SCIENCE ▪ PASSION ▪ TECHNOLOGY

SAXS applications in life science and material science using synchrotron

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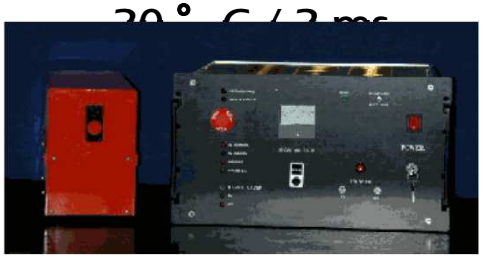
Sample Environment

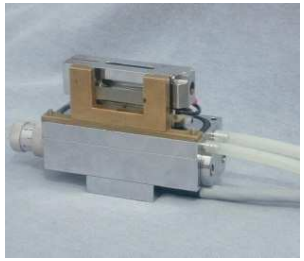
Temperature

-195 ° C to 1100 ° C

20 ° C / 3 ms



IR-Laser

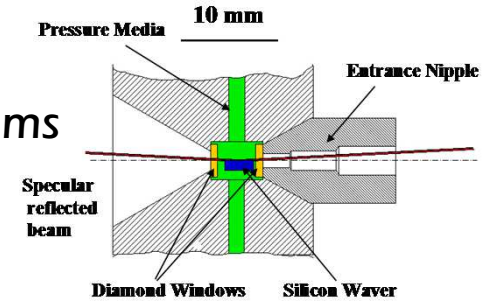


Peltier Moduls / Oxford Cryostream

Pressure

0 - 3 Kbar

3000 bar/ 10 ms



Hydrostatic HP-Cell

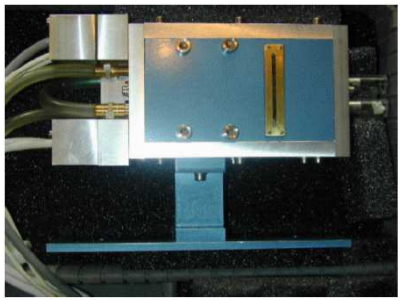
Liquids, Solids, Powders, Films, Gas-phase

Heat capacity

-40 to 150° C

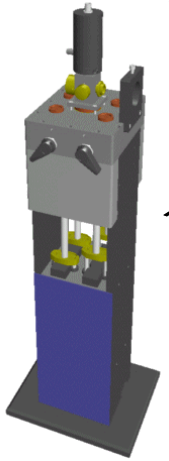
1° C /min

DSC Microcalix

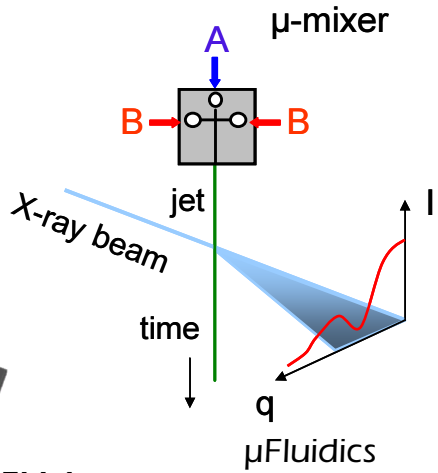


Chemical Potential

50 ms /70 µs

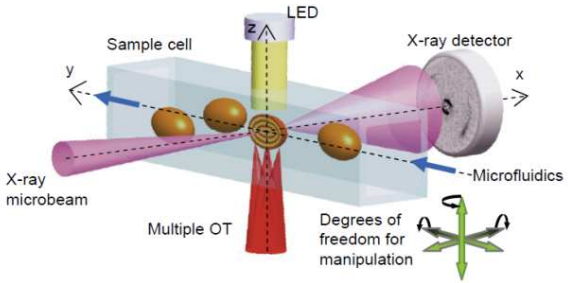


Biologic SFM-4



µFluidics

Single Particle Experiments



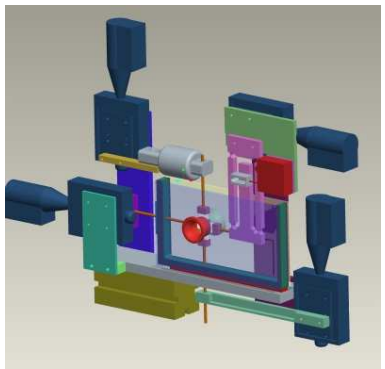
Mechanical Parameters

Force, Extension

Biaxial device

20 µs, s

physiological conditions



Simultaneous characterization: IR-Spectroscopy, UV-vis

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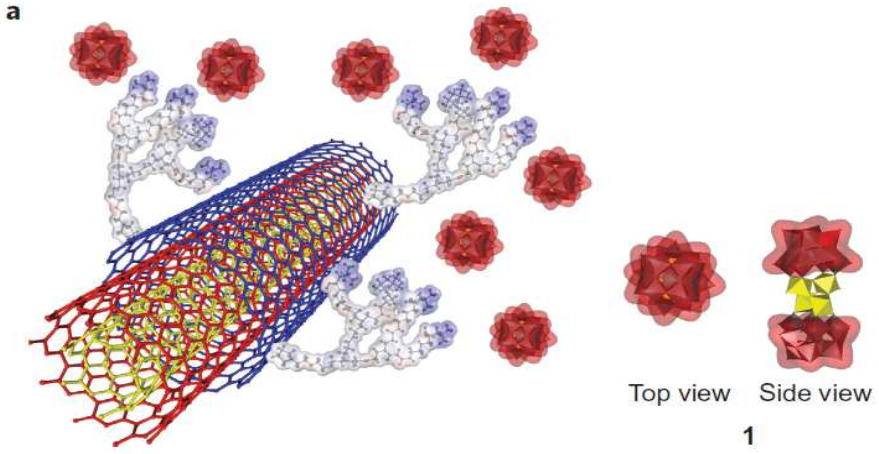
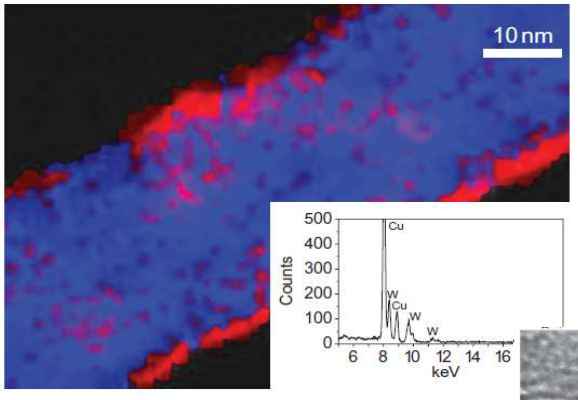
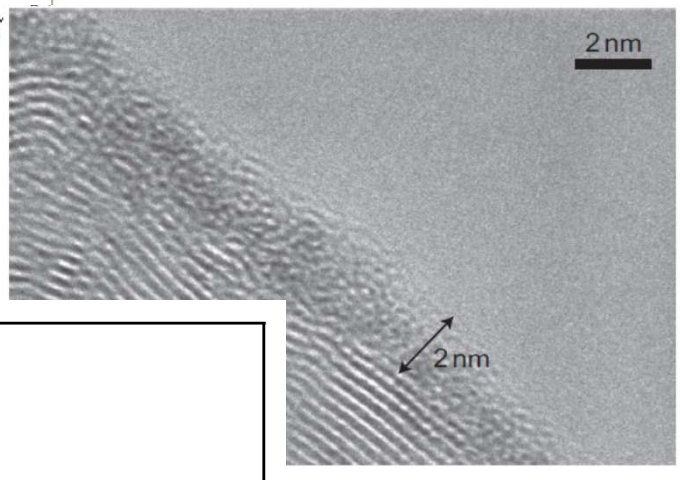
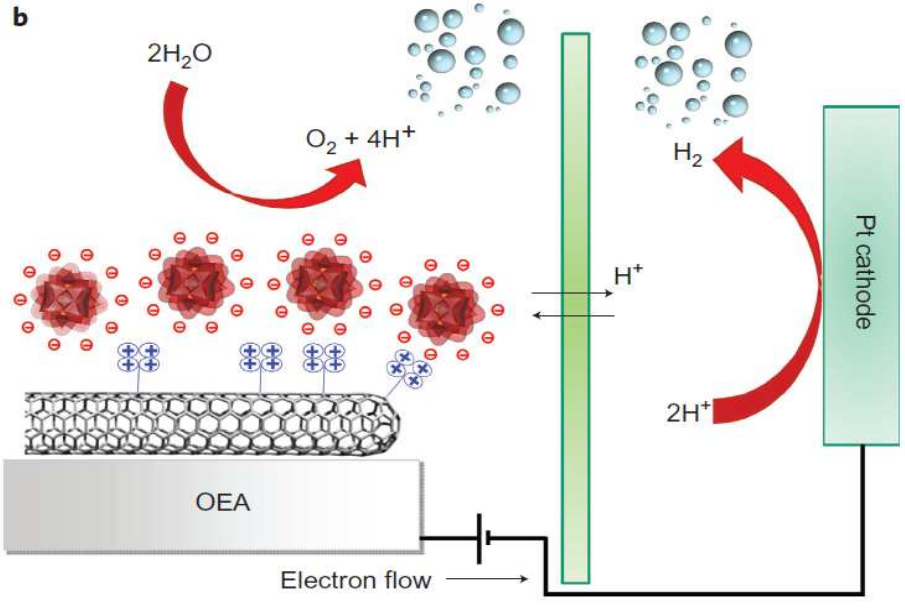
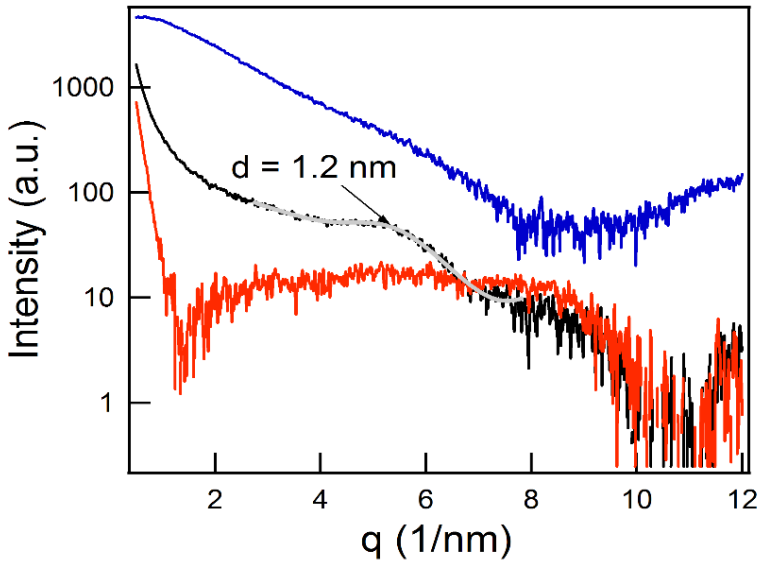
F.Toma, et al., Nature Chemistry, (2010), 10.1038/NCHEM.761











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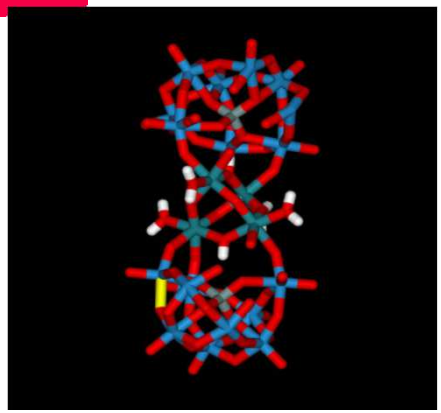
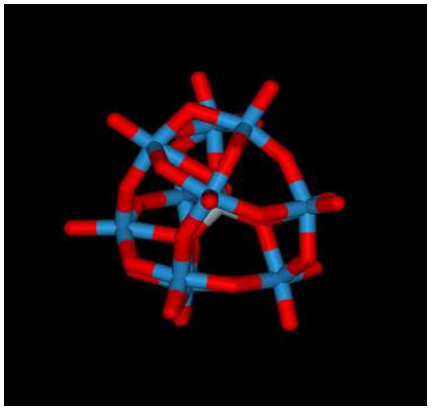
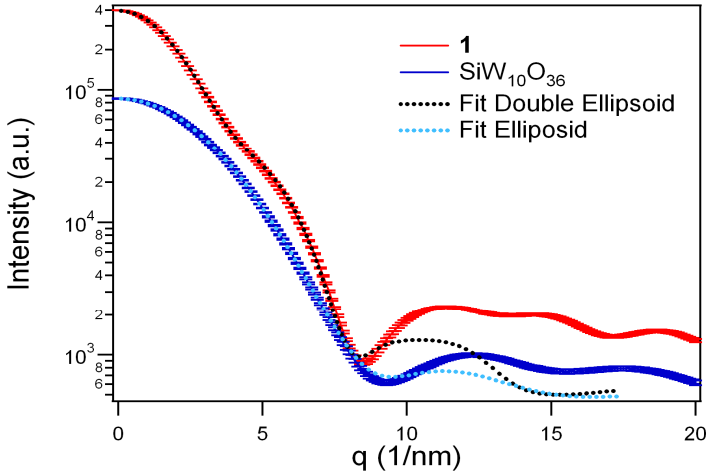


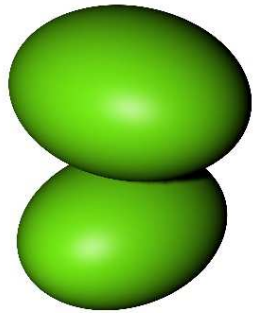
Ru4POM



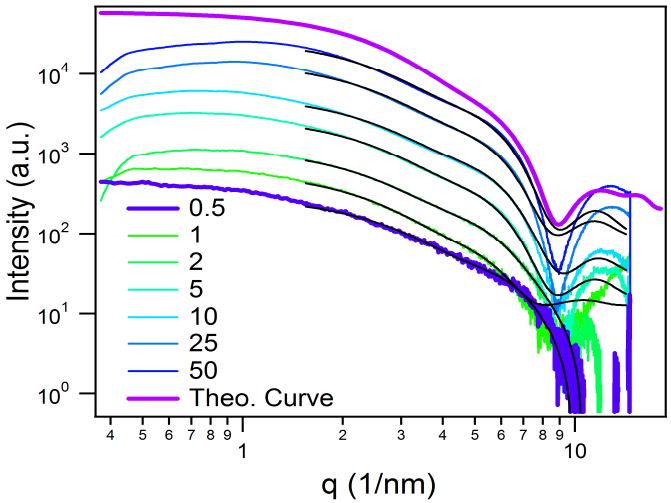
Precursor $SiW_{10}O_{36}$

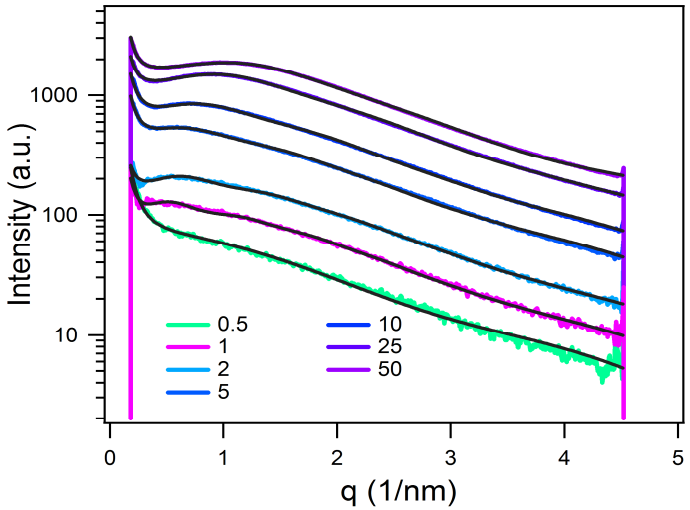


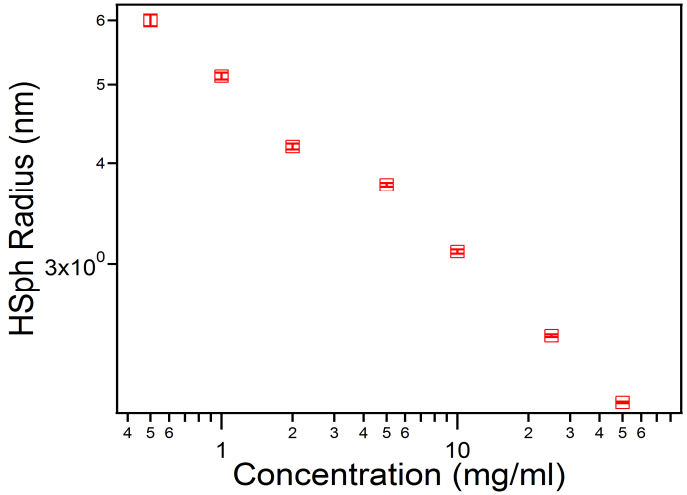
Simulation Crysol ATSAS D.Svergun



Double ellipsoid model







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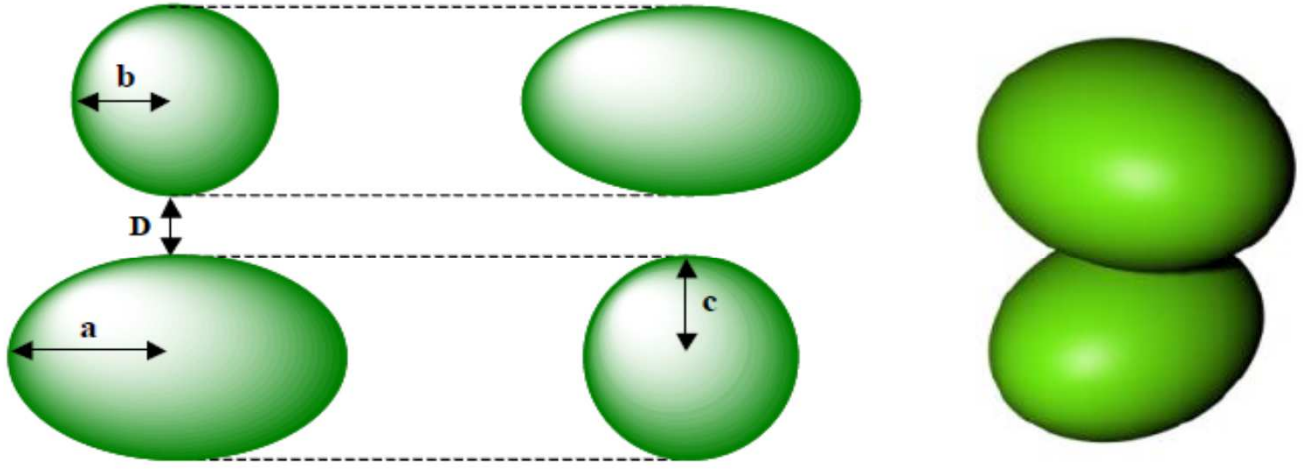

Front view Side view 3D model

$$I_{scat} = I_0 \cdot \frac{1}{\pi} \int_0^{\pi} d\beta \int_0^{\pi/2} d\alpha \cdot \sin(\alpha) \cdot \frac{1}{4} \cdot F_{2ellip}(q,a,b,c,D,\alpha,\beta)^2 \qquad (1)$$

$$F_{2ellip}(q,a,b,c,R,\alpha,\beta)^2 = \left(F_{ellip}(R_1,q) + F_{ellip}(R_2,q)\right)^2 \cdot \cos\left(q \cdot (D/2+c) \cdot \cos(\alpha)\right)^2 +$$

$$+ \left(F_{ellip}(R_1,q) - F_{ellip}(R_2,q)\right)^2 \cdot \sin\left(q \cdot (D/2+c) \cdot \cos(\alpha)\right)^2$$

$$F_{ellip}(R,q) = 3 \cdot \frac{\sin(q \cdot R) - q \cdot R \cdot \cos(q \cdot R)}{(q \cdot R)^3}$$

$$R_1 = \sqrt{\left(a^2 \cdot \sin(\beta)^2 + b^2 \cdot \cos(\beta)^2\right) \cdot \sin(\alpha)^2 + c^2 \cdot \cos(\alpha)^2}$$

$$R_2 = \sqrt{\left(b^2 \cdot \sin(\beta)^2 + a^2 \cdot \cos(\beta)^2\right) \cdot \sin(\alpha)^2 + c^2 \cdot \cos(\alpha)^2}$$

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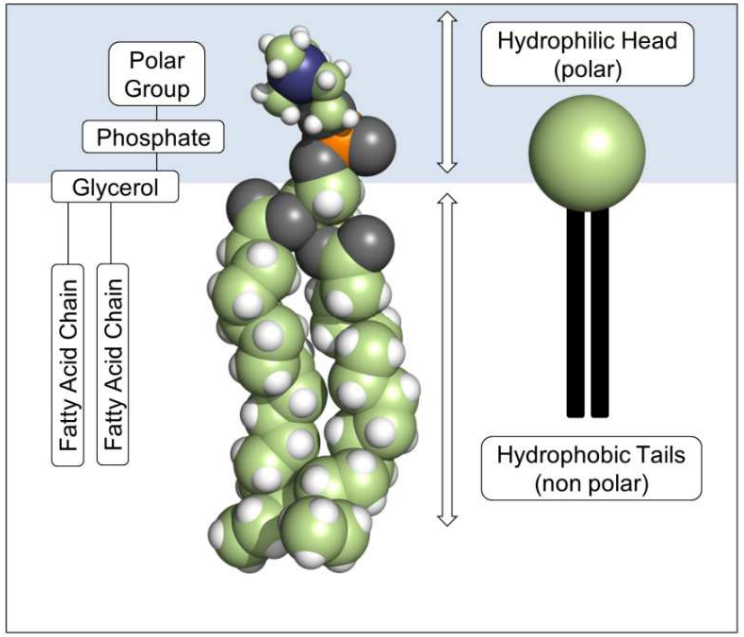
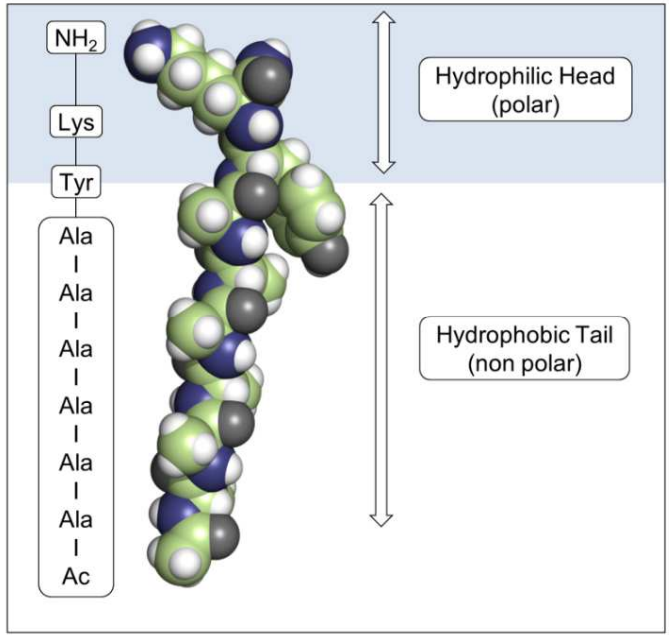
Amphiphilic designer-peptides

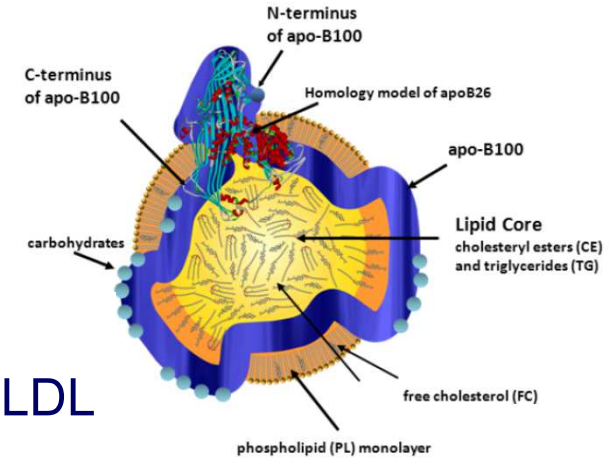


a phospholipid

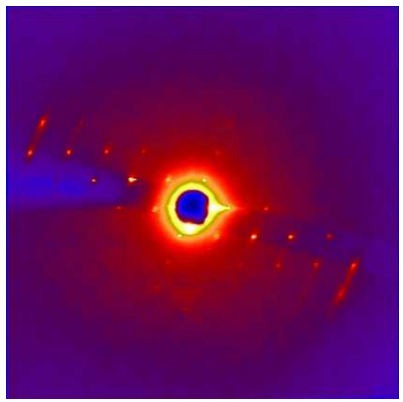


an amphiphilic designer-peptide

a6yk



LDL



Gazit, E. *Chem. Soc. ReV.* **2007**
Cherny, I.; et al., *Angew. Chem., Int. Ed.* **2008**

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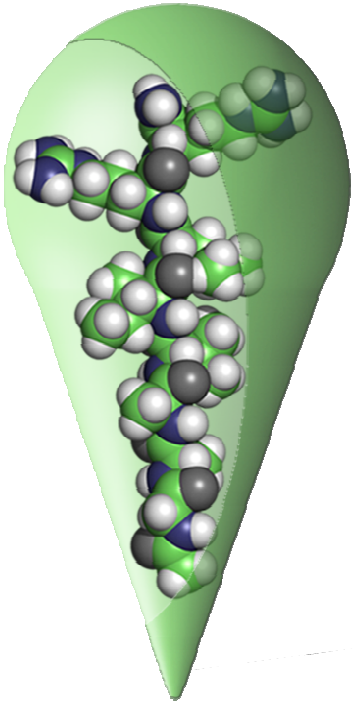
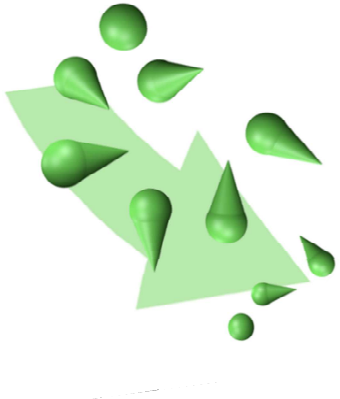
Self-assembly

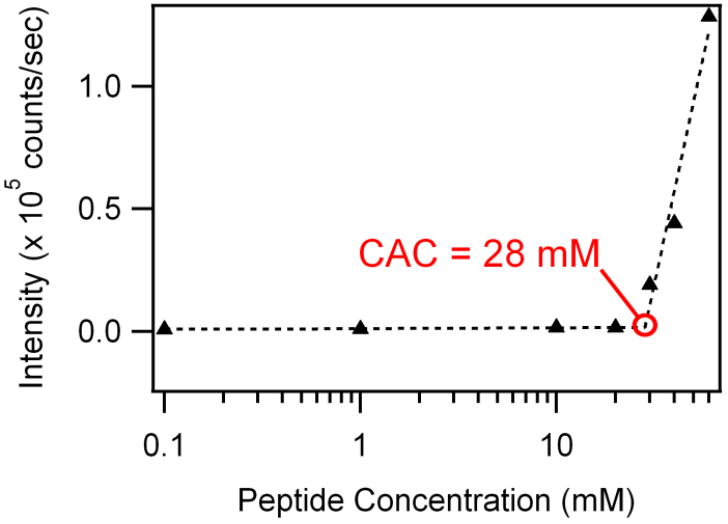




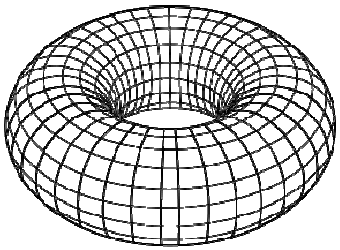
Spherical Micelles

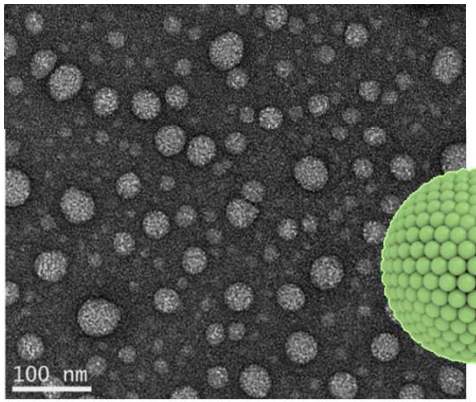
Vesicles

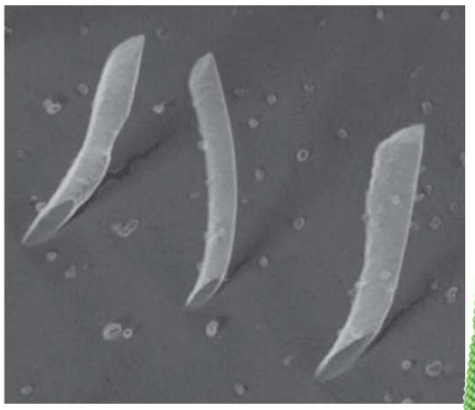
Bilayers

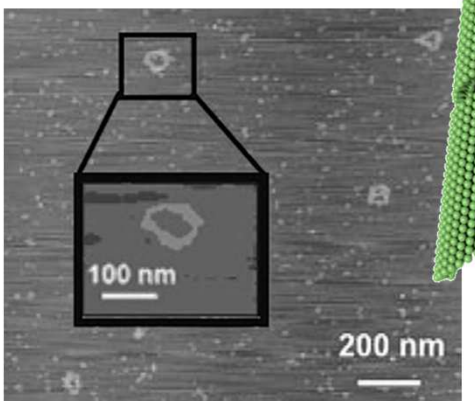


Self-Assembly









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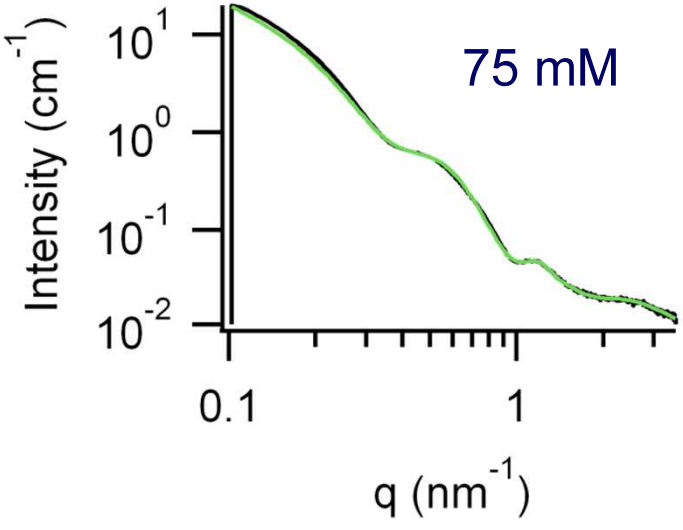
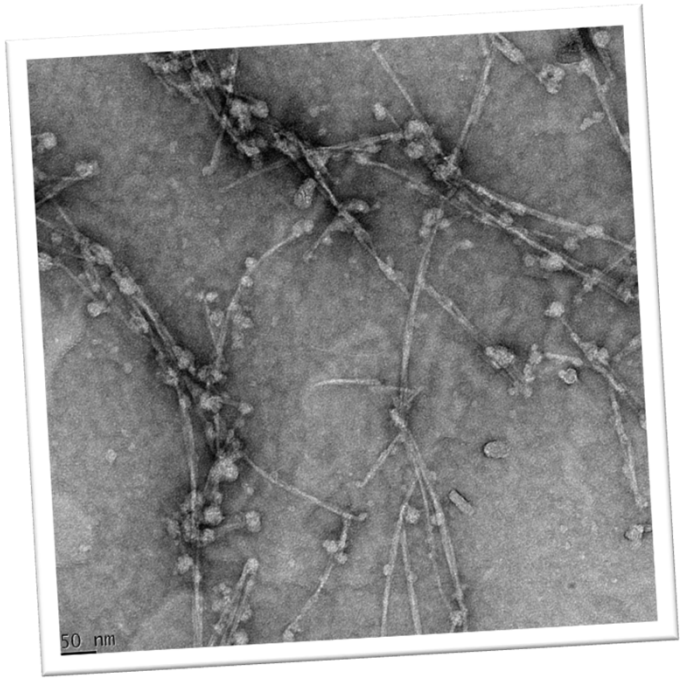
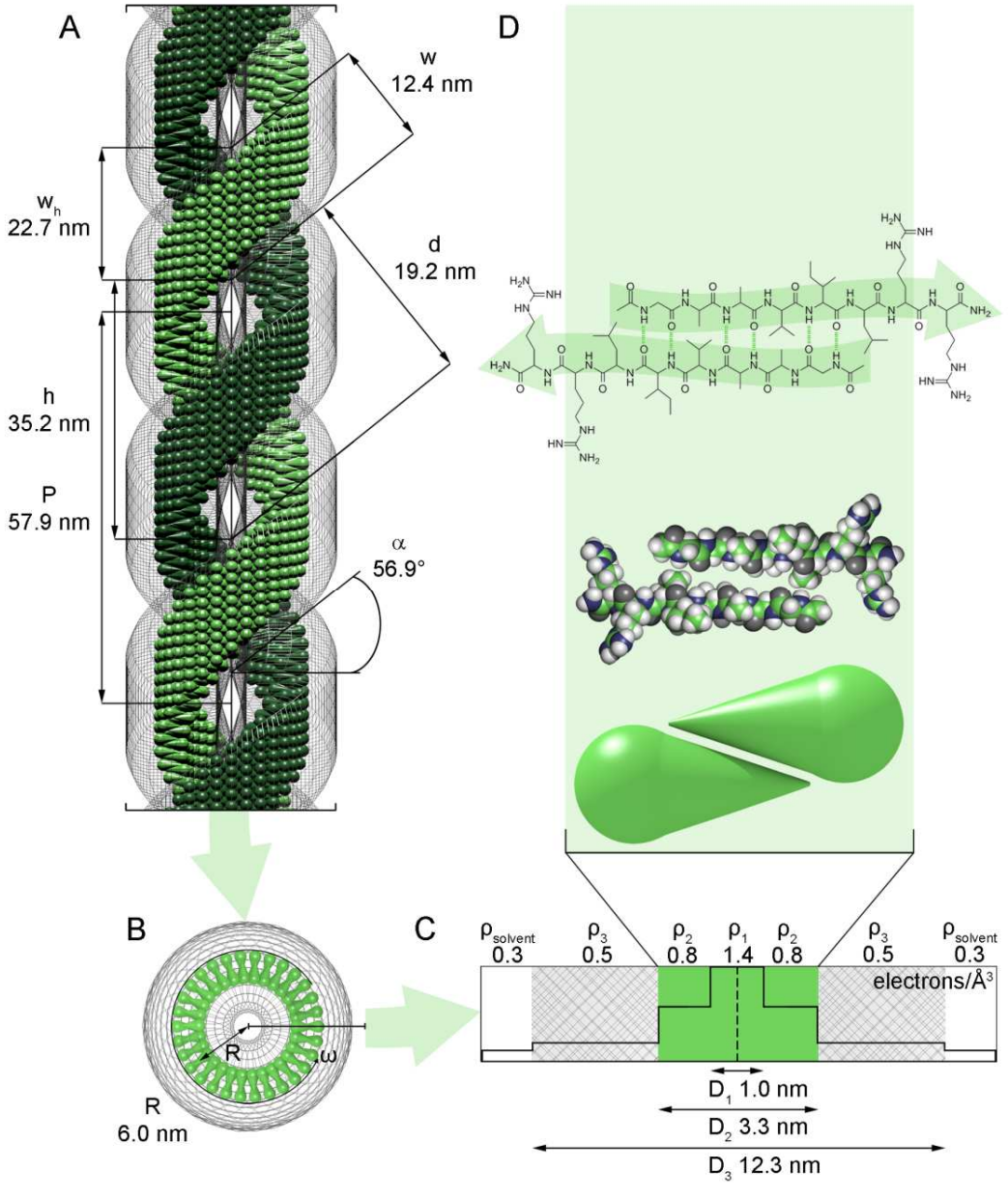
It‘s a double helix!







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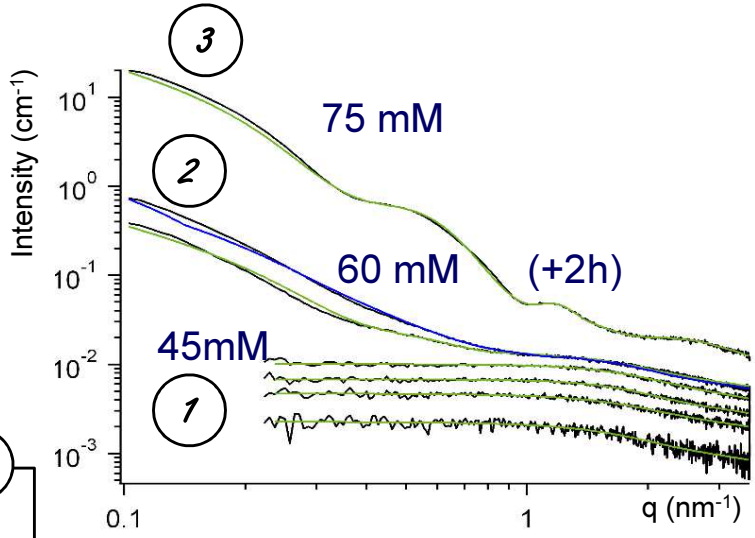
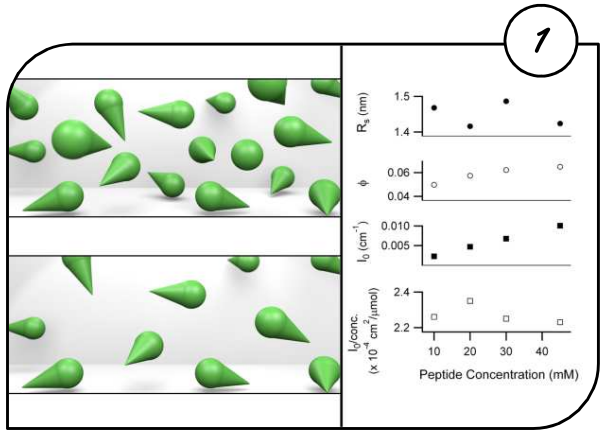
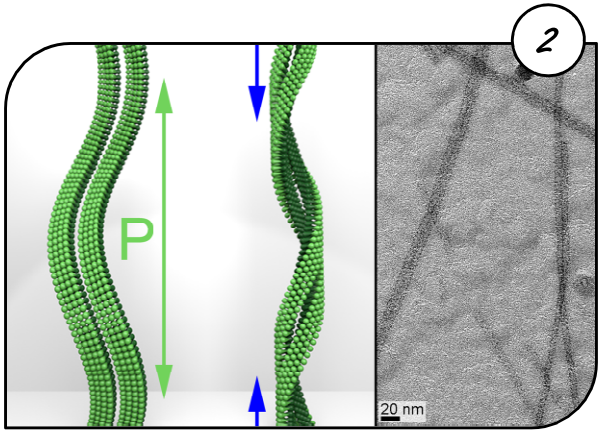
The self-assembly process

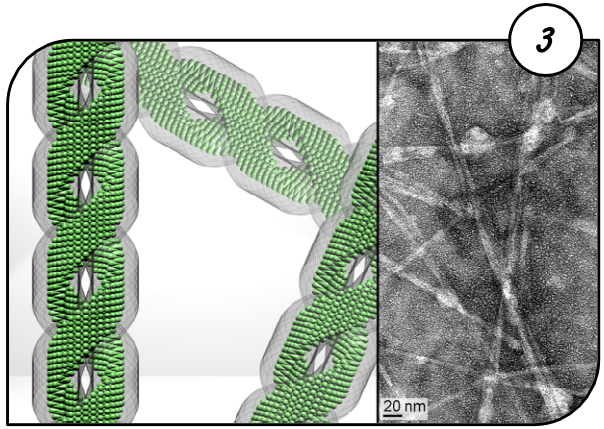




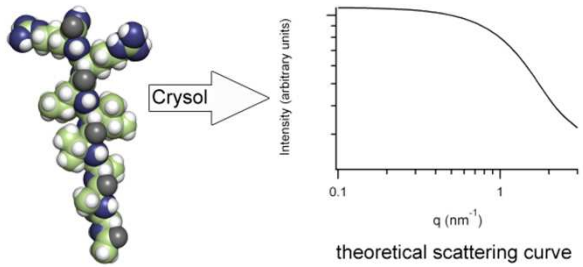
10-45 mM Monomers



60m M 3-layered single helical tape



75 mM 3-layered double helical tape



Pontoni D. et al., J. Chem, Phys, 2003
Svergun D.I. et al., J. Appl. Cryst., 1995

K.Kronmüller et al. sub to JACS (2013)

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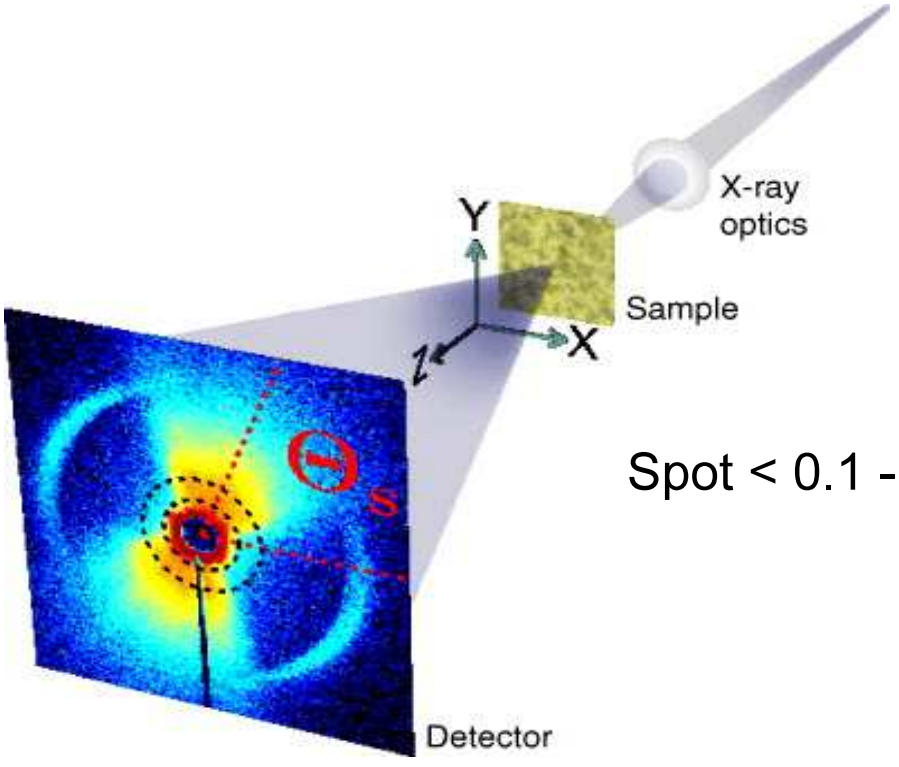
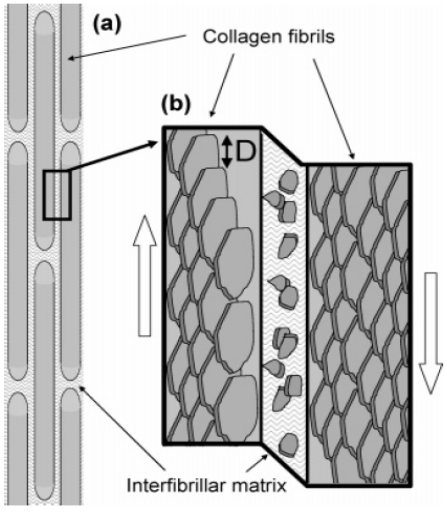
Scanning SAXS - Biomaterials



Spot < 0.1 -1 µm

Bone

P.Fratzl

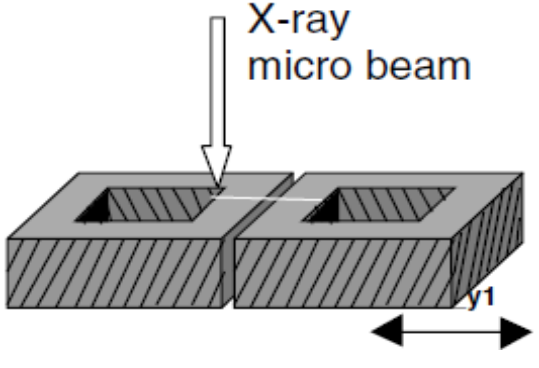


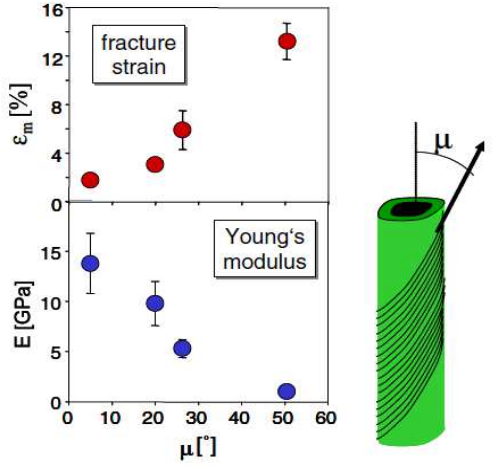
Wood

P.Fratzl

Pic. O.Bunk, et al. New J. Phys. **11** (2009) 123016

Silica-Sponges, Shells,
Tooth, Lobster, Worms,
Starch, Eyes……,





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Scanning SAXS-Integral Parameters

Integated Intensity

$$I = \int_{q\,\min}^{q\,\max} \int_{\chi_1}^{\chi_2} I(q, \chi) q^2 \, \mathrm{d}q \, \mathrm{d}\chi.$$

Porod Invariant

$$\tilde{I} = \int I(\mathbf{q}) \, \mathrm{d}^3 q = \int_0^{\infty} q^2 \, \mathrm{d}q \int_0^{\pi} \sin\psi \, \mathrm{d}\psi \int_0^{2\pi} I(q, \psi, \chi) \, \mathrm{d}\chi$$

$$= 2\pi^2 \varphi_1 \varphi_2 (\Delta\rho)^2,$$

T-Parameter

$$T = \frac{4}{\pi P} \int_0^{\infty} I(q) q^2 \, \mathrm{d}q = 4 \frac{\varphi_1 \varphi_2}{\sigma}$$

Porod (1951,1952)

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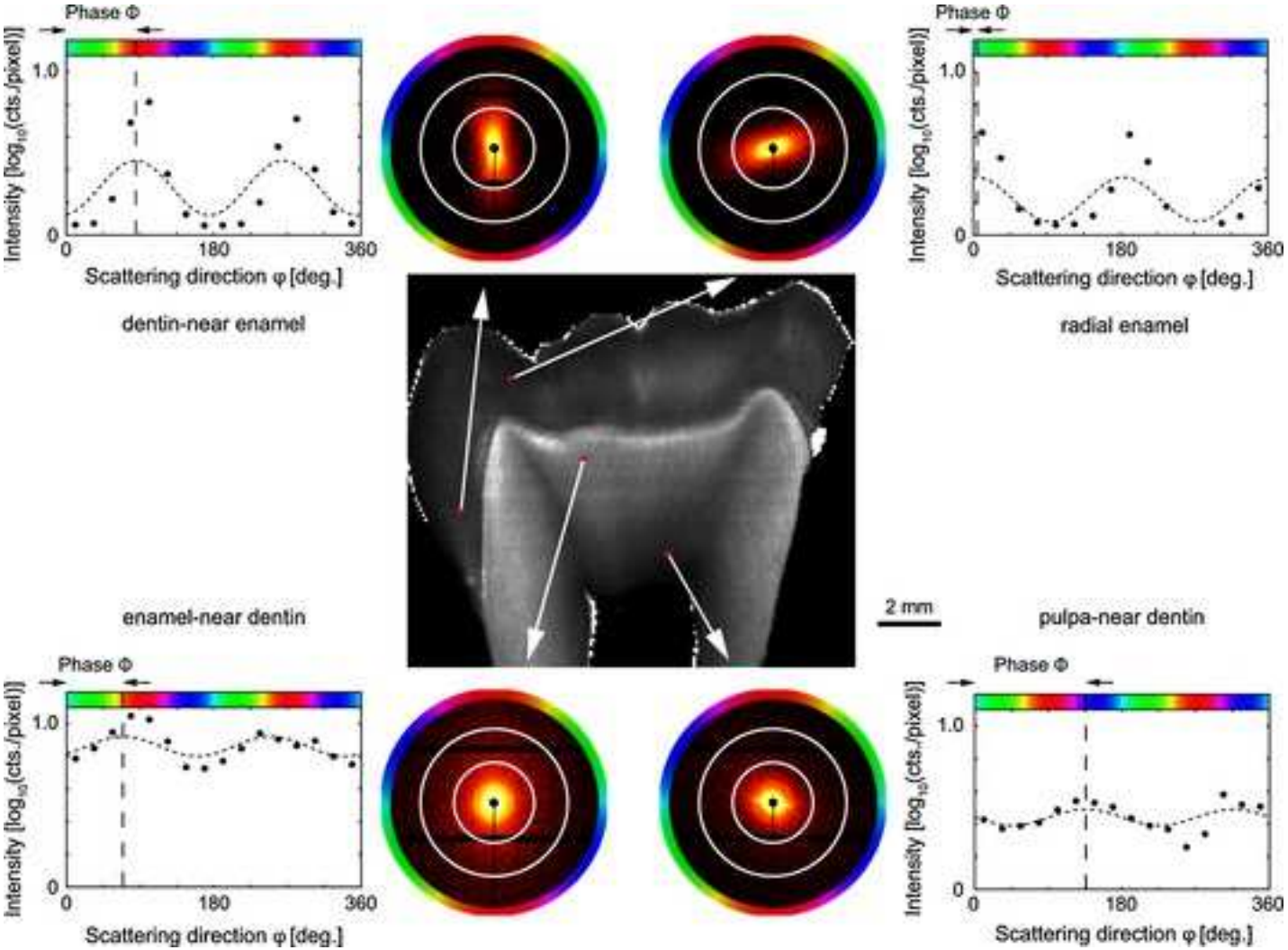
Scanning SAXS - Orientation



Geiser S. et al., Biointerphases
Journal for the Quantitative Biological Interface Data, 2012

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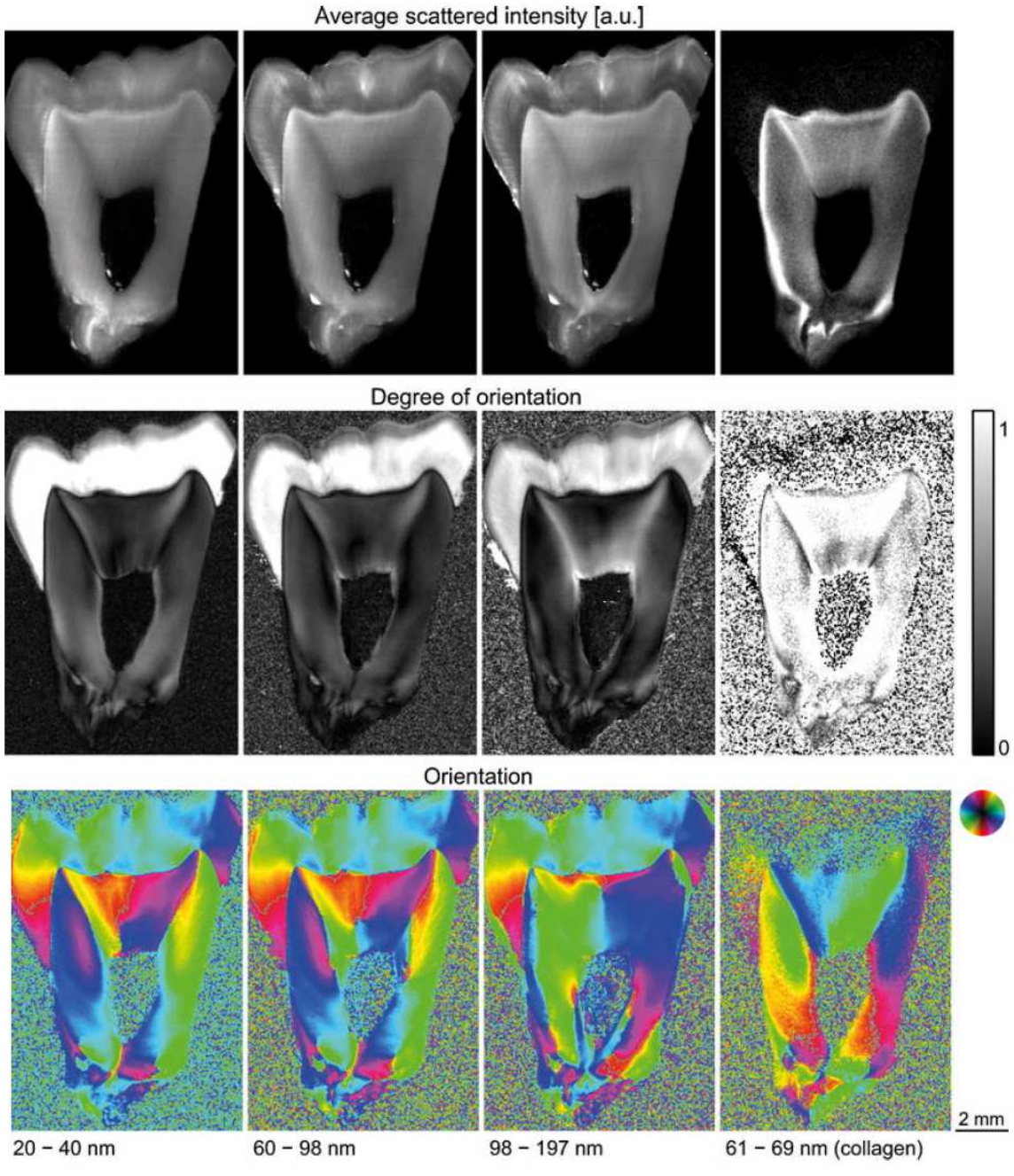
Scanning SAXS - Tooth



Biomechanics on Human Aorta: Motivation

Macroscopic response ⟷ **Model** ⟷ Micro- and nano-structure

Pathology, Clinics

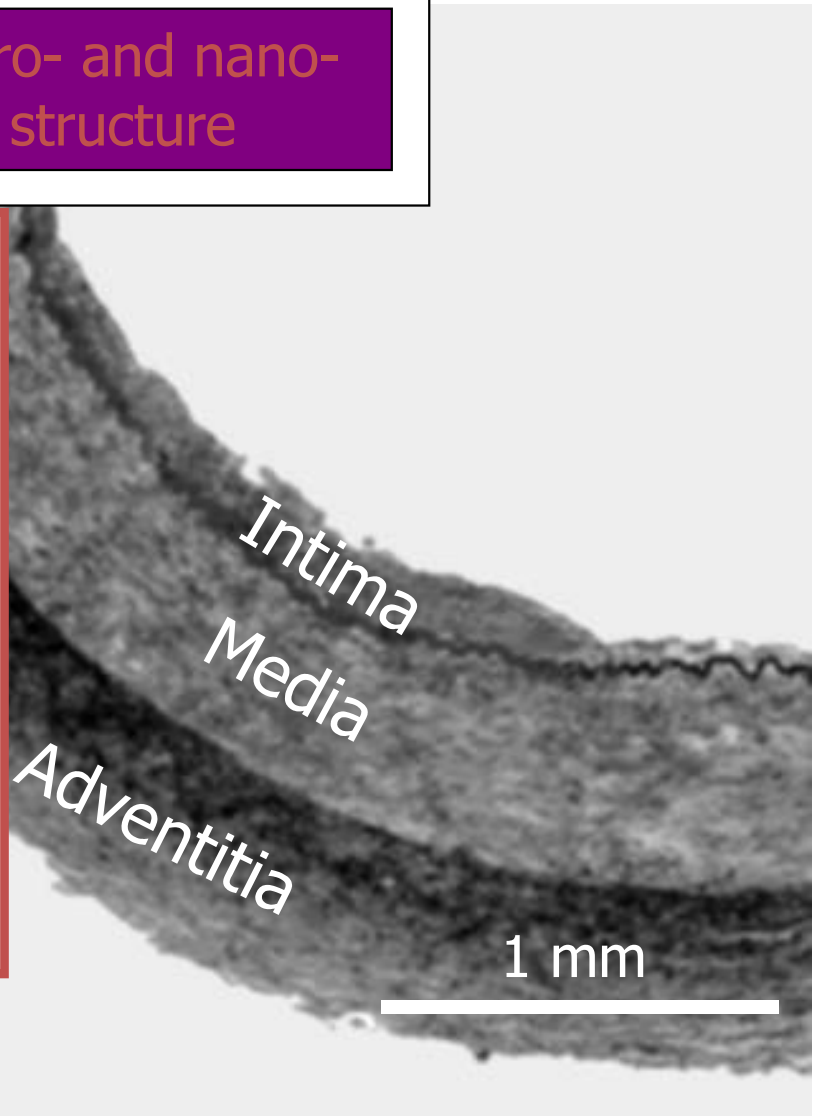
Characterization of vascular disease
Effects of aging
Identification of therapeutic targets (Balloon Angioplasty)

Graft design

Biomimetic materials

Functional tissue engineering

Mechanobiology



Cross section of a human artery

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Human Aorta: Mechanical Parameters

Macroscopic

geometric deformation

stress

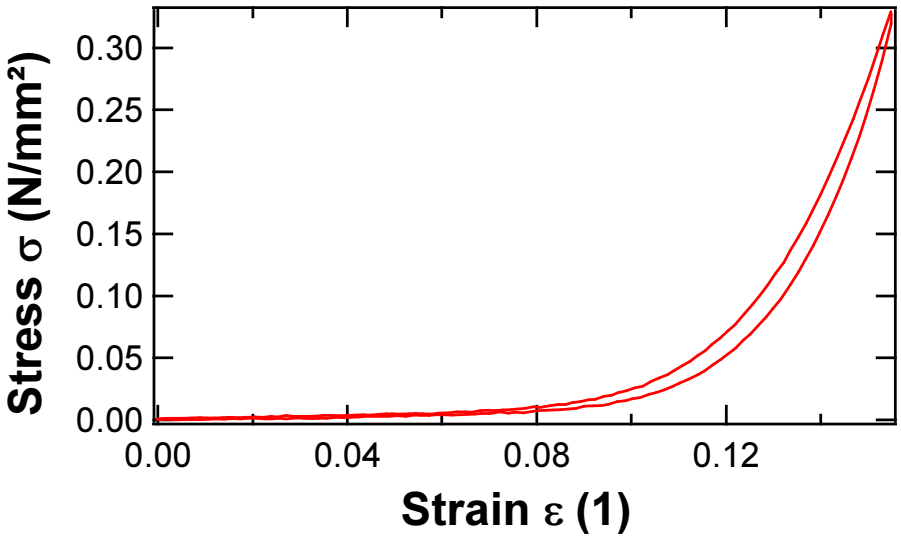
strain

Nanoscopic

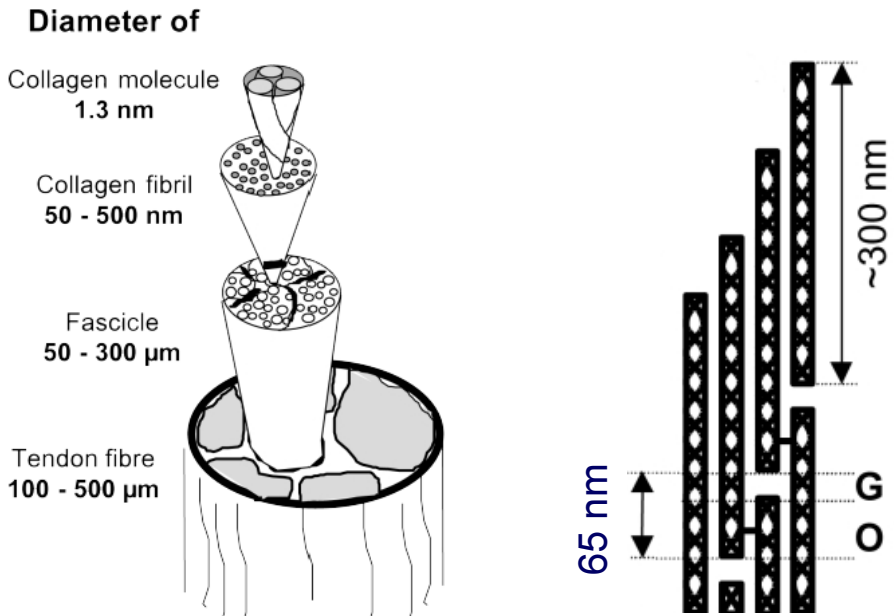
fiber – matrix composite

fiber alignment

fiber strain



Collagen - The most abundant protein



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

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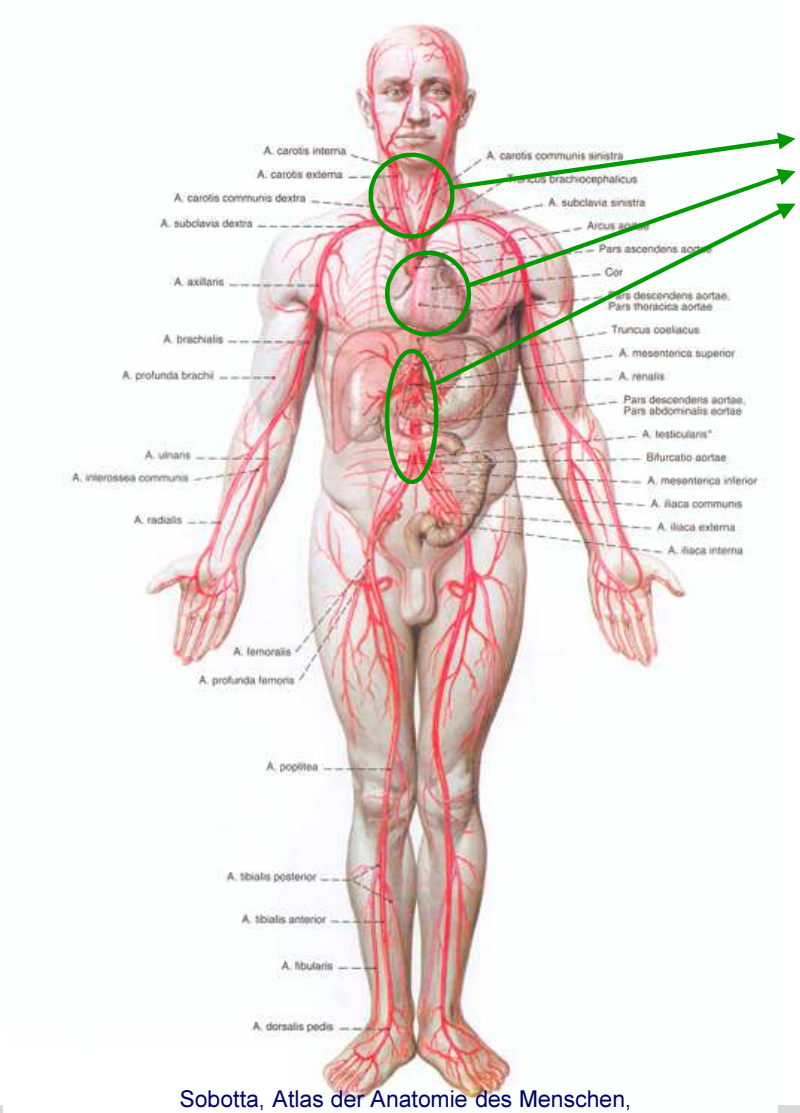
Human Aorta: Sample Preparation



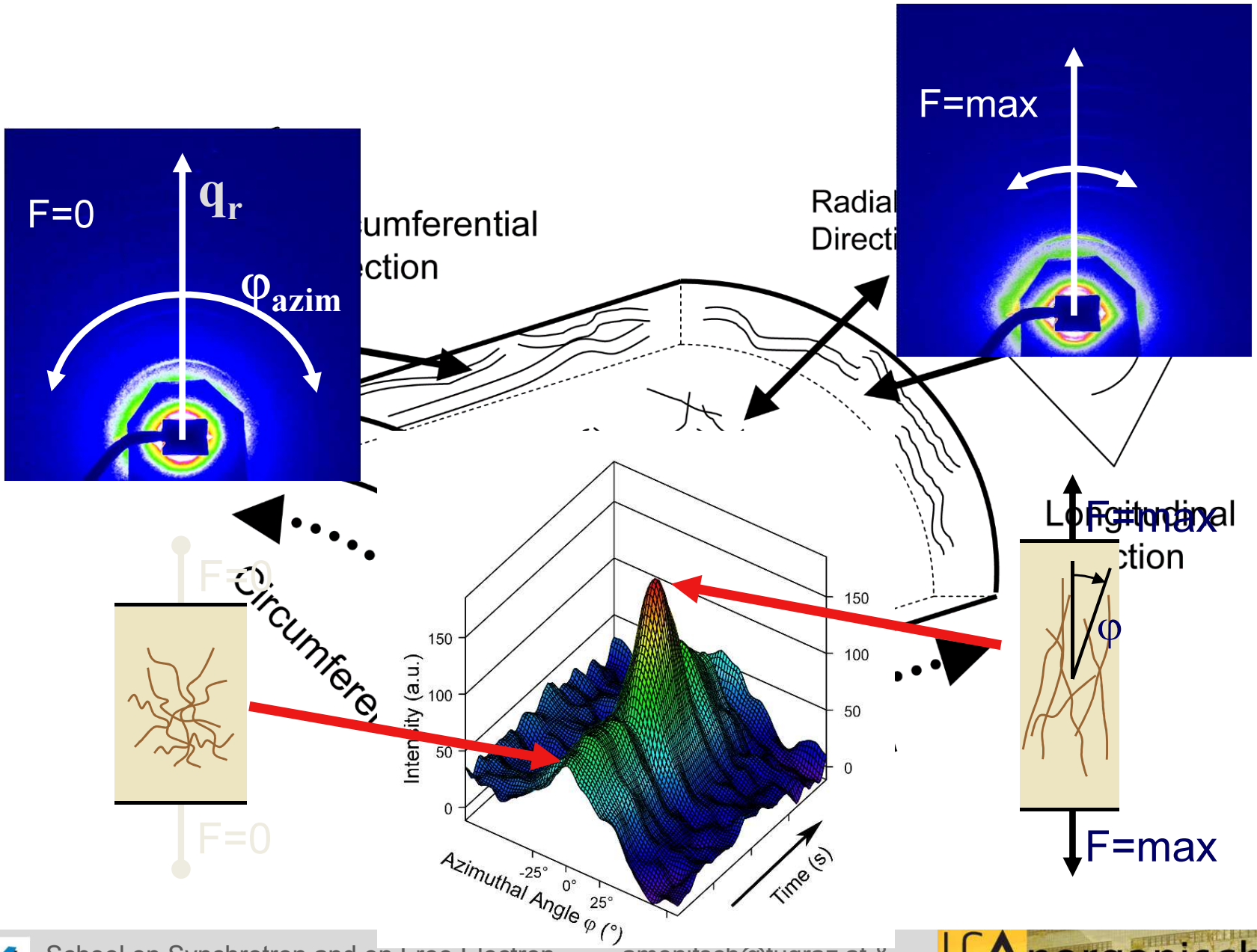
Sobotta, Atlas der Anatomie des Menschen, Band 2, 20. Auflage, S.14, Abb31

An artery, cleaned from surrounding tissue

After dissection into its major layers

The final sample

Human Aorta: Collagen Fiber Orientation



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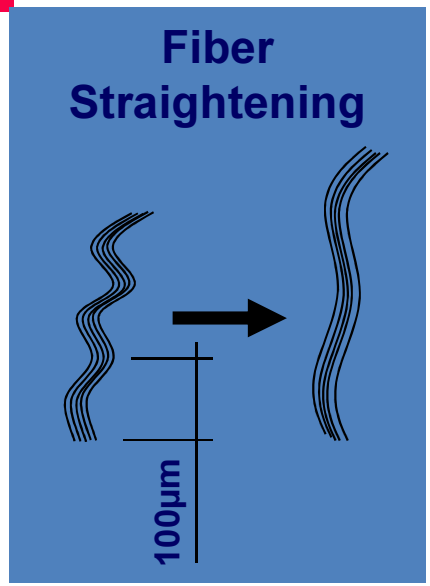
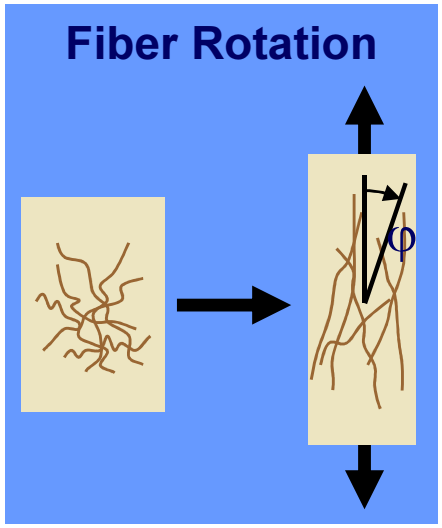
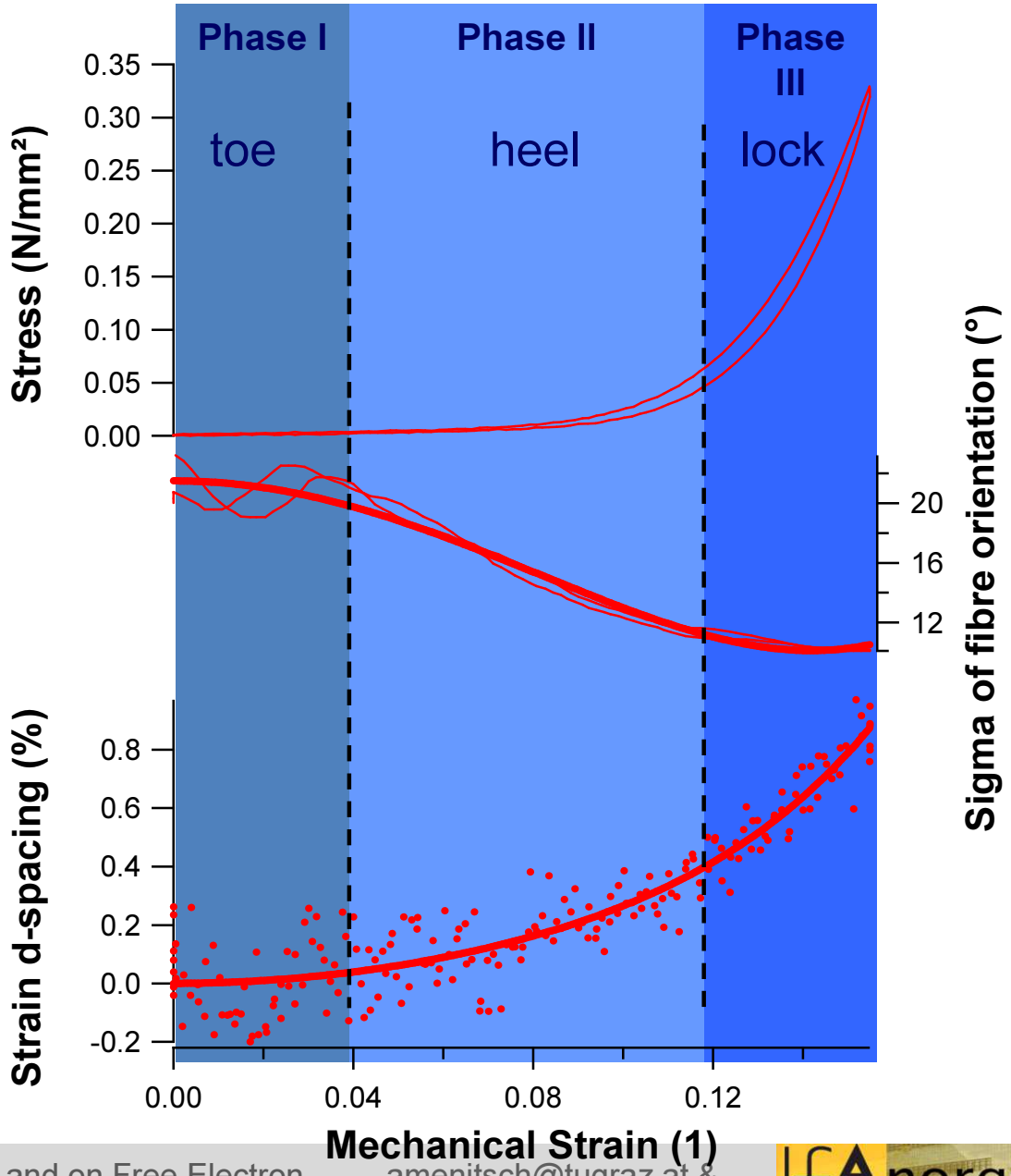
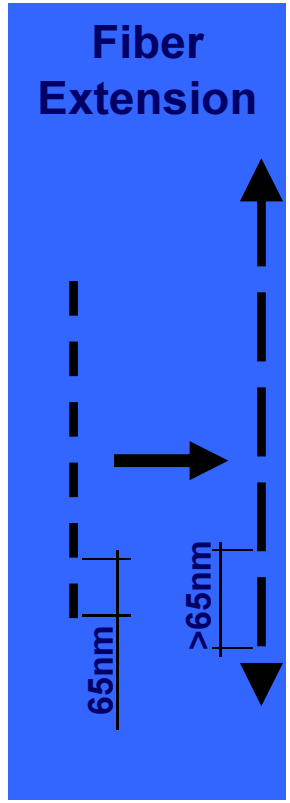
Nano – Macro Coupling









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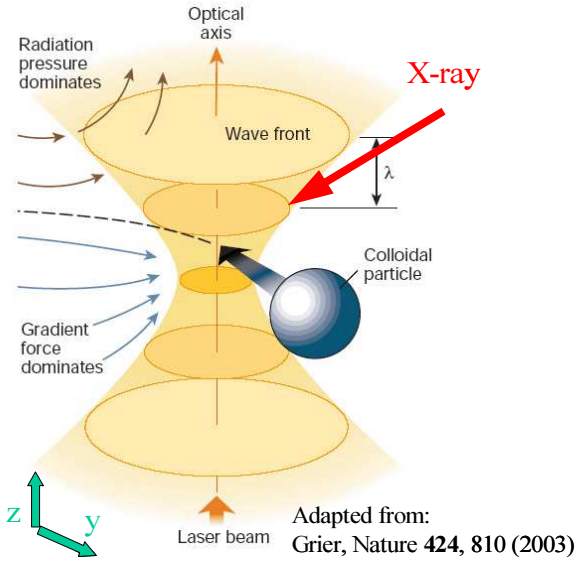
F. Schmid et al., J.Synchr.Rad., 2016, in press

Motivation for combined approach: LT & SWAXS

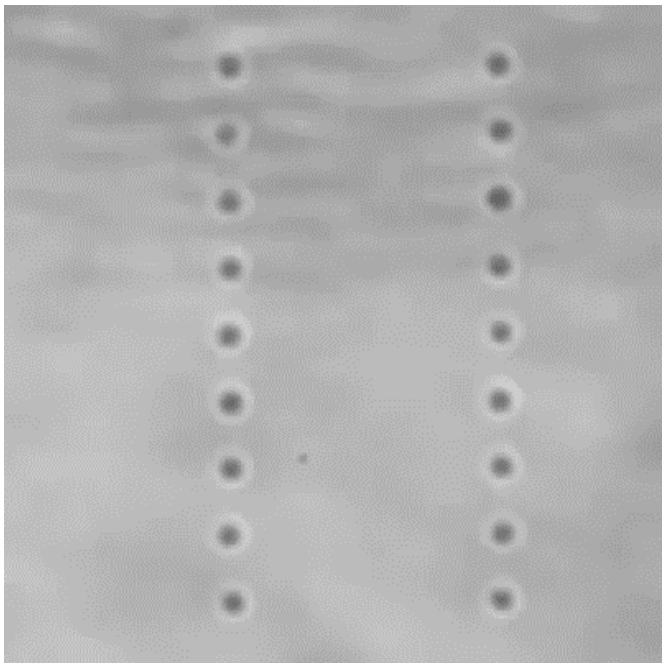


Adapted from:
Grier, Nature **424**, 810 (2003)

Bulk: time and assemble averaged properties

Single Particle: local fluctuations
μ-shape nanostructure corr.
single particle chemistry

Multiple Particle Trapping: local information on interactions
single shot experiments



18 silica micro-beads trapped and manipulated to form the vortices of a Diamond cell.

(M. Padgett group @ Univ. St. Andrews UK)

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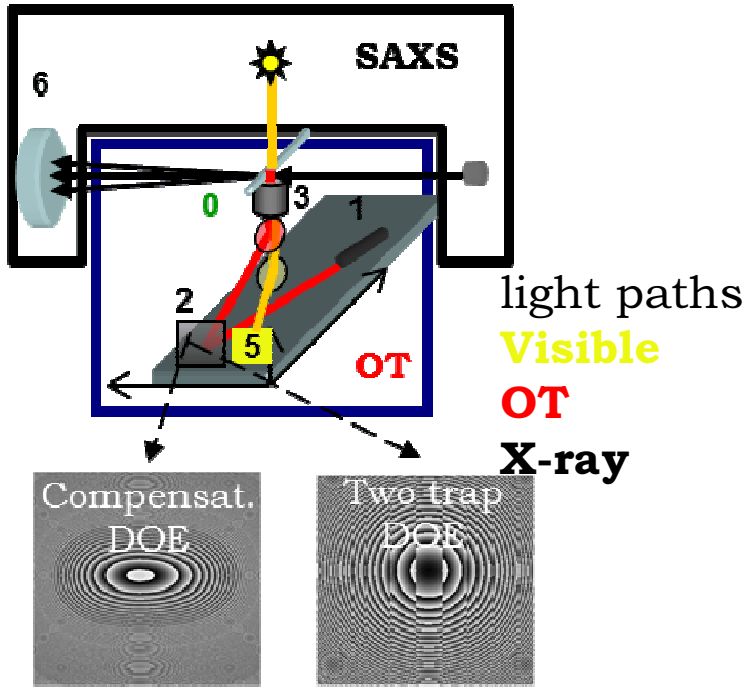
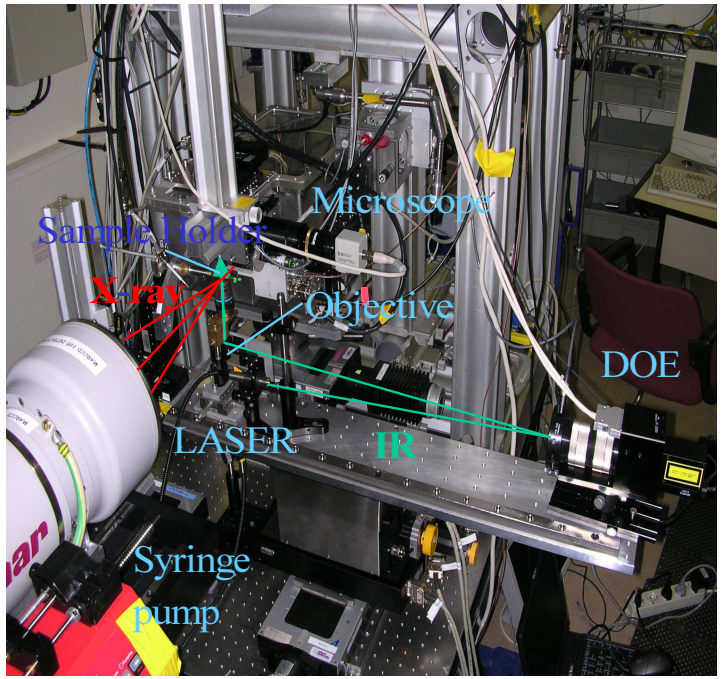
Schematic of the optical manipulation for SAXS



light paths
Visible
OT
X-ray

DOE = Diffractive Optic Element



ESRF: ID13
@46 m & @100 m
KB Mirror
Ref. Lenses
Beam size: ~1 µm
X-rays: ~13.0 keV
(λ = ~0.94 Å)
Detectors:
Mar165
Frelon

0 – sample cell (capillary connected to µfluidics)
1 - IR laser @ 1064 nm
2 - Phase Programmable Modulator (PPM) Hamamatsu
3,4 - microscope objectives, Nikon, Olympus
5,6 - CCDs

D. Cojoc *et al.*, *Proc. SPIE* 6326, 63261M (2006)
H. Amenitsch, *et al.*, CP879, *SRI:Ninth International Conference, AIP*, 1287 (2007)

D. Cojoc et al., *APL, 91, 234107, (2007)*

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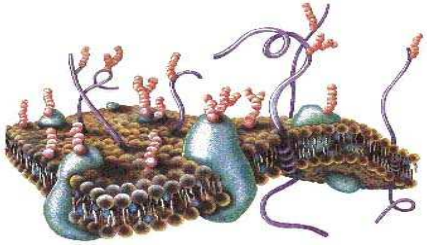
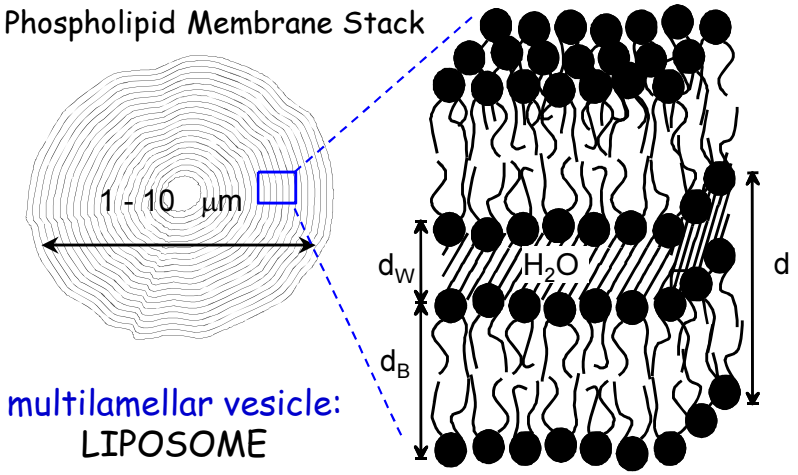
Motivation to combine LT, liposomes and SAXS



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

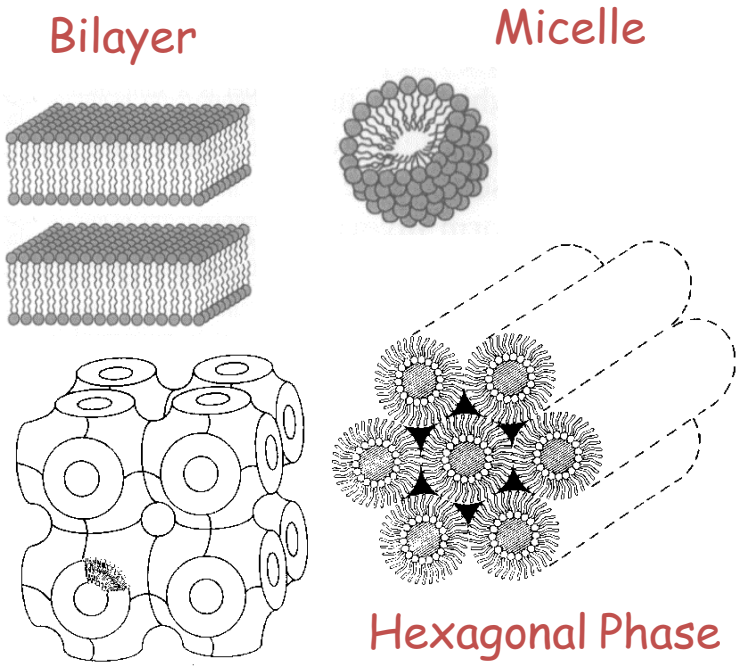
Phospholipids are the major components of biological membranes that form the structural matrix into which proteins are imbedded.

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



multilamellar vesicle:
LIPOSOME

Lyotropic Phases



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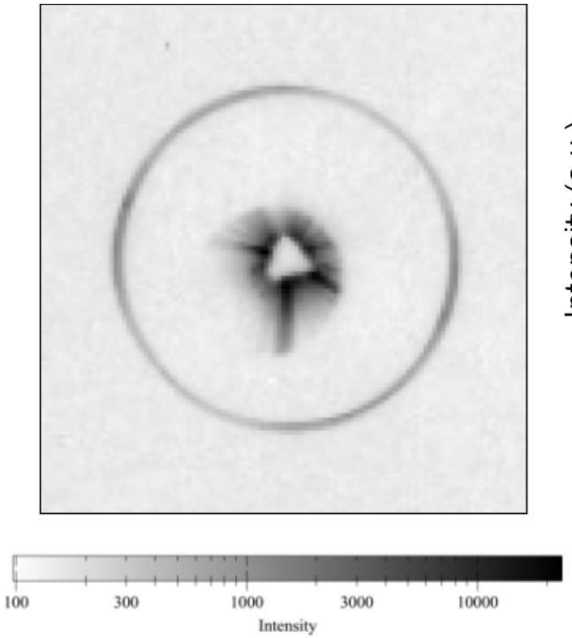
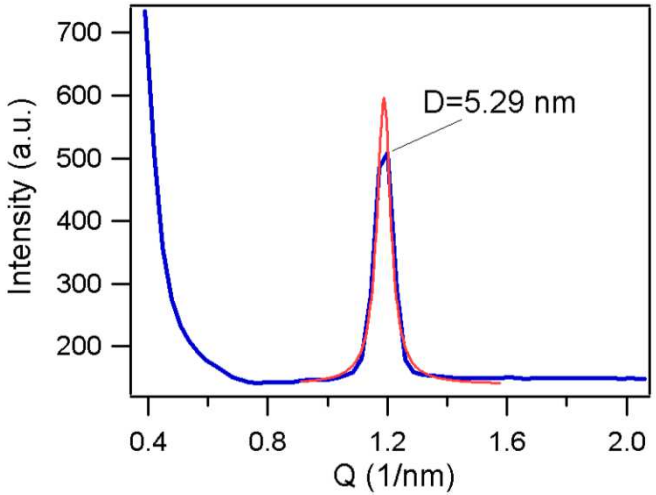
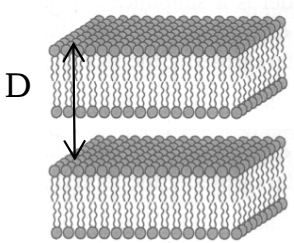
Diffraction from single cluster (10 µm)





Diffraction pattern and azimuthally integrated diffraction pattern



Diffraction image: exposure time 5 s

POPE (Palmitoyl-Oleoyl-Phosphatidyl-Ethanolamine) multilamellar vesicle (1 wt%) in 1 mol $CaCl_2$, Cluster size: 8-10 µm

Liposome size: 1-2 µm, Phase: Liquid crystalline L_α

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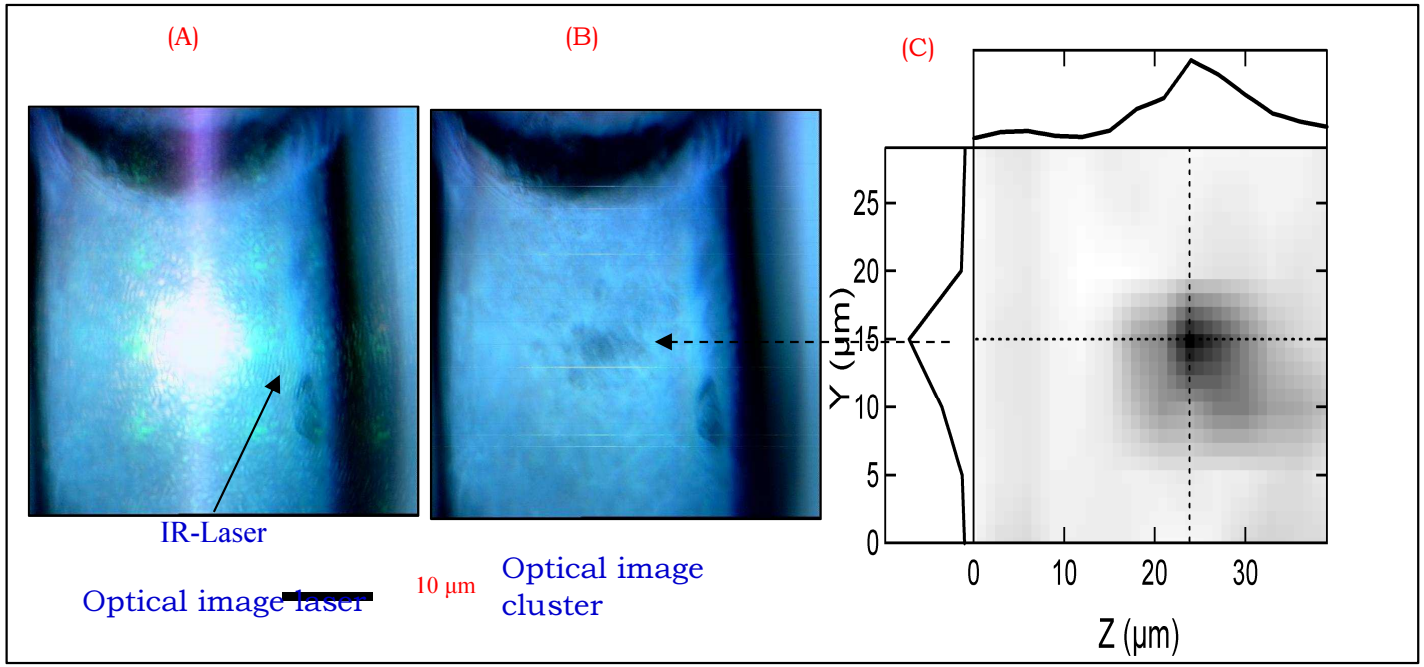
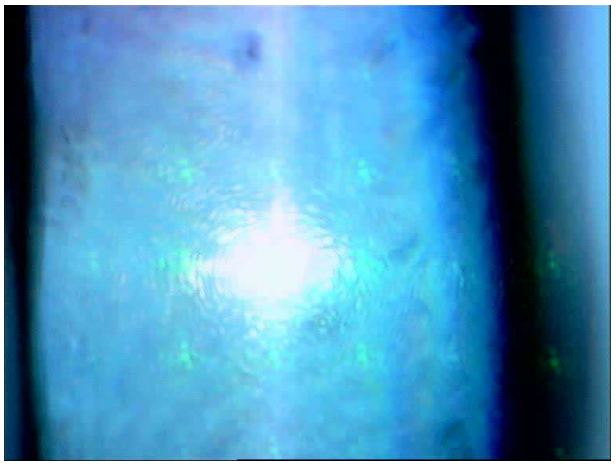
SAXD of optically trapped liposomes



Diffraction from single clusters (8-10 µm)

Step: 2.5 x 5 µm²



‚Diffraction image' (1st order reflection) of the cluster

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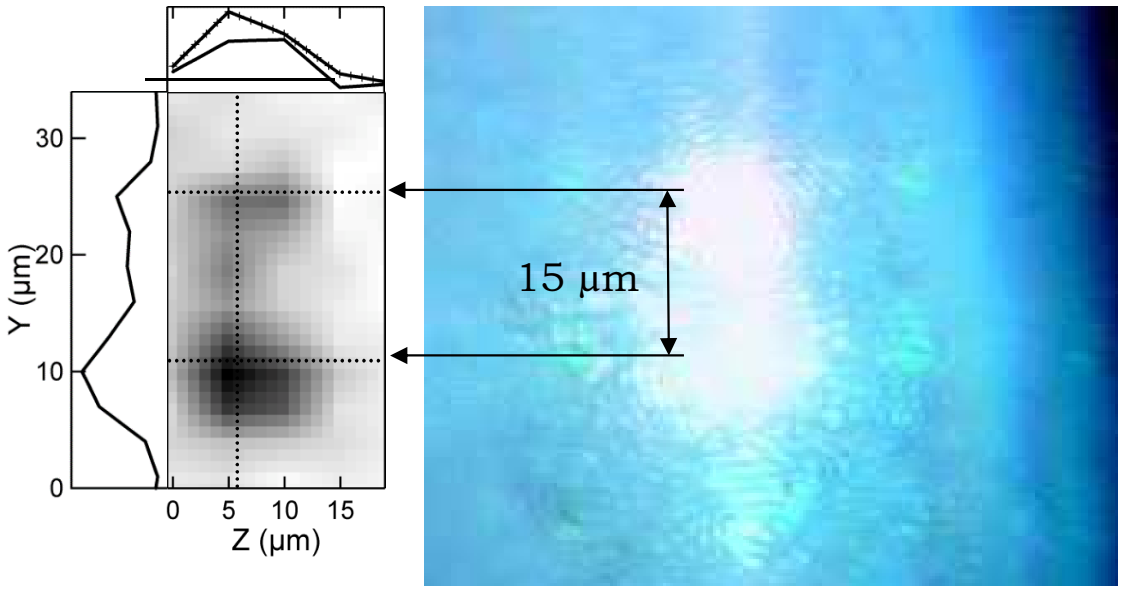
Scanning Diffraction from two clusters multiple trapping

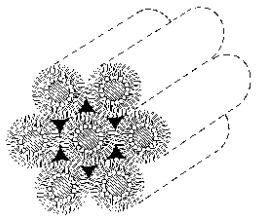


‚diffraction image‘ of two clusters

Optical image of the clusters + laser

Step: 3x5 µm²

DOPE (hexagonal structure)



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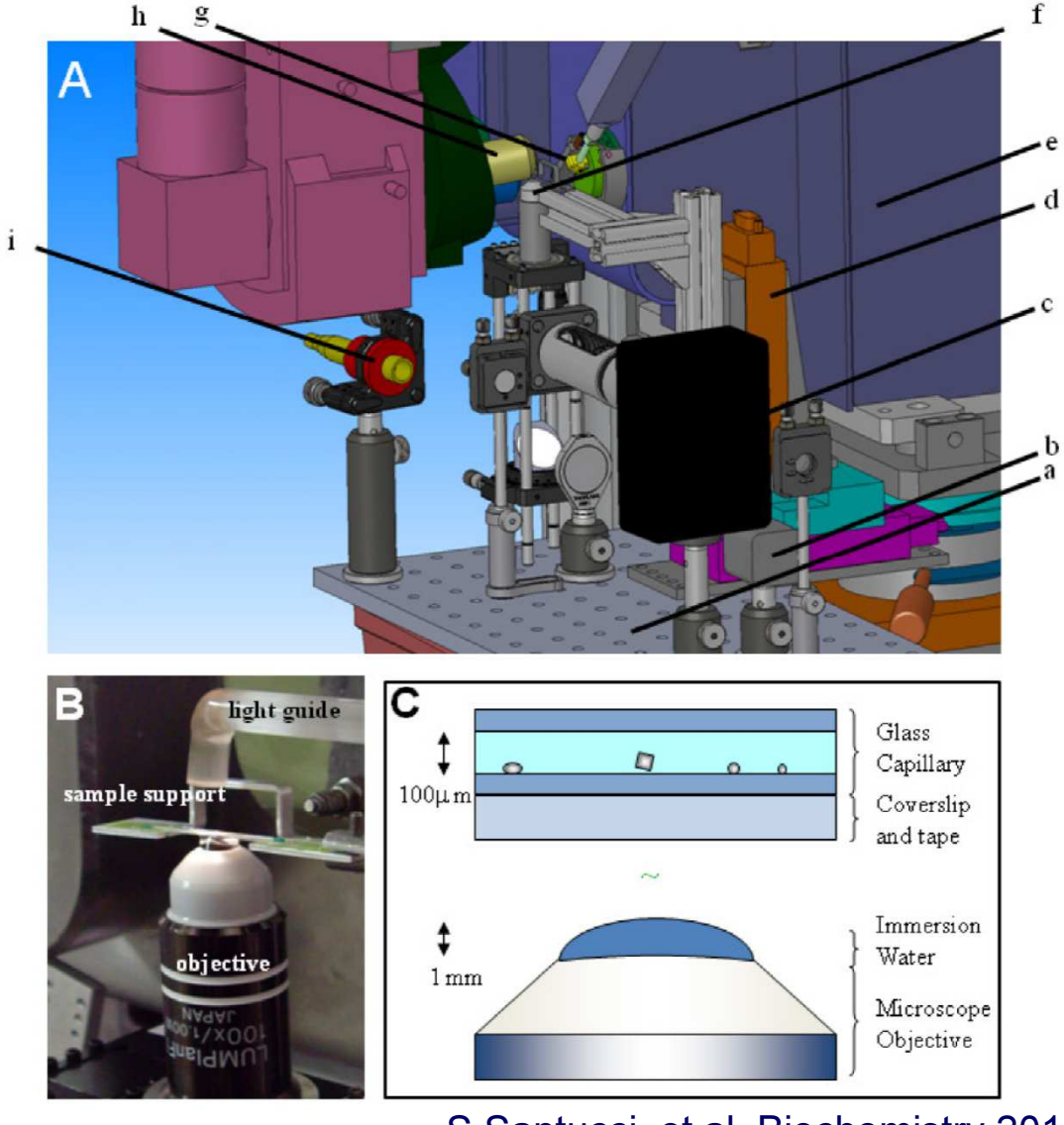
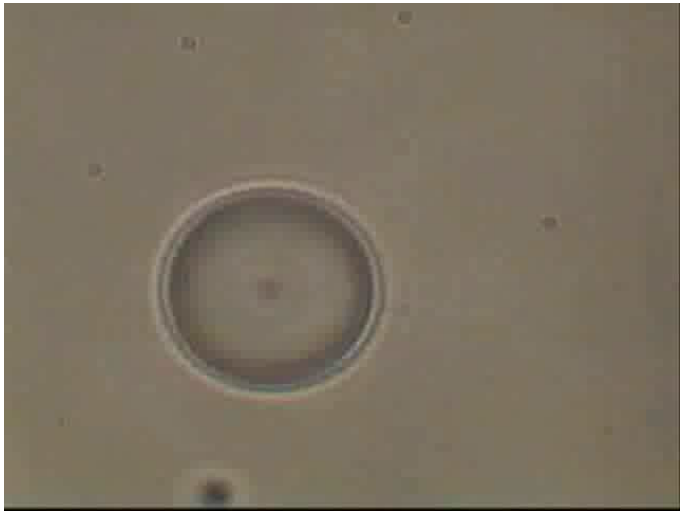
New LT set-up

Improved Sample container

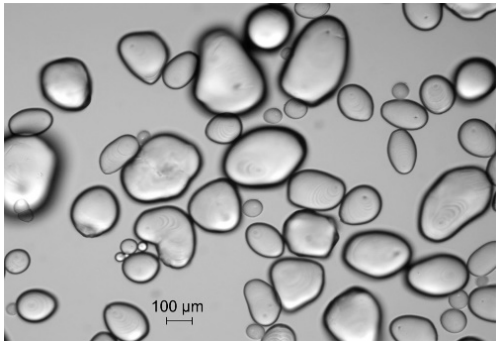




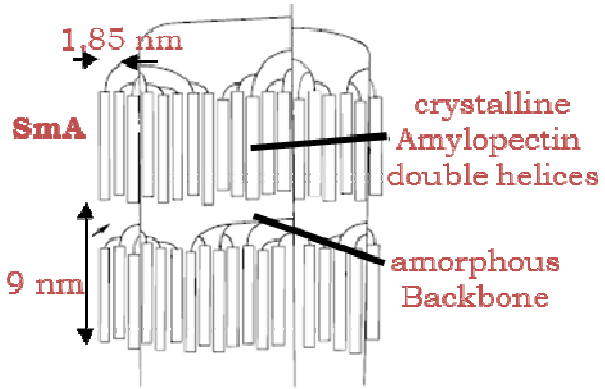
S.Santucci, et al. Biochemistry 2011

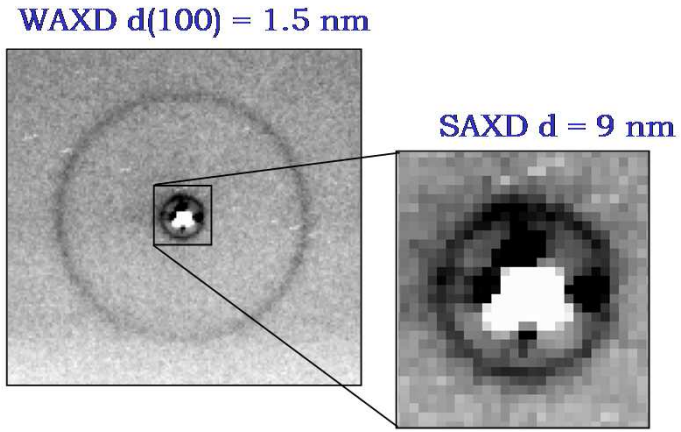
X-ray Diffraction from Starch

Phase Contrast Image of Potato Starch Granules



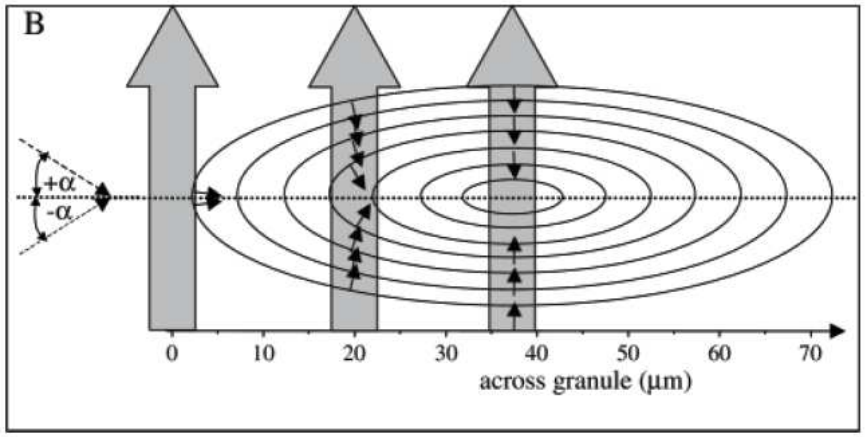
Cartoon Amylopectin Structure

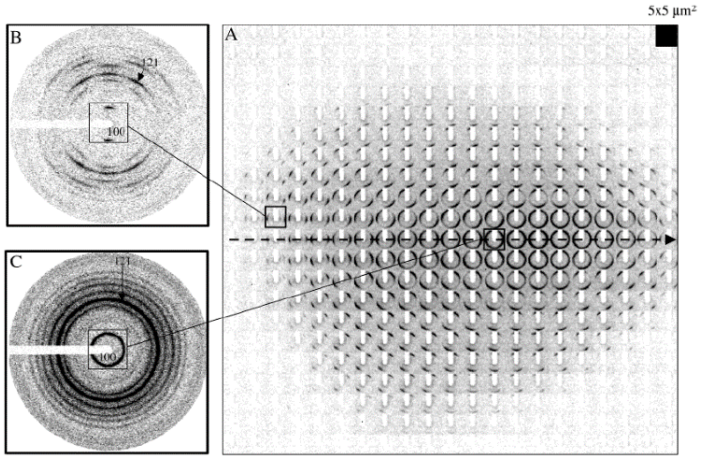




Waigh T et al., *Macromolecules, 1997*

Cartoon Starch Ganule





H. Lemke et al., Biomacromolecules **2004,** 5, *1316-1324*

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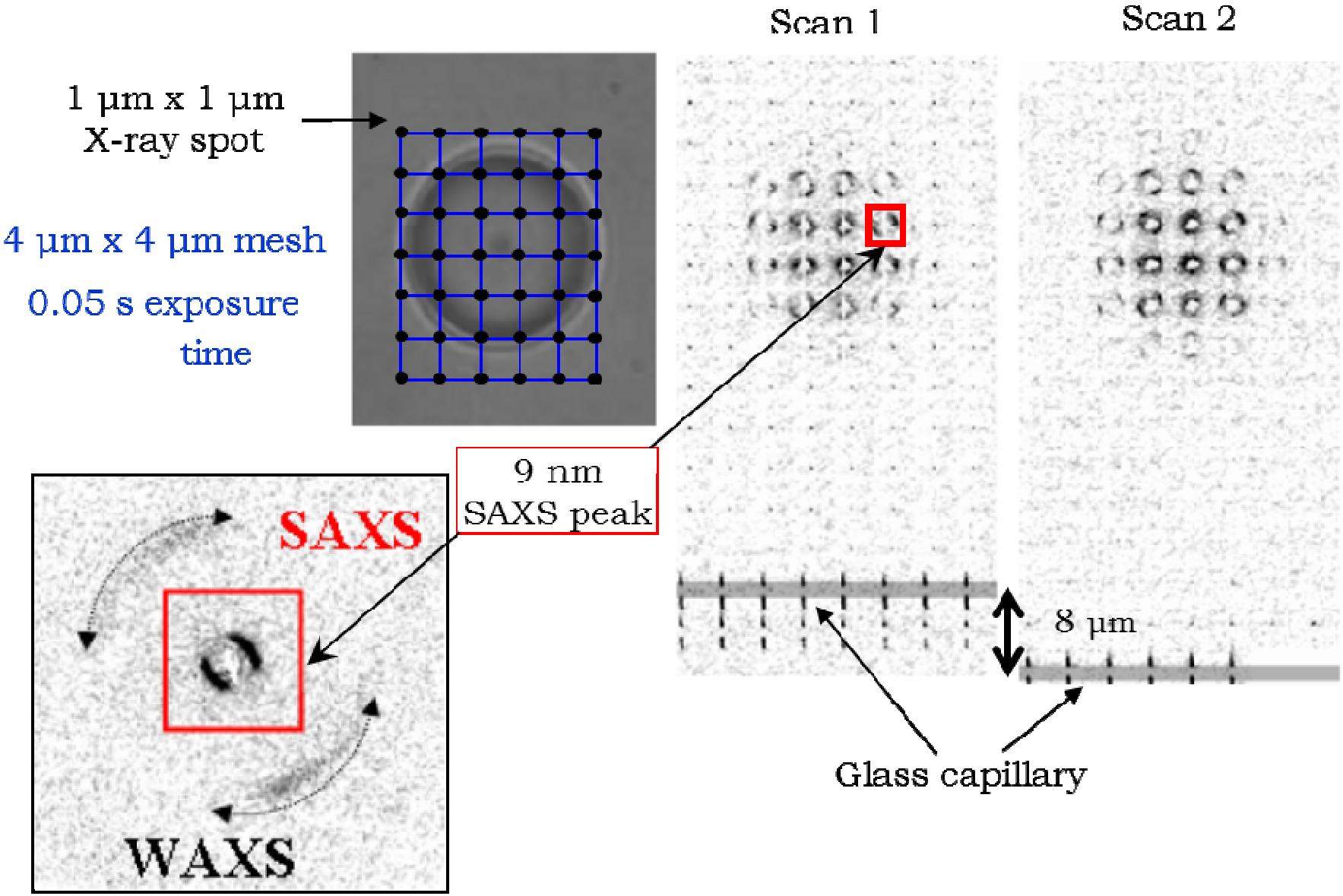
Scanning diffraction experiment



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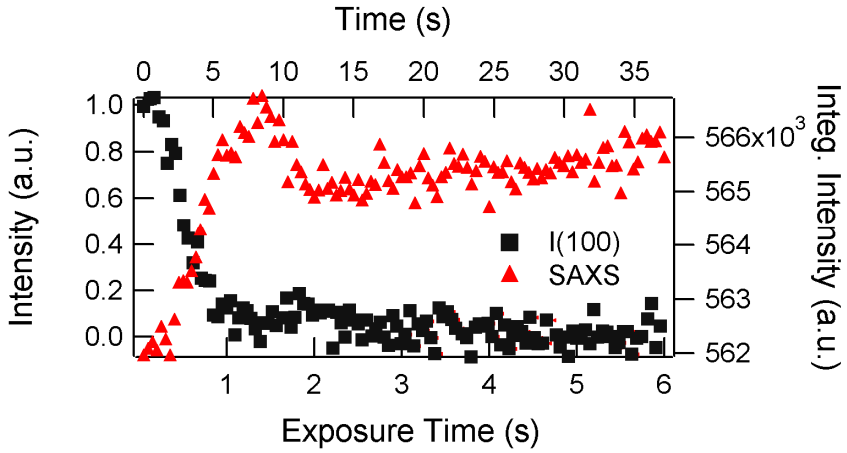
SAXS of optically trapped starch granules (from potato)

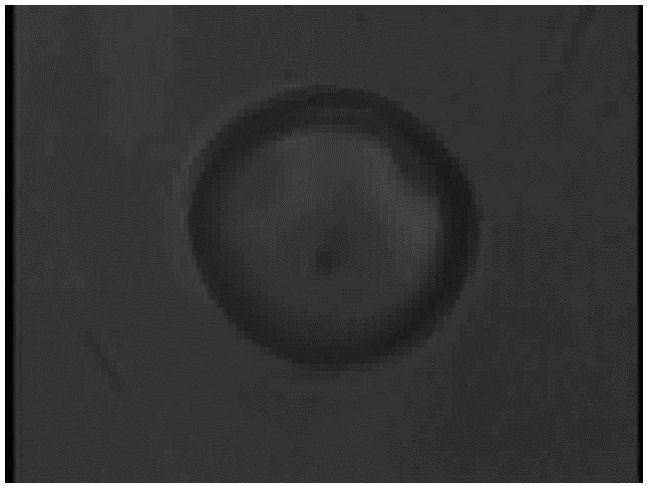
Integrated Intensity & I(100) Reflection

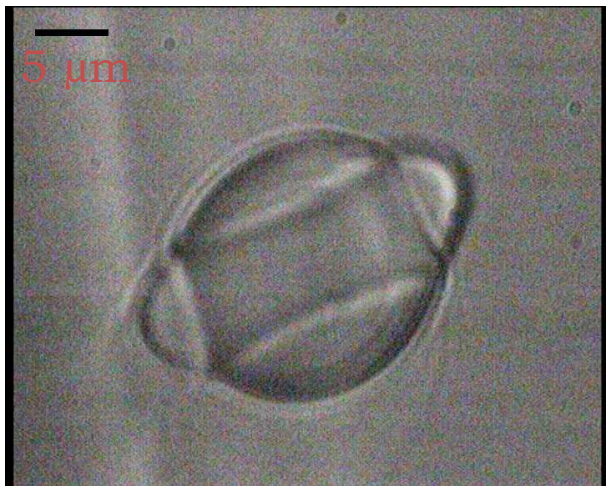
Max. exp.
Time: 200 ms!!!

Time: 1.5 s !!!



FoV 40 x 30 µm





2.12 min x 4 accel.

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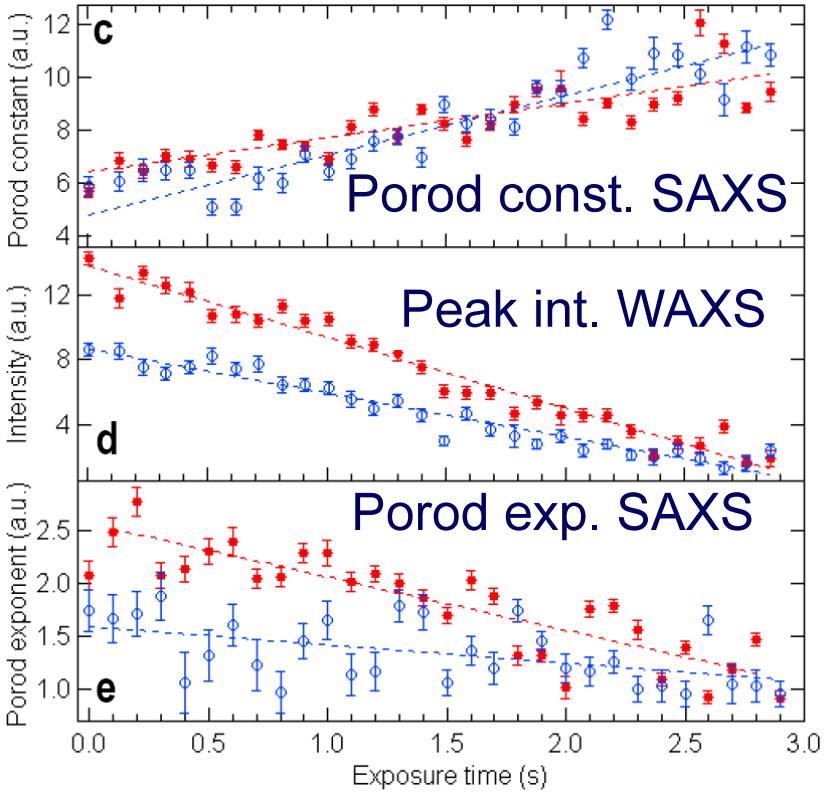
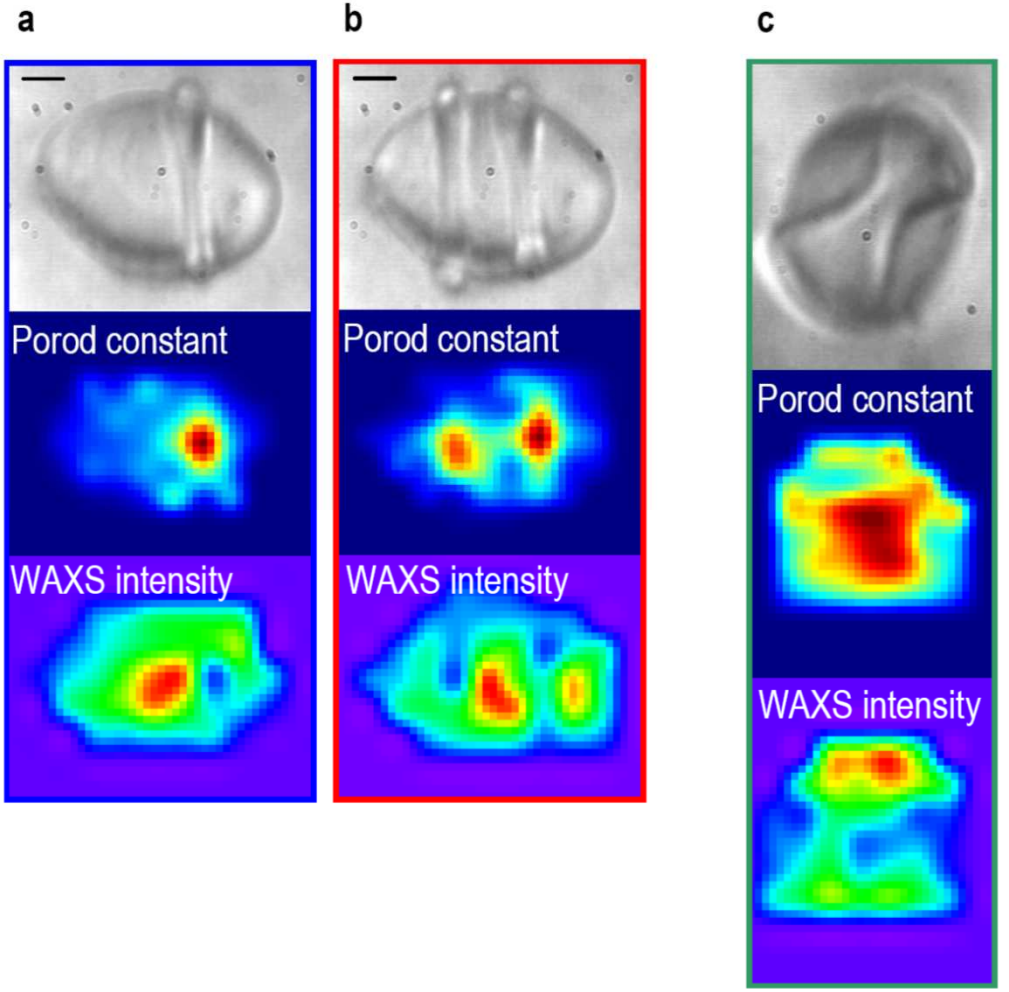
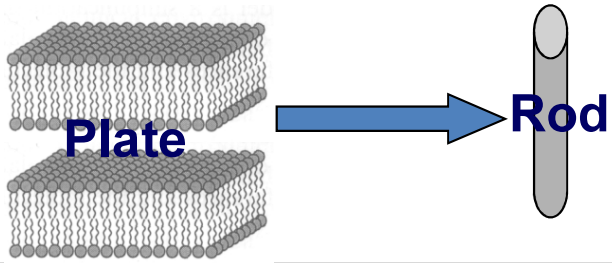
Radiation Damage

Simultaneous Fitting SAXS and WAXS
Porod & Lorentzian Peak

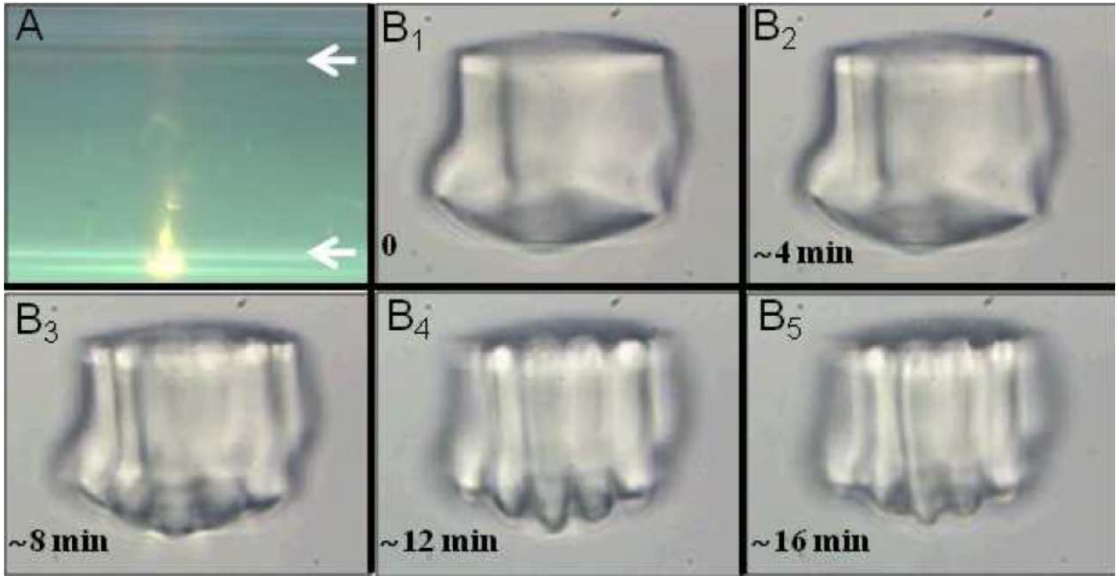




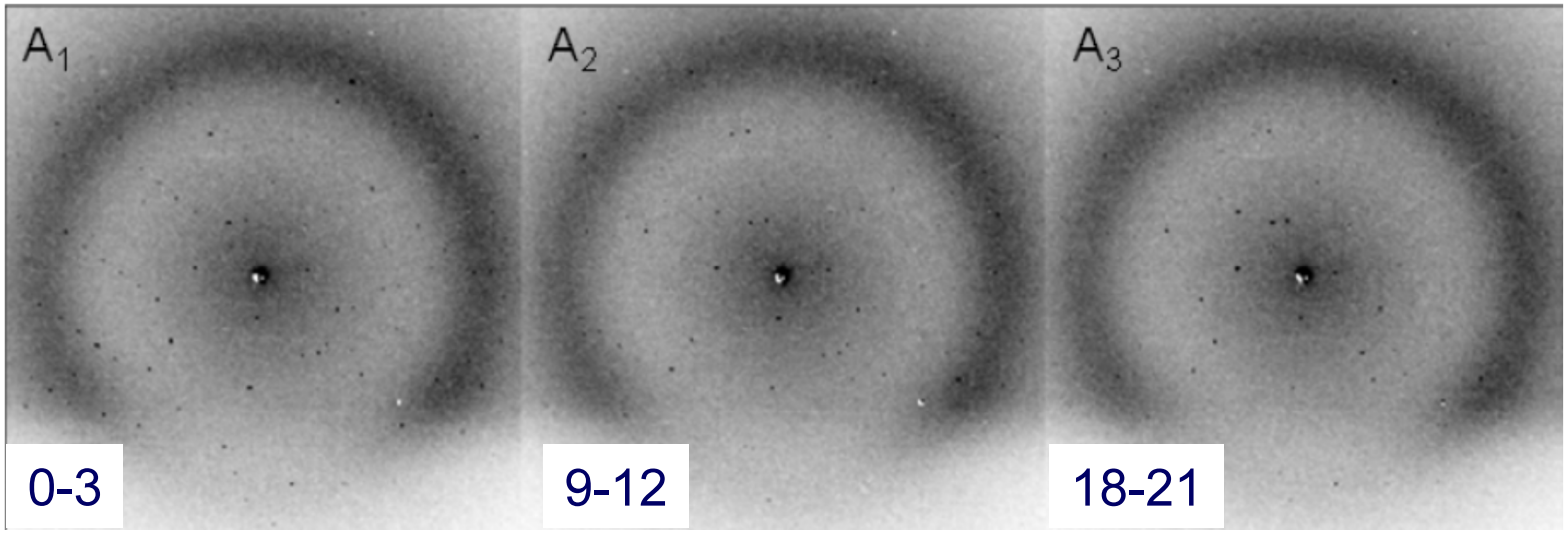


D.Cojoc, H. Amenitsch et al., APL, 2010

Laser Tweezers Protein Crystals

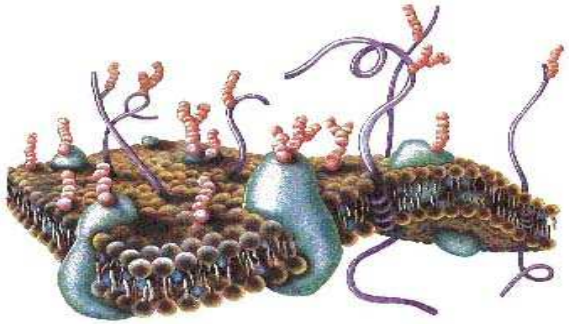


Insulin Crystal



S.Santucci, C.Riekel et al. Biochemistry 2011

Liposomes and SAXS

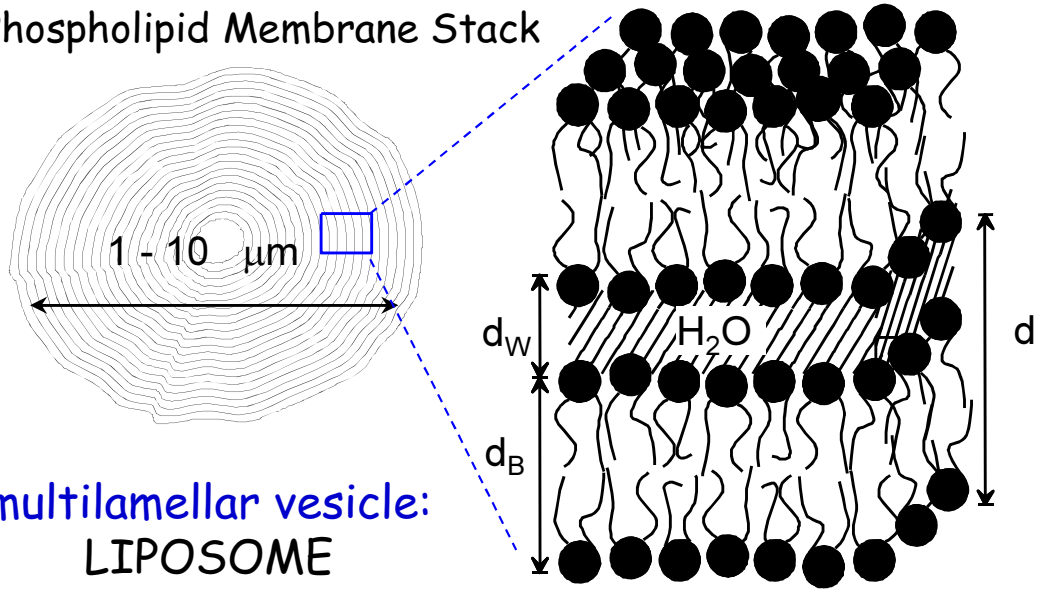


The boundaries of cells are formed by biological membranes, the barriers that define the inside and the outside of a cell.

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

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

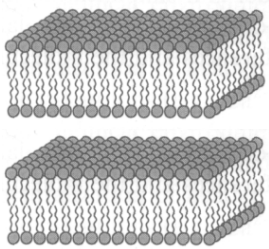
Phospholipid Membrane Stack



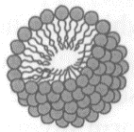
multilamellar vesicle:
LIPOSOME

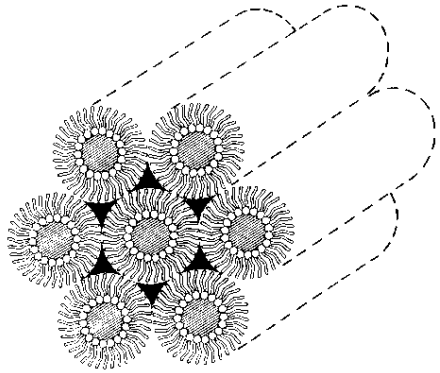
Lyotropic Phases

Bilayer

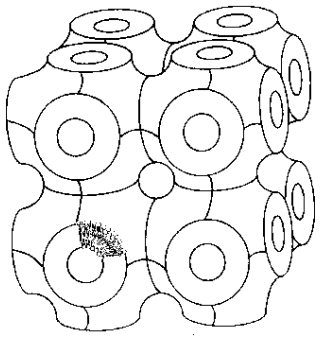


Micelle





Hexagonal Phase



Cubic Phase

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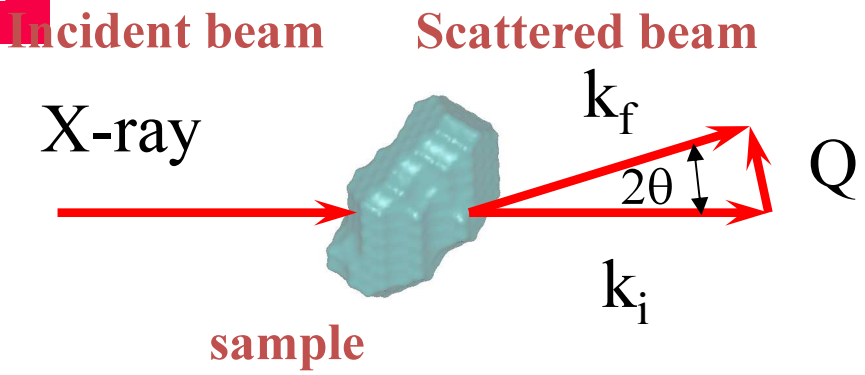
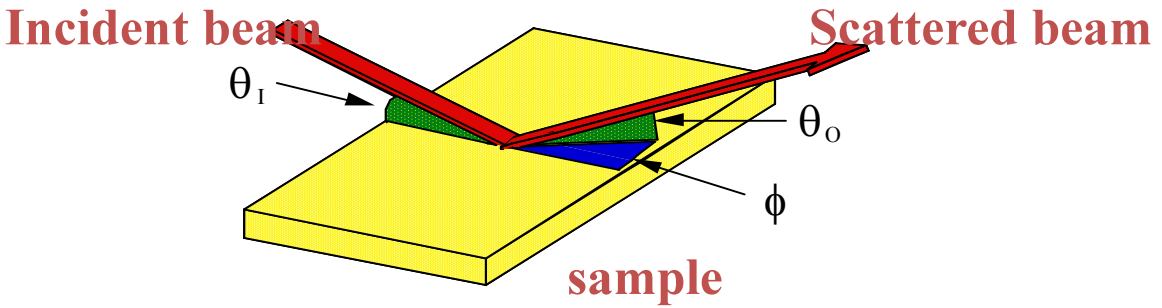
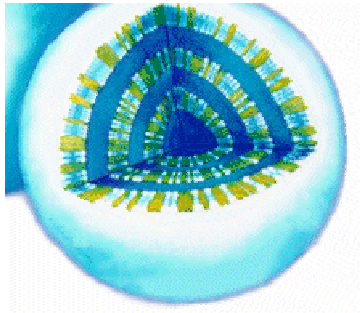
Small Angle Scattering - Surface Diffraction

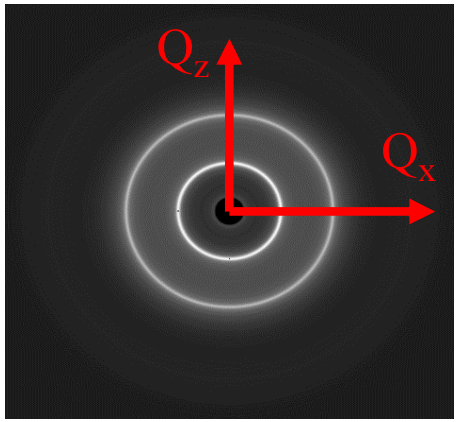


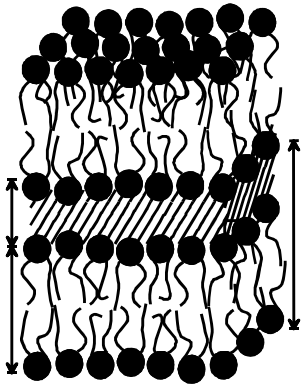
Small-Angle Scattering (Diffraction)

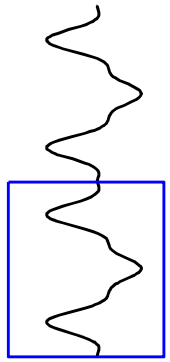


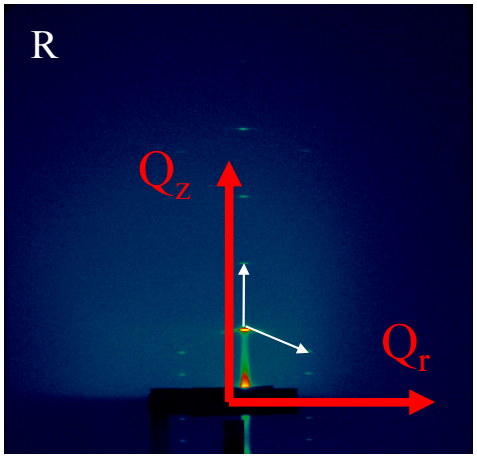
Grazing Incidence Small-Angle Scattering (GISAS) + Reflectometry











$$I(Q) = \left\langle \left| \int_V d^3r \cdot \rho(\vec{r}) \cdot \exp(-i \cdot \vec{Q} \cdot \vec{r}) \right|^2 \right\rangle \qquad I(Q_z, Q_r) = \left\langle \left| \int_V d^3r \cdot \rho(\vec{r}) \cdot \exp(-i \cdot \vec{Q} \cdot \vec{r}) \right|^2 \right\rangle_r$$

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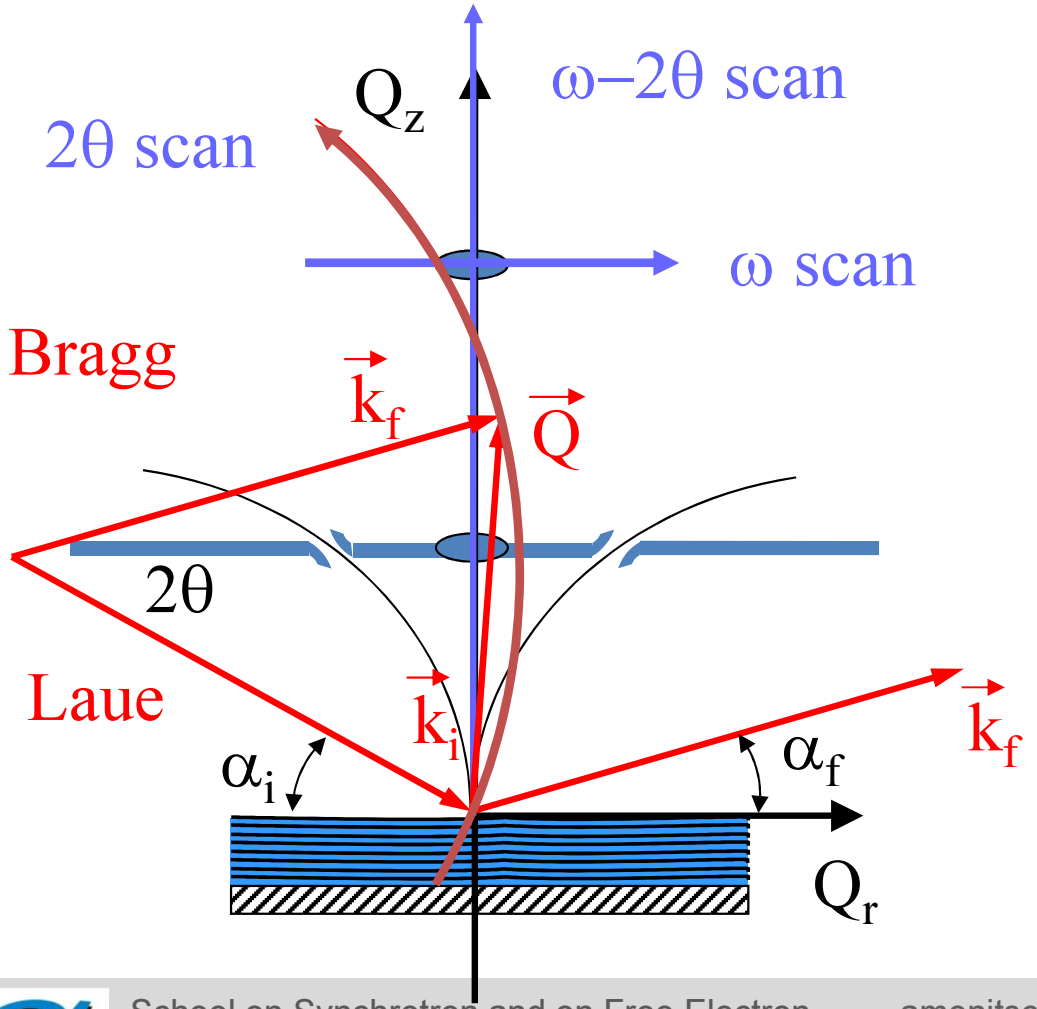
amenitsch@elettra.trieste.it

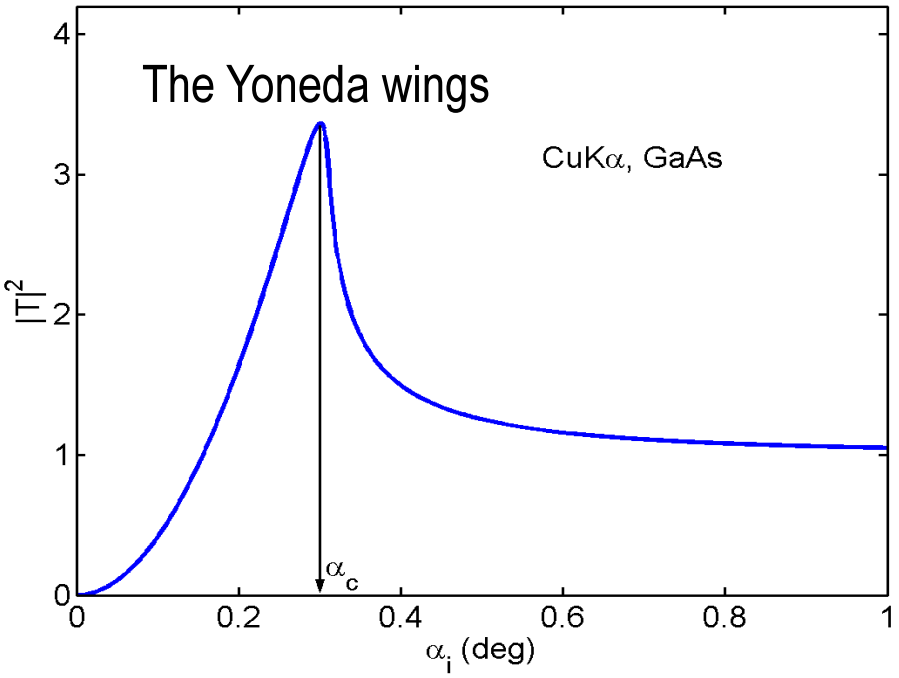
Distorted Wave Born Approximation

Vineyard (1982), Shinha et.al. (1988)

$$I(Q_z, Q_r) = |T_i(\alpha_i)|^2 \left\langle \left| \int_V d^3r \cdot \rho(\vec{r}) \cdot \exp(-i \cdot \vec{Q} \cdot \vec{r}) \right|^2 \right\rangle_r |T_f(\alpha_f)|^2$$



Refraction Effects



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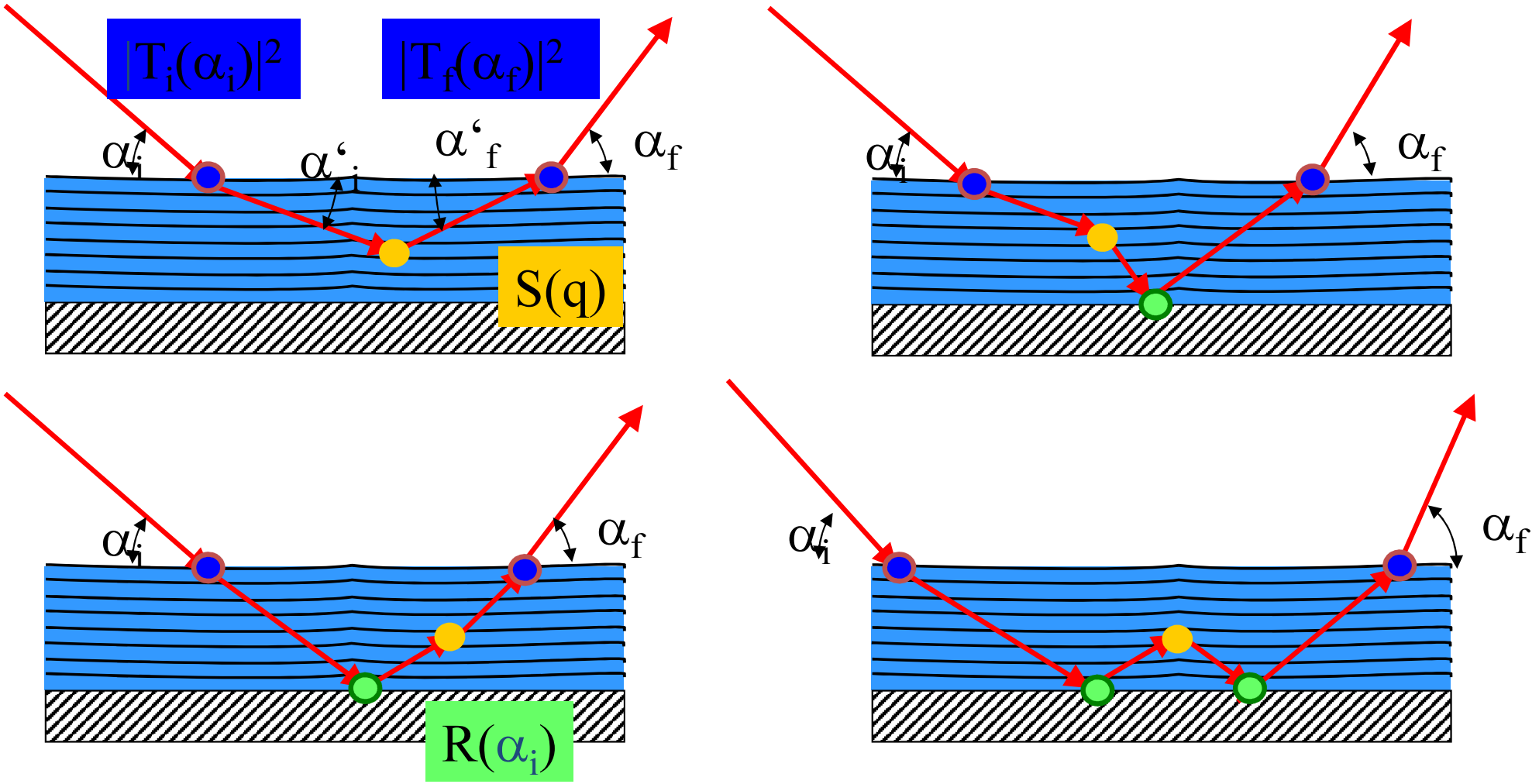
„Higher Orders“ of DWBA



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

M.P.Tate et al., J.Phys.Chem, 2006

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Distorted Wave Born Approximation

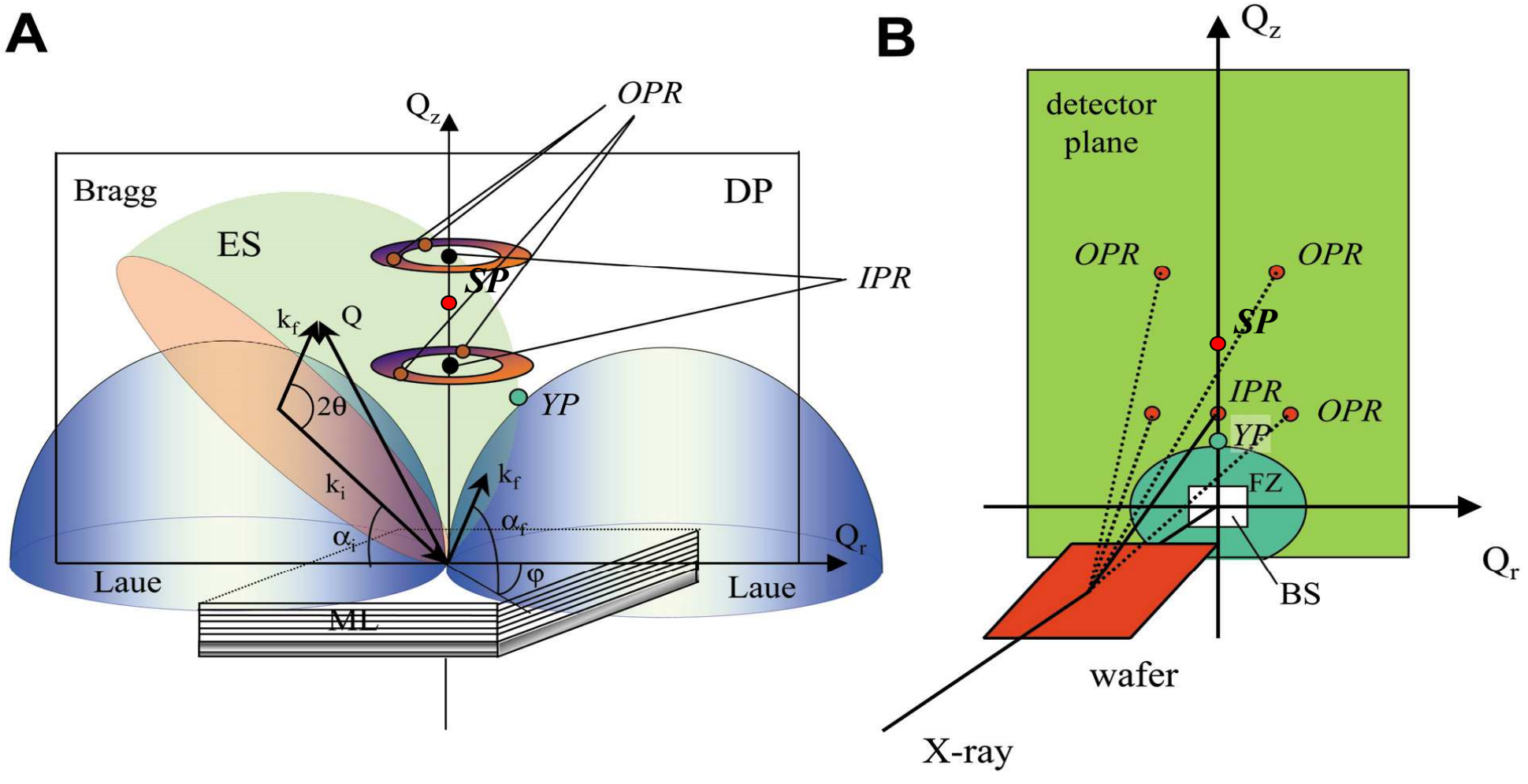

Fig. (A) the scattering geometry in reciprocal space. (B) Scattering geometry in real space. The abbreviations are: (ES) Ewald sphere, (DP) diffraction plane, (OPR) out-of plane reflections, (IPR) in-plane reflections, (ML) multi-layer, (FZ) forbidden zone, (BS) beam stop.

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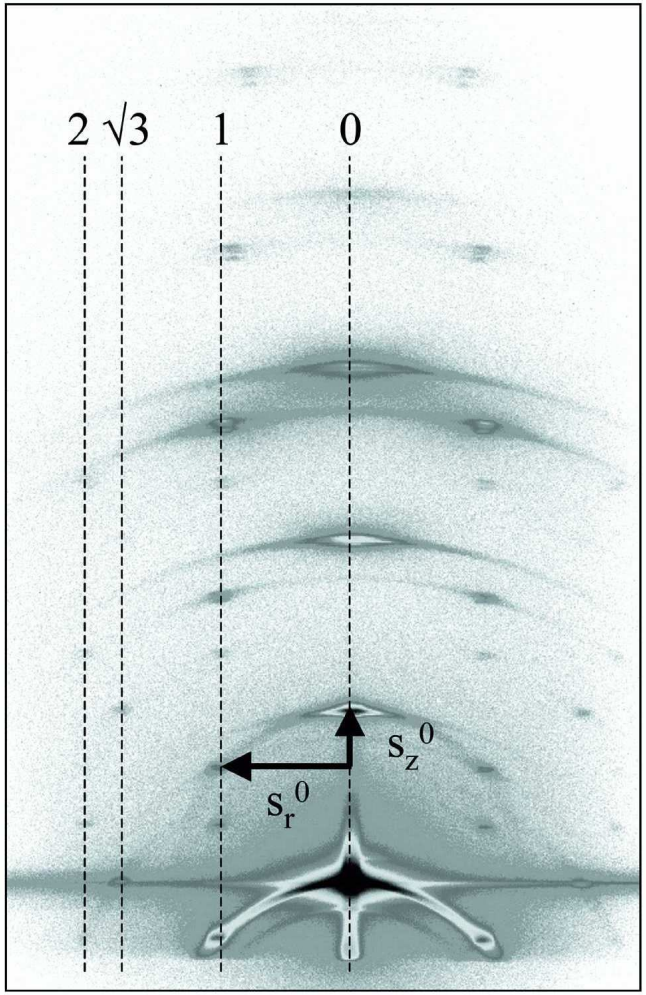
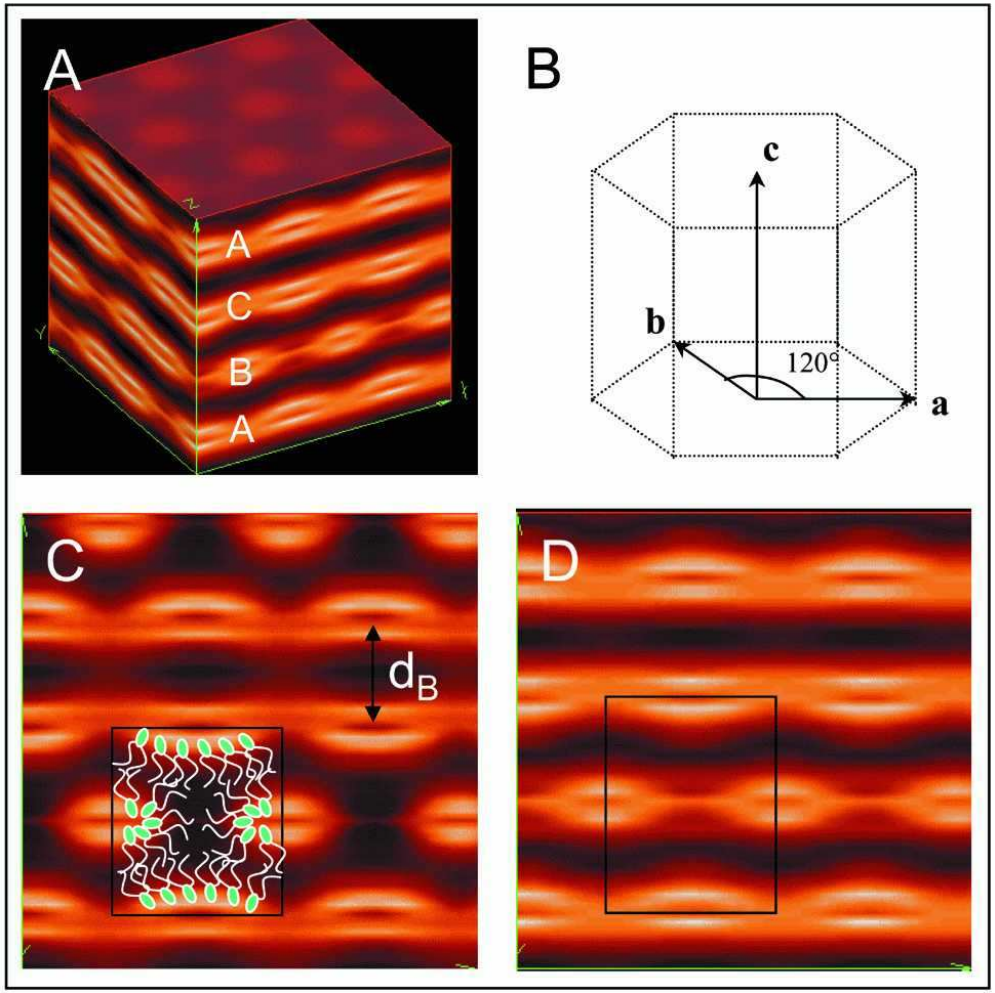
Surface Diffraction Lipids – Rhombohedral Phase





Diffraction Pattern DOPC @ 25° C, 35% rel. humidity

Electron Density Reconstruction: -C DPhPC (d_B= 44.3 Å) -D DOPC (d_B= 48.7 Å), but a= 67 Å / 68 Å

Rappolt,M, et.al., Adv. Coll. and Interf. Science, 111 (2004)

L. Yang, H.W. Huang, Biophys. J. 84 (2003)

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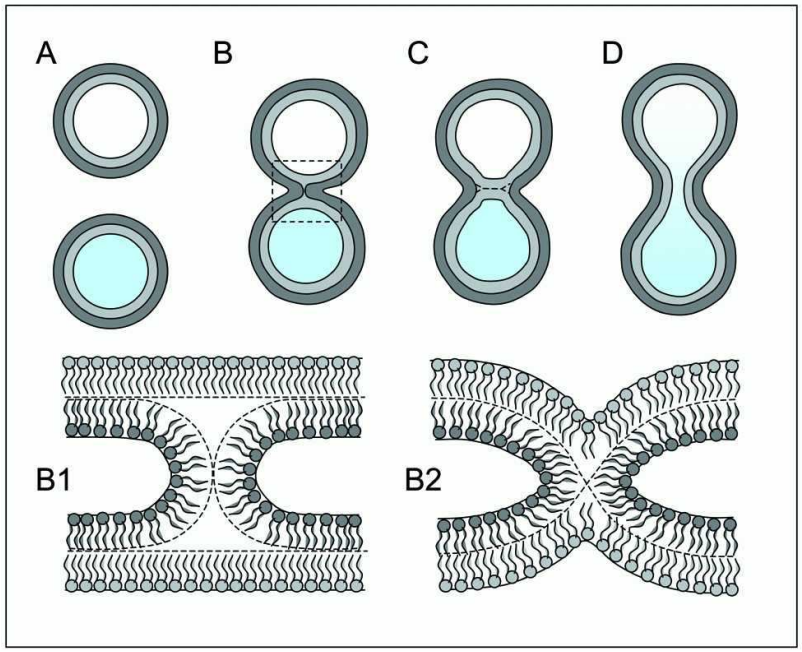
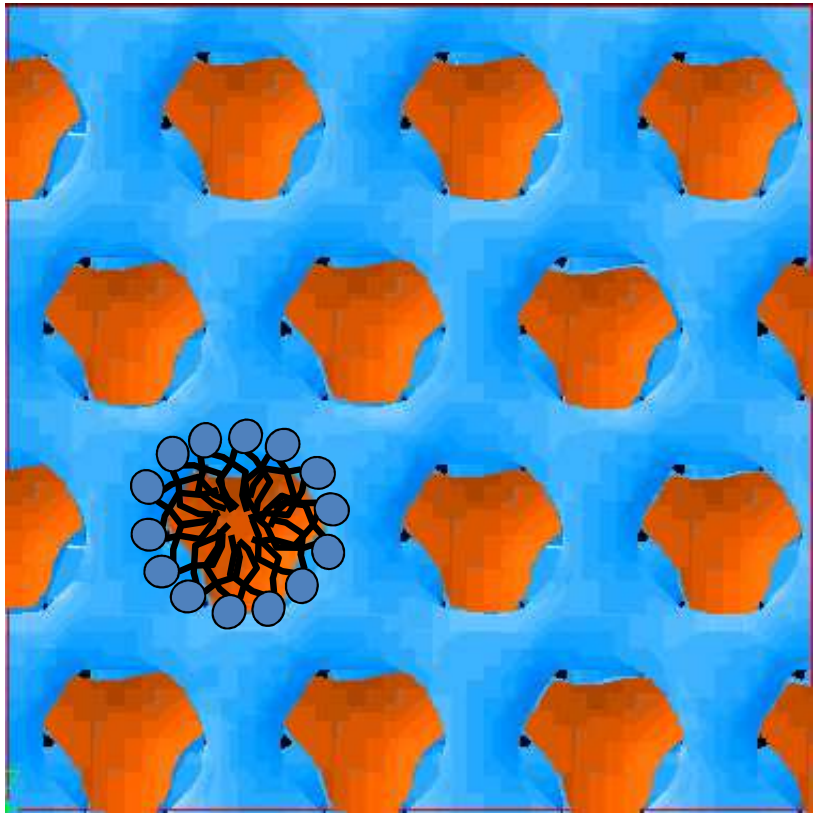
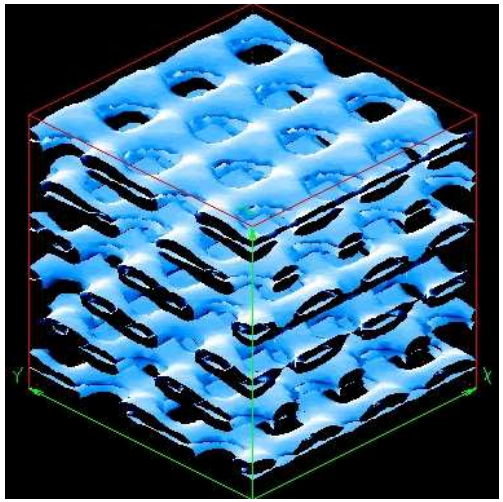
What do we learn? Membrane Fusion







The radius of the torus seems to be confined by the head-group size…

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Surface diffraction: Formation of aligned mesoporous thin films

Sol-gel chemistry
+
surfactant mesophase

Self-assembly

Mesostructured hybrids

Treatment

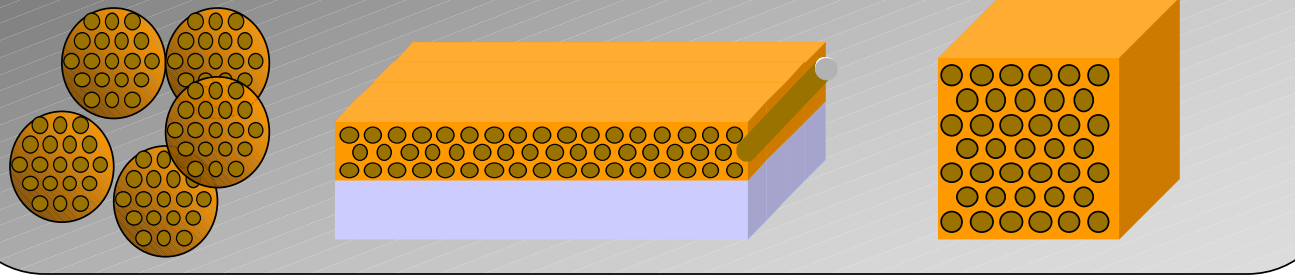
Mesoporous materials with organised porosity

C. J. Brinker et al. Adv. Mater., 1999, 11, 579.

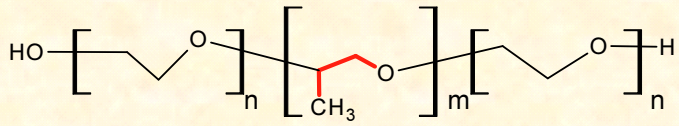
Particles made by aerosols

Films and **fibres** made by liquid deposition

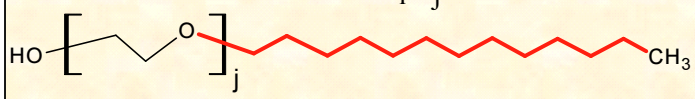
Monoliths made by controlled evaporation



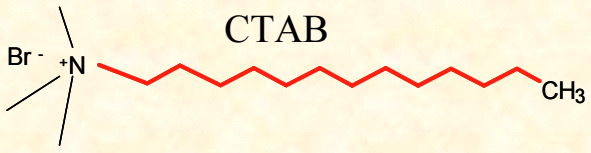
Triblocks POE-POP-POE



Diblocks C_iE_j



CTAB



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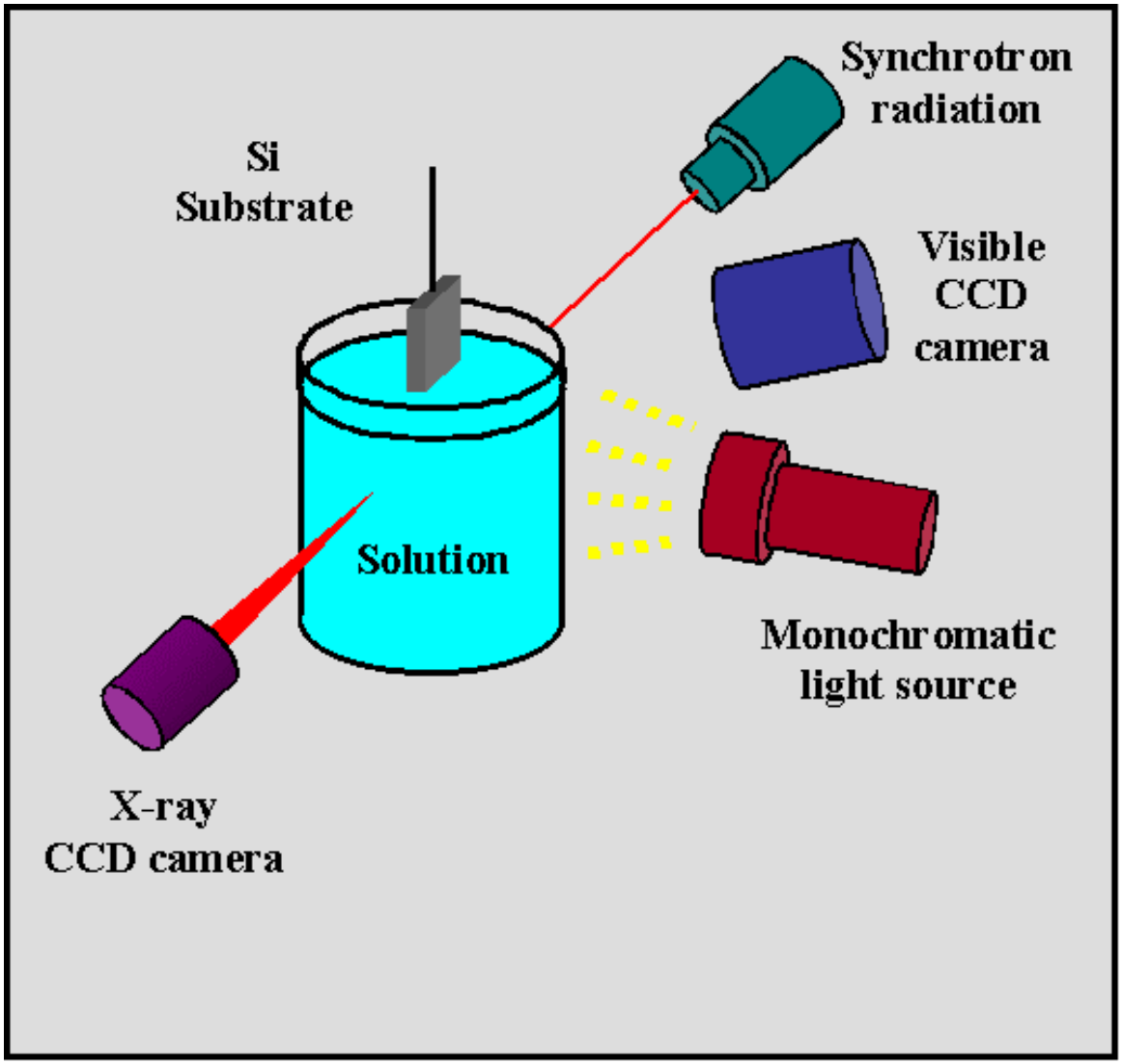
The Self-Assembly of thin films as seen by In- Situ SAXS and interferometry



Film mesostructure

Film thickness profile

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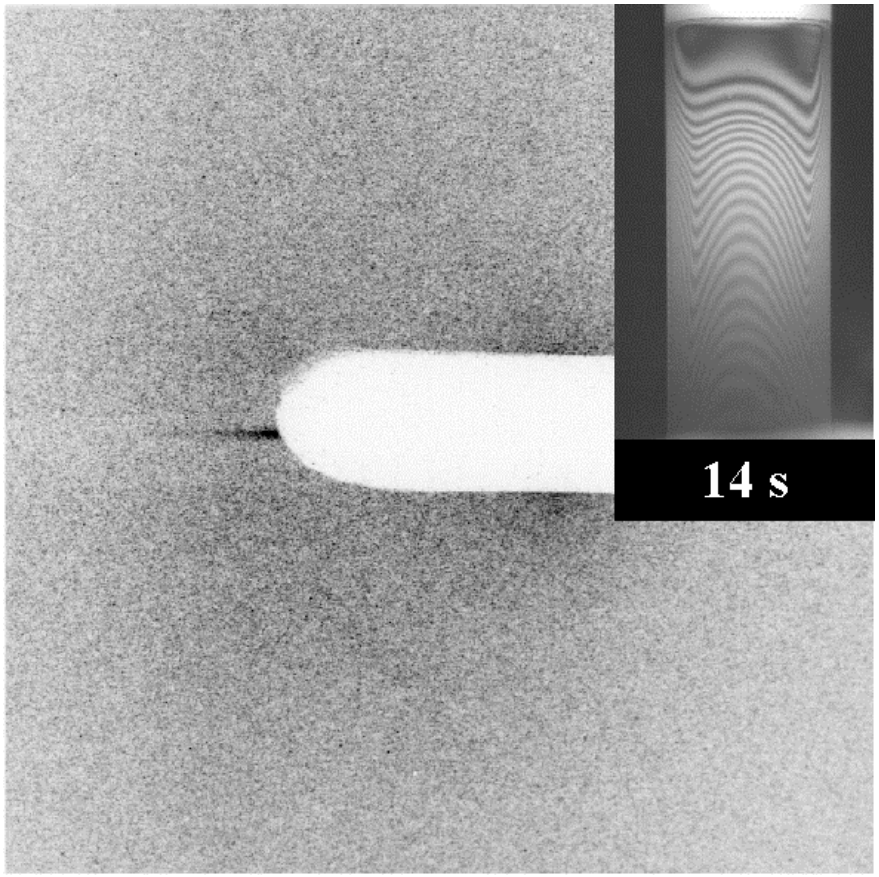
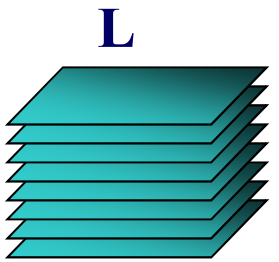
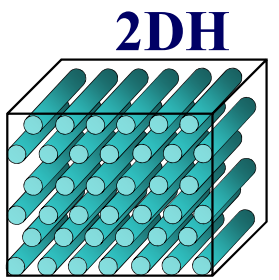
Surface diffraction: Formation of aligned mesoporous thin films

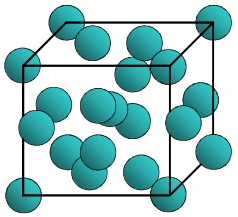
CTAB / Si = 0,18
H_2O / Si = 5
HCl / Si = 0.15
Ageing time
Relative Humidity







P6m



Pm3n
Im3m

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

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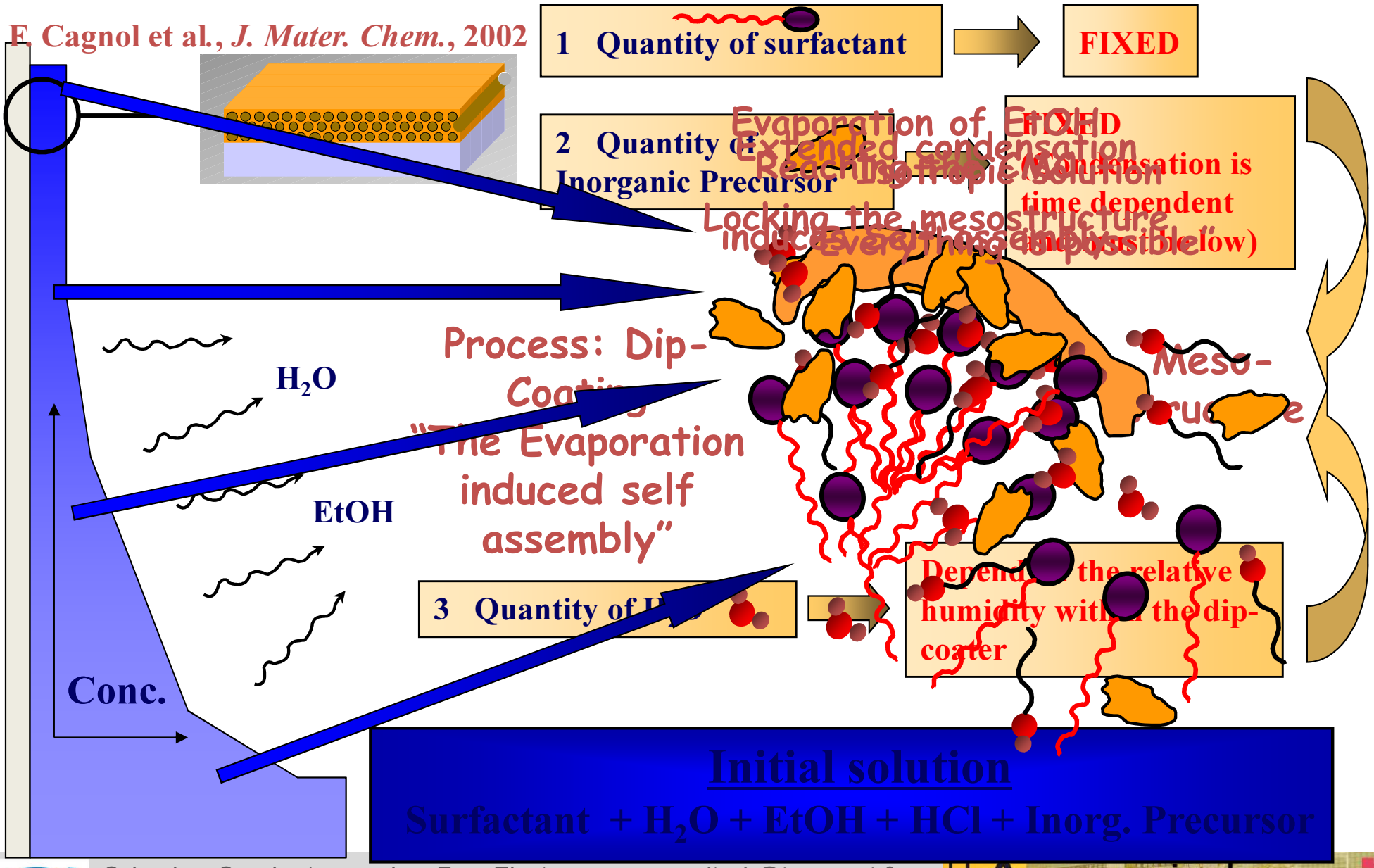
The Modulable Steady State



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Nanoimprinting and Hybrid Solar Cells

ACS APPLIED MATERIALS & INTERFACES

Research Article

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(2014)

Nanoimprinted Comb Structures in a Low Bandgap Polymer: Thermal Processing and Their Application in Hybrid Solar Cells

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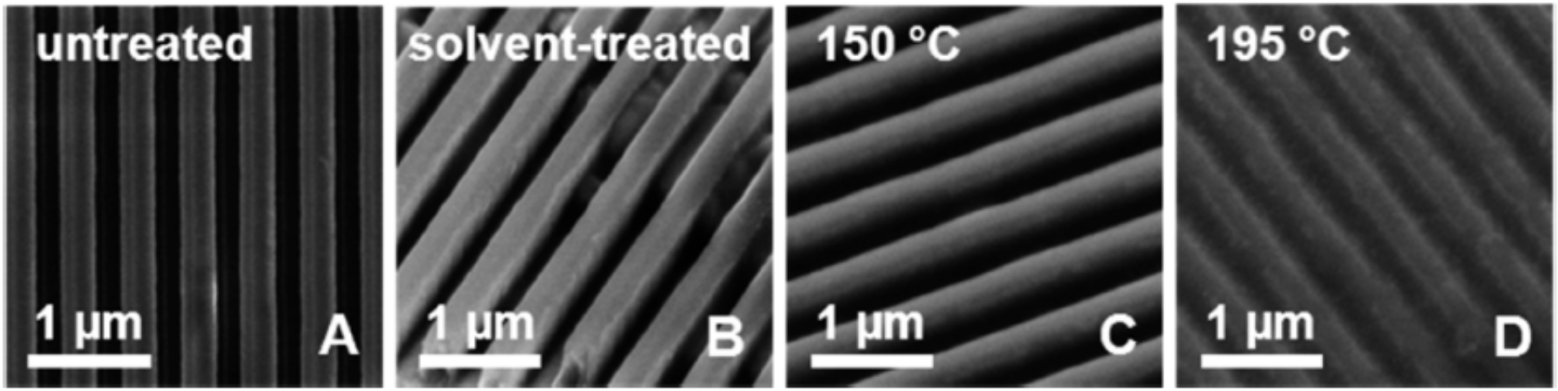
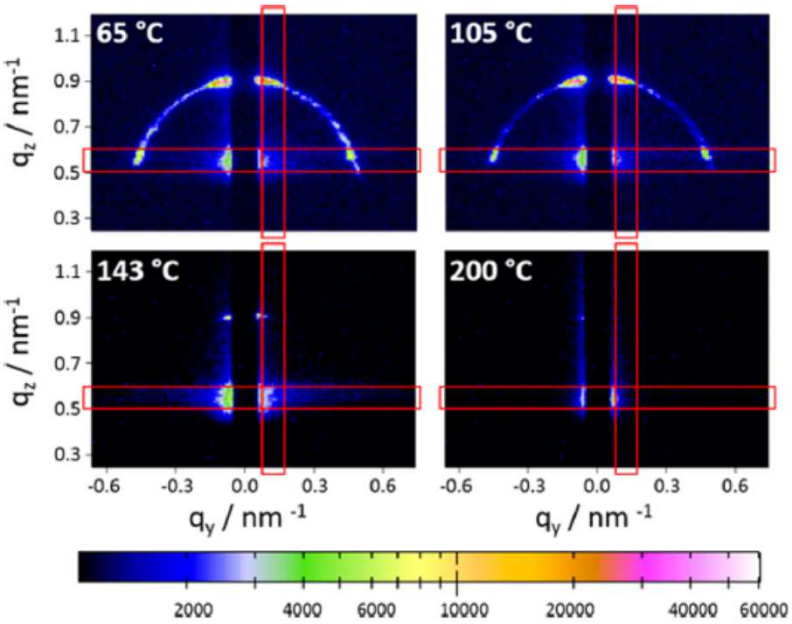
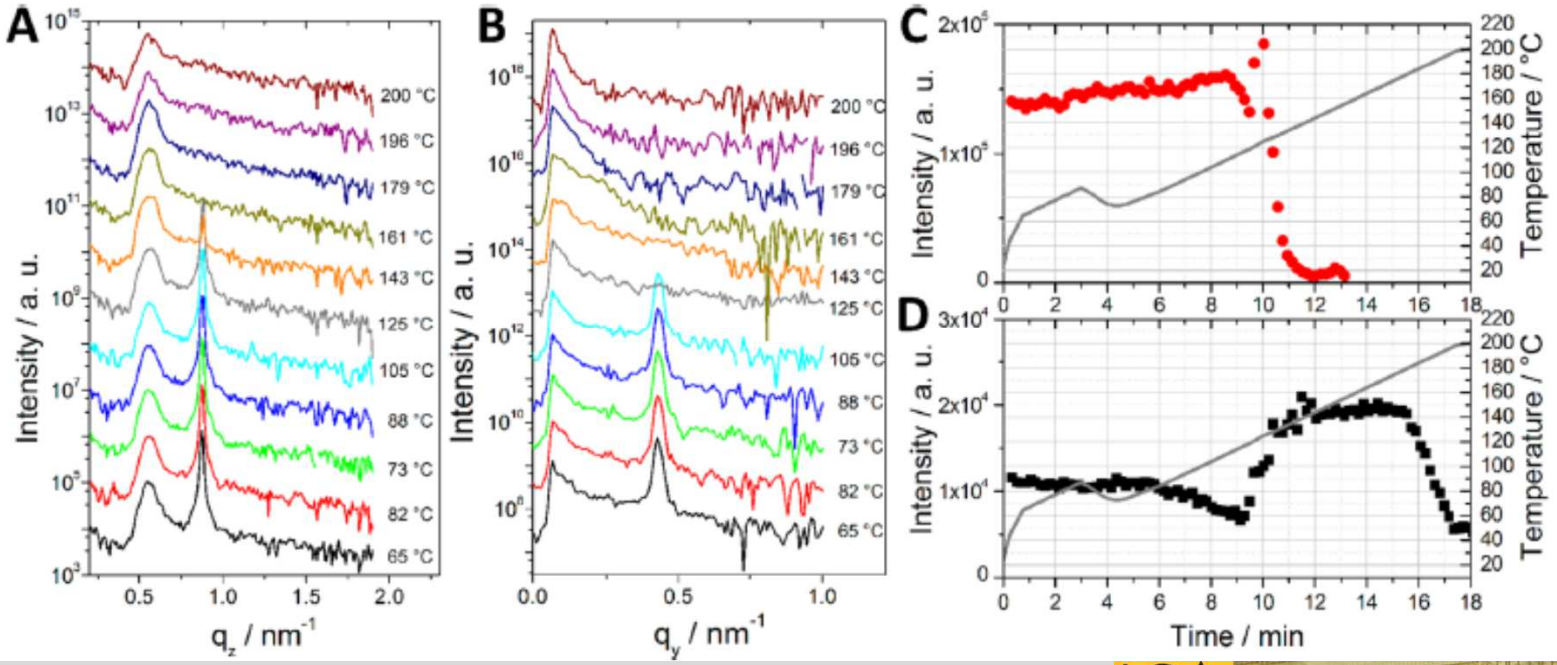


Nanoimprinting and Stability





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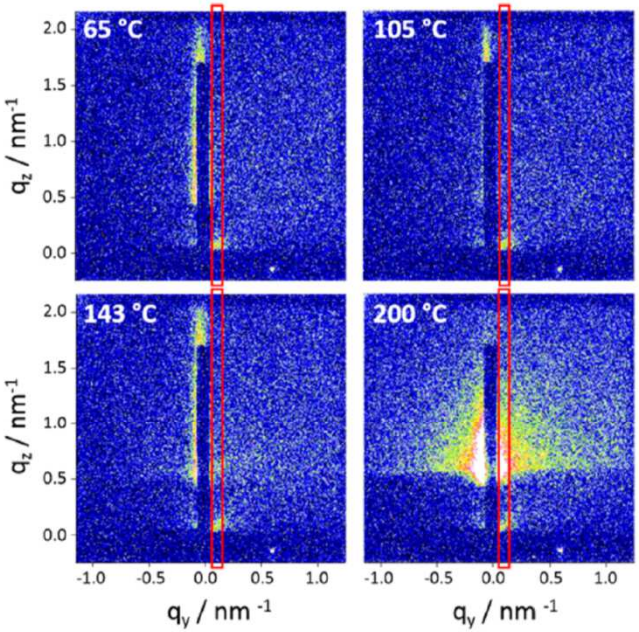
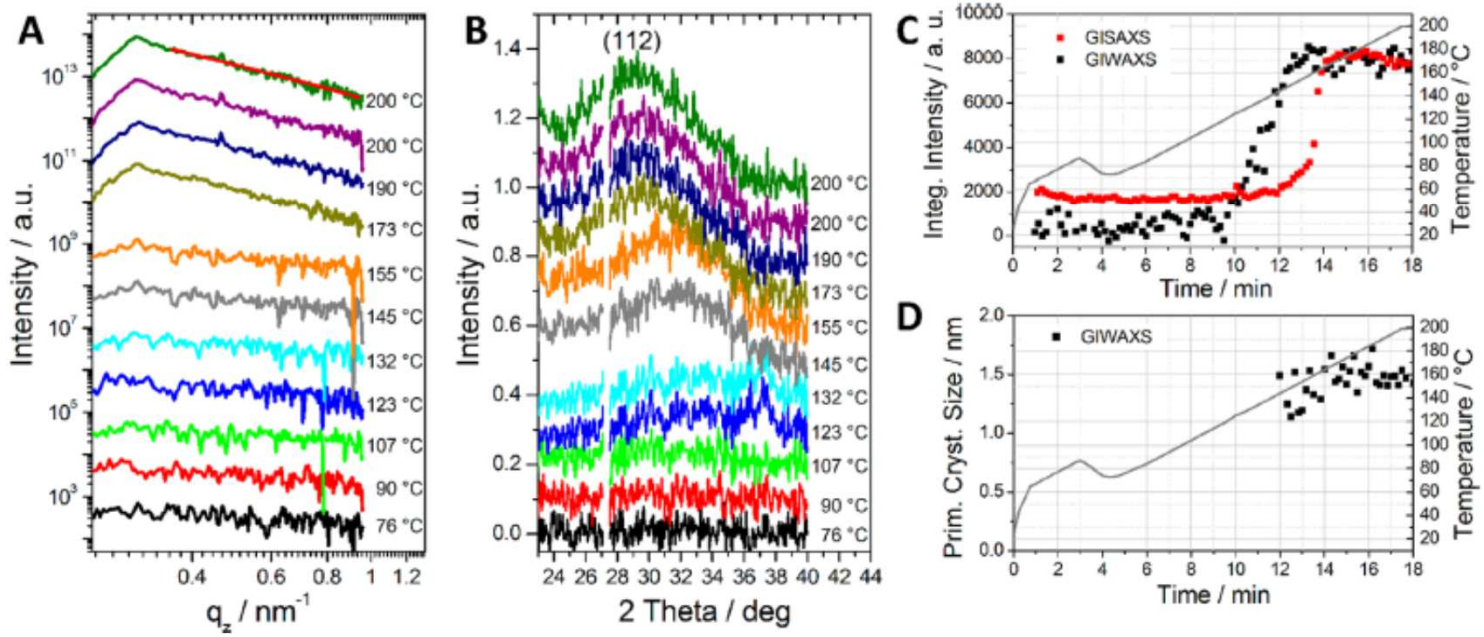
Making of Hybrid Solar Cells





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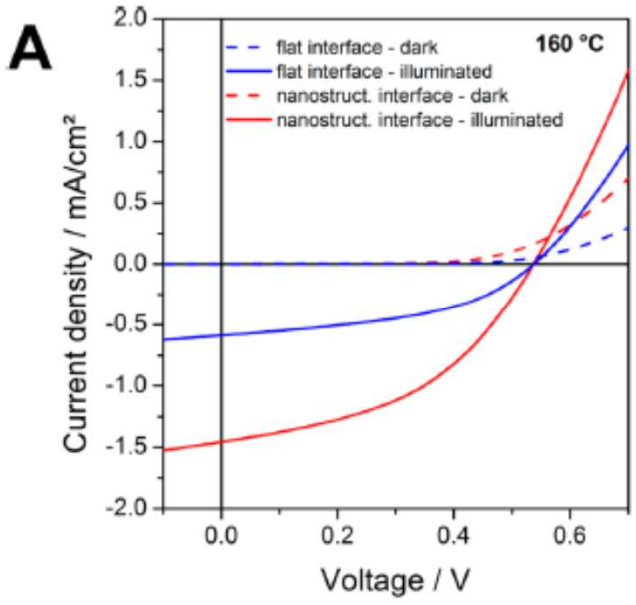
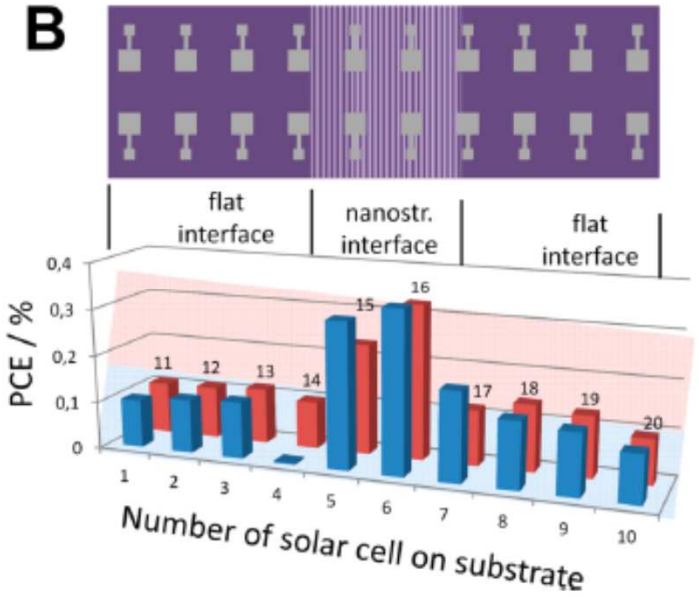
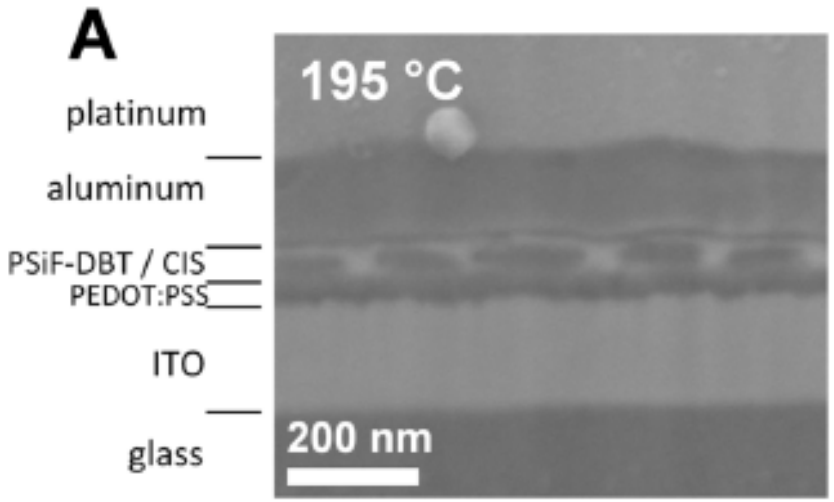
NIL Hyprid Solar Cells: Power conversion







Out come:

(i) Improvement up 3 times in PCE

(ii) Lower annealing temperatures better PCE 3 at 160° C to 1.5 at 195° C

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Conclusion

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