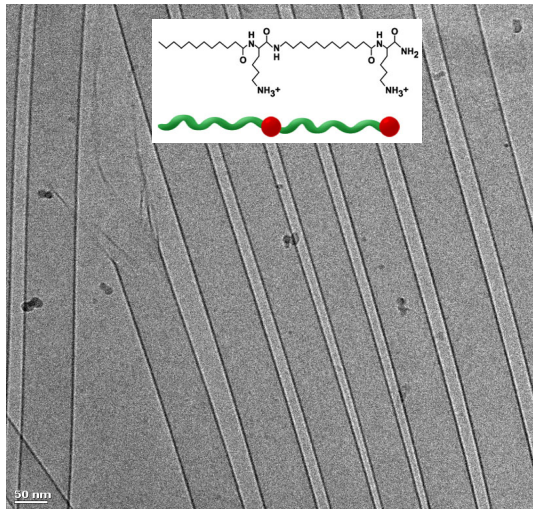
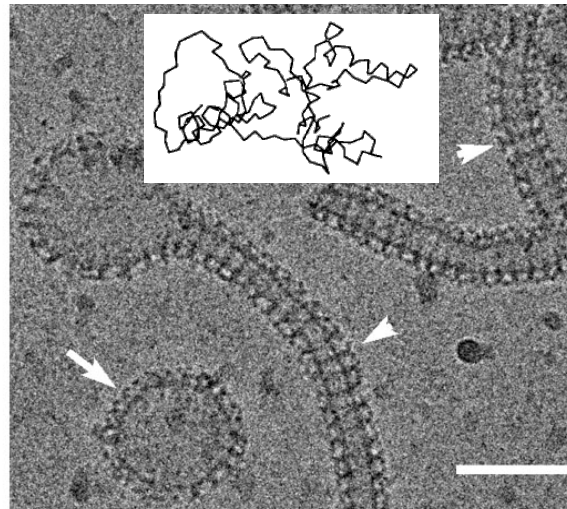




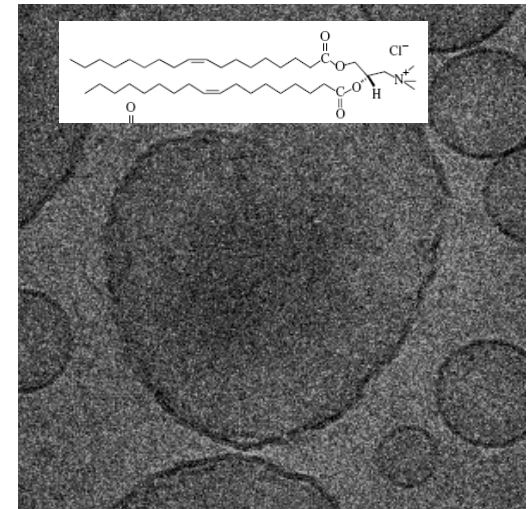
Spatial and Temporal CryoEM of Soft Molecular Assemblies and other Colloids



peptides



proteins



lipids

Dganit Danino

Dept. of Biotech and Food Eng, and The Russell Berrie
Nanotechnology Institute, Technion- Israel institute of Technology

CryoEM@Technion.ac.il

ESRF & ILL, Grenoble, France, Feb 2019



Old campus,
established in
1912

Technion

12,000 undergrad students
2,000 graduate students

Branches:
GTIT, Cornell-TECH



Technion

3 Nobel Prize laureates in Chemistry in 7 years

Distinguished Profs.

Aaron Ciechanover, Avram Hershko 2004

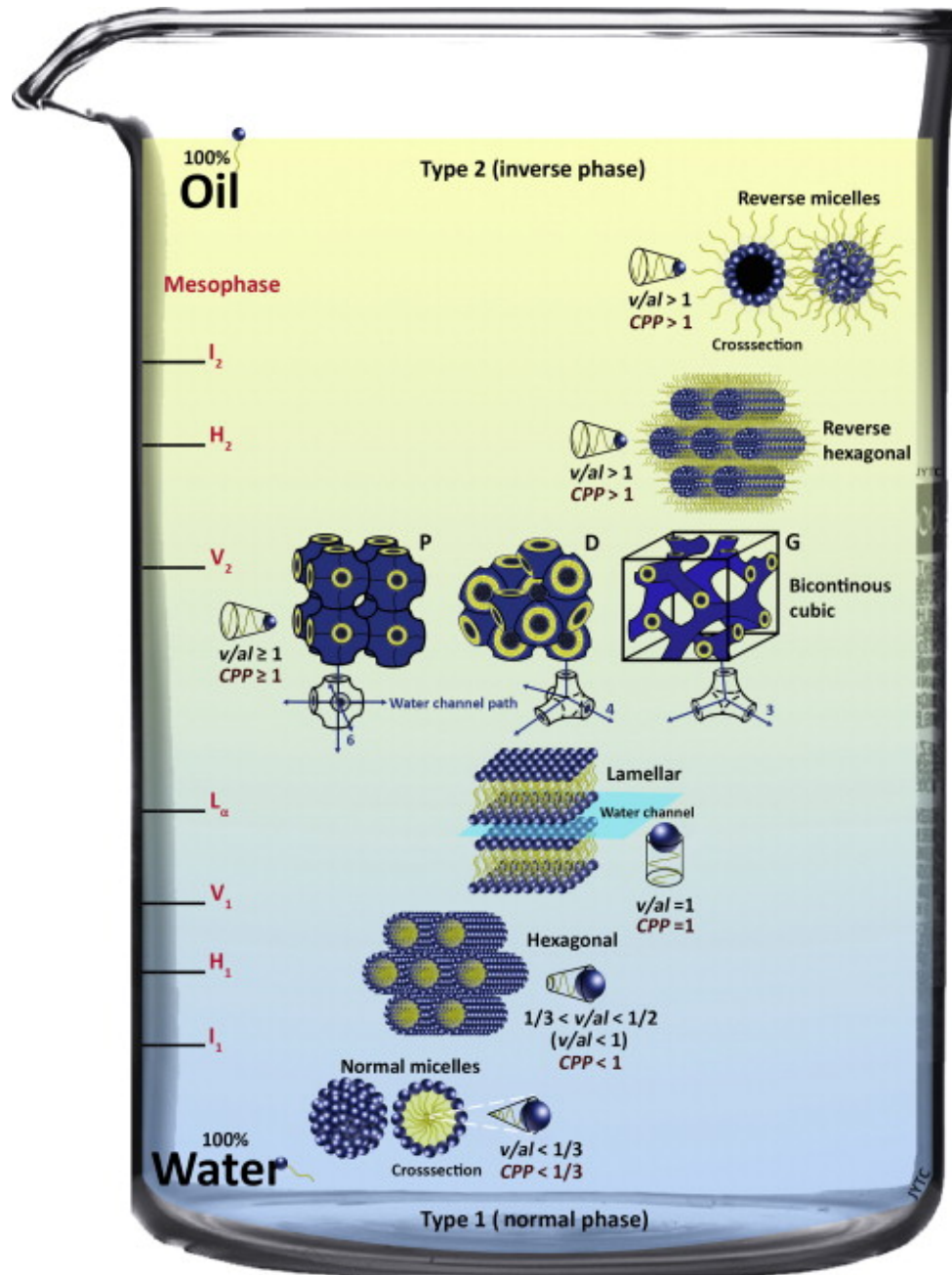
“for the discovery of ubiquitin-mediated protein degradation”

Distinguished Prof. Dan Shechtman 2011

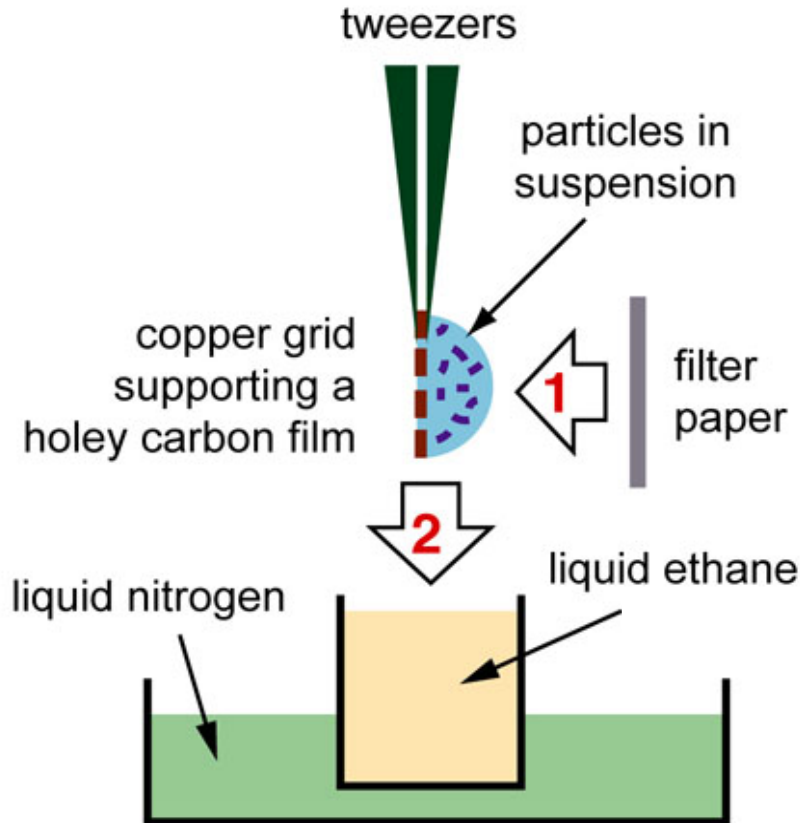


“for the discovery of quasicrystals”

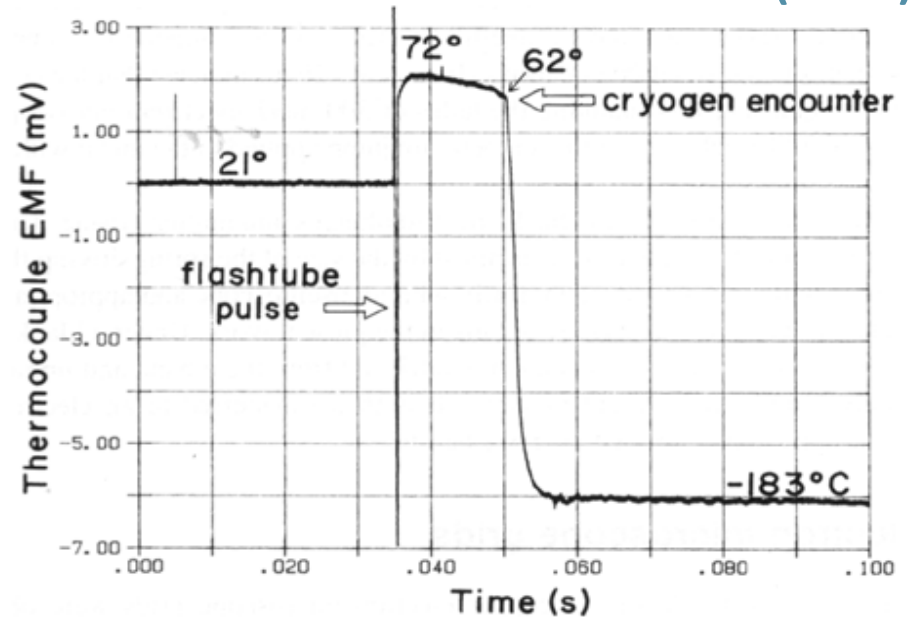




Vitrification - Transforming a liquid sample to a vitreous (amorphous) specimen by rapid cooling



Chestnut et al (1992)



$\Delta T = 245\text{ }^{\circ}\text{C}$

50-300 nm

$\Delta t = \sim 4\text{ ms}$

LN₂ - inert, inexpensive but bad coolant

Freezing temp $-210\text{ }^{\circ}\text{C}$

Boiling temp $-196\text{ }^{\circ}\text{C}$

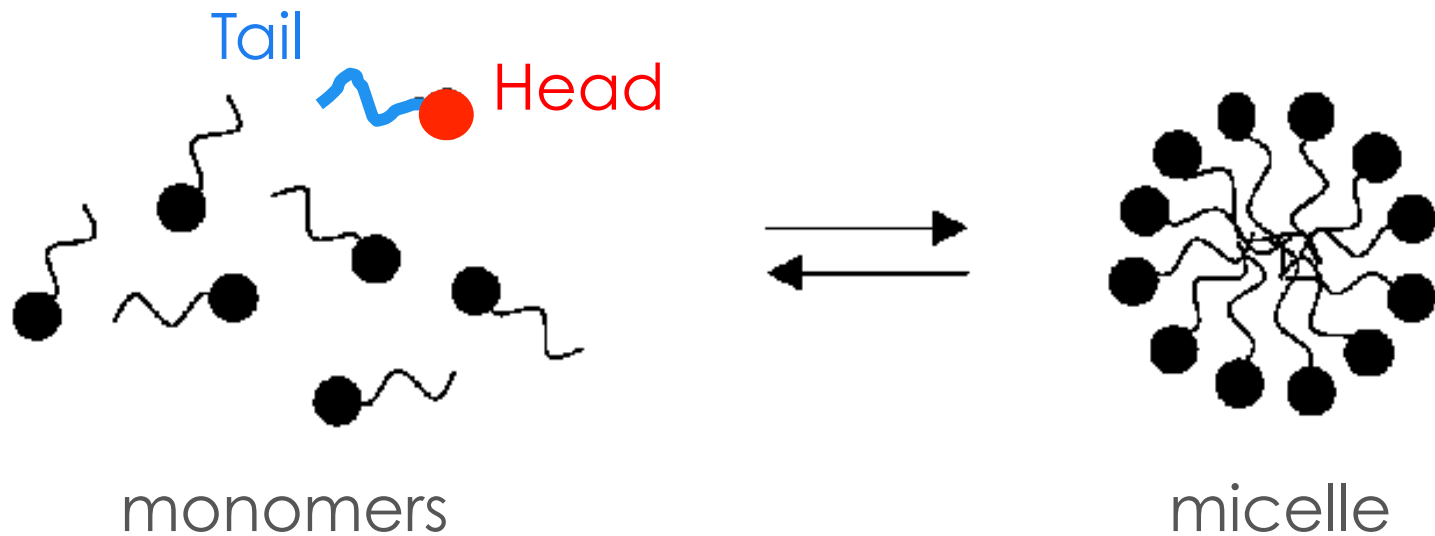
Ethane - excellent coolant

Freezing temp $-183.2\text{ }^{\circ}\text{C}$

Boiling temp $-88.6\text{ }^{\circ}\text{C}$

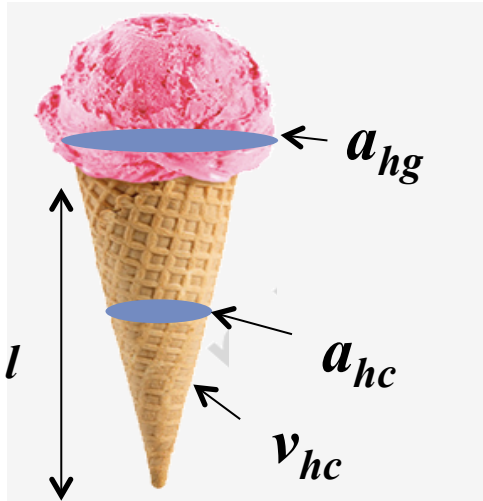
Fact # 1:

above the CMC amphiphiles form spherical micelles

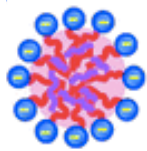


Fact # 2:

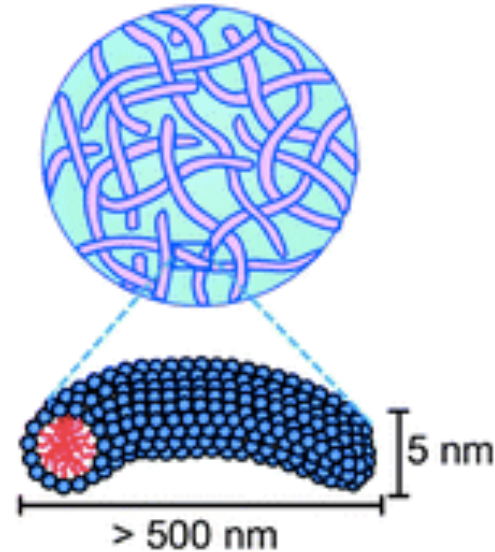
micelles can grow from spherical to wormlike/threadlike



$$P = \frac{a_{hc}}{a_{hg}} = \frac{V_{hc}}{l \cdot a_{hg}}$$



temperature
concentration
ionic strength

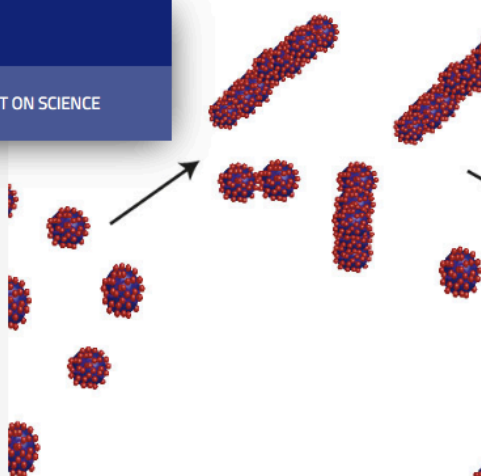


Long worms

Spheres becoming worms: elucidating the transformation of surfactant micelles

News

SPOTLIGHT ON SCIENCE



26-09-2014

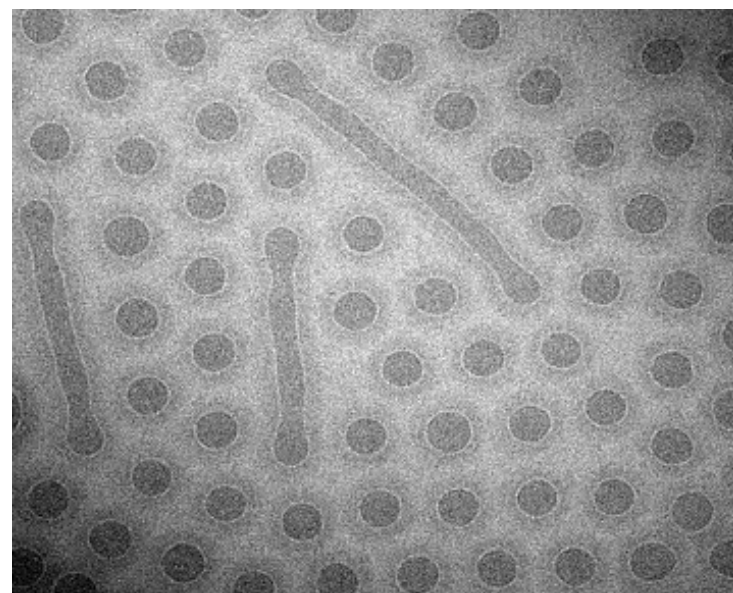
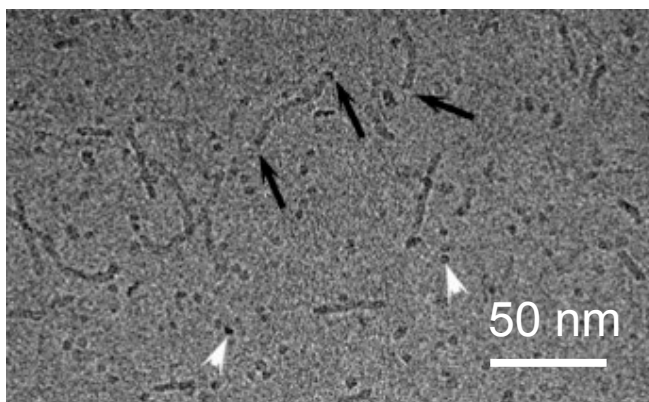
The morphological transformation pathways in micellar systems are still not well understood despite over a century of research in surfactant science. This is mainly due to lack of sufficiently fast methods that capture structural changes underlying such transformations. In the present work, an archetypical, simple surfactant system consisting of sodium dodecyl sulphate in aqueous salt solutions was investigated using time-resolved small-angle X-ray scattering (SAXS) combined with stopped-flow rapid mixing. The results show that upon rapid change in salt concentration, "worm-like" micelles are formed by the fusion of globular and transient elongated micelles on a millisecond time scale in a similar fashion to a step-like polymerisation process.

Share [in](#) [twitter](#) [facebook](#)

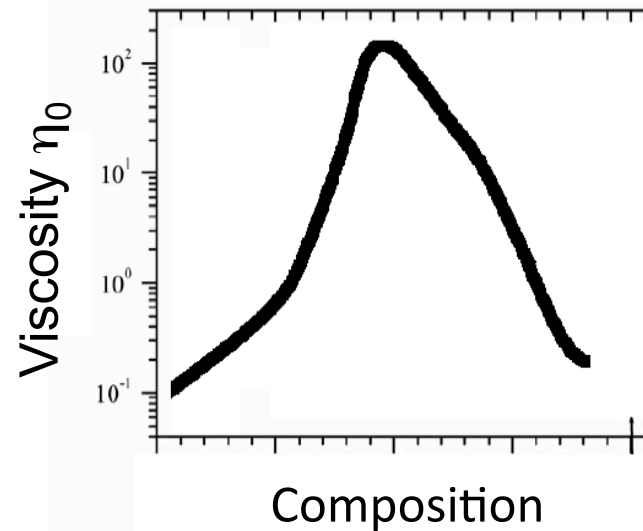
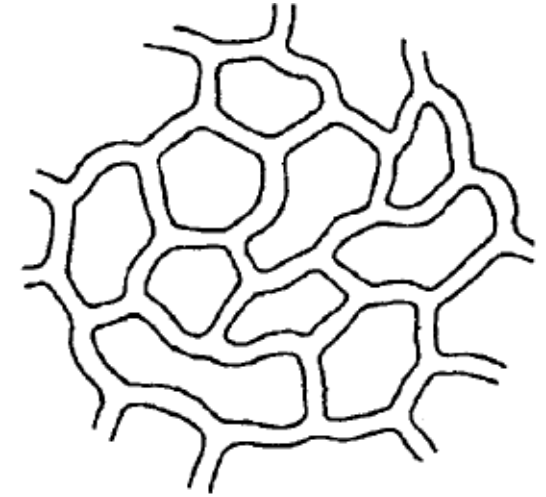
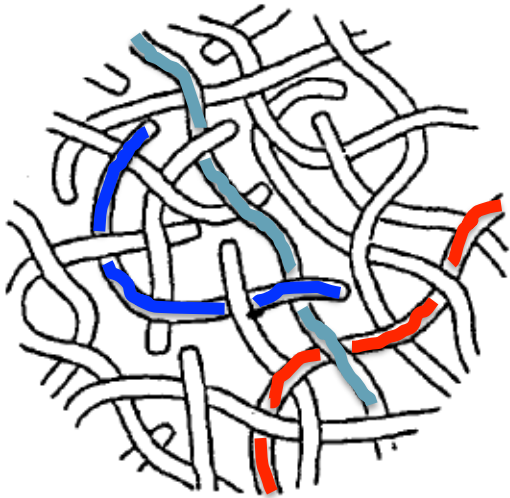
Cryo-TEM reveals

- ✓ core-shell structure
- ✓ spheres to worms
- * ✓ swollen endcaps

Shimoni & Danino, Langmuir, 2006

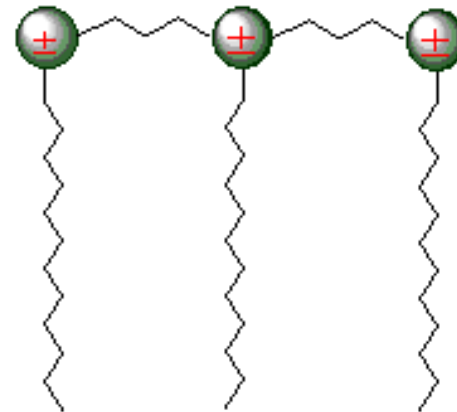
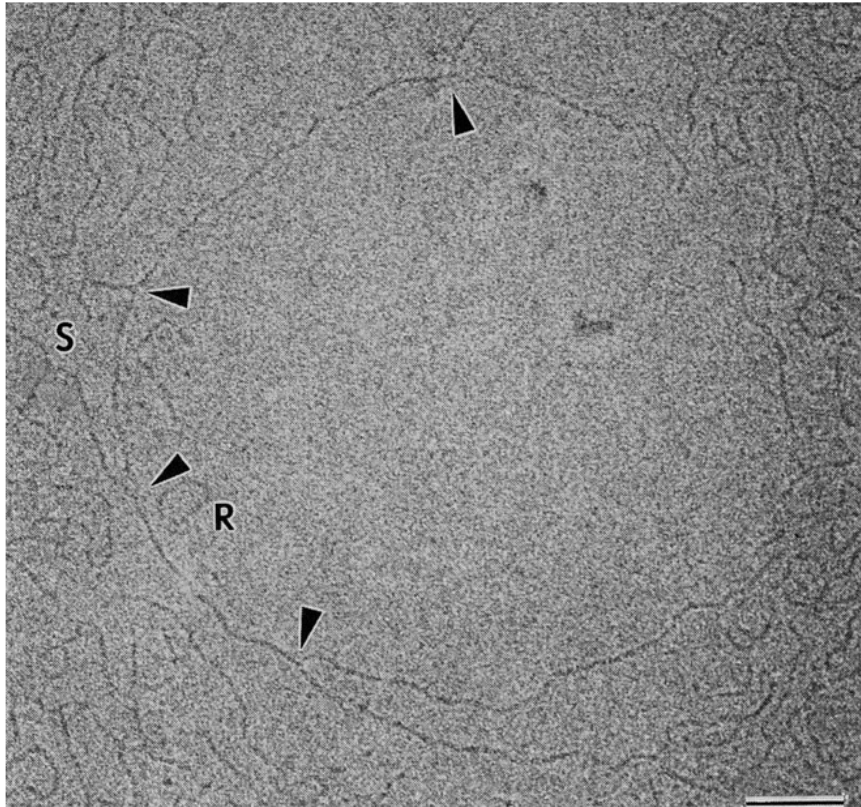


Micelles ('living polymers') – dynamic entities presenting high viscosity upon growth (confirmed) and - low viscosity upon branching (speculated)



Candau et al
J Phys IV (1983)

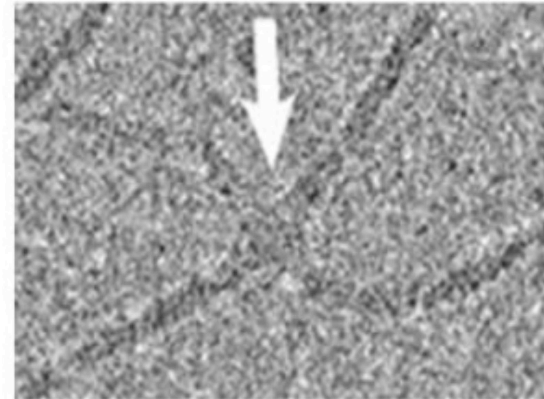
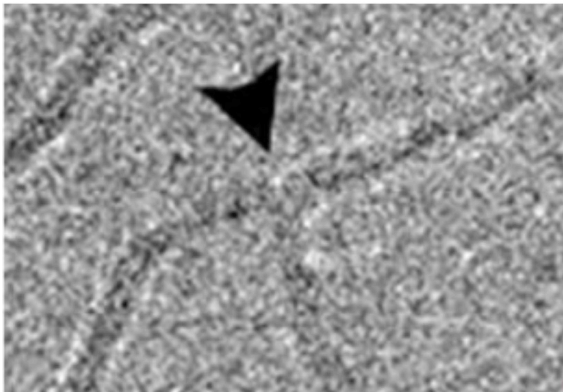
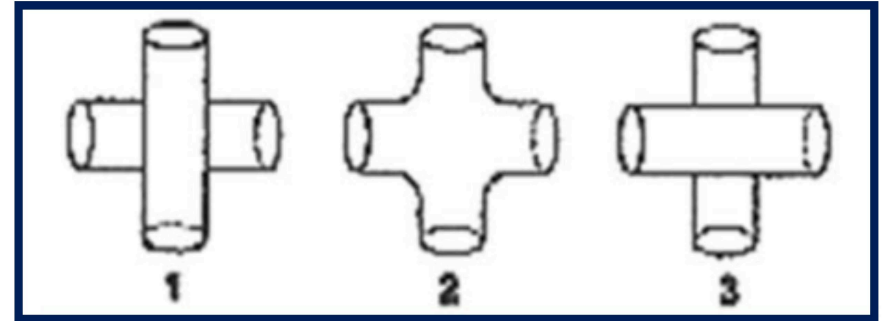
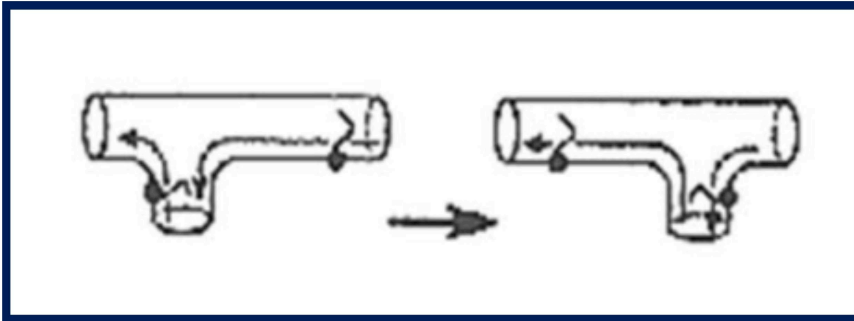
Cryo-TEM provides conclusive evidence of branches

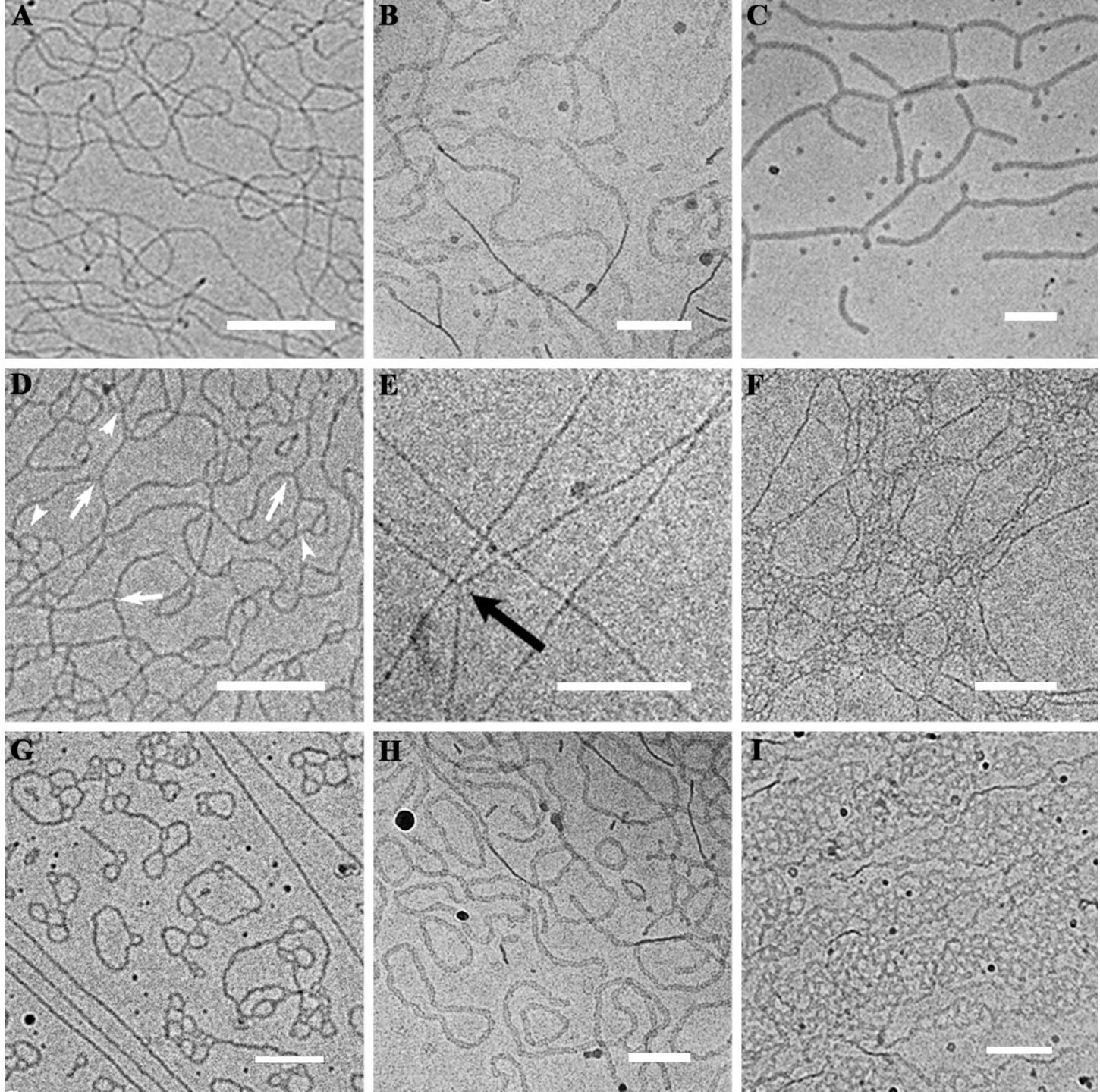


Danino et al, Science (1995)



Branching - dynamic, fluid, transient interconnections

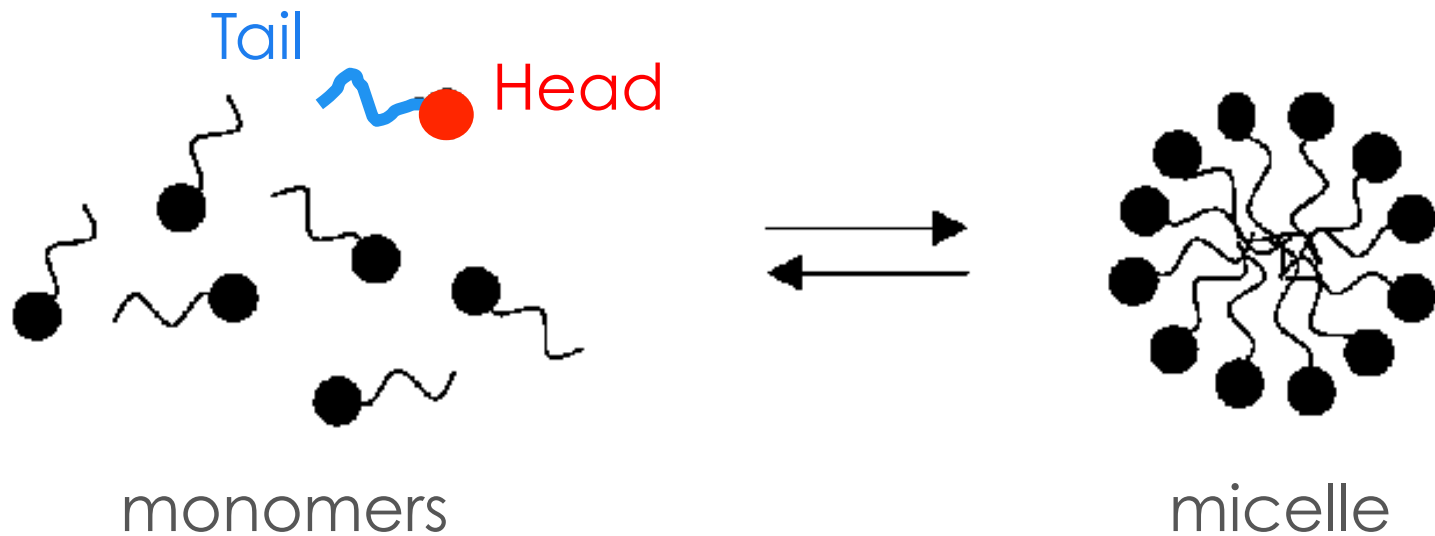




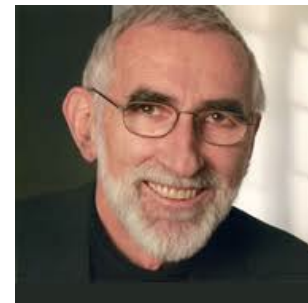
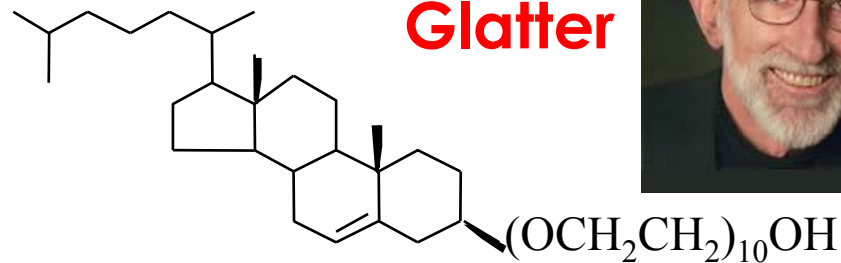
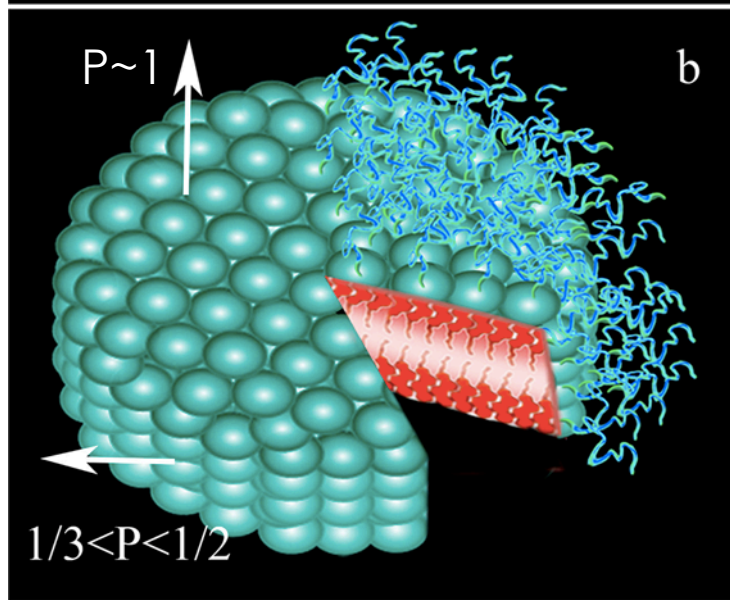
Danino, in
"Wormlike
micelles"
(2017)

Fact # 1:

above the CMC amphiphiles form spherical micelles

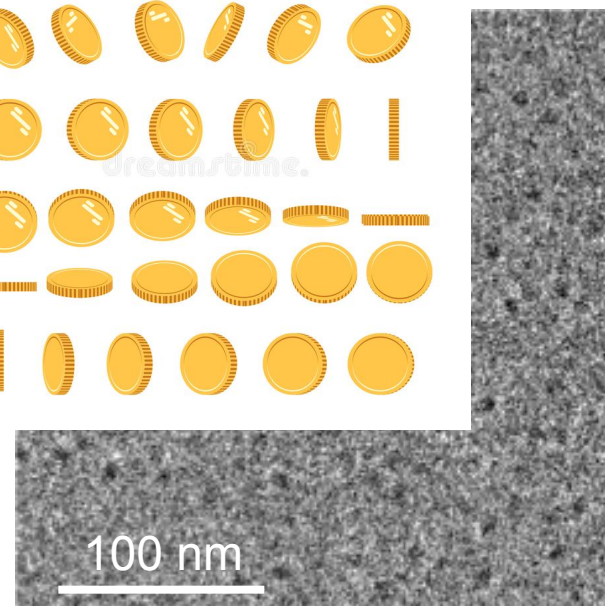
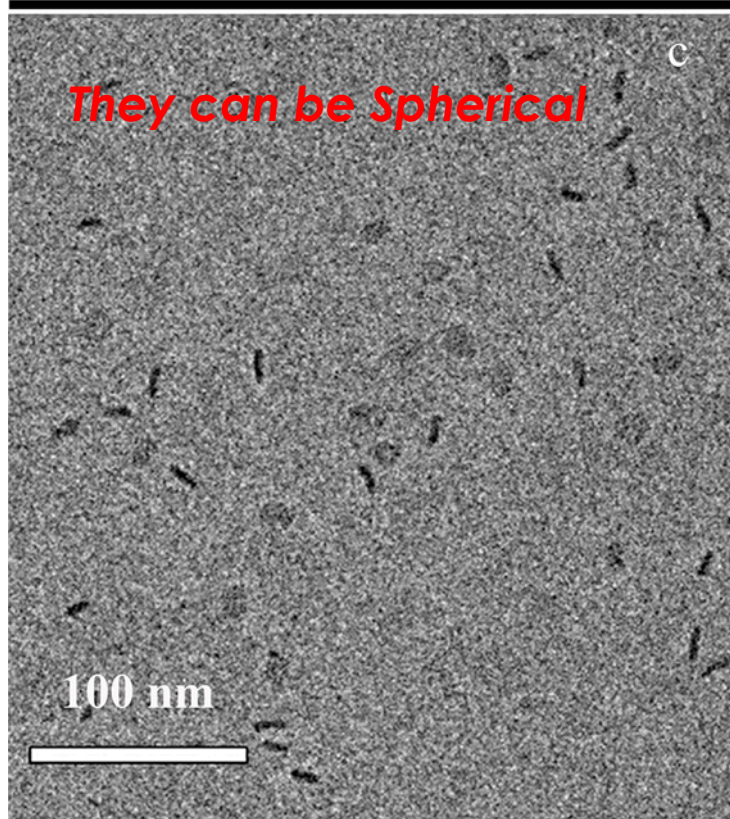
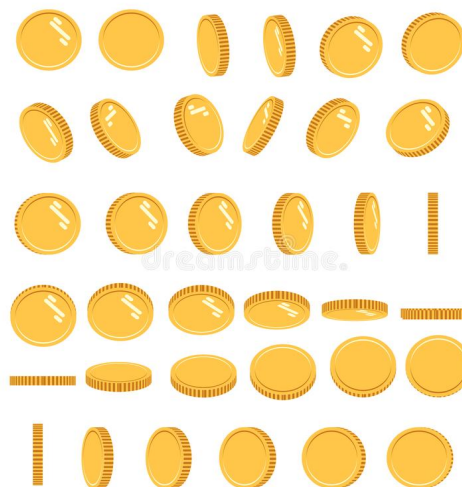


Always Spherical Micelles?



D ~ 13 nm

t ~ 4-5 nm

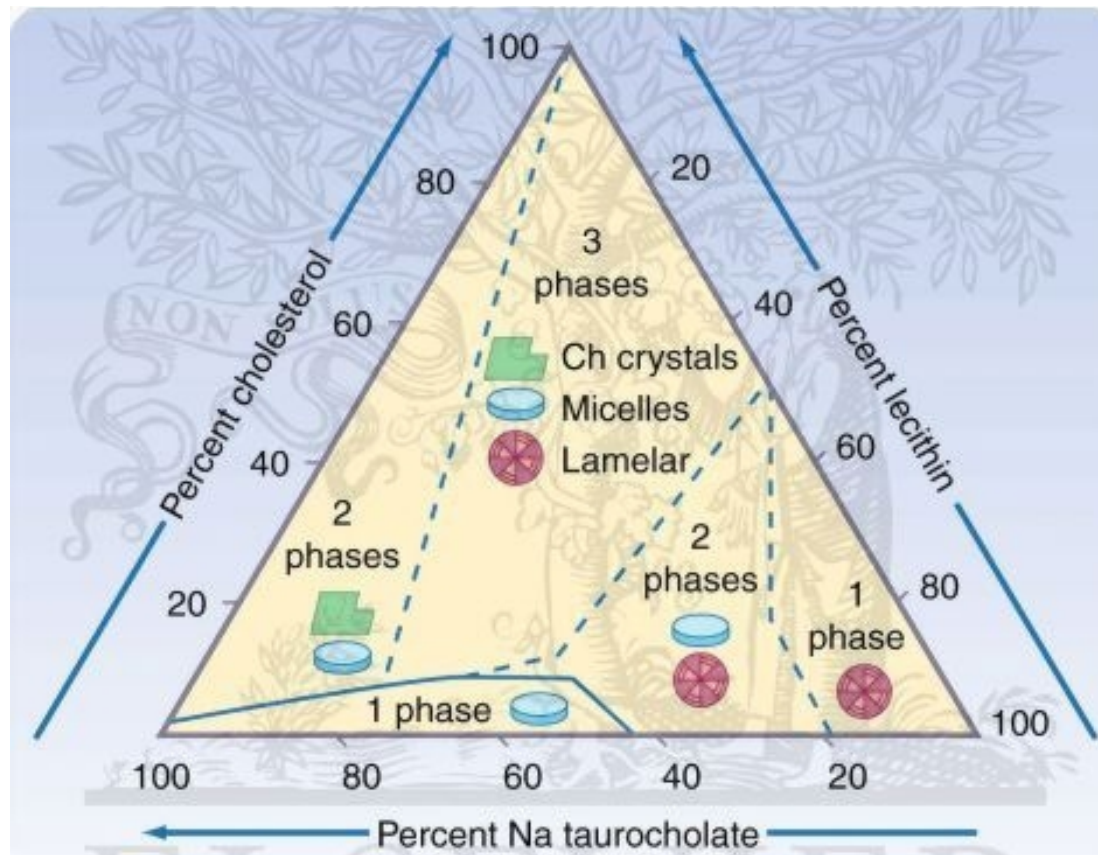


ChEO₁₀

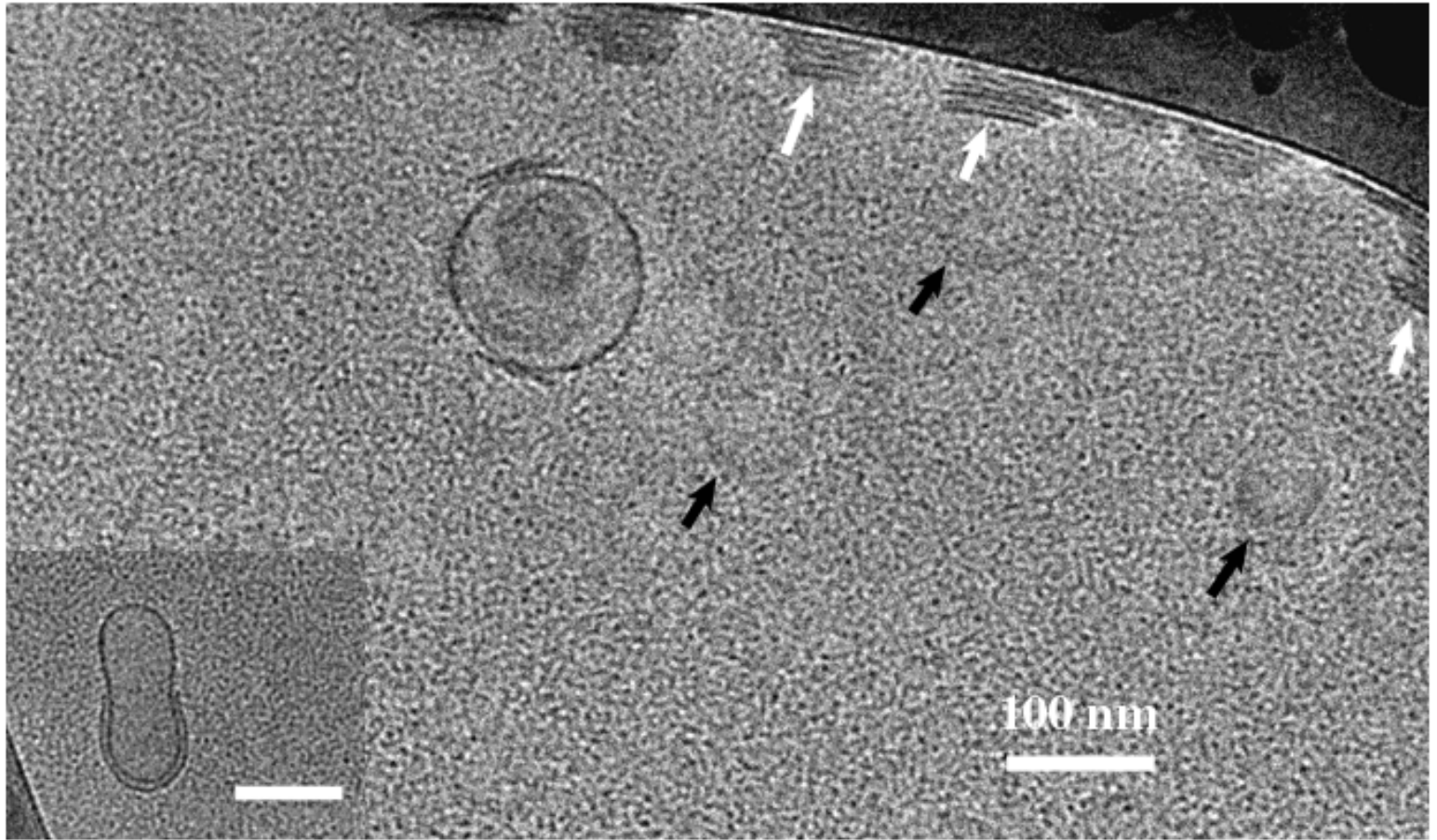
Danino et al, J Phys Chem Lett, 2016



Gallstones



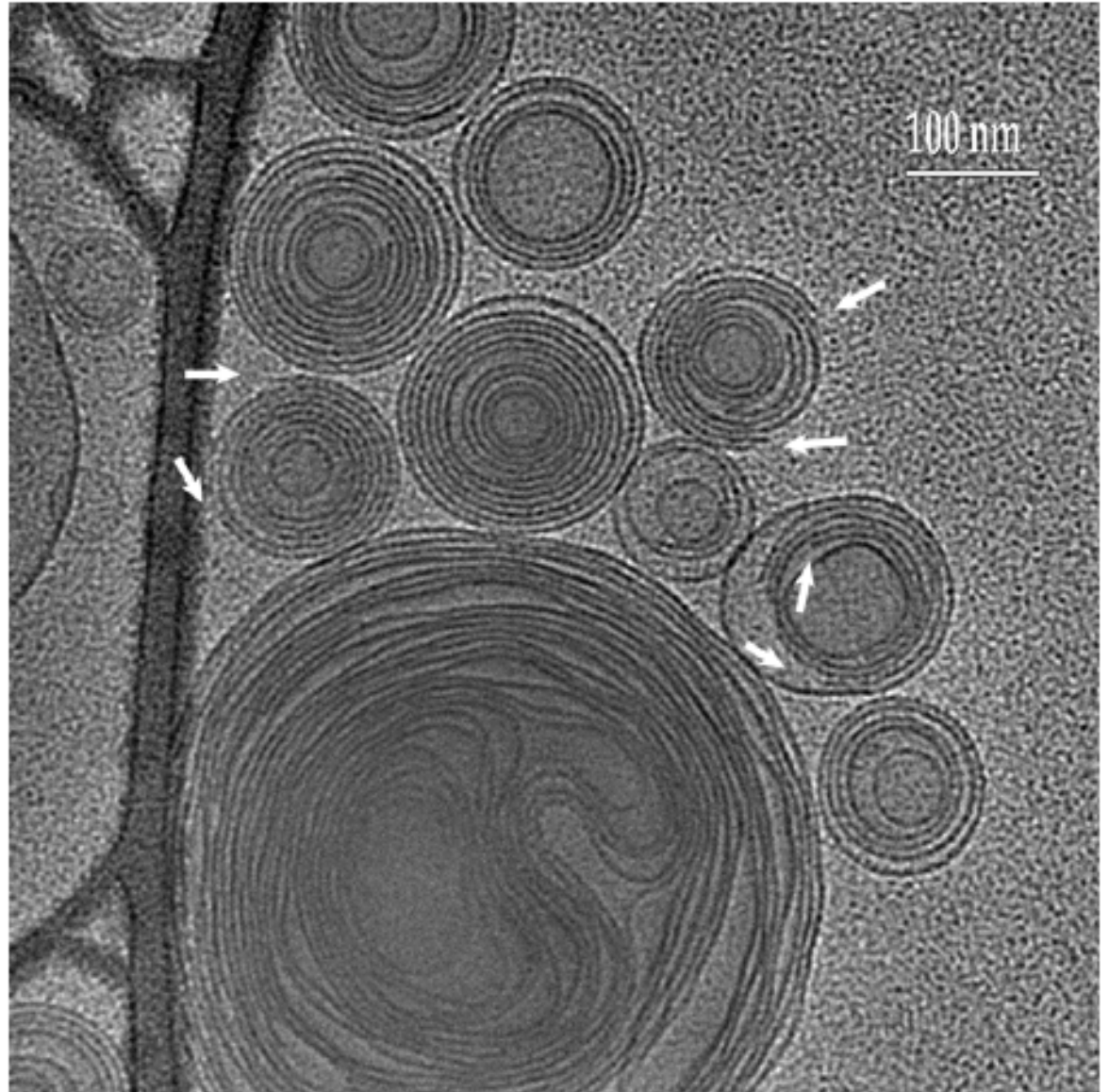
Time-resolution cryo-TEM of cholesterol crystallization in nucleating bile

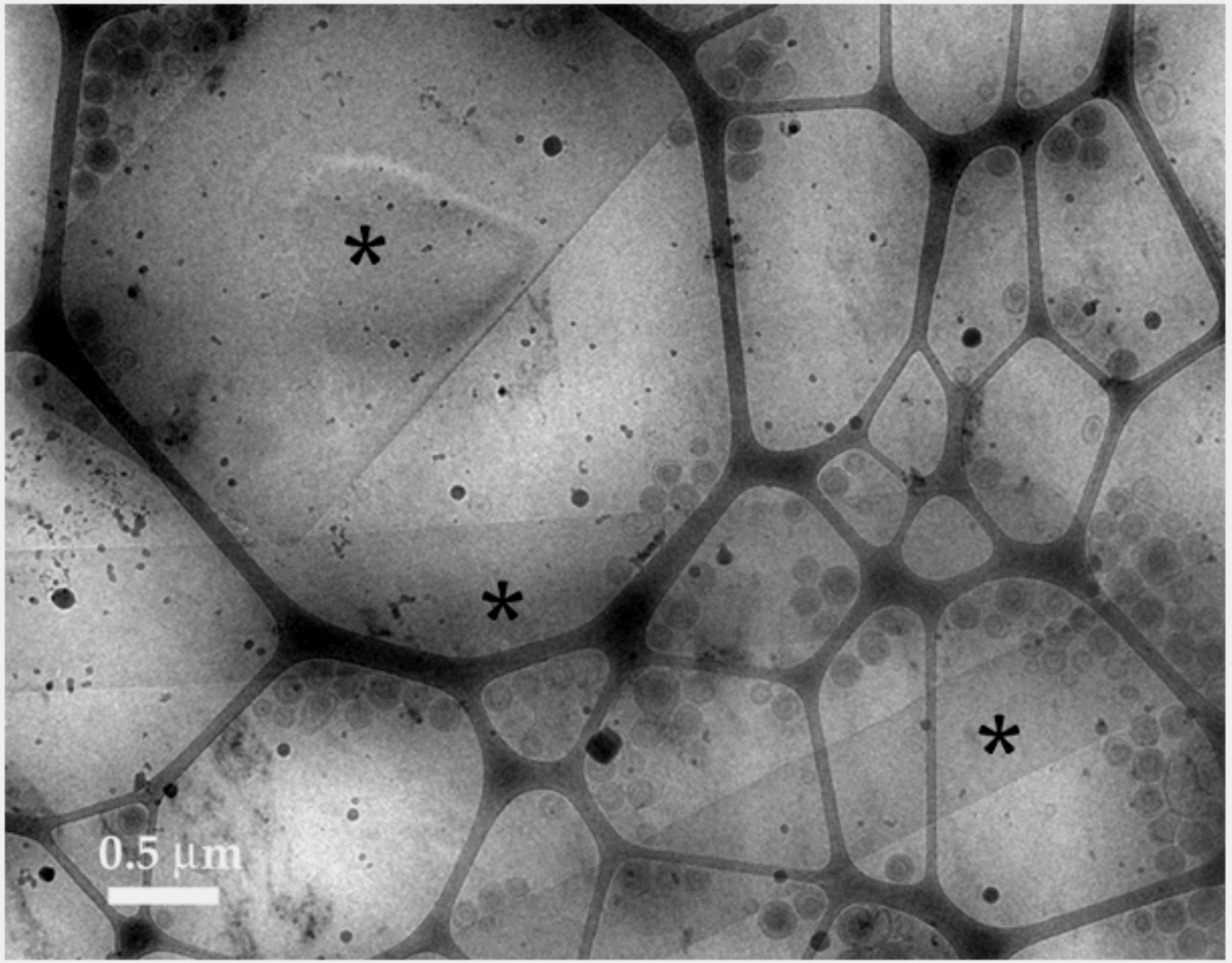


Model Bile, Time Zero

Model Bile

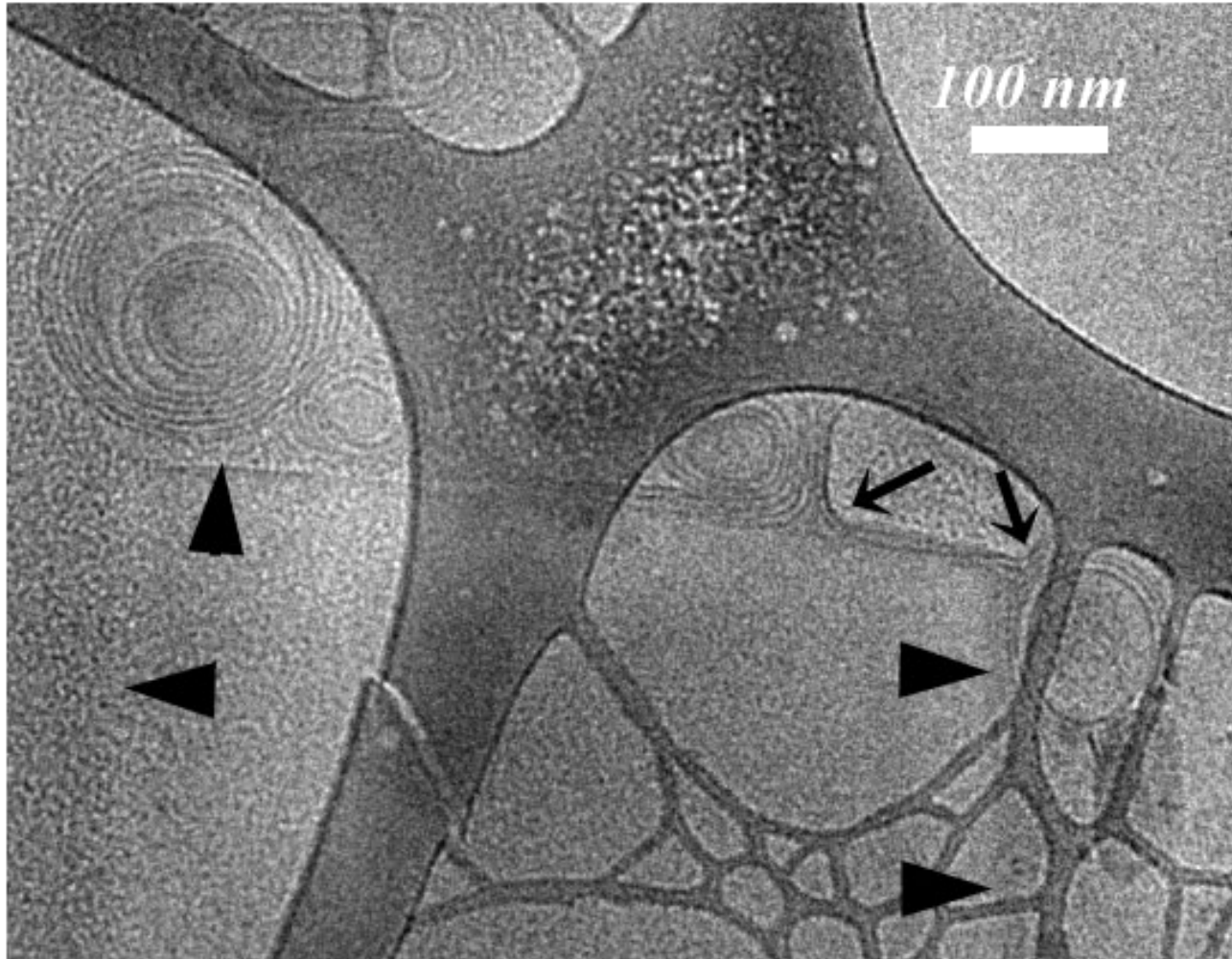
1 hr after
dilution





Model Bile, 42 hrs after dilution

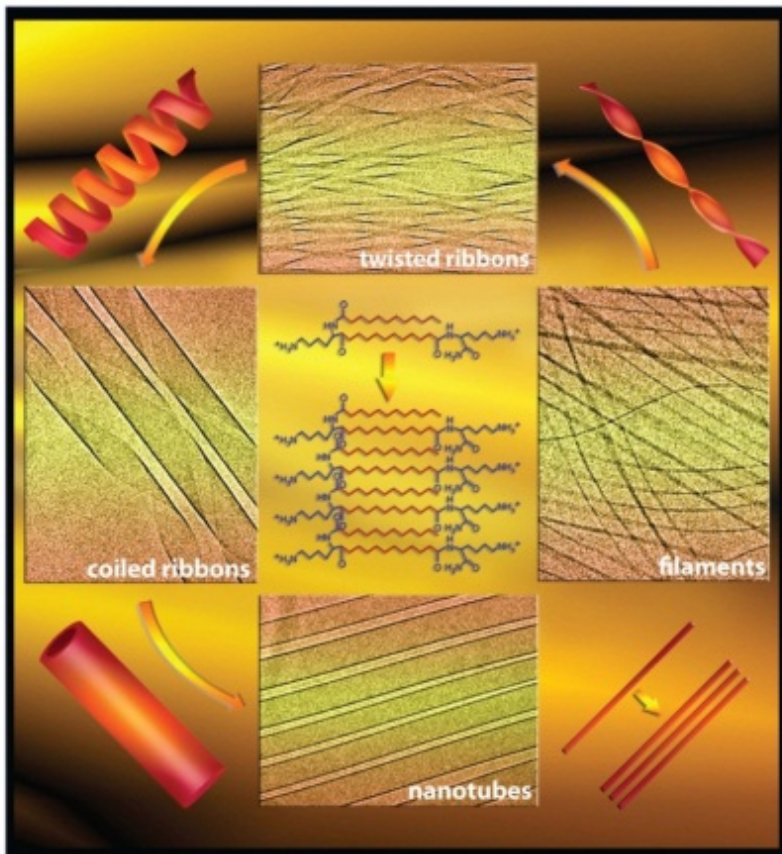
How do the crystals grow ?



Possibly by interaction between the bilayers and the growing crystal

March 2, 2011
Volume 133
Number 8
pubs.acs.org/JACS

J | A | C | S
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY



ACS Publications
MOST TRUSTED. MOST CITED. MOST READ.

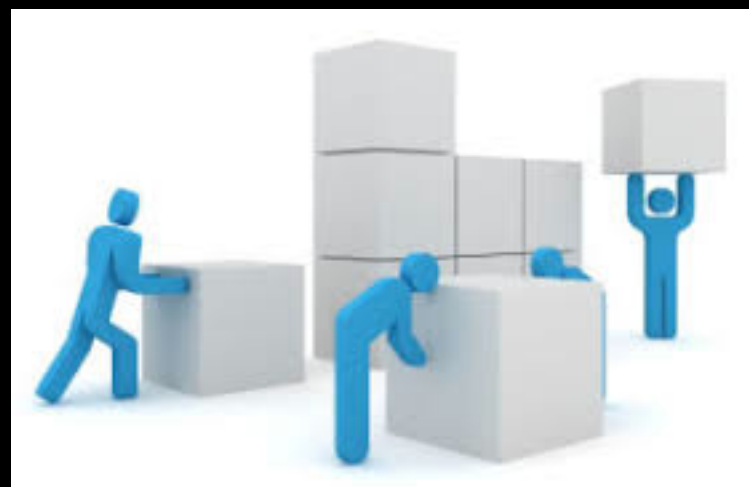
www.acs.org

Chiral Self-Assembly

Bio-Inspired Building Blocks

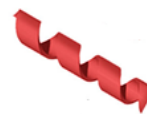
Shape Selection

Design Rules





1D Structures

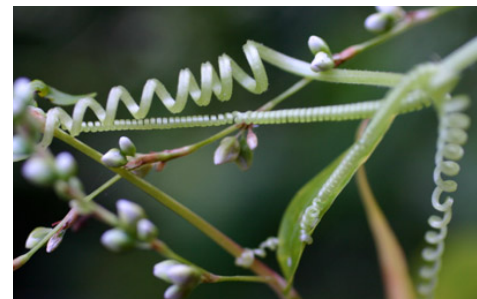


Sea shells



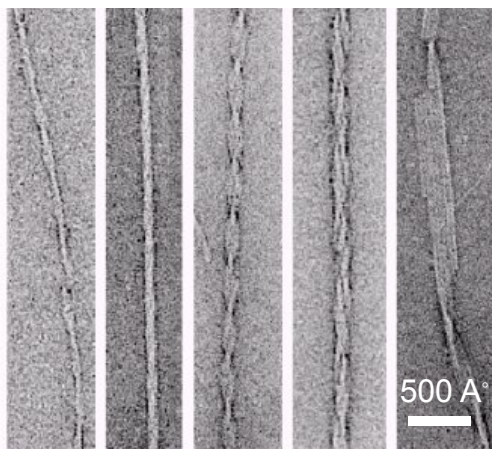
Bauhinia pods

Sharon, Science, 2011



Cucumber Tendril Coils

Mahadevan, Science, 2012



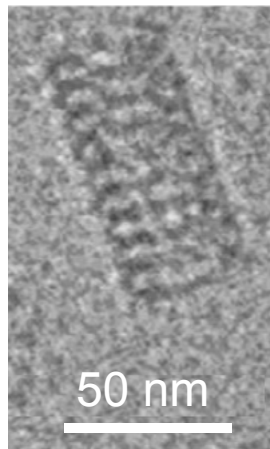
Amyloid fibrils

Saibil, PANS, 2002



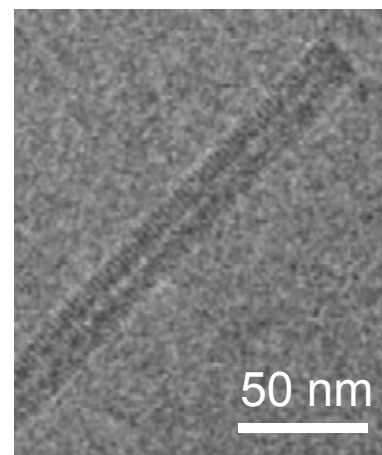
Nucleating Bile

Danino



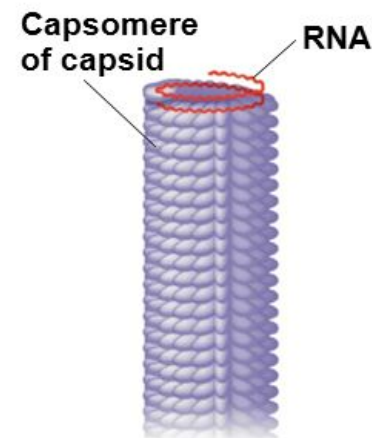
Endocytosis (dynamin)

Danino



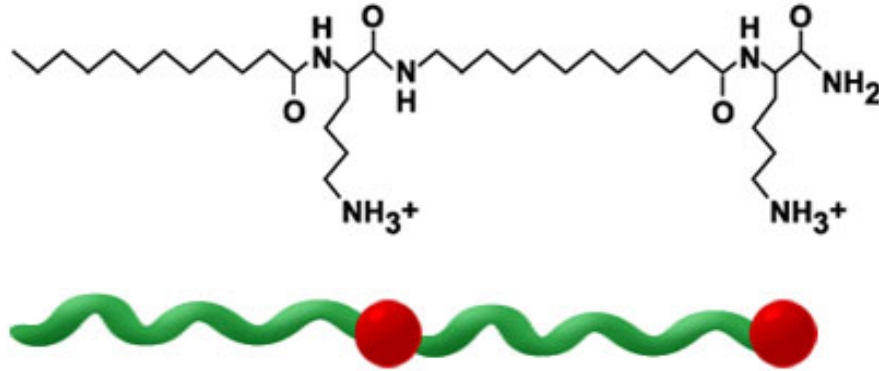
Tobacco Mosaic Virus (TMV)

Sachse et al., J. Mol. Bio. 2007



Mechanisms of Chiral Self-Assembly

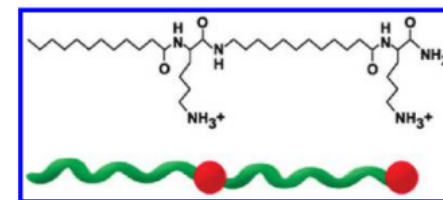
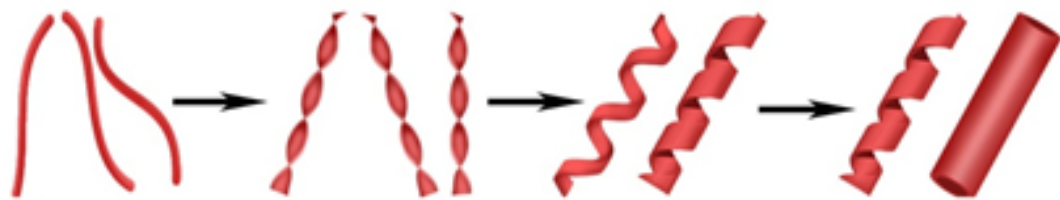
1. Molecular design



Dilauryllysine (C₁₂-β₁₂)

Simple..... and **smart**

Time-resolved cryo-TEM provides the first complete pathway to nanotubes by chiral self-assembly



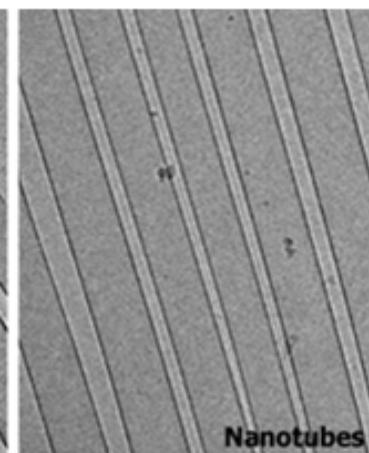
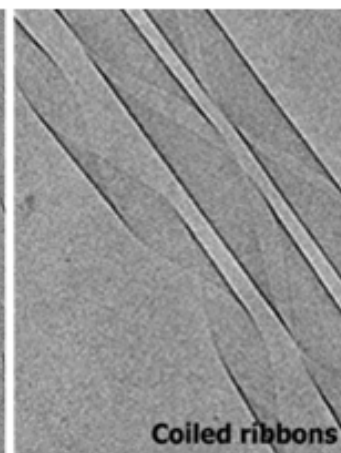
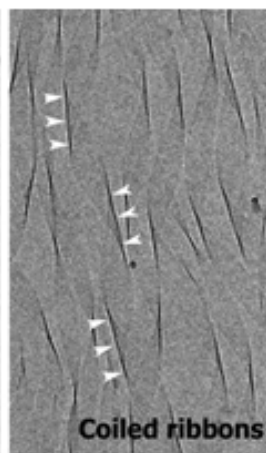
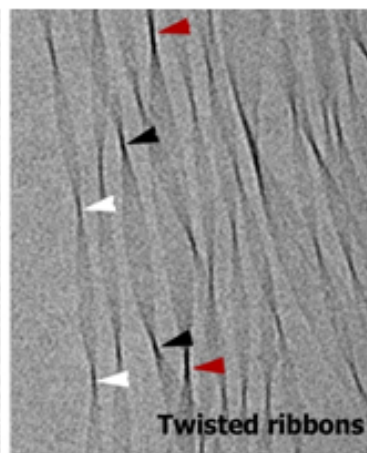
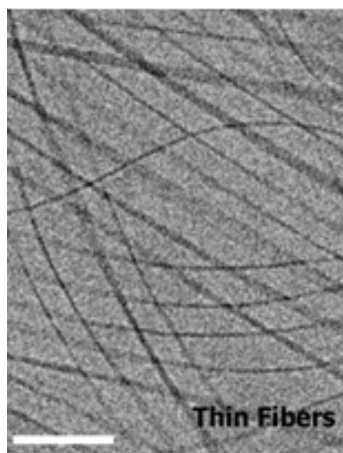
Minutes

24 hours

1 week

4 weeks

4 months

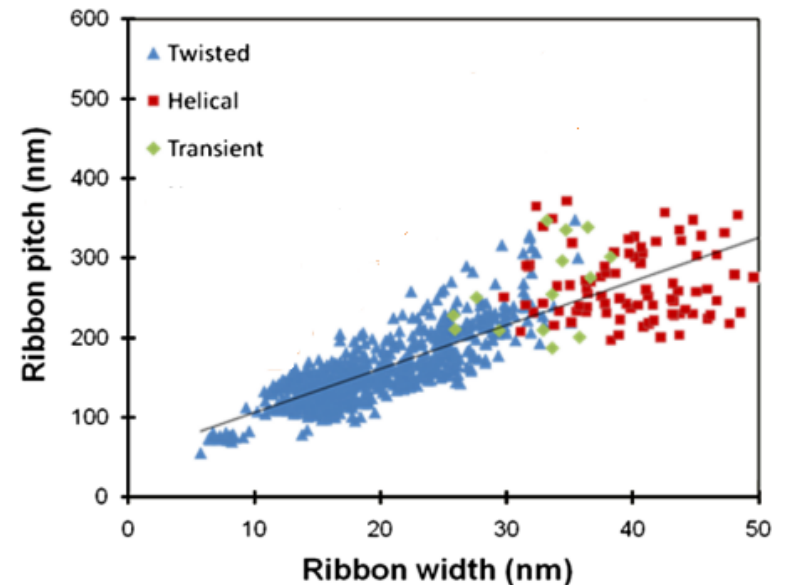
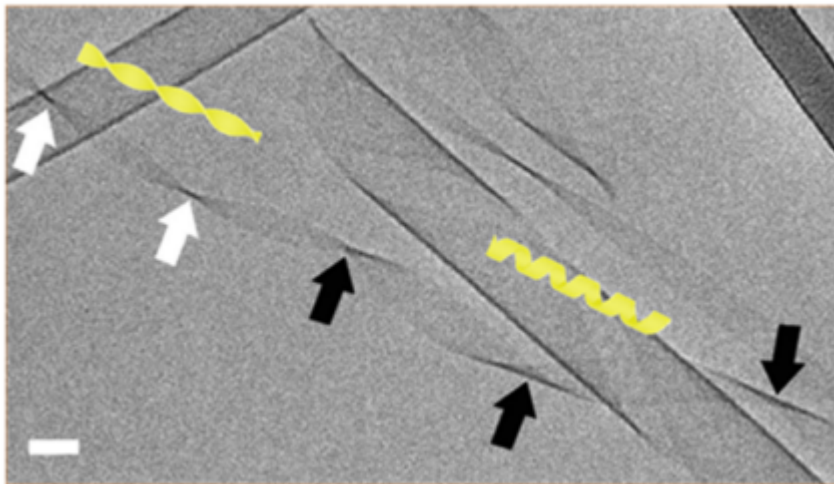
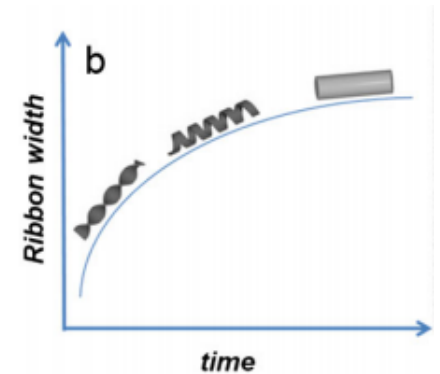


Lior Ziserman,[†] Hee-Young Lee,[§] Srinivasa R. Raghavan,[§] Amram Mor,[†] and Dganit Danino^{*,†,‡}

Shape Selection – dynamic transitions between Gaussian and cylindrical curvature

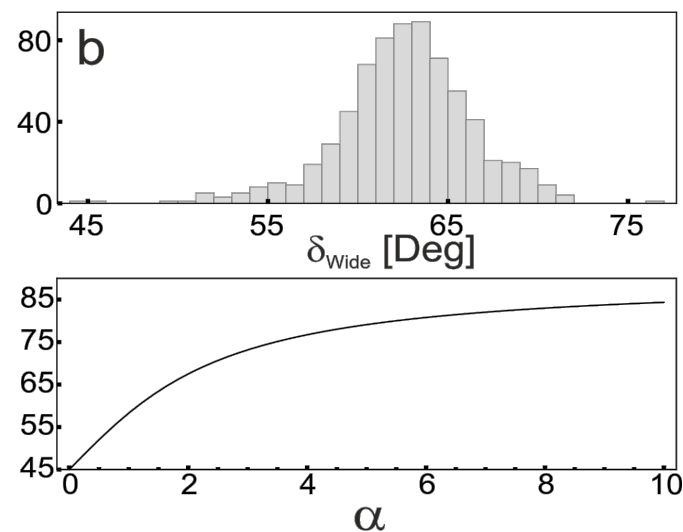
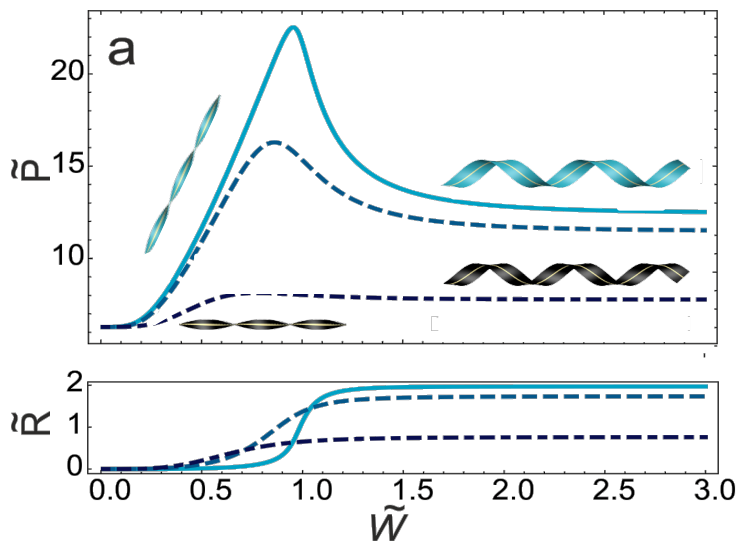
Quantitative cryo-TEM reveals

- ✓ Both width and pitch grow
 - ✓ Twisted ribbons are precursors of helical ones
 - ✓ The transition occurs at a defined ribbon width
- width

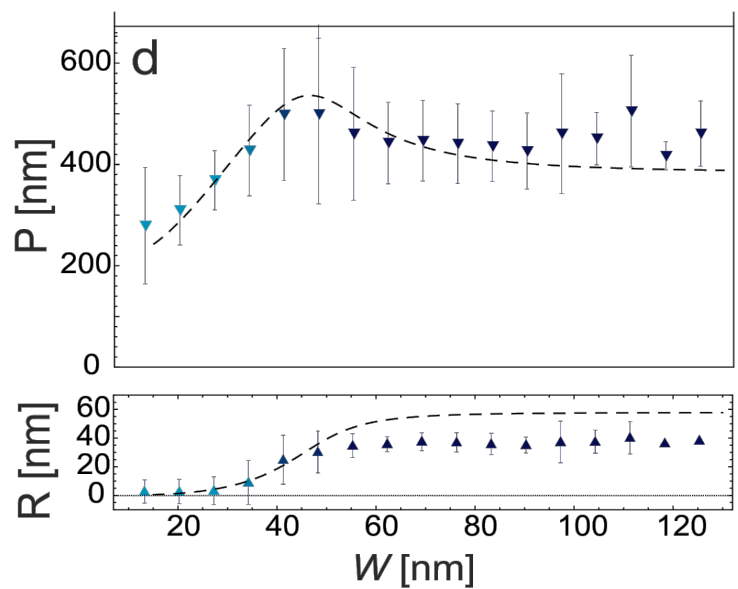
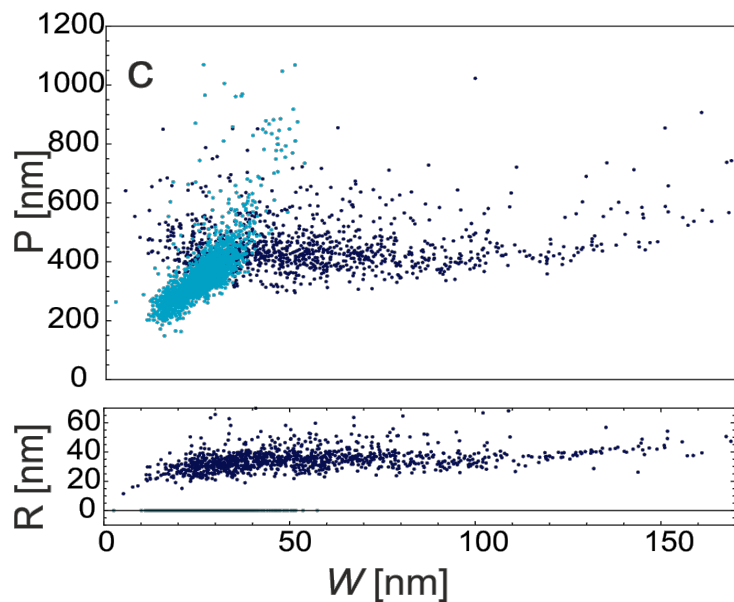


Shape evolution and statistics of self-assembled molecular chiral ribbons, using a reduced model of incompatible elasticity

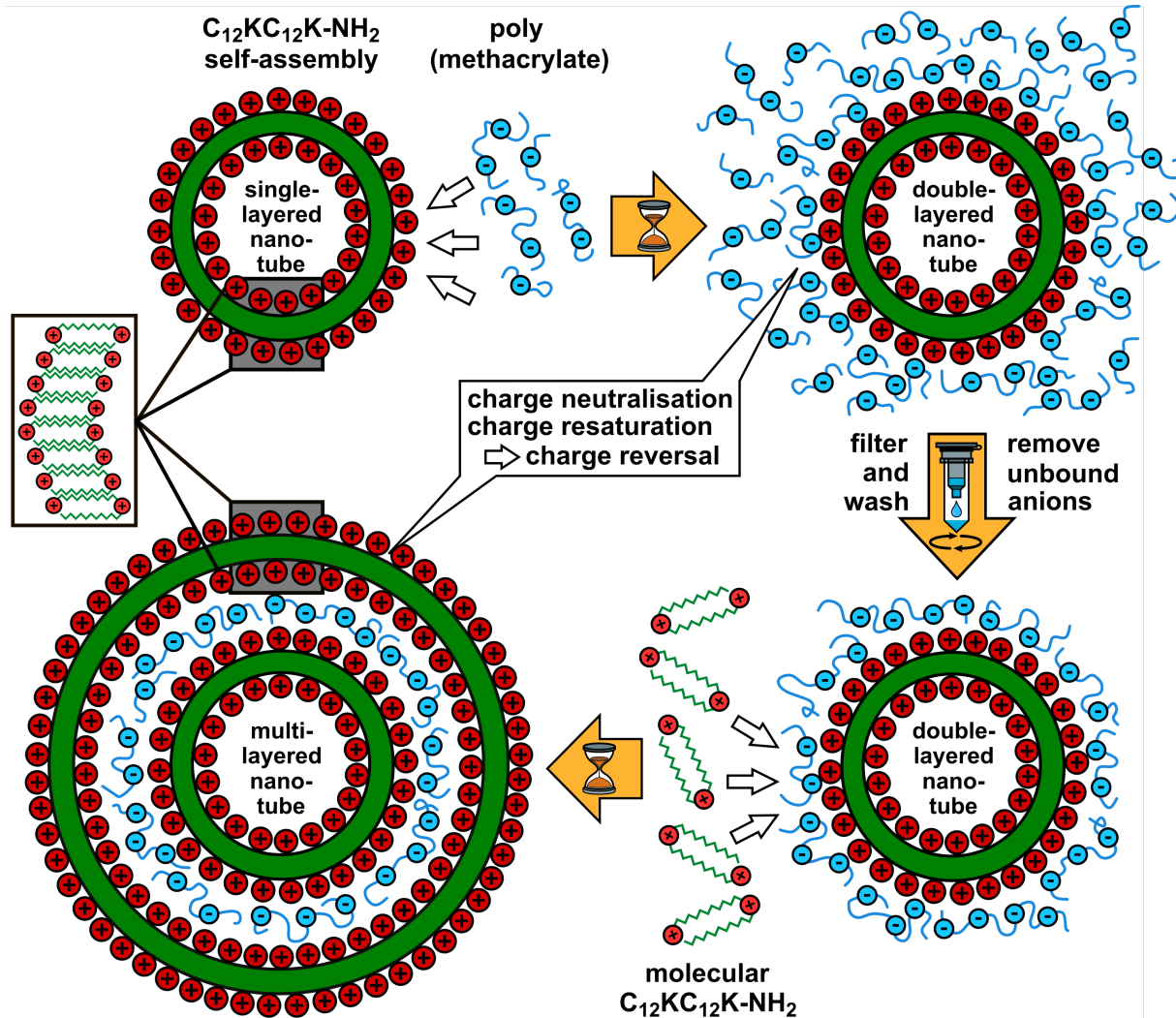
analytic solutions



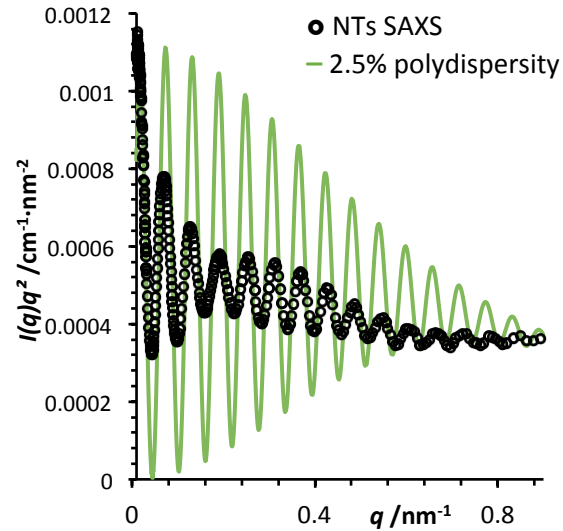
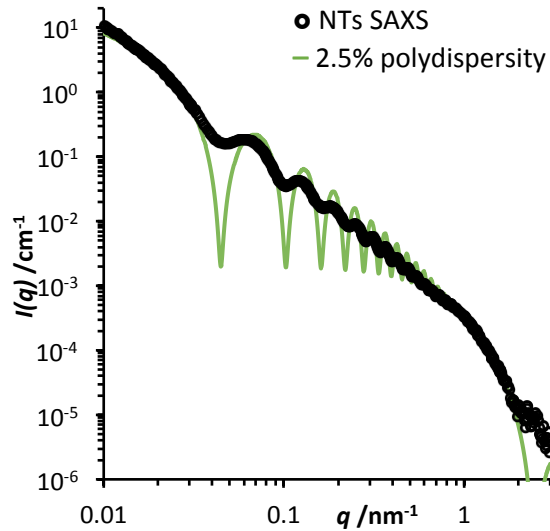
experiment



Can we control multilamellarity?

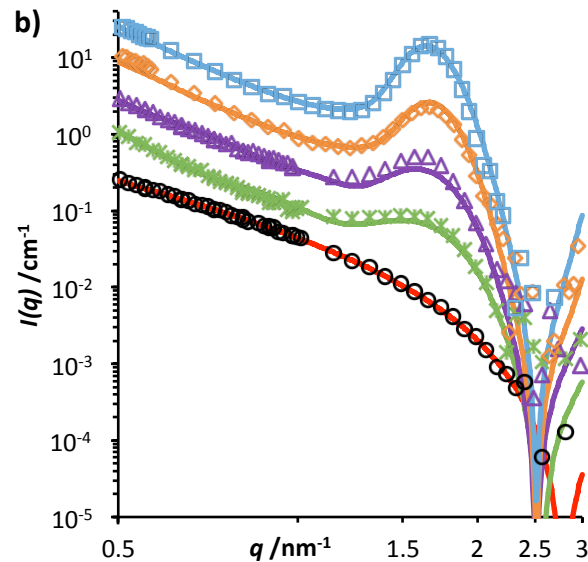
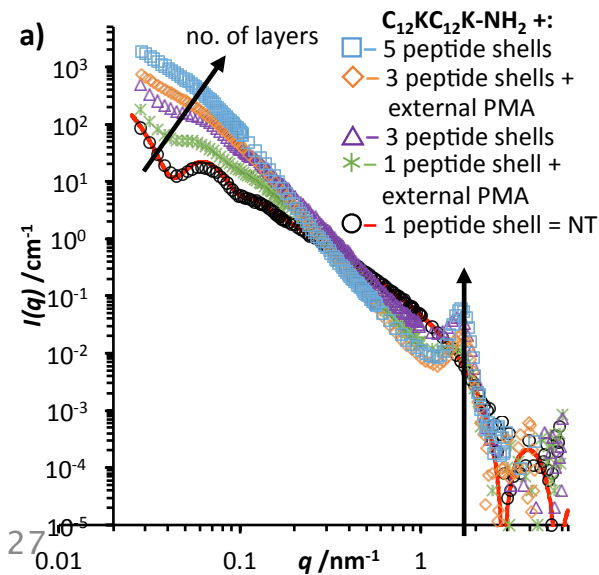


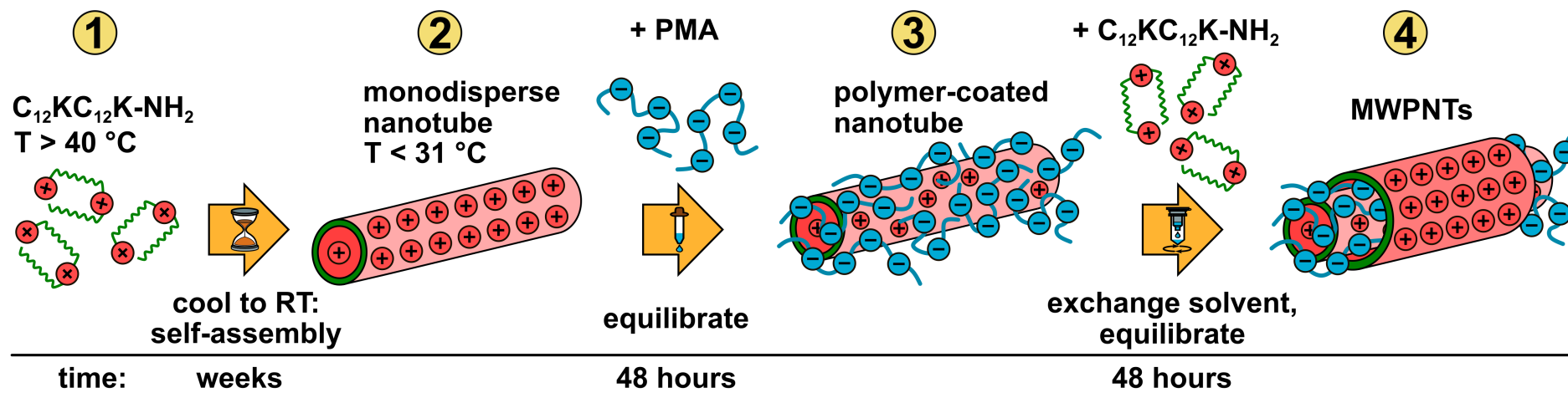
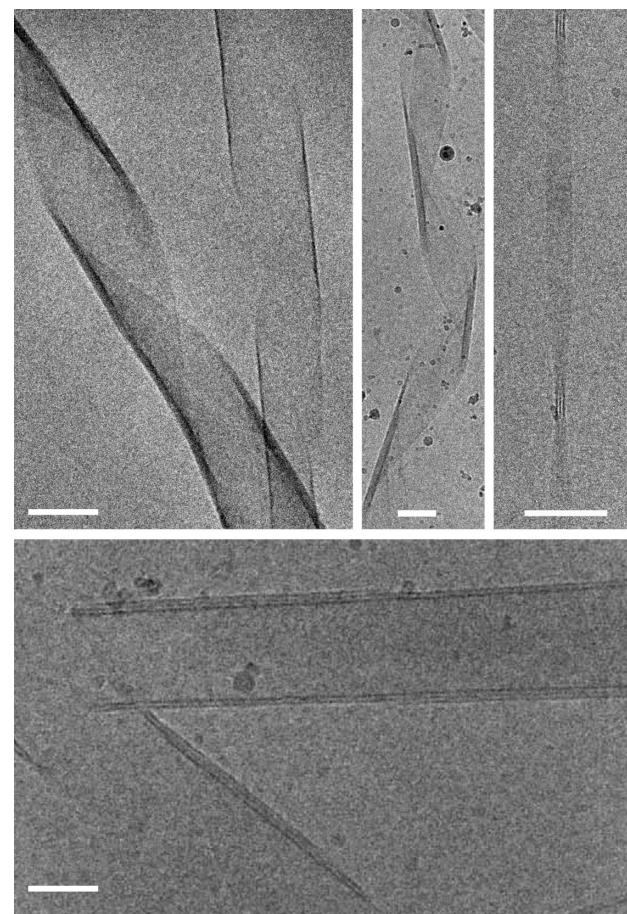
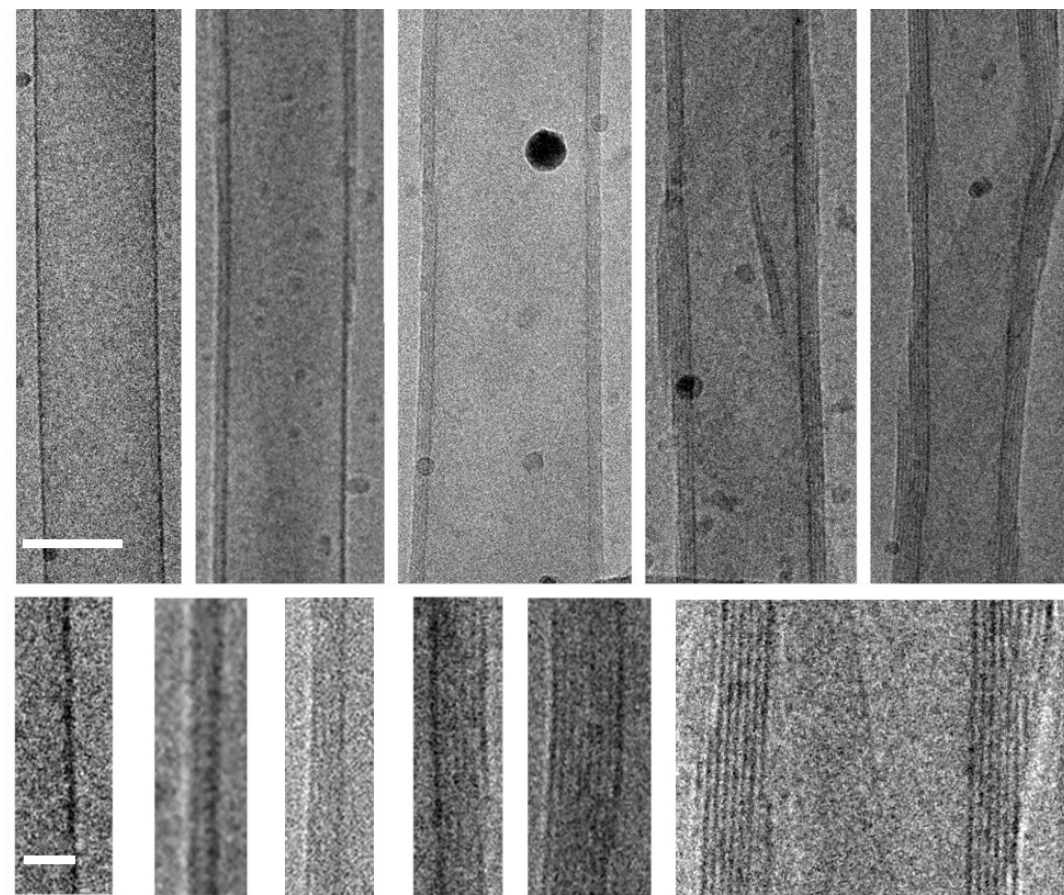
SAXS (ID02@ESRF)



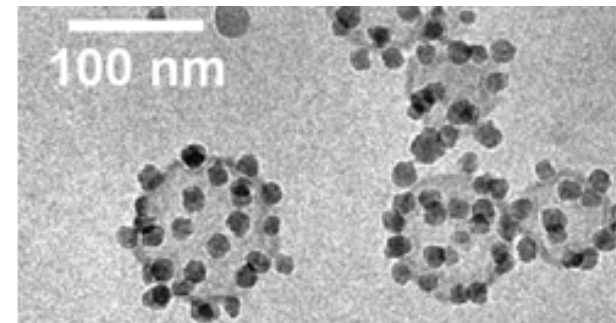
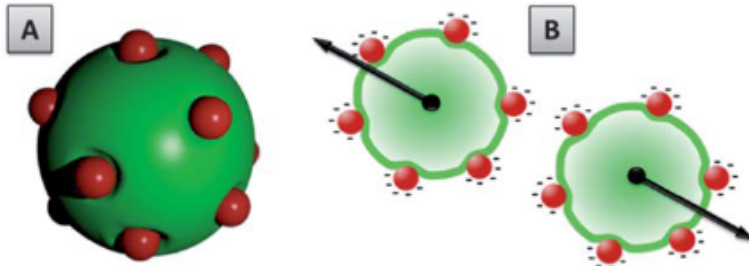
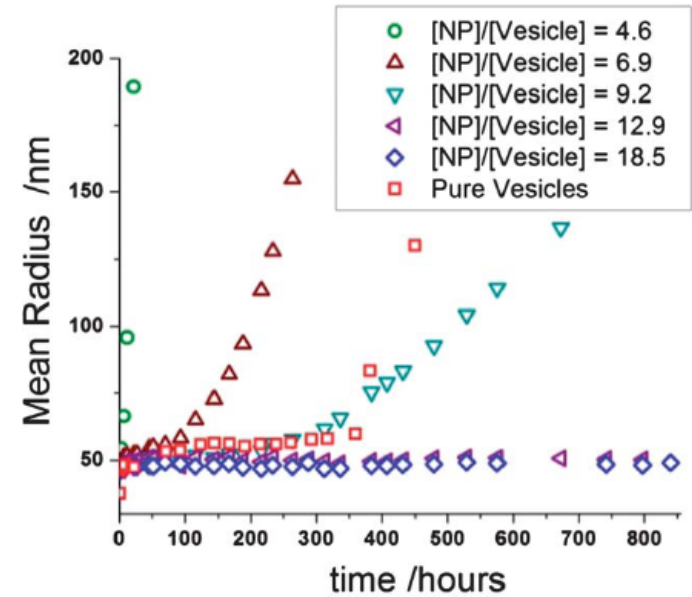
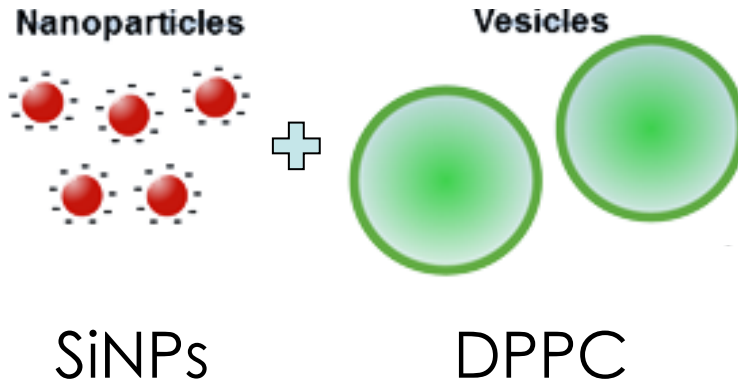
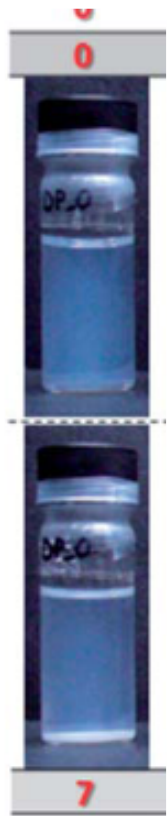
$\text{C}_{12}\text{KC}_{12}\text{K-NH}_2$
 Kratky-Porod plot

SANS analysis following the layering



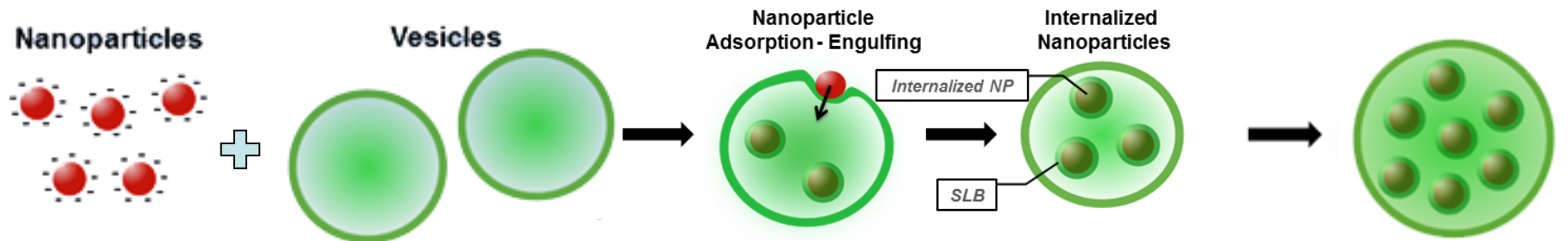


Nanoparticle-lipid interactions ($<T_m=41^\circ\text{C}$)

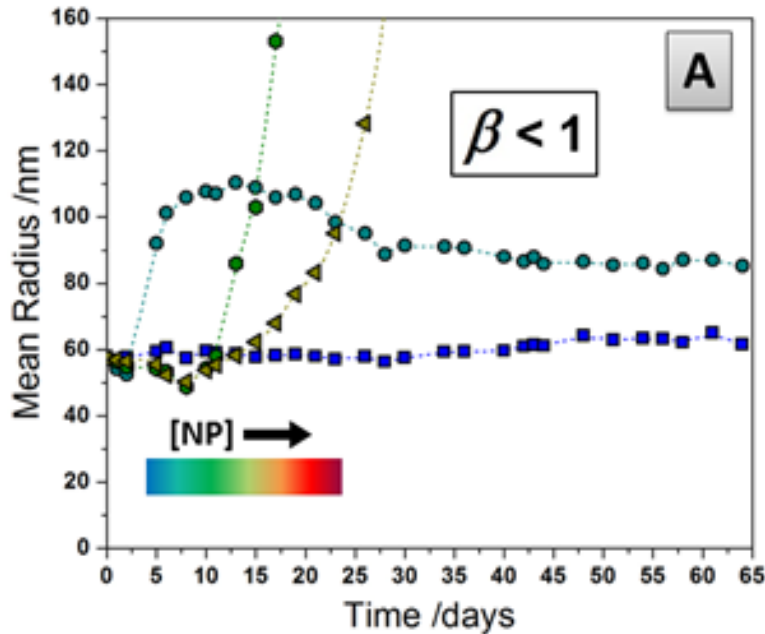


Michel, Gradzielski & Danino,
Soft Matter, 2013,

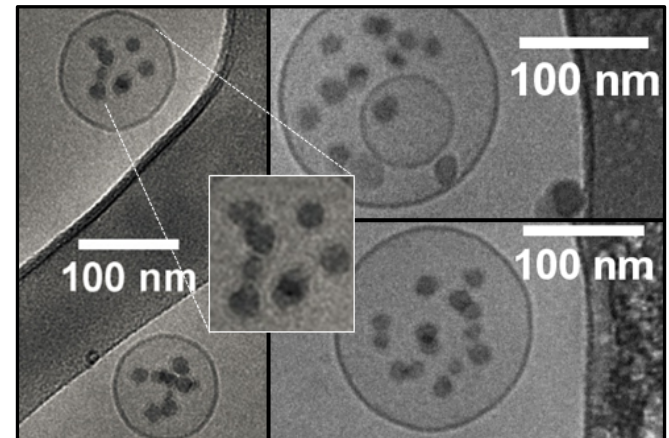
Nanoparticle-lipid interactions ($>T_m = -18^\circ\text{C}$)



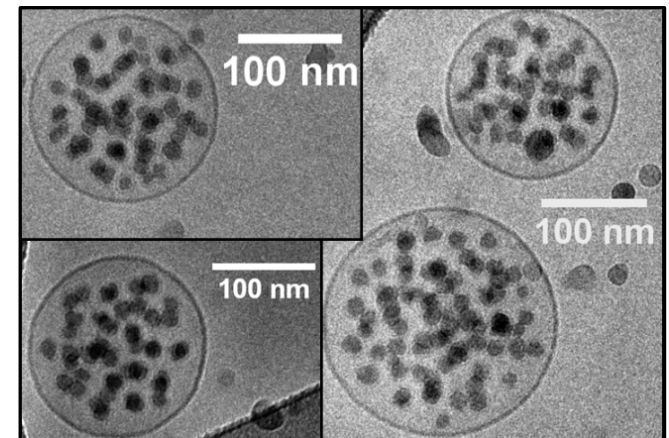
time →



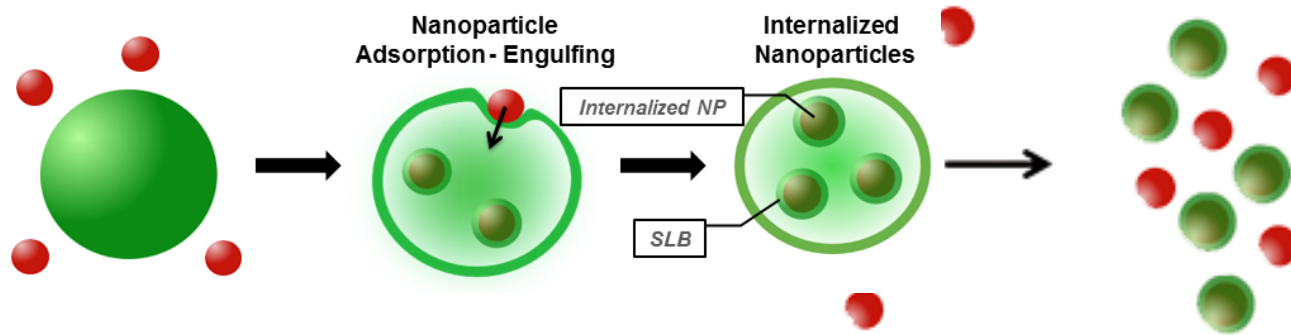
DOPC, SiNPs



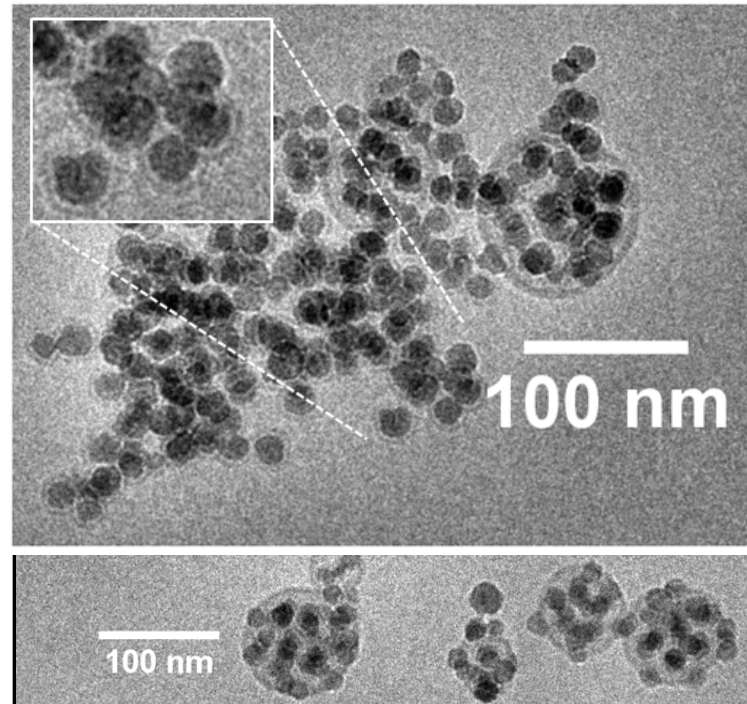
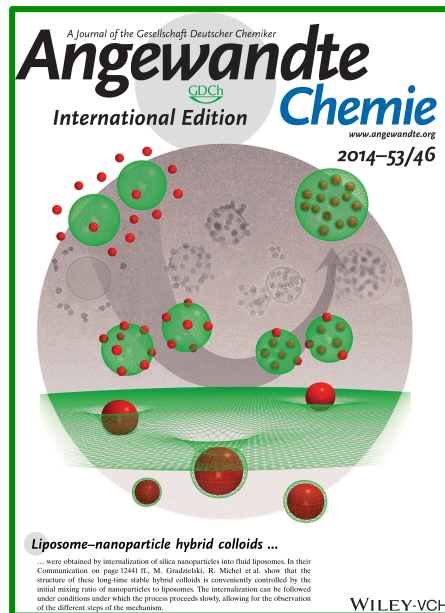
3 weeks



Nanoparticle-lipid interactions ($>T_m = -18^\circ\text{C}$)

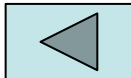


DOPC, SiNPs



2
weeks
 $\beta=1.2$

New hybrid materials combining the vesicles softness with the NPs hard colloid properties



Freeze-fracture and cryo-SEM

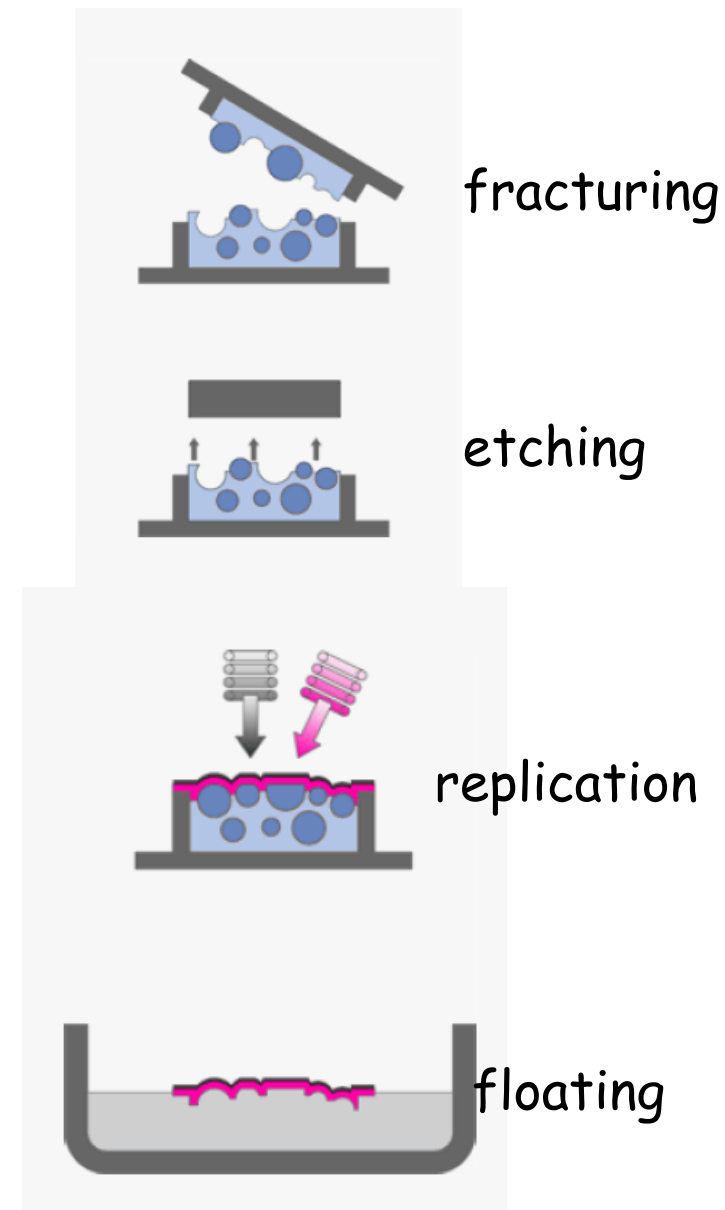
For:

gels, liquid crystals, viscous samples, micro-scale structures

Disclose:

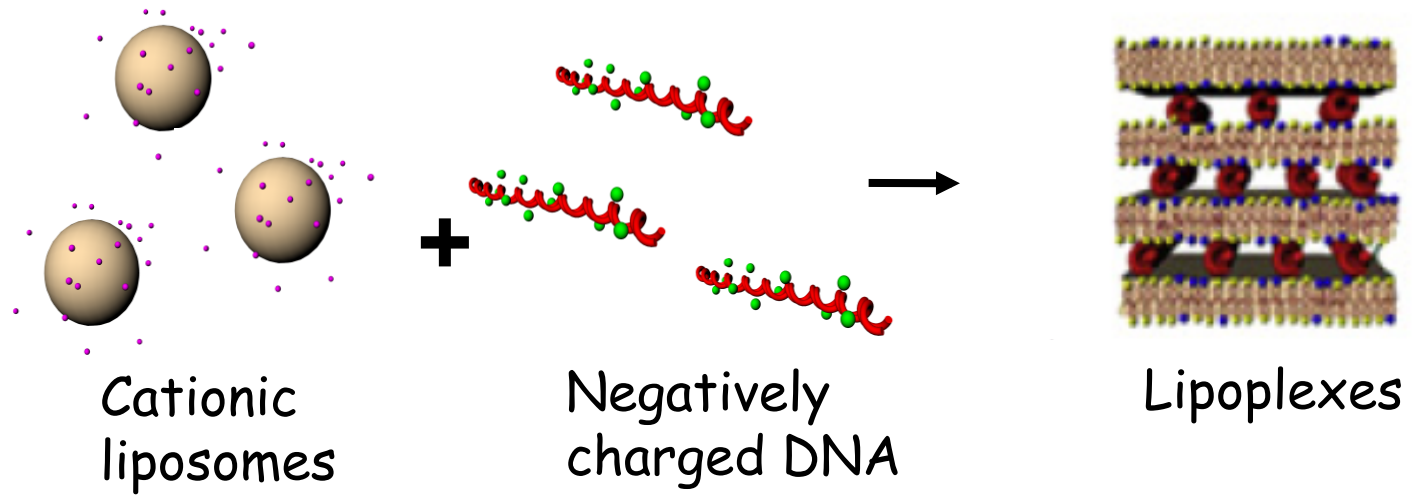
shape, size, morphology
heterogeneity of particles
surface topology
lateral periodicity
symmetry and space group
changes in the lattice
parameter defects

Elemental analysis

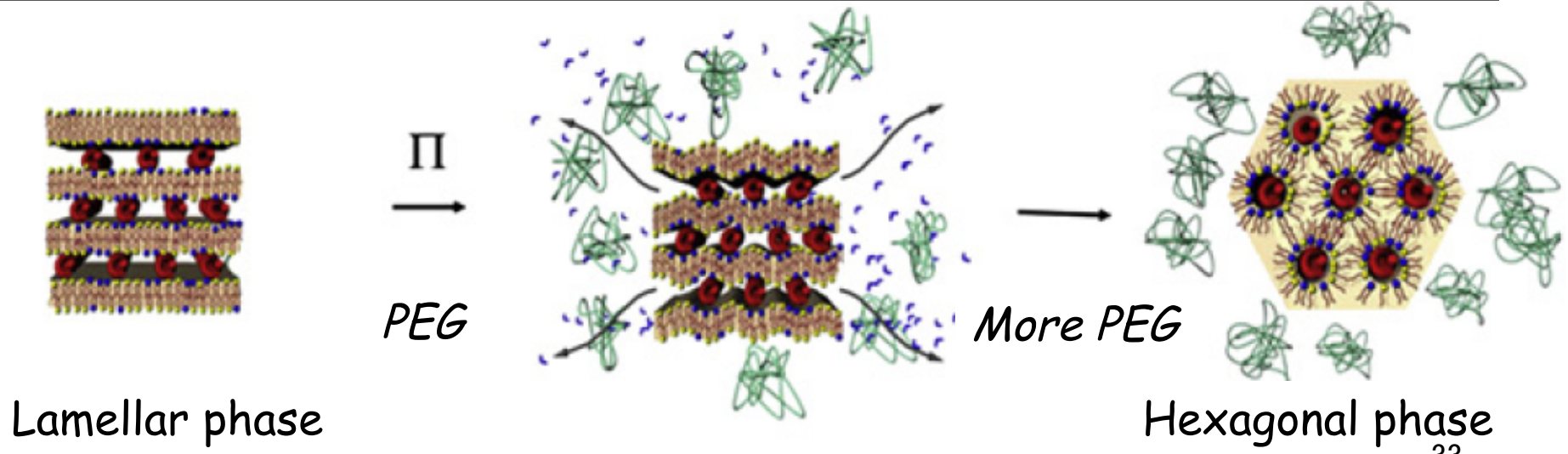


<http://elmilab.com>

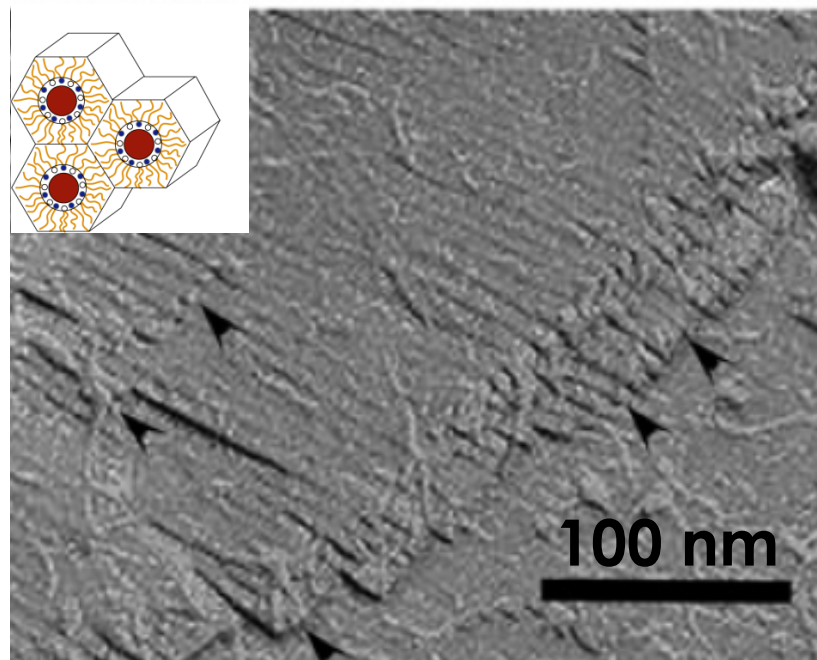
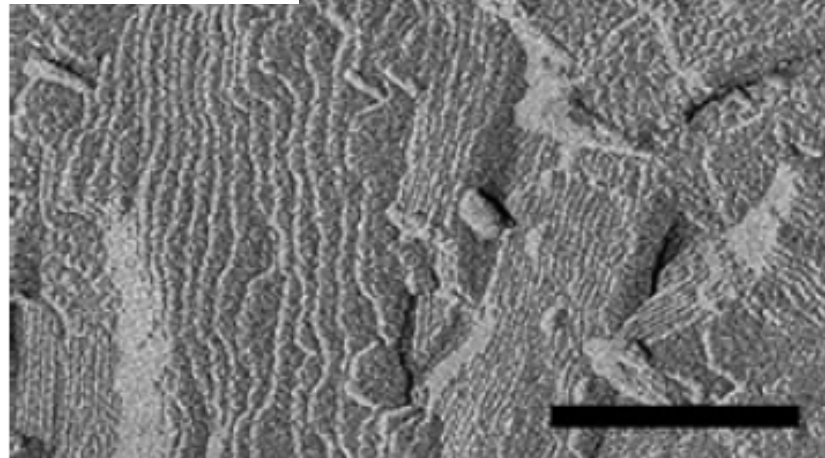
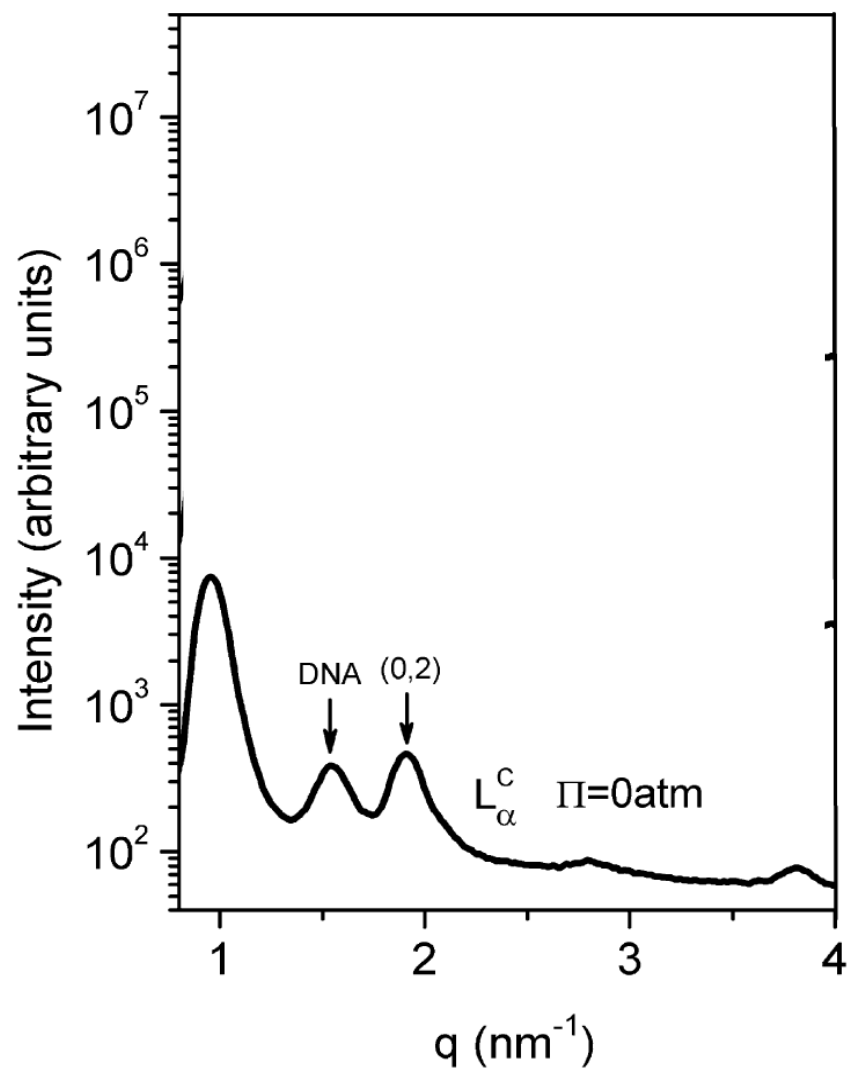
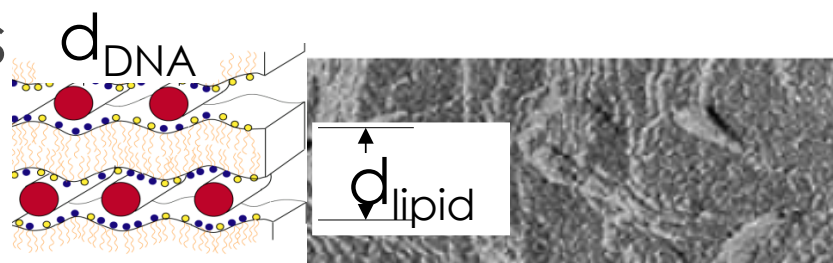
Lipoplex formation



Osmotically-induced transitions in lipid/DNA mesophases

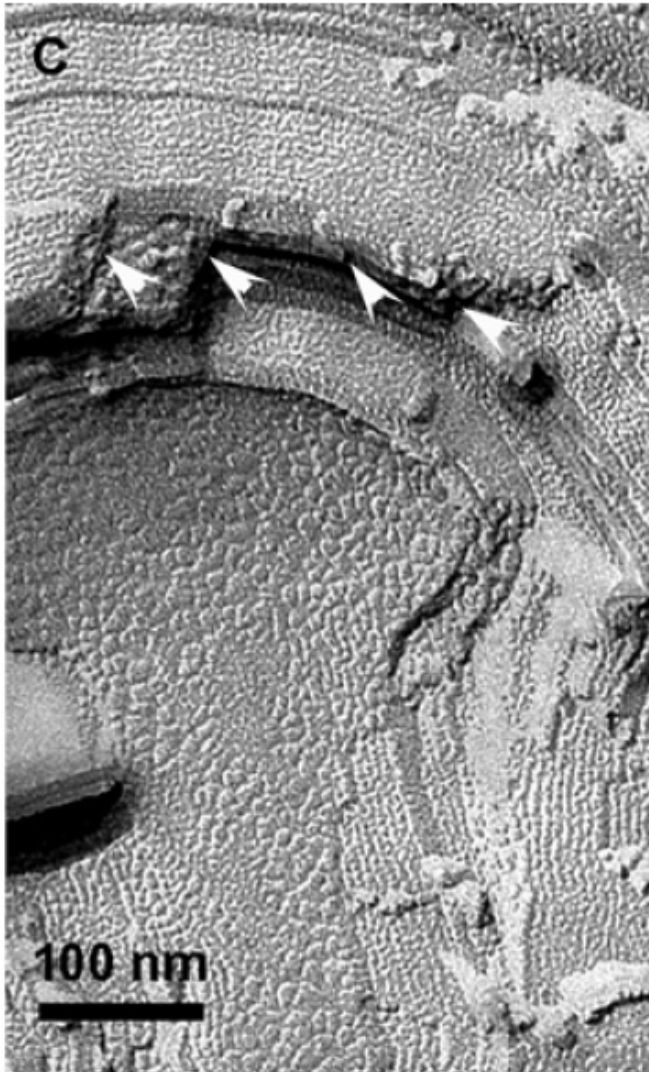


Osmotically-induced transitions between lipoplex mesophases



Effect of cosurfactant

lamellar-to-hexagonal transition at lower osmotic pressure



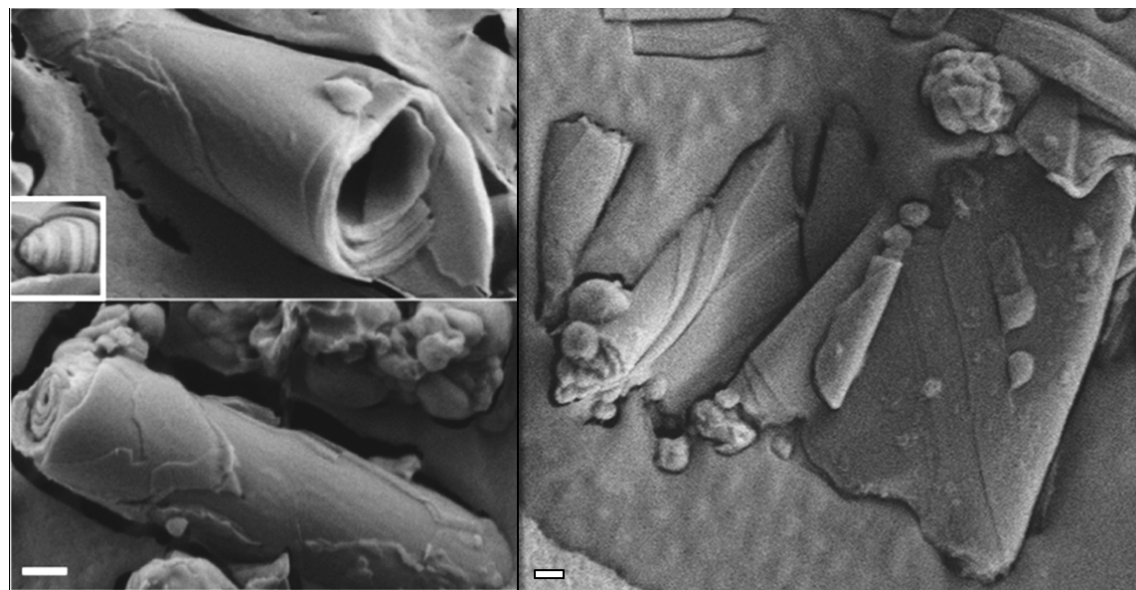
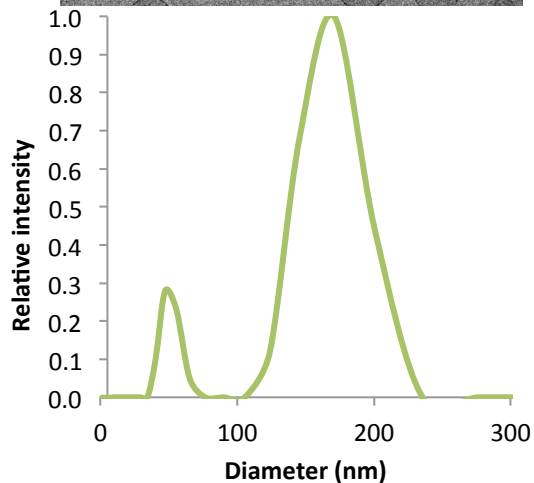
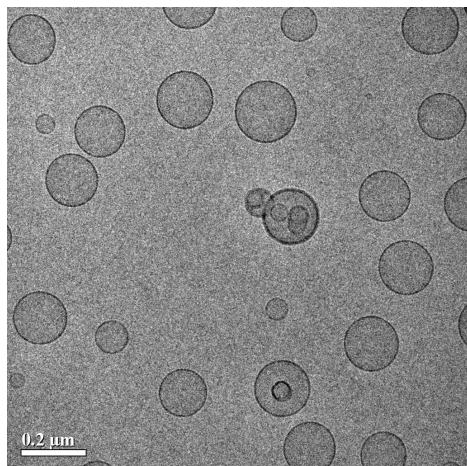
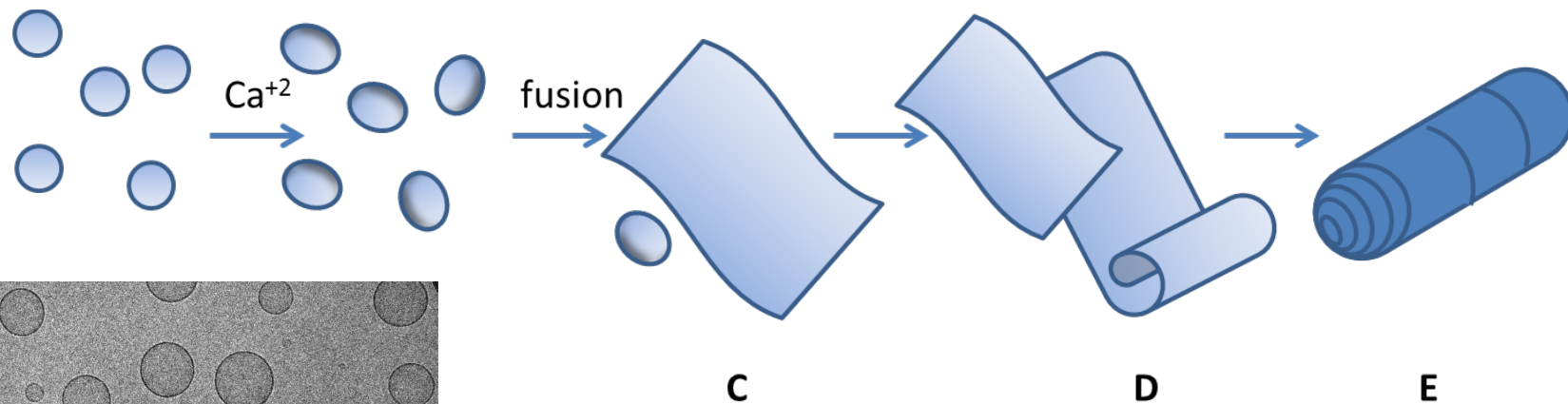
1:5 Hexanol:lipid

P = 45 atm



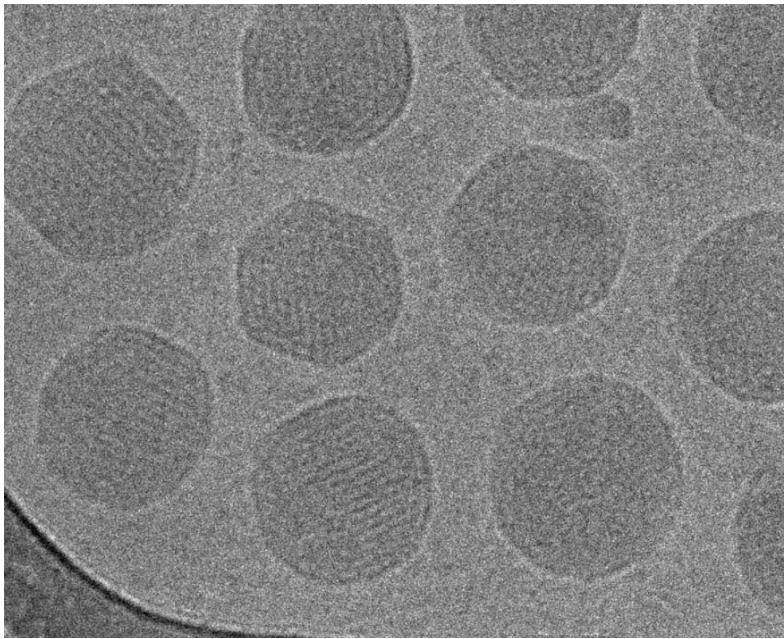
**Danino & Harries,
Biophys J, 2009**

Cryo-SEM: Cochleation with multivalent ions

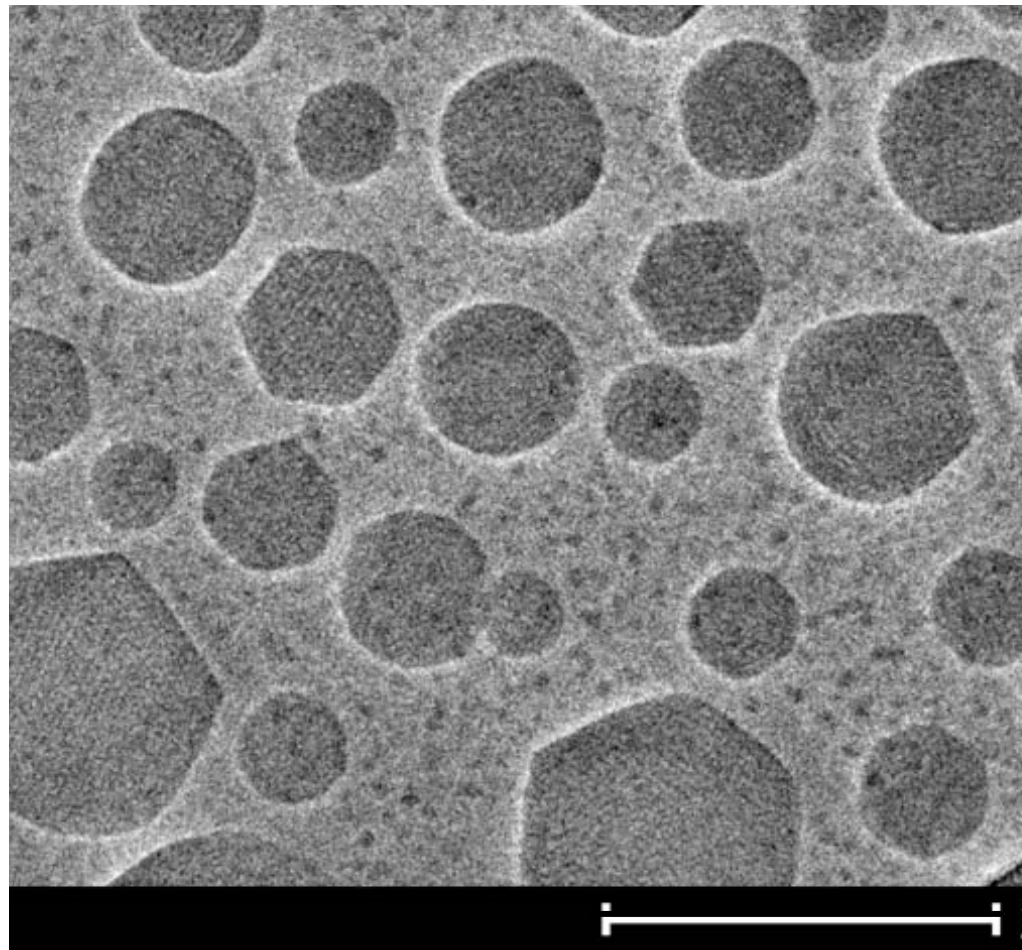


Low Contrast and Beam Damage

↓ CCD, Phase Contrast

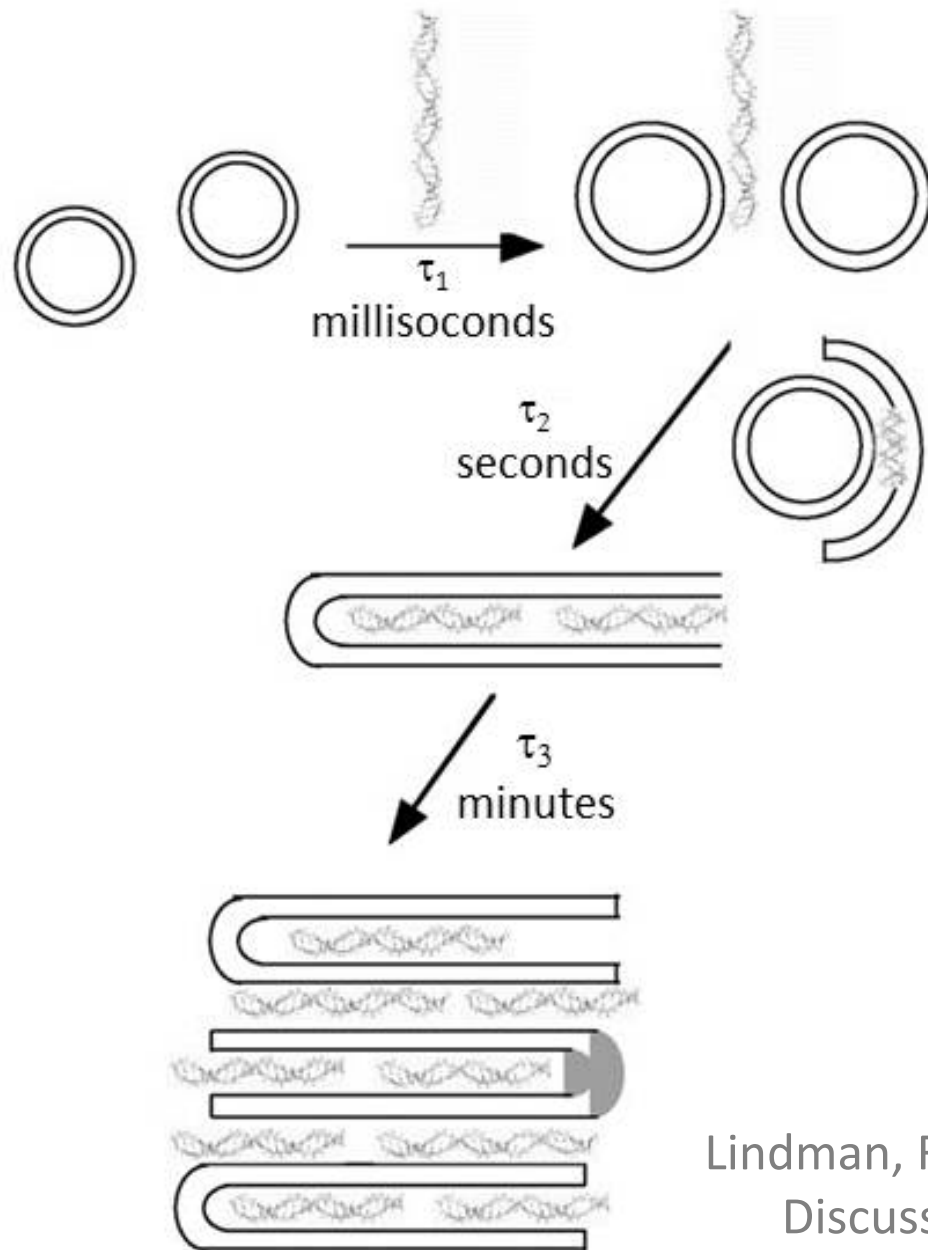


Direct Detector,
Phase Plates



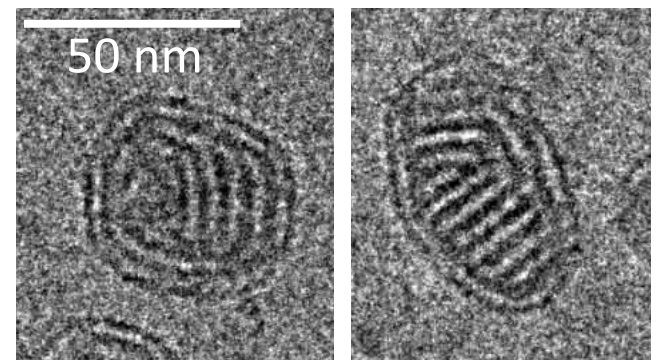
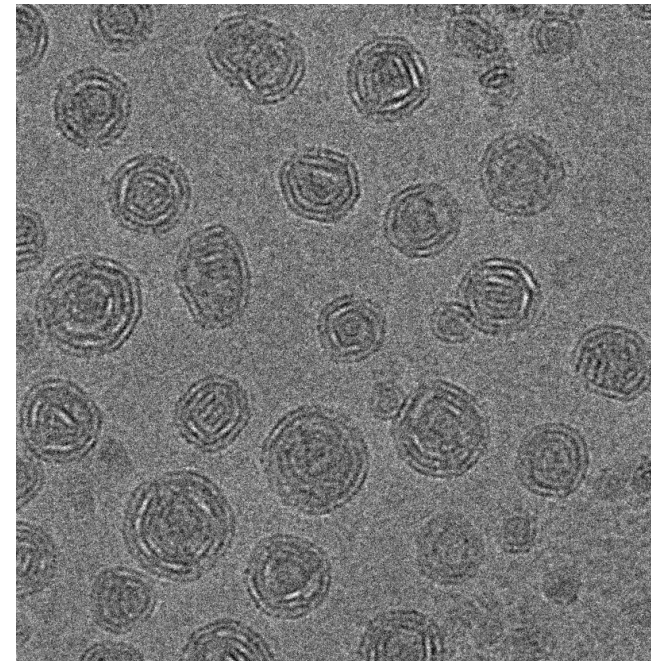
200 nm

Low Contrast



Lindman, Faraday
Discussion

Selective Etching, here to
'expose' encapsulated siRNA



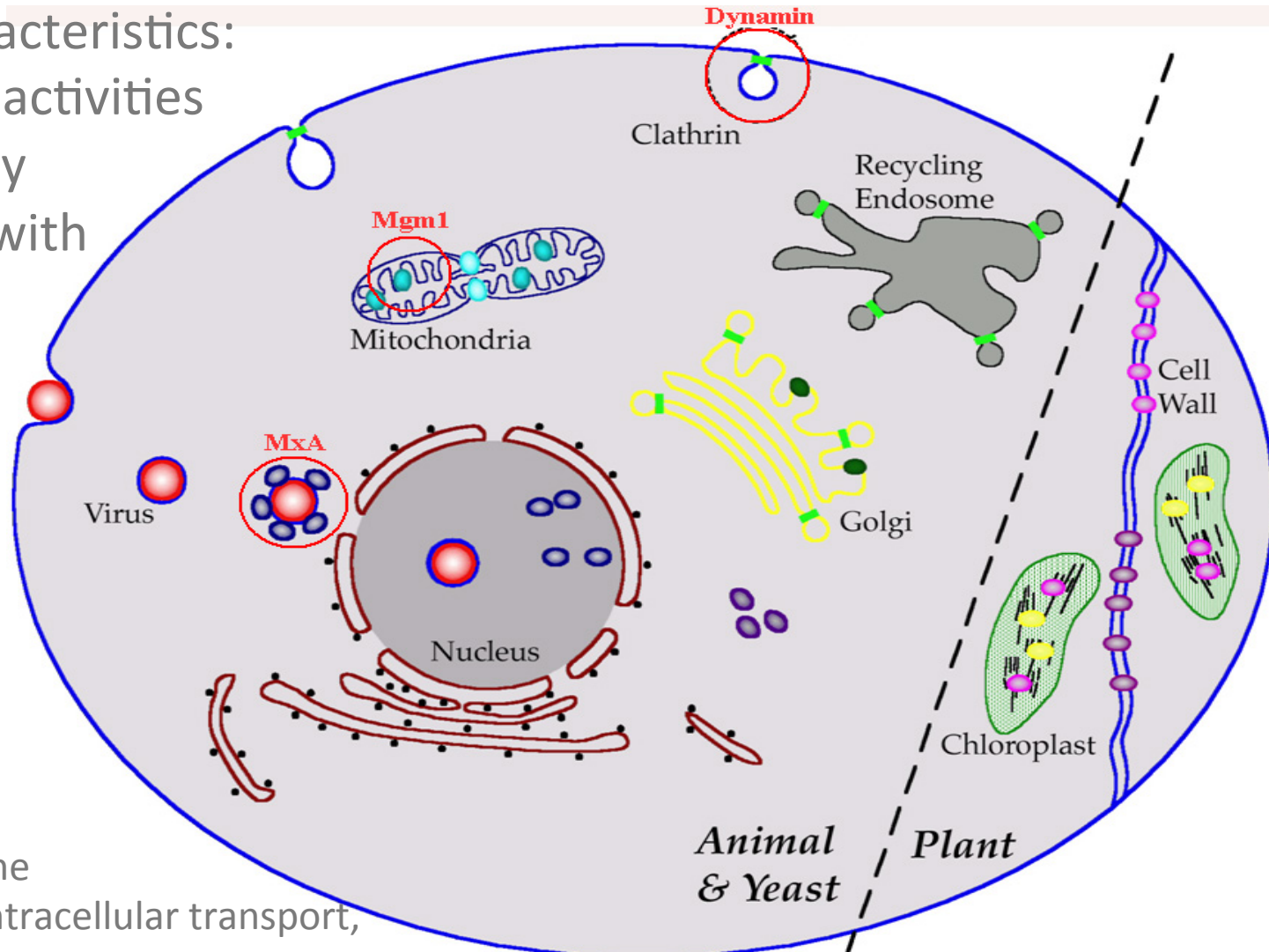
Langer, Danino
Nature Nanotech, 2014
Nano Letters, 2016

Dynamin Family Members

Large GTPases

Common characteristics:

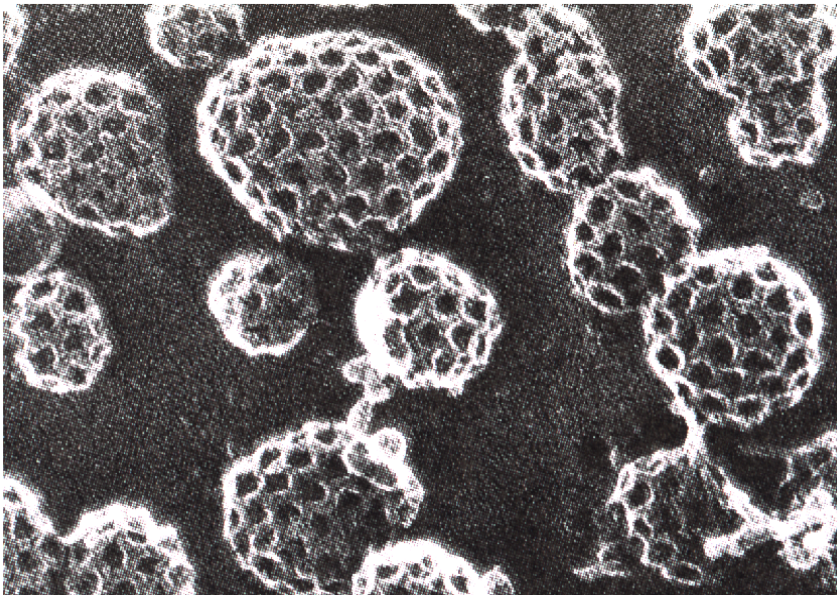
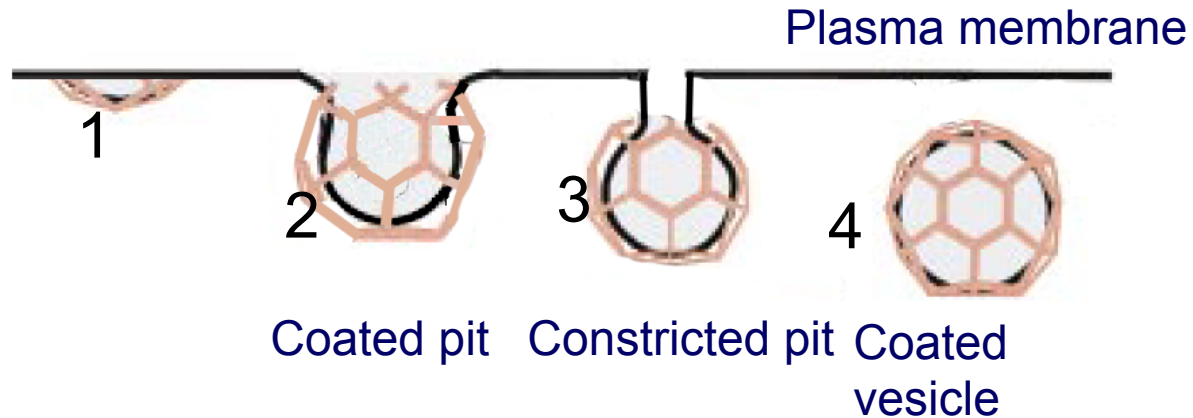
- Biochemical activities
- Self-assembly
- Association with membranes



Involved in membrane fission and fusion, intracellular transport, membrane morphology, antiviral activity,

**Danino & Hinshaw,
Curr Opin Cell Biol, 2001**

Clathrin-Mediated Endocytosis

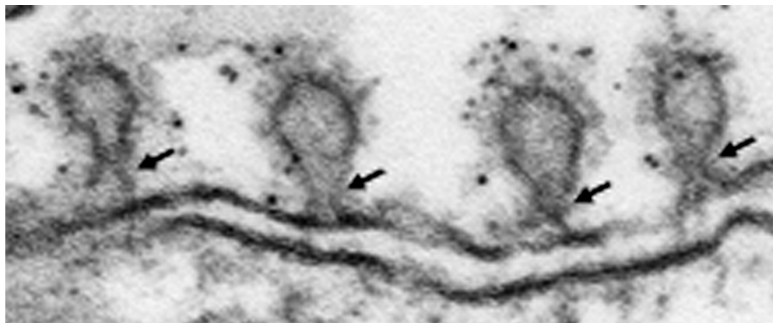
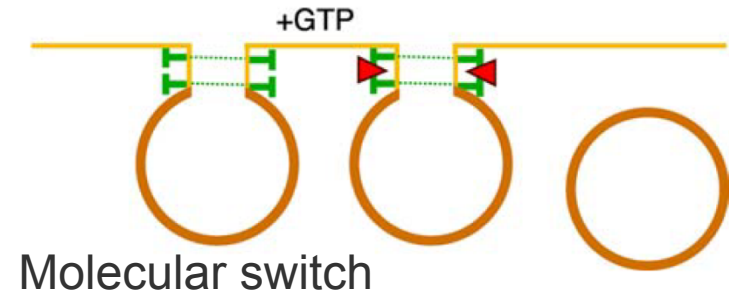
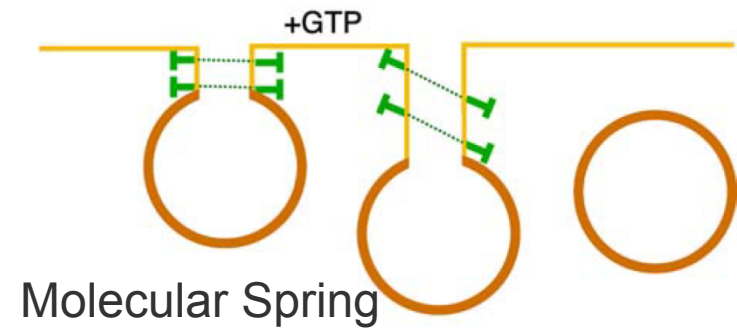
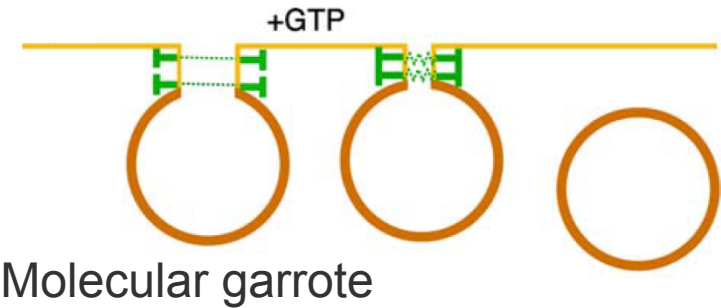


Coats (clathrin baskets)
under the plasma membrane
of fibroblasts

J. Heuser, JCB, 1980

Dynamin

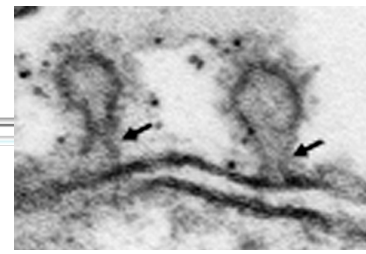
Models for Dynamin Function



Evergren et al., 2007

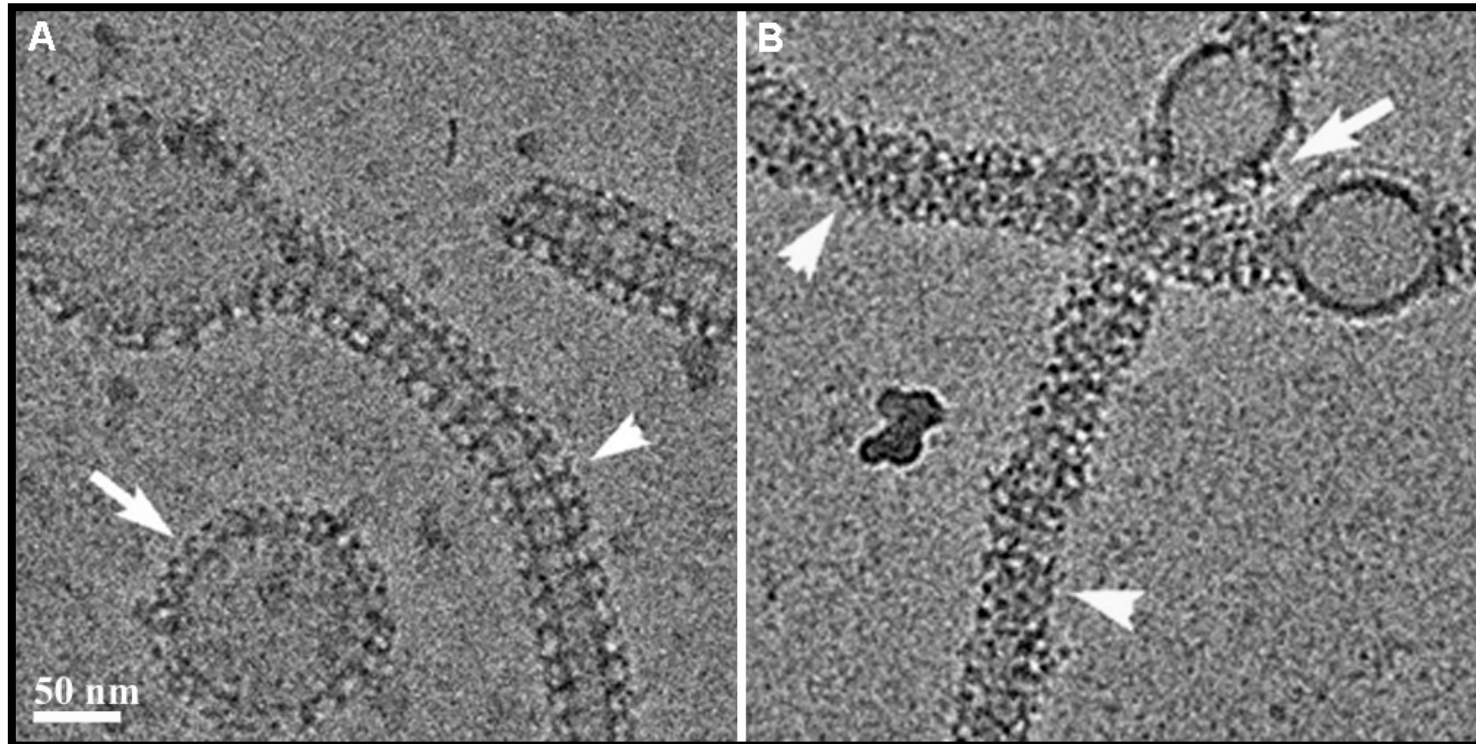
Danino et al JSB 2004


Dynamin

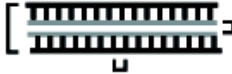


- Dynamin transforms vesicles into long helical tubes
- GTP activity results in membrane constriction

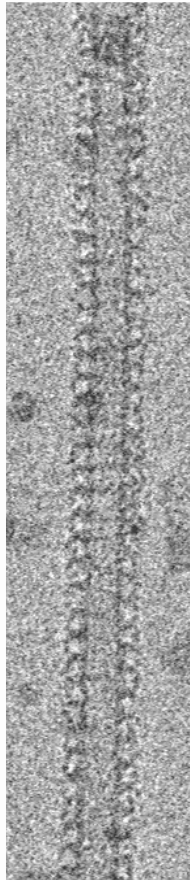
+GTP



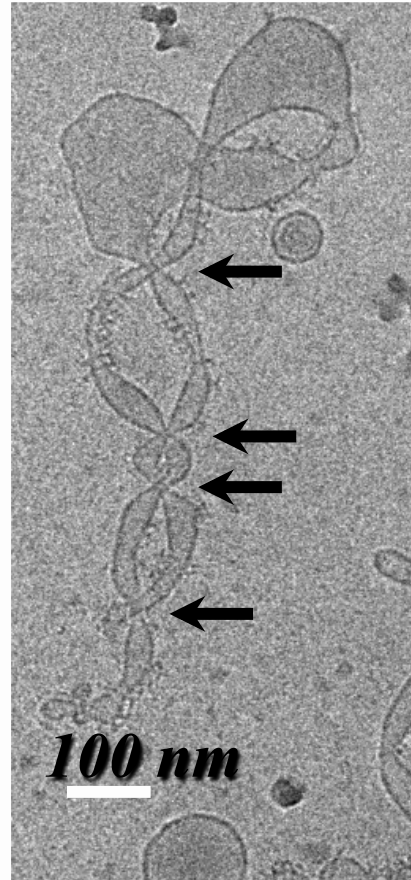
50 nm [] 20 nm
13 nm

40 nm [] 10 nm
9 nm

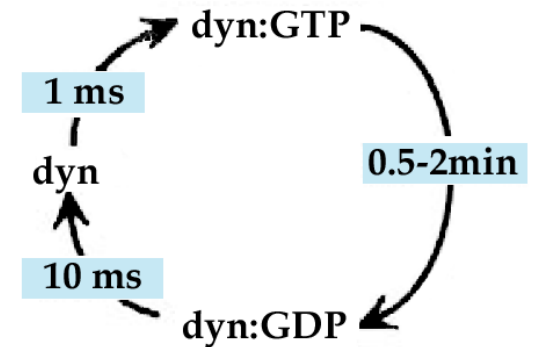
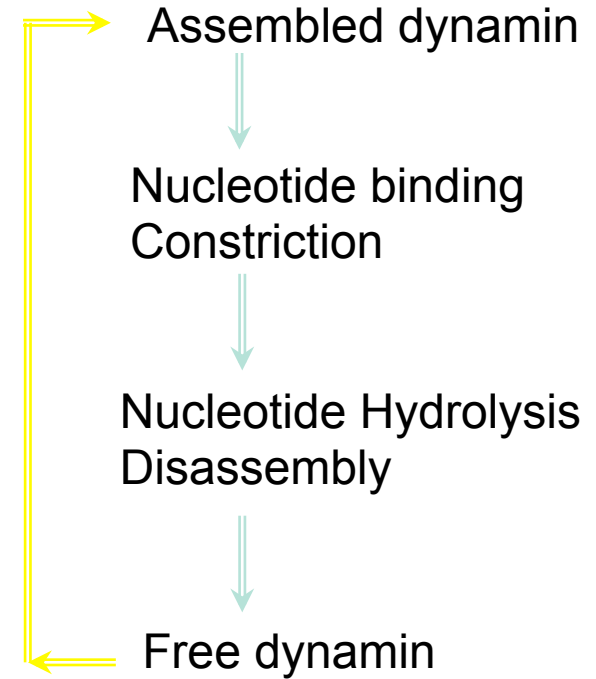
Dynamin: Resolving Conformational Changes along the GTP Cycle



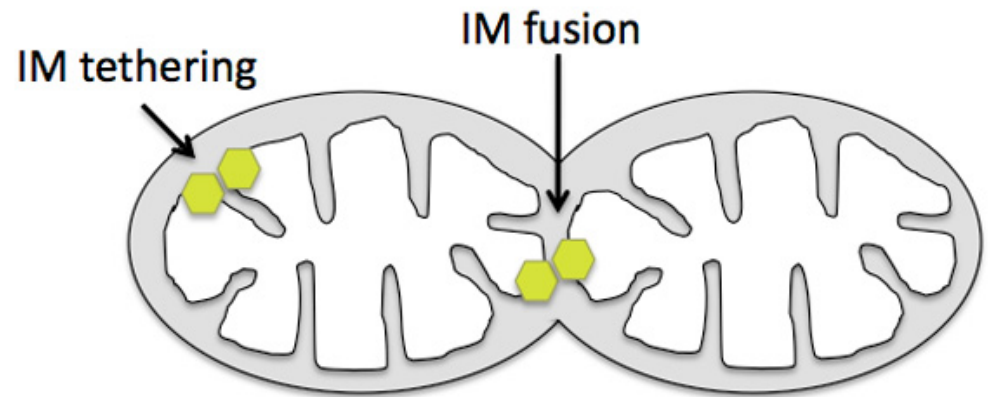
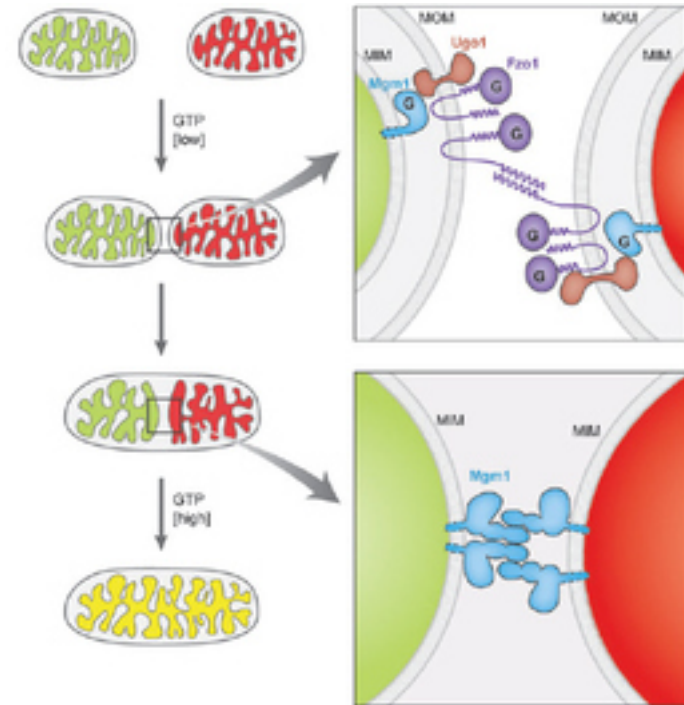
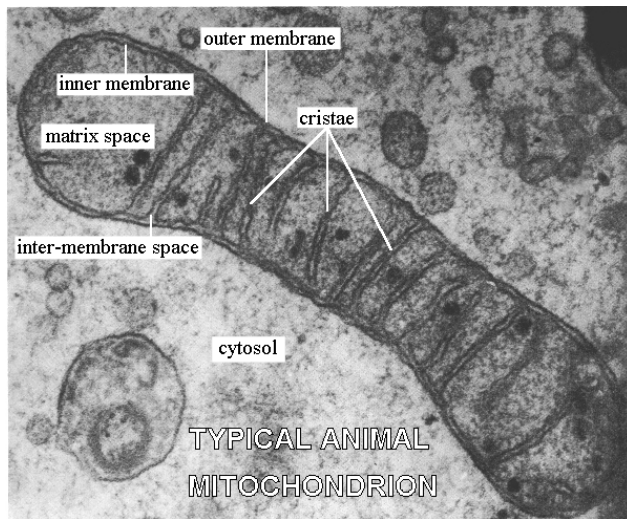
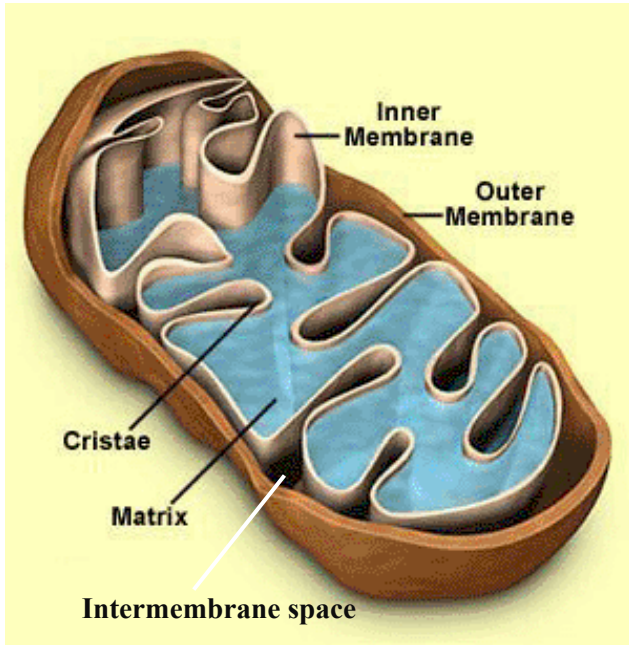
Constriction
5 sec



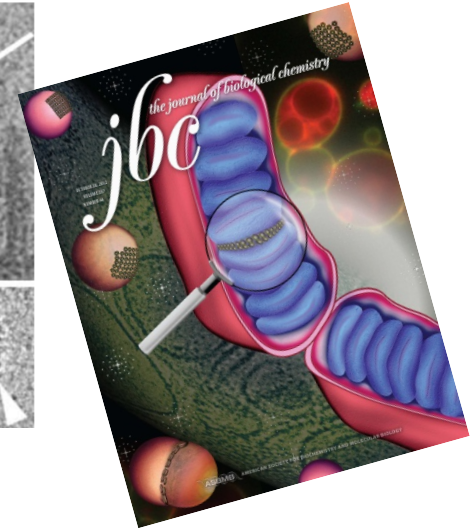
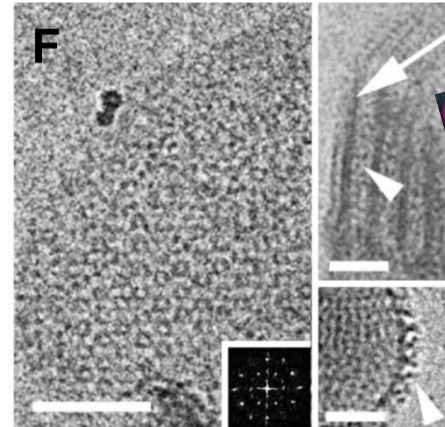
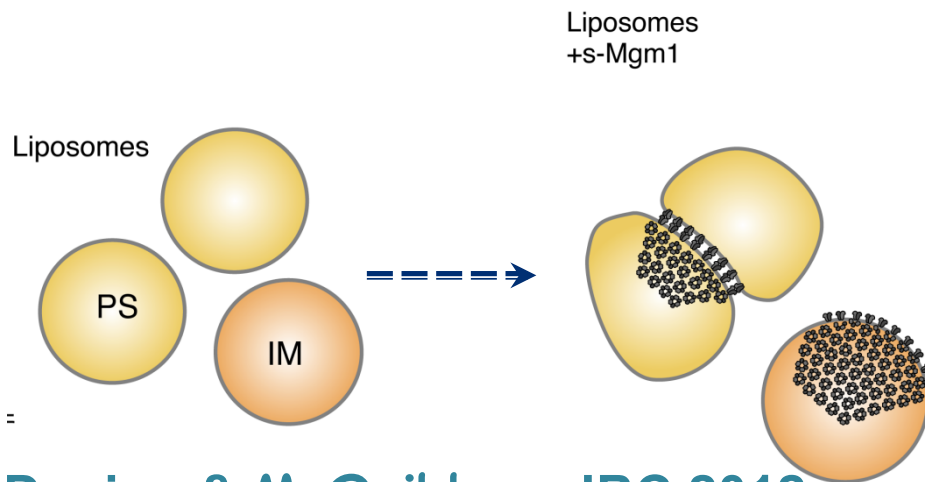
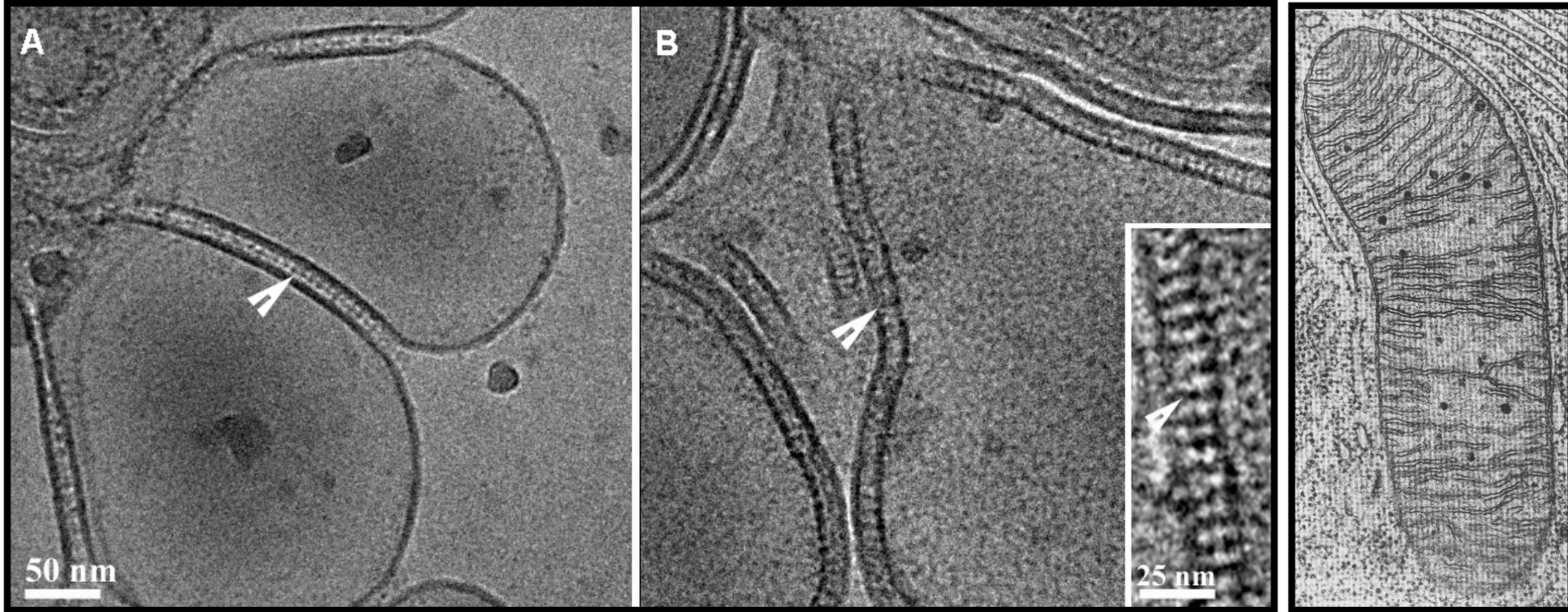
Disassembly
15 sec



Mitochondrion



- The protein-membrane complexes morphology and dimensions are similar to the mitochondrial cristae structures



Danino & McQuibban, JBC 2012

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