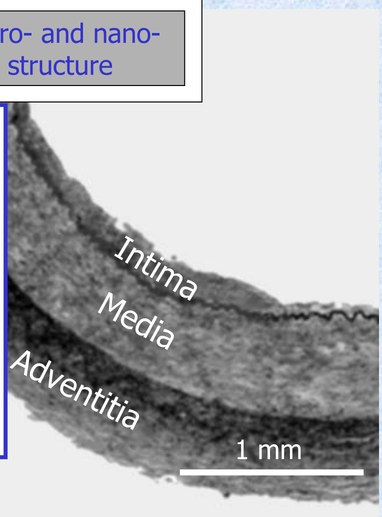
Macroscopic response ⟵ **Model** ⟶ Micro- and nano-structure

Pathology, Clinics
Characterization of vascular disease
Effects of aging
Identification of therapeutic targets (Balloon Angioplasty)

Graft design
Biomimetic materials

Functional tissue engineering

Mechanobiology

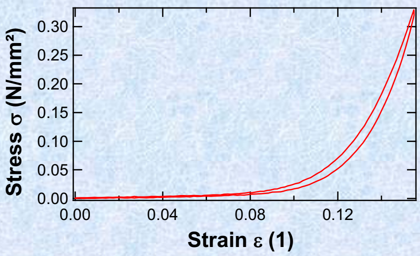


Cross section of a human artery

Human Aorta: Mechanical Parameters

Macroscopic

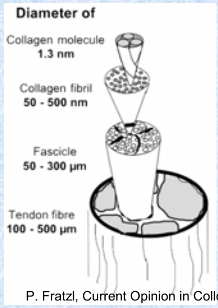
- geometric deformation
- stress
- strain

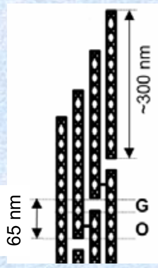


Nanoscopic

- fiber – matrix composite
- fiber alignment
- fiber strain

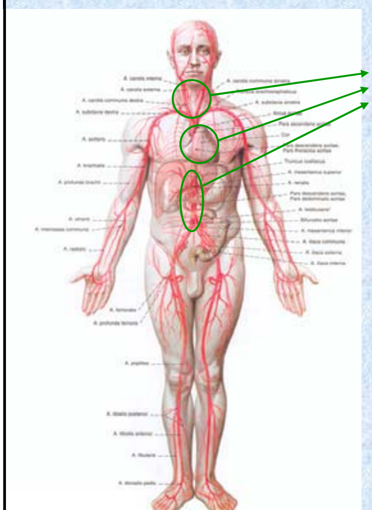
Collagen -
The most abundant protein





P. Fratzl, Current Opinion in Colloid and Interface Science, 2003

Human Aorta: Sample Preparation



Sobotta, Atlas der Anatomie des Menschen, Band 2, 20. Auflage, S.14, Abb31

An artery, cleaned from surrounding tissue

After dissection into its major layers

The final sample

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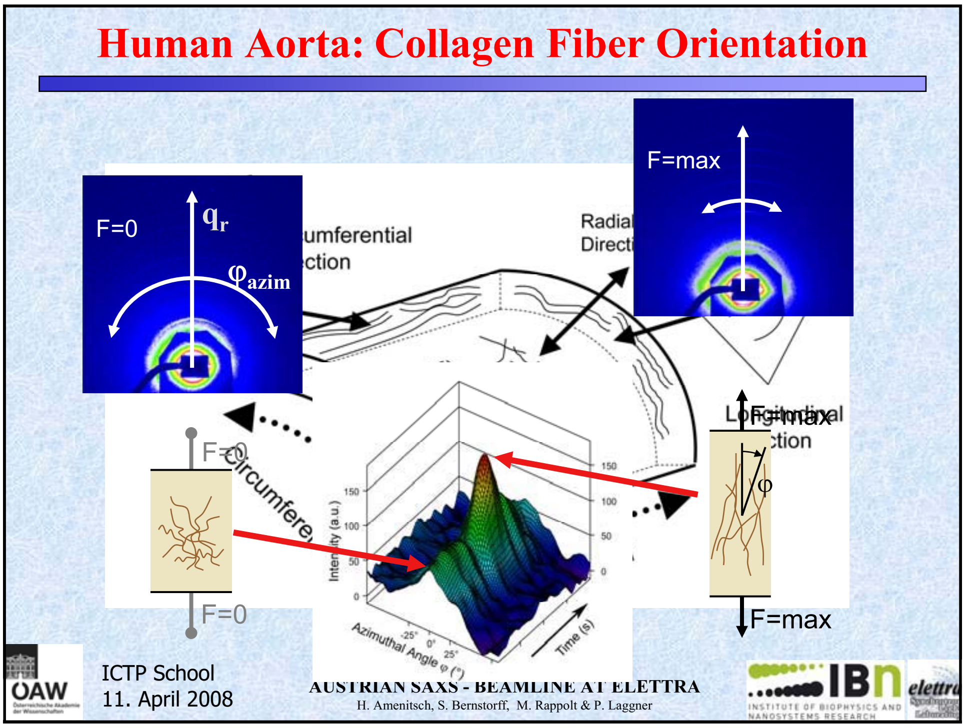
Human Aorta: Collagen Fiber Orientation
F=0
q_r
φ_{azim}
F=max
umferential
ction
Radial
Directi
Longitudinal
ction
F=max
F=0
Circumfere
F=0
φ
Intensity (a.u.)
Azimuthal Angle φ (°)
Time (s)
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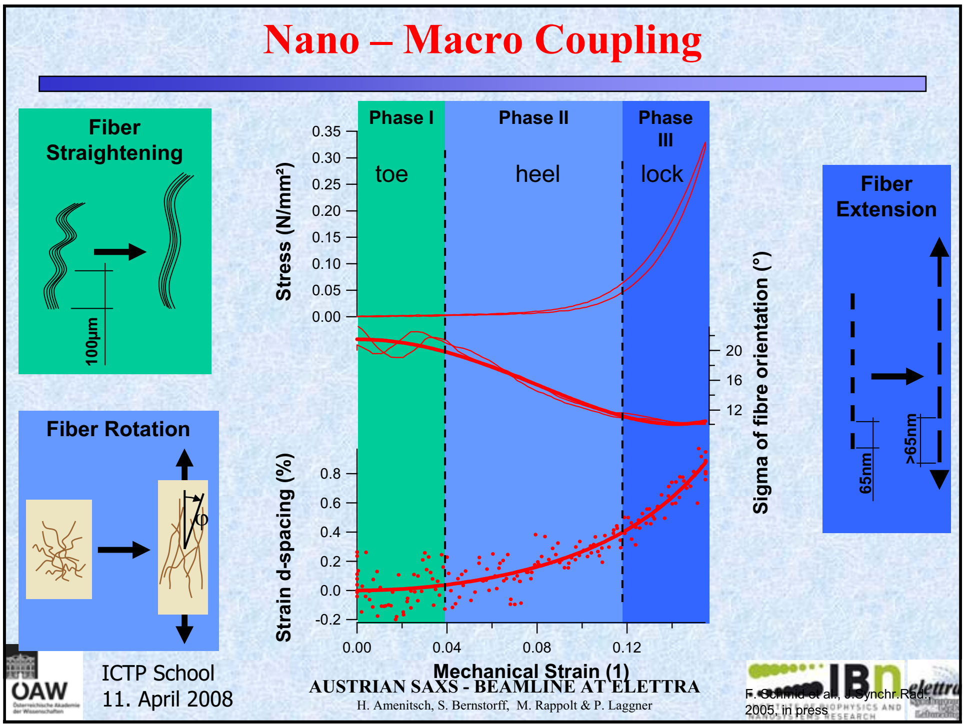
Nano – Macro Coupling
Fiber Straightening
100μm
Fiber Rotation
φ
Phase I
Phase II
Phase III
toe
heel
lock
Stress (N/mm²)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
20
16
12
Sigma of fibre orientation (°)
Strain d-spacing (%)
0.8
0.6
0.4
0.2
0.0
-0.2
0.00
0.04
0.08
0.12
Mechanical Strain (1)
Fiber Extension
65nm
>65nm
F. Schmid et al., J.Synchr.Rad. 2005, in press
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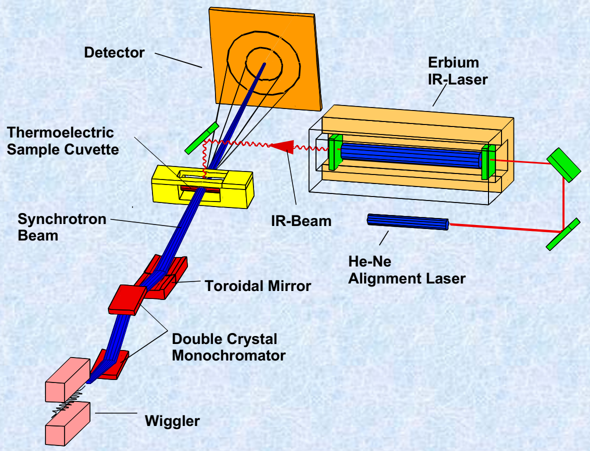
T-jumps: Phospholipid Phase Transition



Technical Data

Power: 4 J / 2 ms

Jump height: 20 K

Wavelength: 1.54 μm

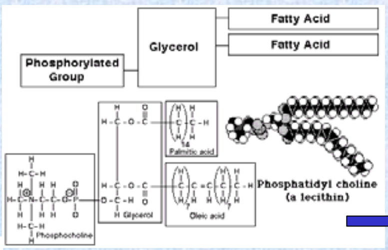
Fig. Experimental set-up of the T-jump device at the SAXS beamline (ELETTRA).

What is a biological / model membrane ?

The boundaries of cells are formed by biological membranes, the barriers that define the inside and the outside of a cell.

Phospholipids are the major components of biological membranes that form the structural matrix into which proteins are imbedded.

STRUCTURE



In aqueous solution: self assembly into, e.g., unilamellar vesicles

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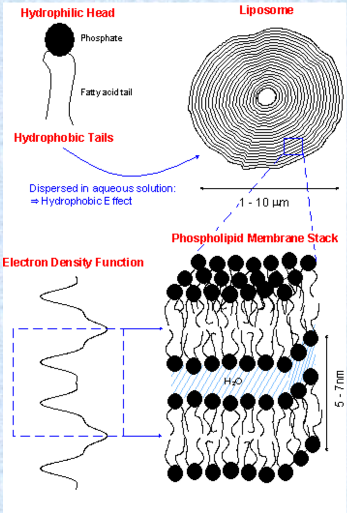
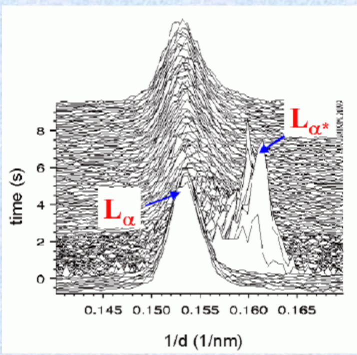
T-jumps: Phospholipid Phase Transition



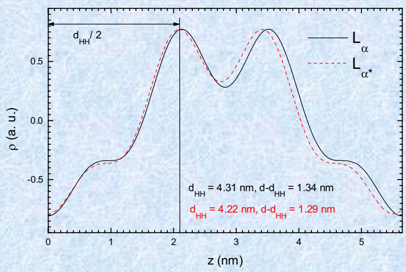
The formation of a phospholipid membrane. Phospholipids aggregate spontaneously into ordered supra-molecular structures in the presence of water. This can be explained in simple terms by the fact that phospholipids feature a hydrophilic headgroup (attracting water) and hydrophobic hydrocarbon-chains. 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.

T-jumps: Phospholipid Phase Transition



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_{α^*} (dashed line) immediately after the laser flash



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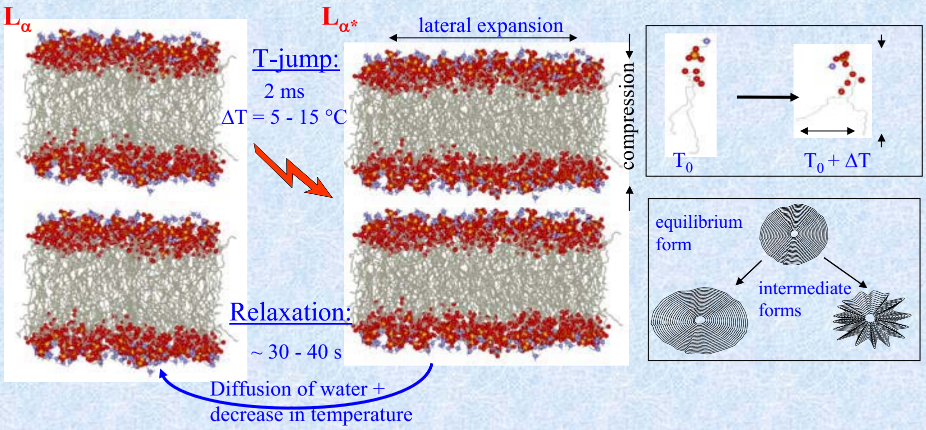
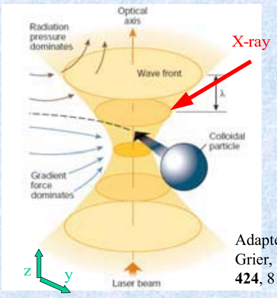
T-jumps: Phospholipid Phase Transition



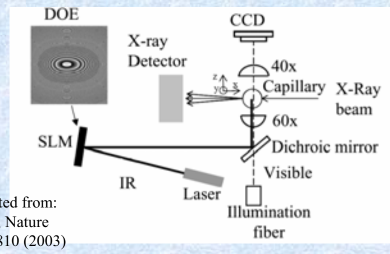
G. Pabst, M. Rappolt, H. Amenitsch, S. Bernstorff & P. Laggner, Biophys. J., (2000)

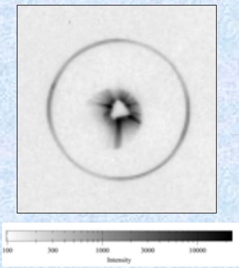
Current Developements: Optical Tweezer

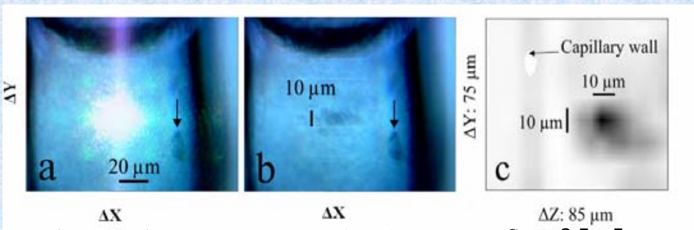


Adapted from:
Grier, Nature
424, 810 (2003)

Sketch:







POPE in Liquid crystalline Phase

Cojoc D. et al. SPIE6326, 63261M, (2006).

Step: 2.5 x 5 µm

Cojoc D. et al. APL, (2007).

Amenitsch H. et al. AIP Conference Proceedings, 879, 1287-1290,(2007).

Mesoporous Materials: Bulk MCM-41

In-situ study of the Formation of the MCM-41 Structures using liquid crystal templating mechanism
P. Ågren, J.Phys. Chem. B, (1999), 103, 5943

Aim:
Influence of the co-surfactant and its concentration on the phase behaviour of the TEOS synthesis.

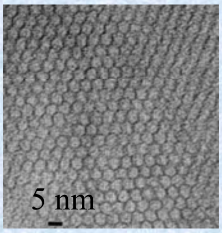
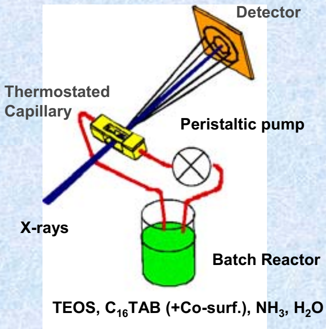


Fig. Representative electron transmission micrograph of a MCM41 structure depicting the mesoporous hexagonal nano-structure.

Experimental set-up



Industrial applications: -adsorbents
-ion-exchangers
-catalysts

Hosts for technologically advanced materials

TEOS Tetraethylorthosilicate
C_{16}TAB Hexadecyltrimethylamonium bromide

Mesoporous Materials: Bulk MCM-41

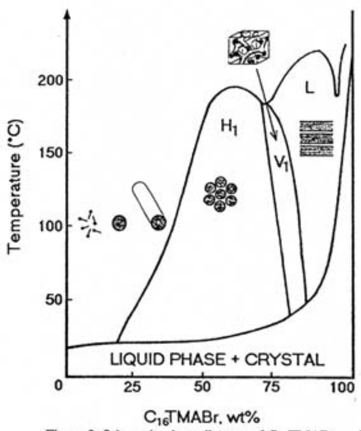


Figure 2. Schematic phase diagram of C_{16}TMABr in water [44].

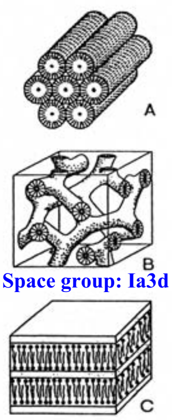


Figure 3. Schematic representation of liquid-crystal structures, (A) hexagonal, (B) bicontinuous cubic, (C) lamellar.

MCM-41

MCM-48

Space group: Ia3d

MCM-50

From: Sayari, Studies in Surface Science and Catalysis (1996), Vol 102, 1

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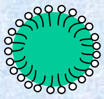
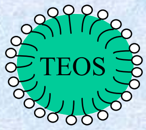
Model for synthesis of mesophases in the system: TEOS/CTAB MCM-41

1
CTAB (surfactant) + H_2O



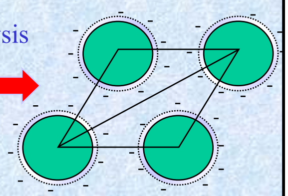
Spherical/elliptical Micells

2
Micelles + H_2O + TEOS (oil)



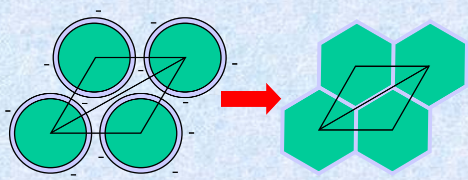
Microemulsion

3
Silanhydrolysis on the surface of the micelles



Formation of mesophases

4
condensation to poly-silicates between the aggregates of micelles

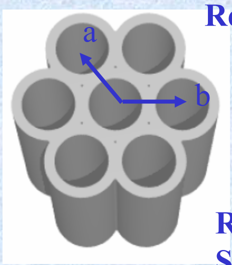


Inter-aggregat condenstation   Intra-aggregat condenstation

How to obtain real space information?

Hex:
P6m

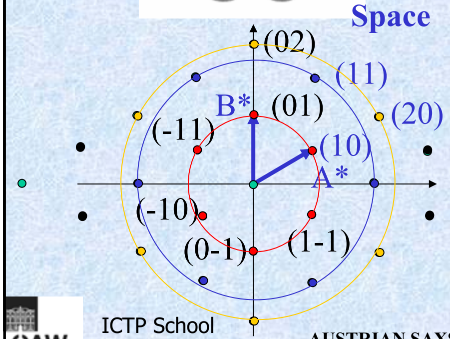
Real Space



$$\rho(\vec{r}) = \rho_{average} + \sum_{\vec{q}} A_{\vec{q}} \cdot \cos(\vec{q} \cdot \vec{r})$$

$$|\vec{q}| = \frac{4 \cdot \pi}{\sqrt{3} \cdot a} \cdot \sqrt{h^2 + k^2 + h \cdot k}$$

Reciprocal Space

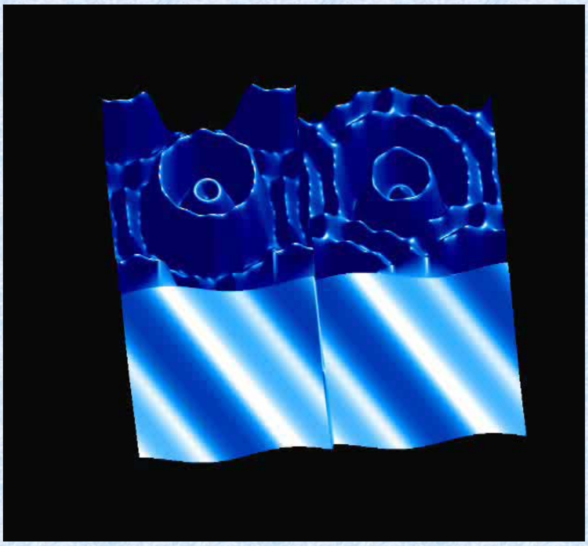


$$I(|\vec{q}|) = K \cdot M(h,k) \cdot |A_{\vec{q}}|^2 \cdot |\vec{q}|^{-2}$$

(10)	6
(11)	6
(20)	6
(21)	12

$\pm A_{\vec{q}} \Rightarrow ?$

How to obtain real space information?



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Mesoporous Materials: Bulk MCM-41

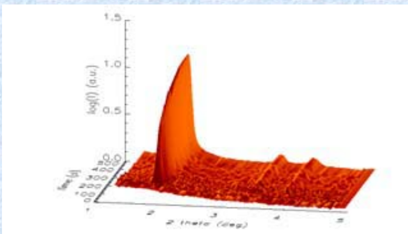
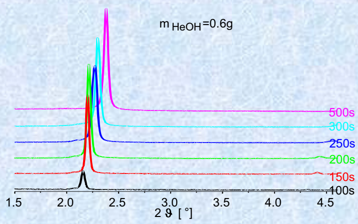
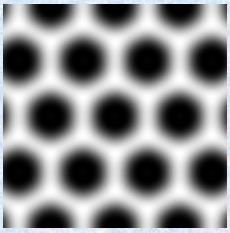


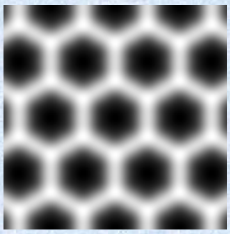
Fig. : Time-resolved diffraction pattern of the TEOS synthesis
Time resolution: 0.3 s/frame, Transition: micellar solution - ordered phases (standard synthesis: hexagonal D = 4.67 nm)



Calculated Electron Density



After 150s



Final Structure

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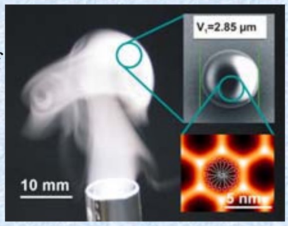
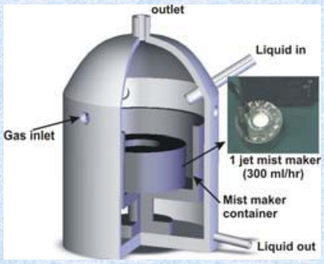
SAXS in Gasphase – Aerosol Synthesis of Mesoporous Materials

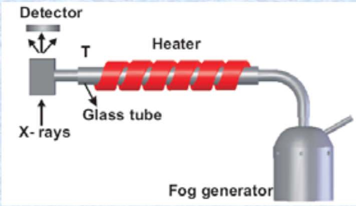
Evaproation Induced Self Assembly (EISA) J.Brinker et.al



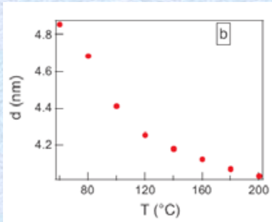
Boississiere,C., et.al. 2003. *Chemical Communications*



Aerosol Generator



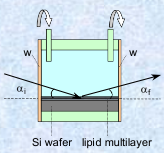
Aerosol Setup

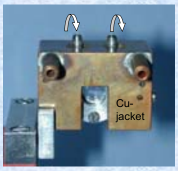


Results

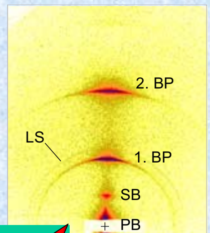
I.Shyjumon et al., Rev.Sci.Instrum., (2008)

Surface Diffraction Lipids – Surface Chemistry

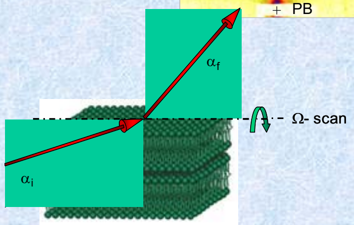


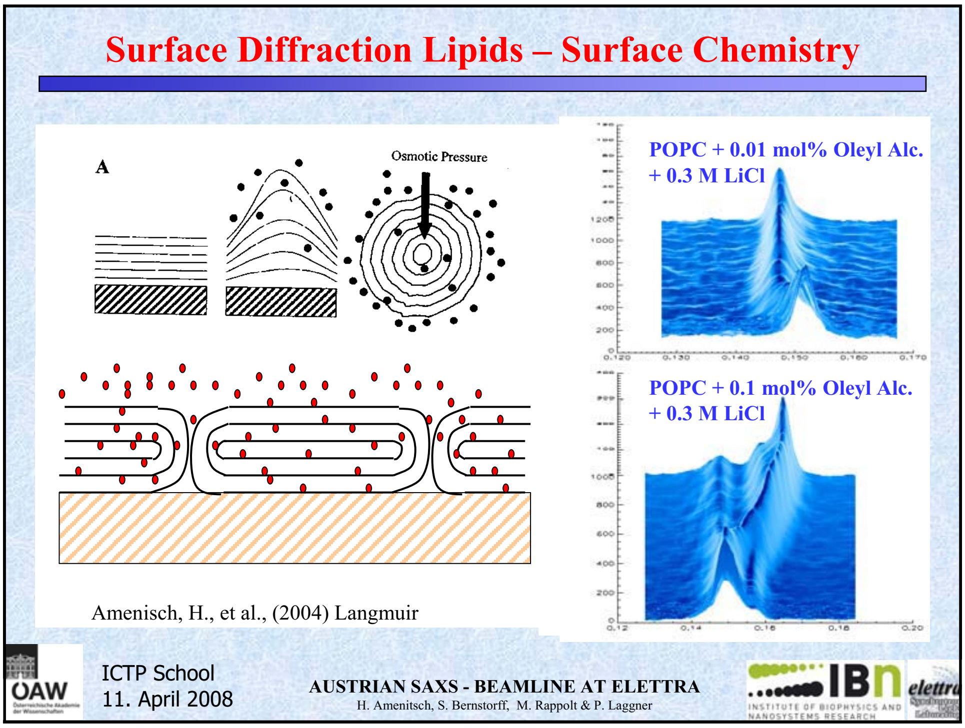


Sketch of the exp. set-up and 2D diffraction pattern of POPC and 0.5 M LiCl



Sketch and photograph of the sample cell in transmission geometry for GISAXS.




Surface Diffraction Lipids – Surface Chemistry
A
Osmotic Pressure
POPC + 0.01 mol% Oleyl Alc.
+ 0.3 M LiCl
POPC + 0.1 mol% Oleyl Alc.
+ 0.3 M LiCl
Amenisch, H., et al., (2004) Langmuir
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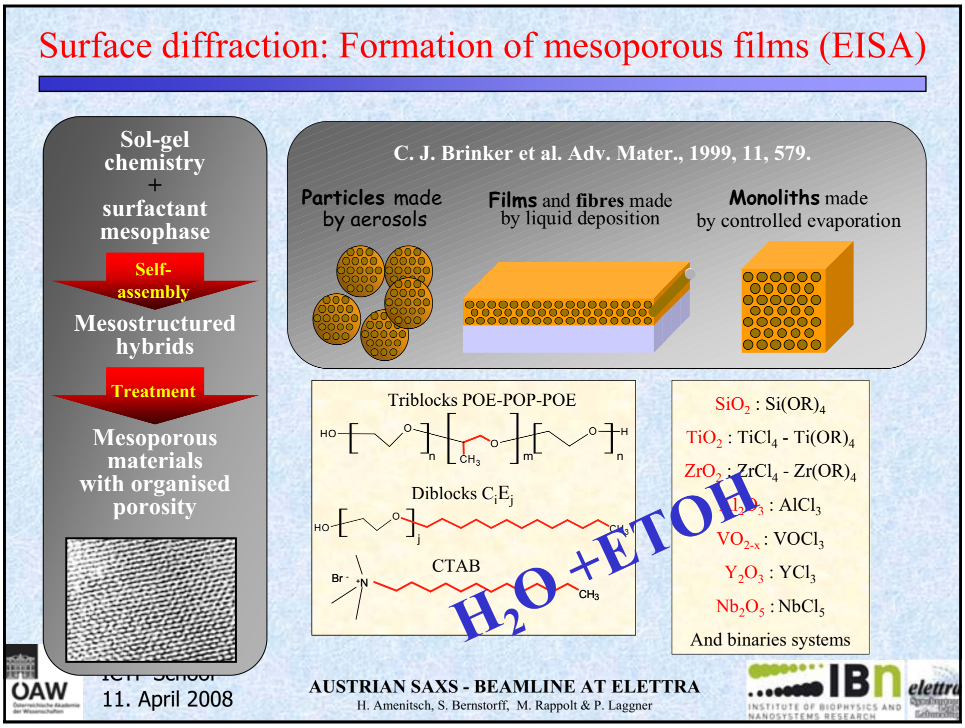
Surface diffraction: Formation of mesoporous films (EISA)
Sol-gel chemistry + surfactant mesophase
Self-assembly
Mesostructured hybrids
Treatment
Mesoporous materials with organised porosity
C. J. Brinker et al. Adv. Mater., 1999, 11, 579.
Particles made by aerosols
Films and fibres made by liquid deposition
Monoliths made by controlled evaporation
Triblocks POE-POP-POE
Diblocks C_iE_j
CTAB
H_2O +ETOH
SiO_2 : $Si(OR)_4$
TiO_2 : $TiCl_4$ - $Ti(OR)_4$
ZrO_2 : $ZrCl_4$ - $Zr(OR)_4$
Al_2O_3 : $AlCl_3$
VO_{2-x} : $VOCl_3$
Y_2O_3 : YCl_3
Nb_2O_5 : $NbCl_5$
And binaries systems
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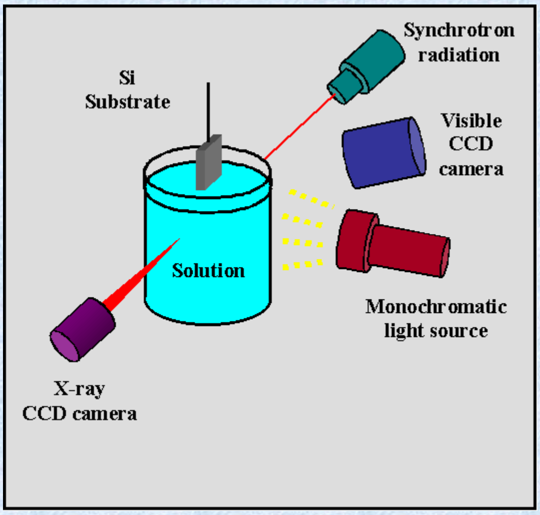
The Self-Assembly of thin films by *in situ* SAXD and interferometry

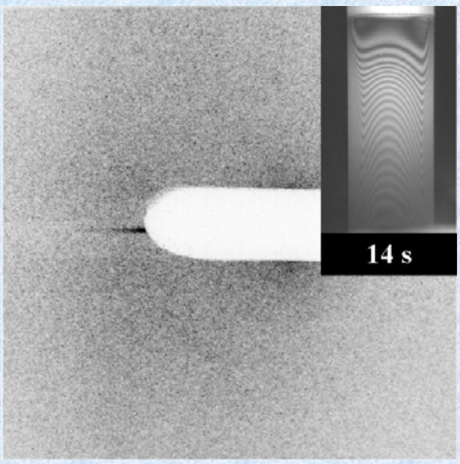
Film mesostructure

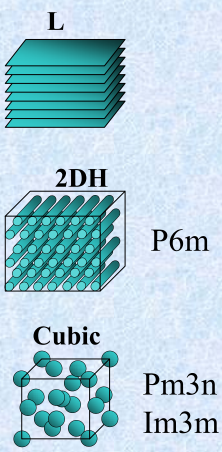


Film thickness profile

Surface diffraction: Formation of aligned mesoporous thin films

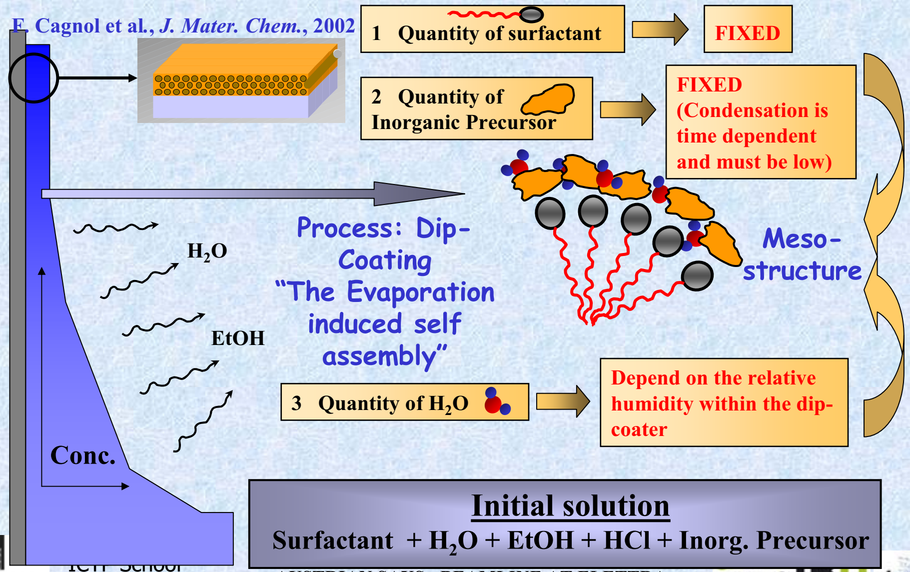
CTAB / Si = 0,18
H_2O / Si = 5
HCl / Si = 0.15
Ageing time
Relative Humidity





Grosso D, et.al., CHEMISTRY OF MATERIALS *14,* 931,(2002)
Grosso D, et.al., Nature Materials, (2004)

The Adjustable Steady State



Further Reading

Annual Reports SAXS Beamline:
- Time resolved studies
- Surface diffraction
- GISAXS

SAXS:

Books: Guinier
Glatter, Kratky
Feigin, Svergun

SAXS Conferences: every 3-4 years

Software: D.Svergun, **ATSAS**, O.Glatter, **GIFT**
J.Ivlasky, **Basic Suit**

Surface Diffraction/GISAXS

Book: M. Tolan, X-ray scattering from soft-matter thin film. Springer Tracts in Modern Physics 148, Springer, Berlin, 1999.

Paper: Rauscher M, Salditt T, Spohn H
GISAXS –Distorted Born Wave Approximation
PHYS REV B 52, 16855 (1995)

Software: Lazzari R, ISGISAXS: program, J APPL CRYSTALLOGR 35: 406, (2002)
http://www.esrf.fr/computing/scientific/joint_projects/IsGISAXS/isgisaxs.htm

Conclusion

Biology: Single Muscle Fiber Diffraction
Human Aorta
Biomembranes

Nucleation and Growth

Mesoporous Materials: Bulk, Surfaces

-Outlook: USE of NEW DETECTORS!
Use of coherence in SAXS!
(photon correlationspectroscopy, single particle imaging)
Use of new sources FEL's, new nm-beams

Think for yourself of new ways
to use SAXS and SR

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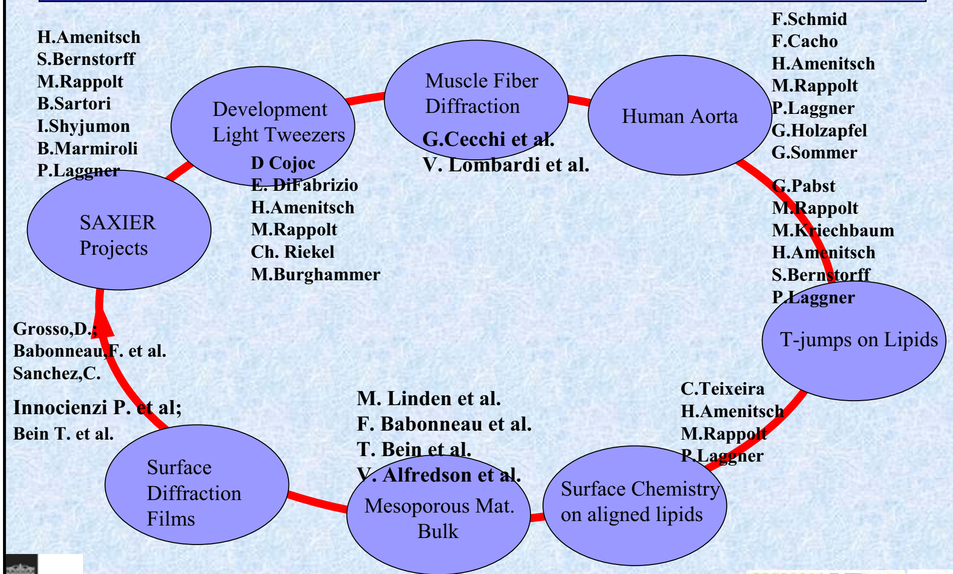
Acknowledgement



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Acknowledgement