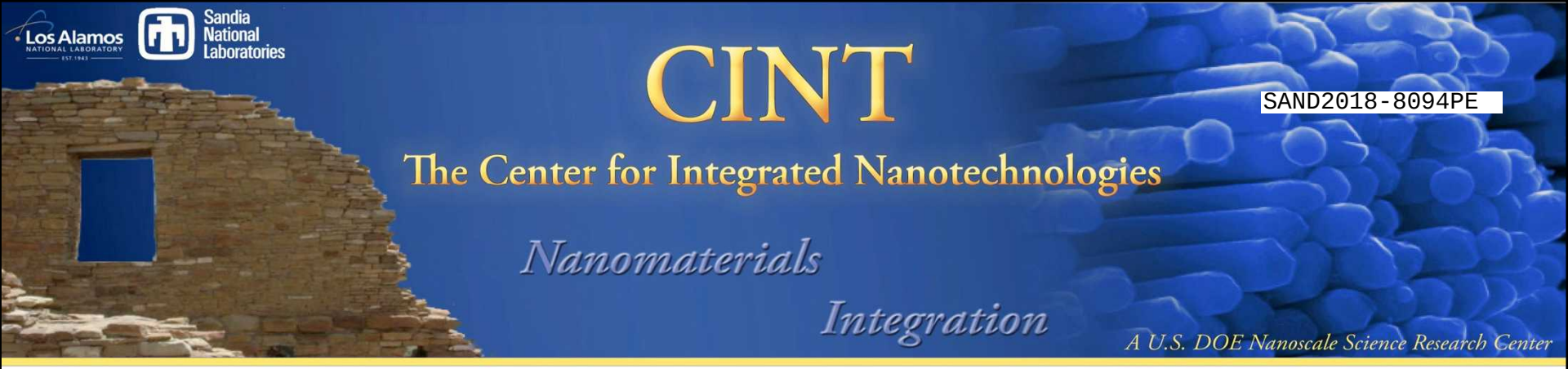

SAND2018-8094PE

CryoEM for fundamental study of soft/bio and inorganic nanomaterials

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Center for Integrated Nanotechnologies (CINT)
Sandia National Laboratories

Interview Seminar
August 29th, 2018

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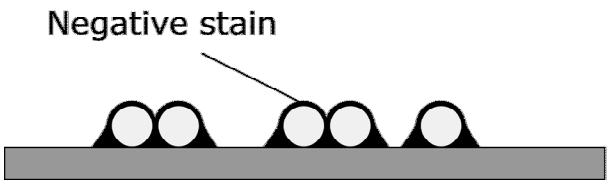
Los Alamos NATIONAL LABORATORY

U.S. DEPARTMENT OF ENERGY | Office of Science

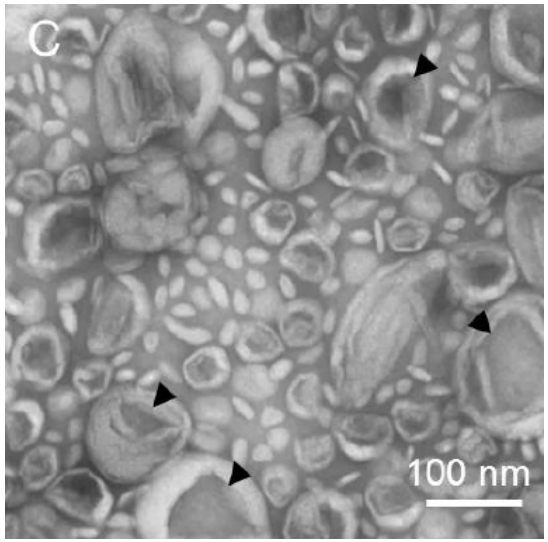
Sandia National Laboratories

What is cryo electron microscopy (EM)?

Stained specimen for conventional EM

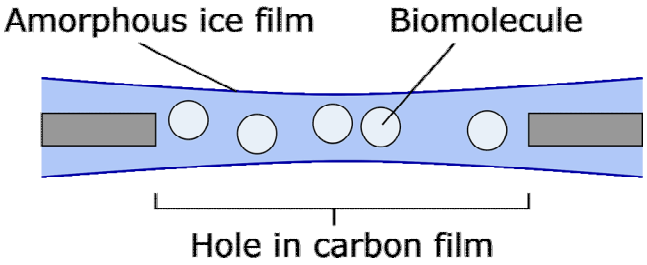


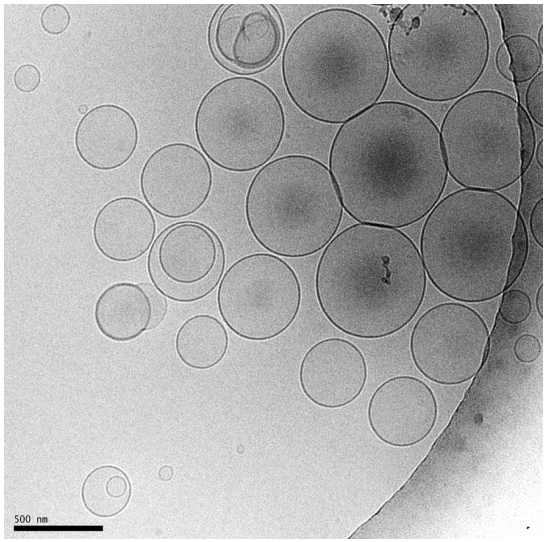
Liposome



Polymers 9, 521 (2017)

Vitrified specimen for cryoEM





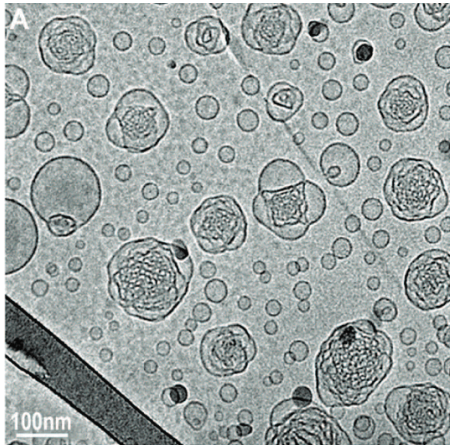
Liposome

www.cicbiogune.es

What is cryoEM used for?

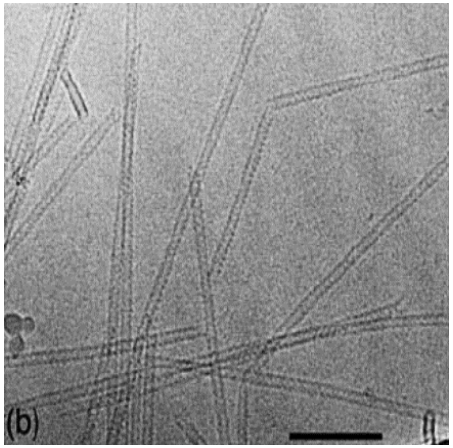
Analyzing complex, flexible structures in their native, hydrate states

Lipid superstructure



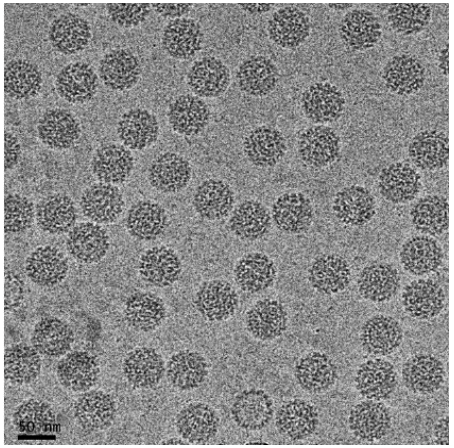
Langmuir 25, 1316-1326 (2009)

Peptide nanotube

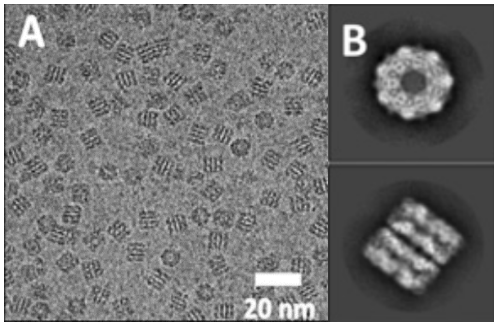


Structure 23, 280-289 (2015)

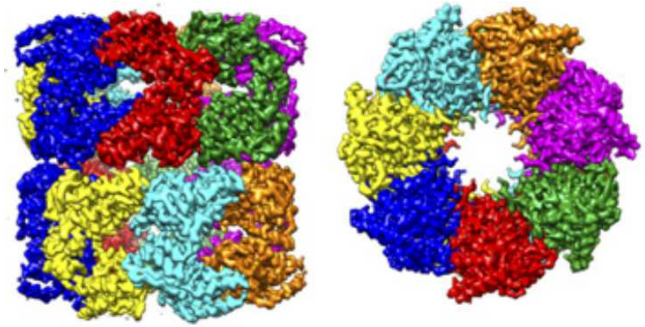
Human papillomavirus



www.phys.org



GroEL protein



PNAS 114, 8259-8264 (2017)

Overview

Part 1. Self-assembly of inorganic nanoparticles in solution

Part 2. Interaction between inorganic-organic materials

Part 3. Research plan

Part 1. Self-assembly of inorganic nanoparticles in solution

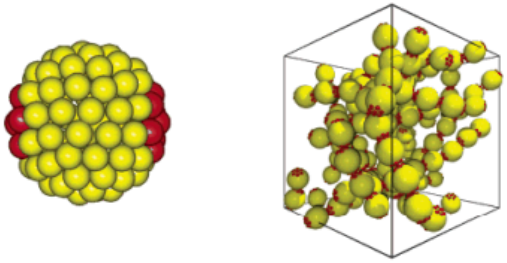
Part 2. Interaction between inorganic-organic materials

Part 3. Research plan

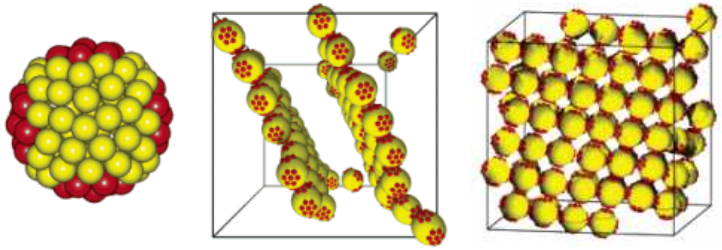
Self-assembly of patchy particles

Directed self-assembly of particles with fixed patches

n=2



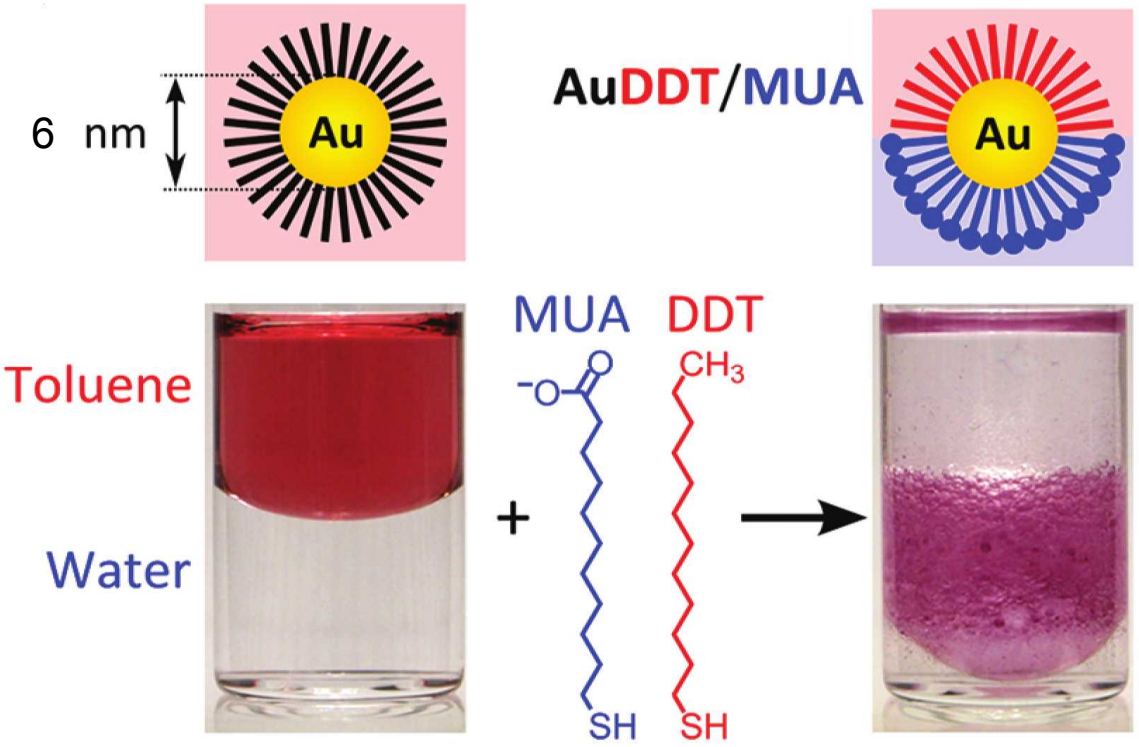
n=4



Nano Lett., 4,1407-1413 (2004)

What happen if the patches rearrange on the particles' surface?

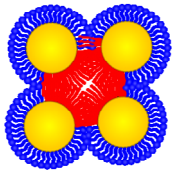
Amphiphilic nanoparticles (NPs) with adaptive surface chemistry

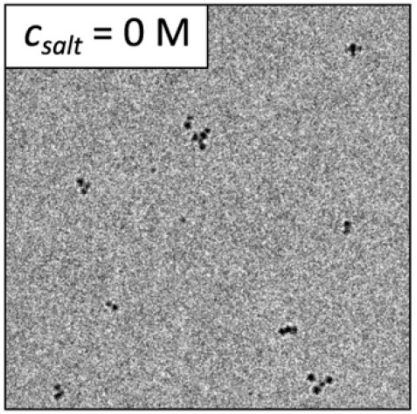


Andala, Shin et al. *ACS Nano*, 6,1044-1050 (2012)

Self-assembly of same NPs into small cluster and chain

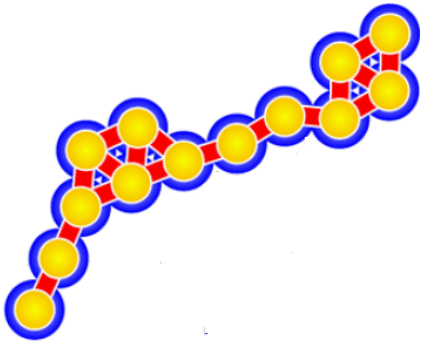
Small cluster

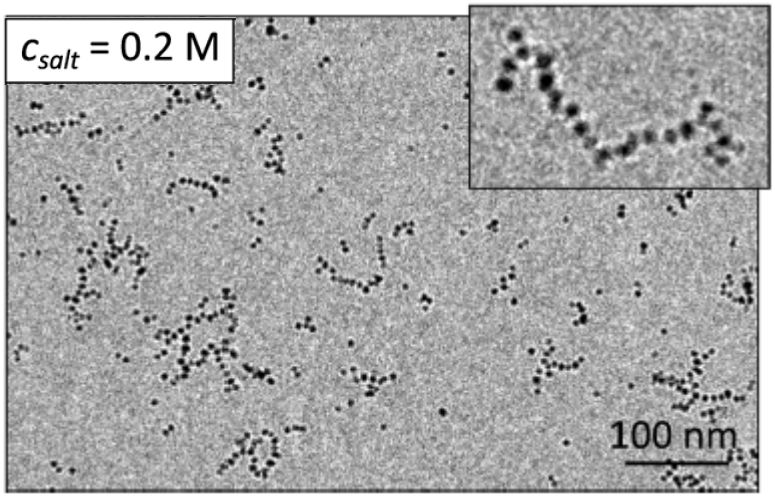




CryoEM

Chain-like structure





CryoEM

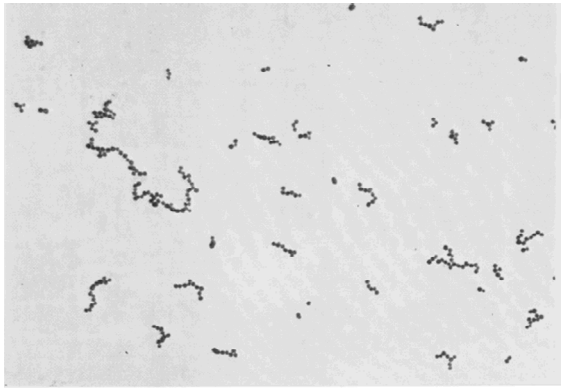
Lee, Shin et al. *ACS Nano*, 8,9979-9987 (2014)

Several routes to particle chains

Crosslinking divalent NPs

Science 315, 358-361 (2007)

Diffusion-limited aggregation

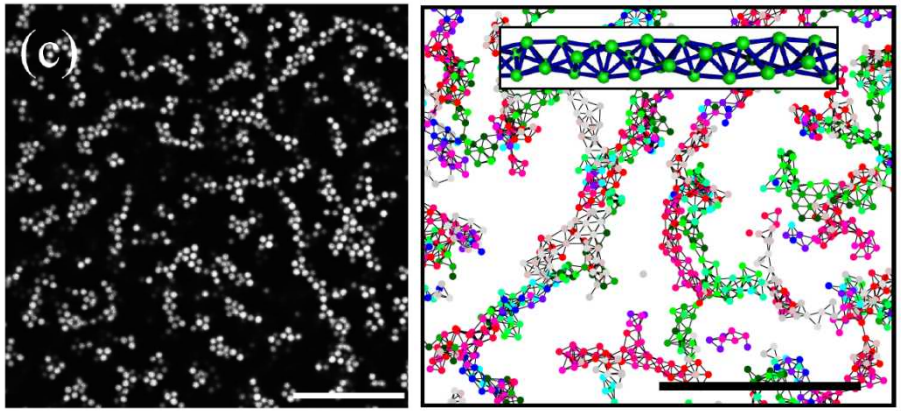


JACS. 85, 3317 -3328 (1963)

Dipolar interactions

Science 297, 237-240 (2002)

Short-ranged attraction, long-ranged repulsion

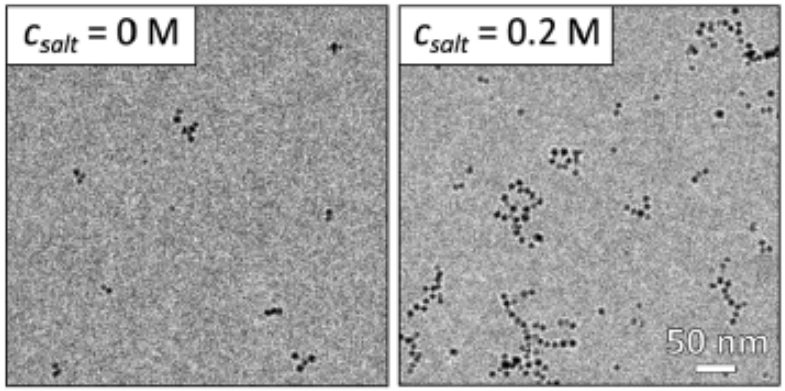


Phys. Rev. Lett. 94, 208301 (2005)

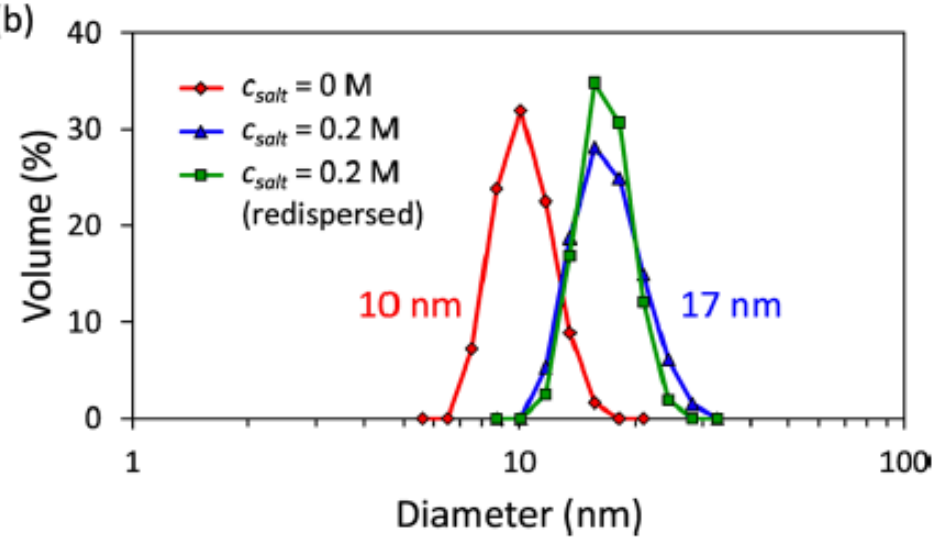
Chains form only in salty conditions

(a)

CryoEM



(b)



Short-ranged interactions

$$\kappa^{-1} = \sqrt{\frac{\varepsilon\varepsilon_0 k_B T}{2e^2 c_{salt}}} < 1 \text{ nm}$$

Equilibrium structures
(not kinetic aggregates)

Several routes to particle chains

Crosslinking divalent NPs

Science 315, 358-361 (2007)

Diffusion-limited aggregation

JACS. 85, 3317 -3328 (1963)

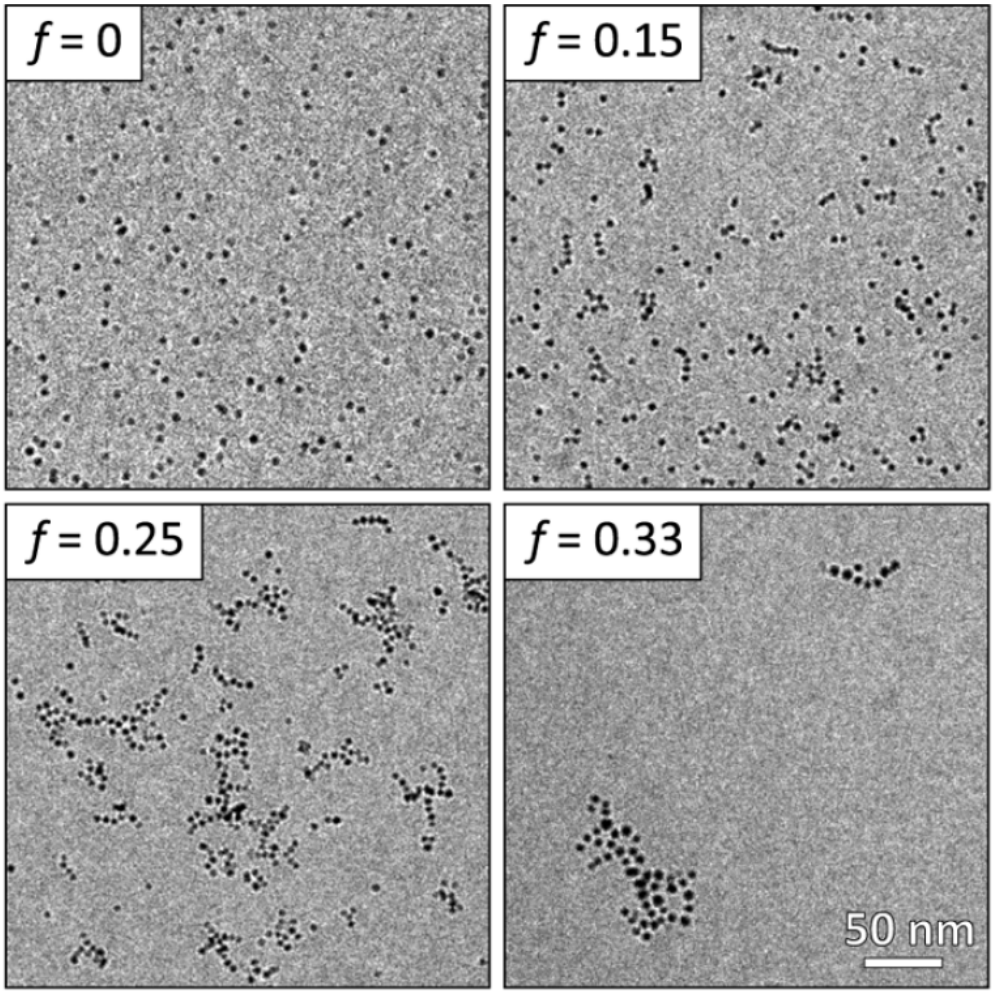
Dipolar interactions

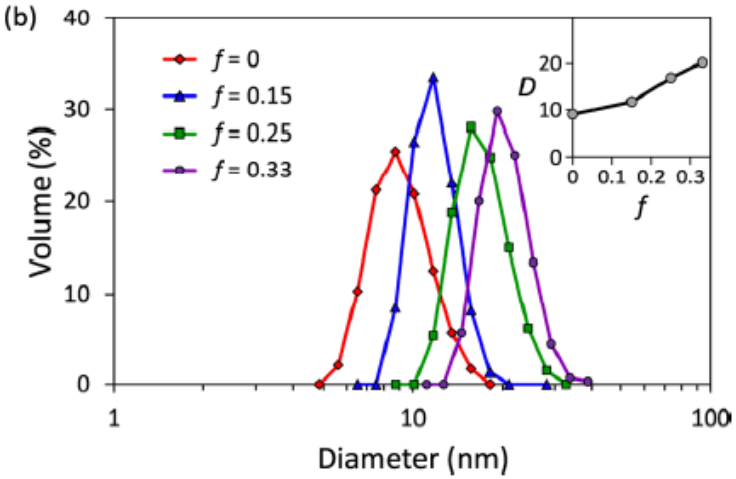
Science 297, 237-240 (2002)

Short-ranged attraction, long-ranged repulsion

Phys. Rev. Lett. 94, 208301 (2005)

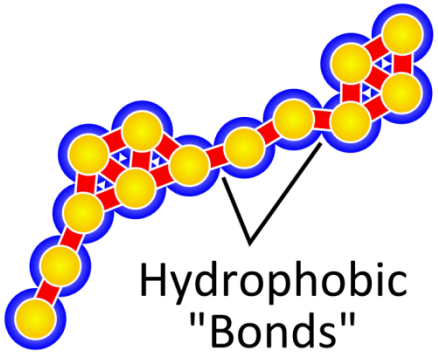
Amount of hydrophobic ligand (*f*)





CryoEM, C_{salt} = 0.2 M

Adaptive hydrophobic bonding model



- Short-ranged interactions (electrostatic and hydrophobic)
- Dynamic rearrangement of ligands

$$E_{hp} = \varepsilon_{hp} \sum_{i=1}^{N_P} H_{ii}$$

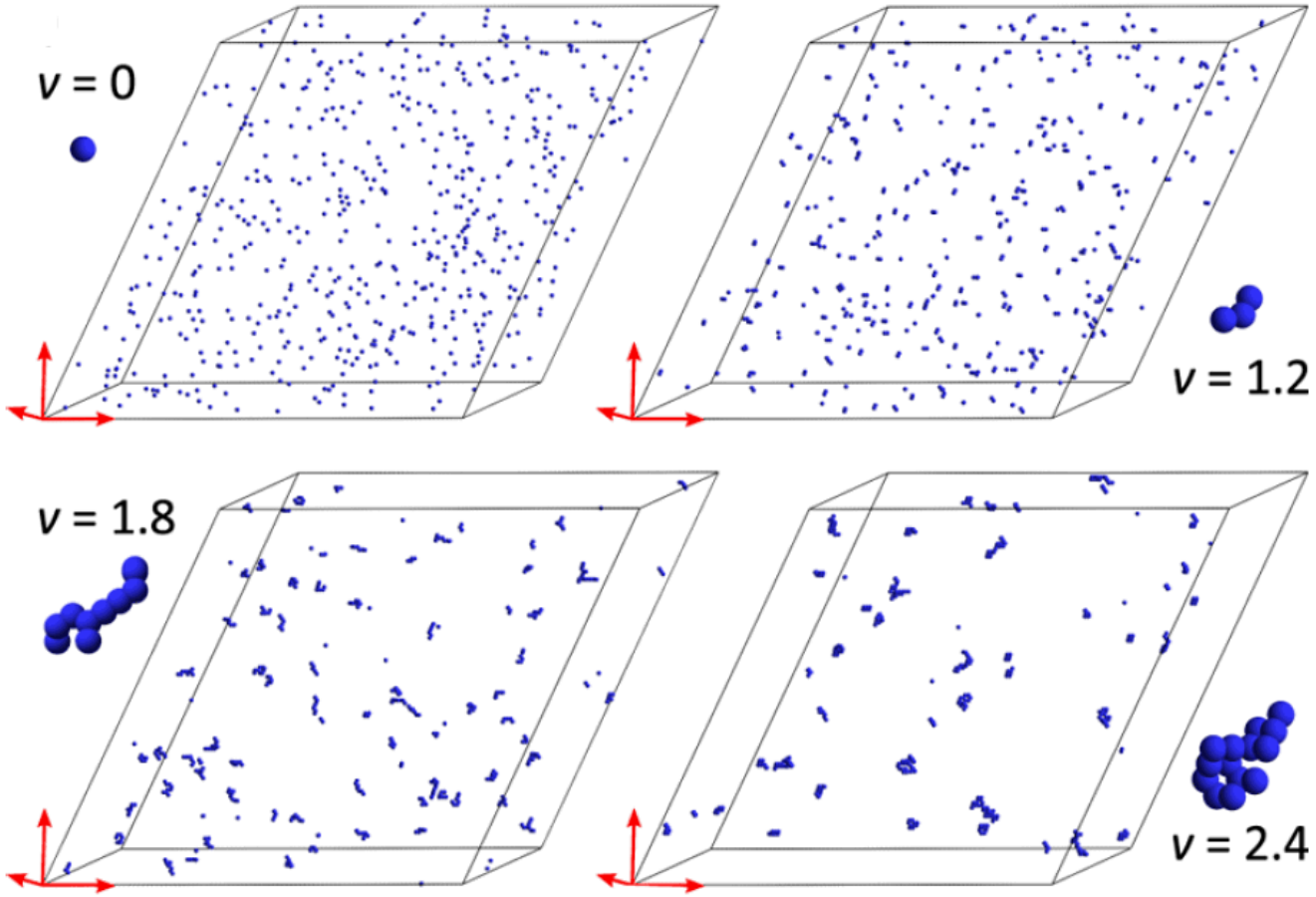
Minimize

ε_{hp} Energy due to contact between a hydrophobic ligand and water

N_P Total number of particles

H_{ii} Number of "nonbonding" hydrophobic ligands

3D Monte Carlo simulation

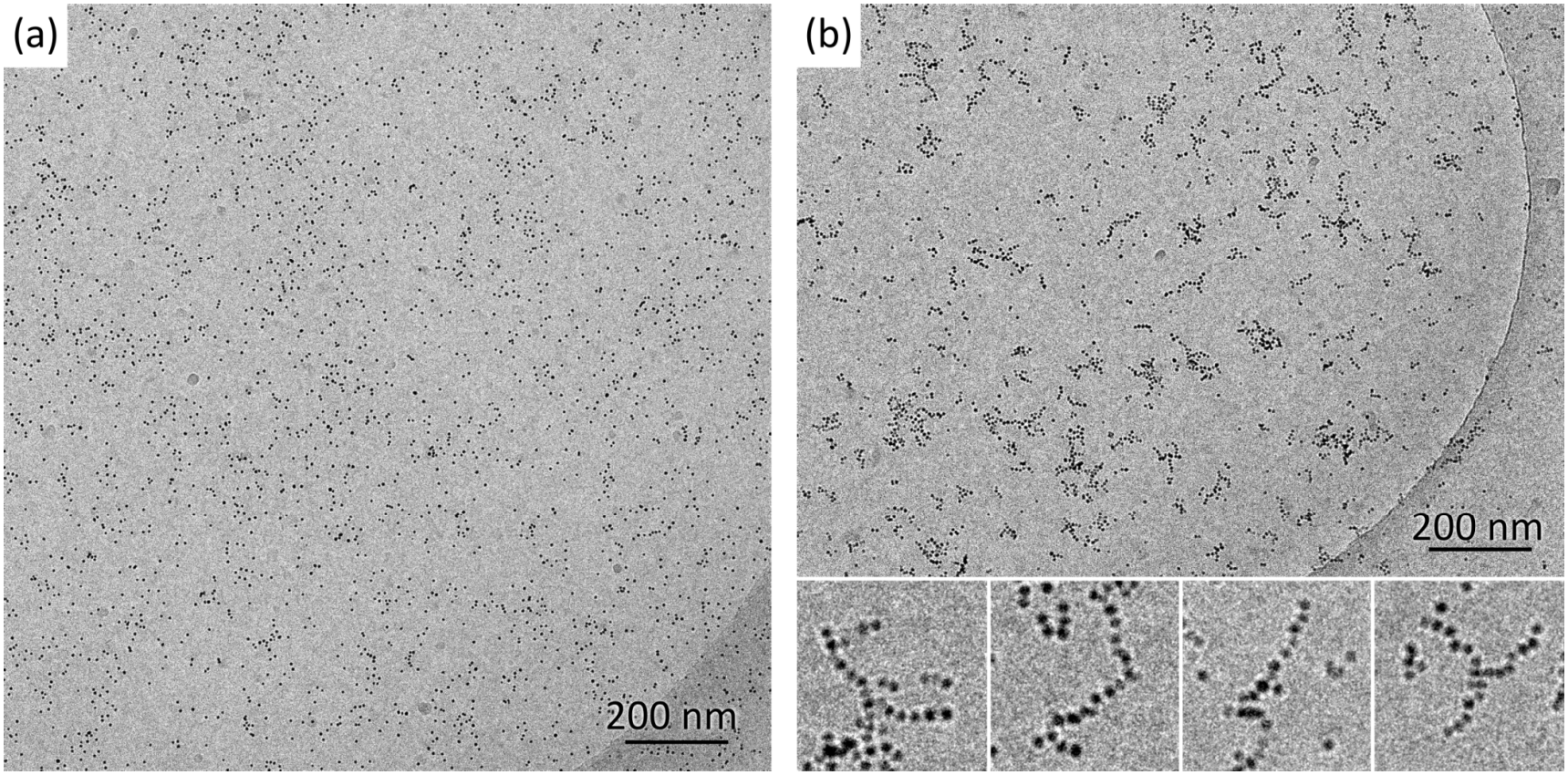


v corresponds to *f* (hydrophobic coverage)

Time scale of ligand reorganization

~ 1 min

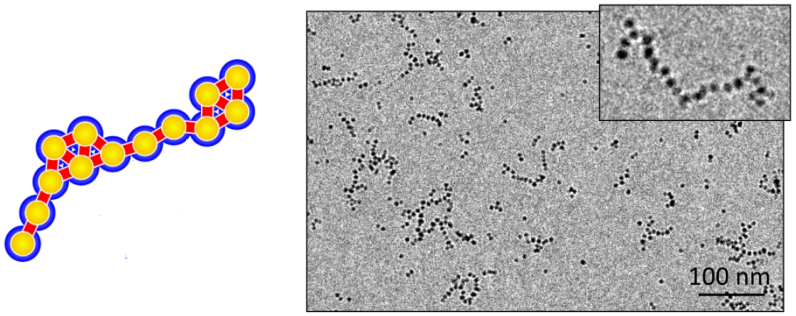
~24 h



CryoEM, C_{salt} = 0.2 M, f = 0.25

Conclusion

Part 1. Self-assembly of inorganic nanoparticles in solution



- Formation of chain structure via short-ranged electrostatic/ hydrophobic interaction
- Dynamic rearrangement of ligands
- 3D simulations capture experimental trends

Part 1. Self-assembly of inorganic nanoparticles in solution

Part 2. Interaction between inorganic-organic materials

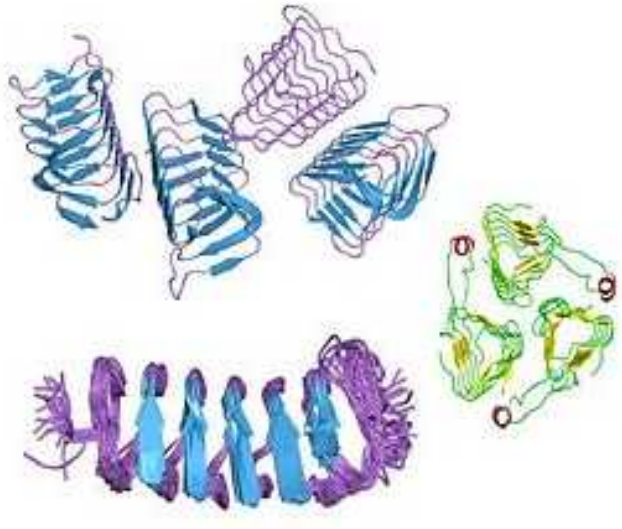
2.1. Selective binding of nanoparticles to open edges

2.2. Formation of nanoparticle-supported bilayers

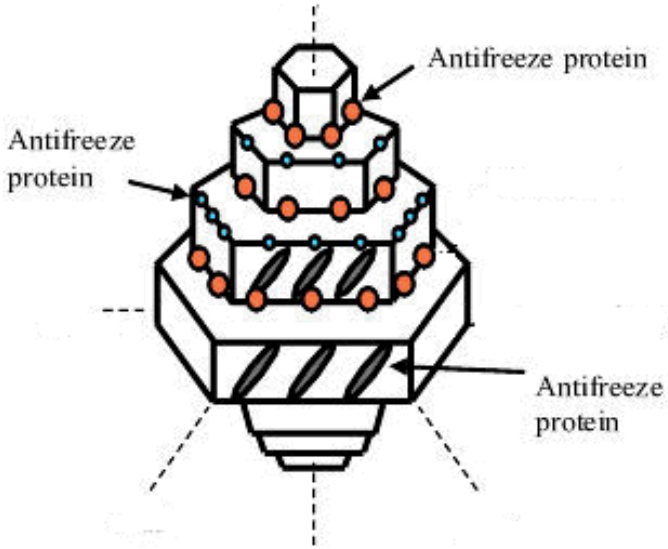
Part 3. Research plan


www.mbri.org; www.fishinging a.com

Antifreeze proteins as kinetic capping agents



Antifreeze protein

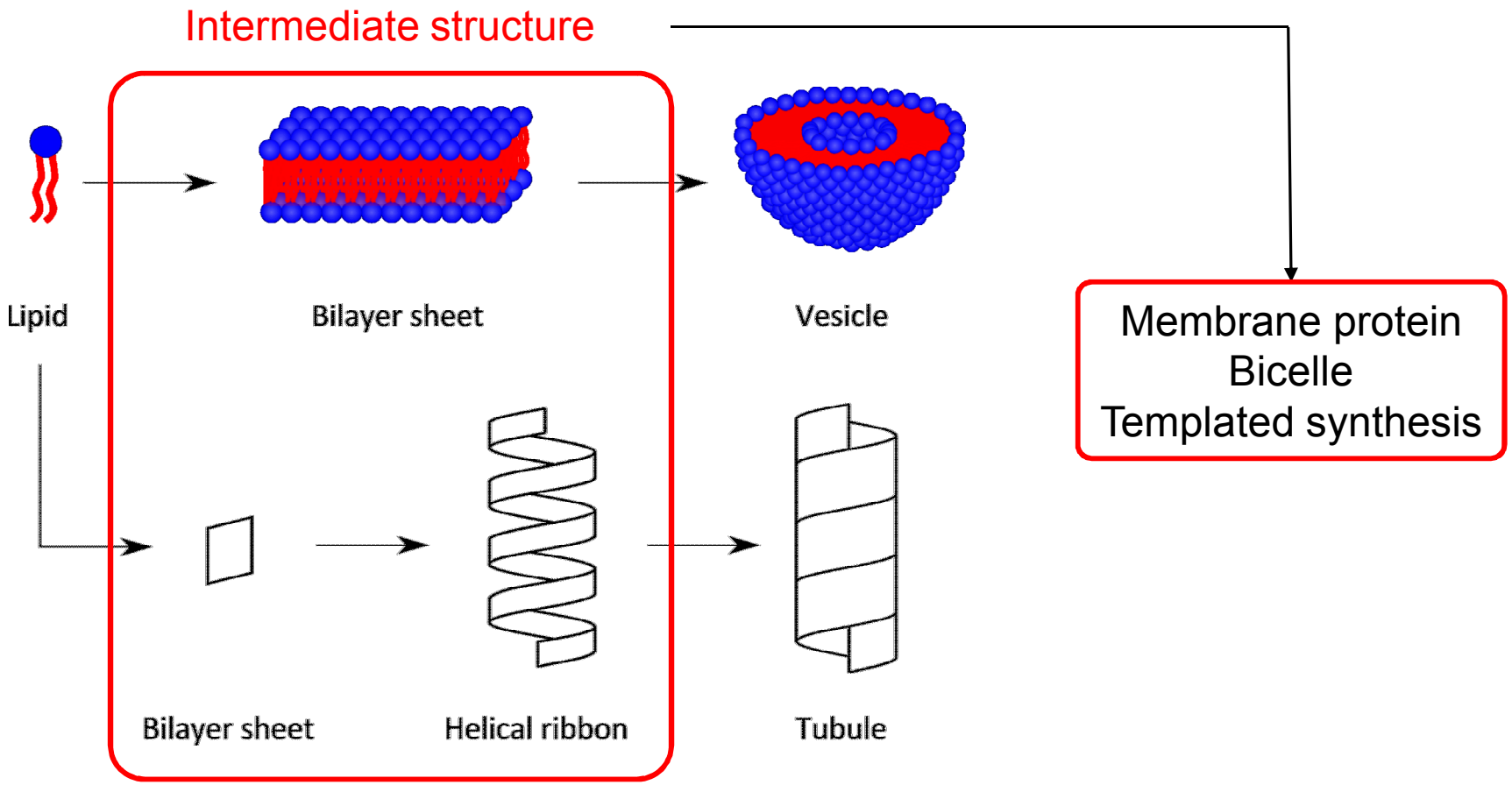


Ice crystal

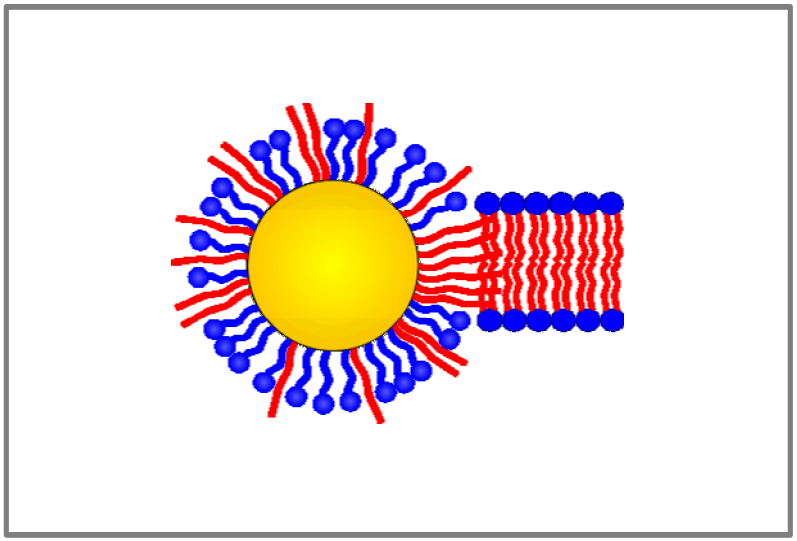
SKIM MILK, SUGAR, CORN SYRUP, POLYDEXTROSE, MALTODEXTRIN, PROPYLENE GLYCOL MONOESTERS, MONO AND DIGLYCERIDES, CELLULOSE GUM, CREAM*, CAROB BEAN GUM, GUAR GUM, NATURAL FLAVOR, CARRAGEENAN, ICE STRUCTURING PROTEIN, VITAMIN A PALMITATE, ANNATTO (FOR COLOR) * ADDS A DIETARILY INSIGNIFICANT AMOUNT OF FAT. Ingredients and Nutrition Facts are current as of 3/1/13. Please see shelf packaging for any changes. Nutrition Facts may vary in high altitude areas.

Chem. Rev., 96, 601-618 (1996)
www.wikipedia.org; www.intechopen.com

Intermediate structures with open edges



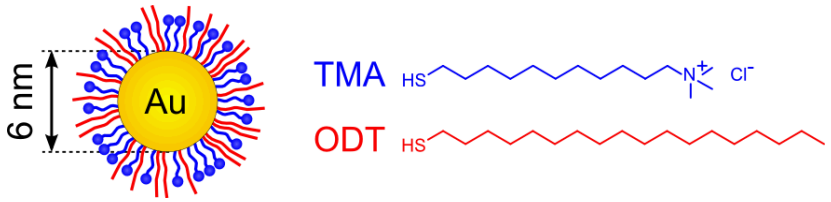
NP amphiphiles bind selectively to open edge of bilayers



1) Controlling growth of lipid tubules

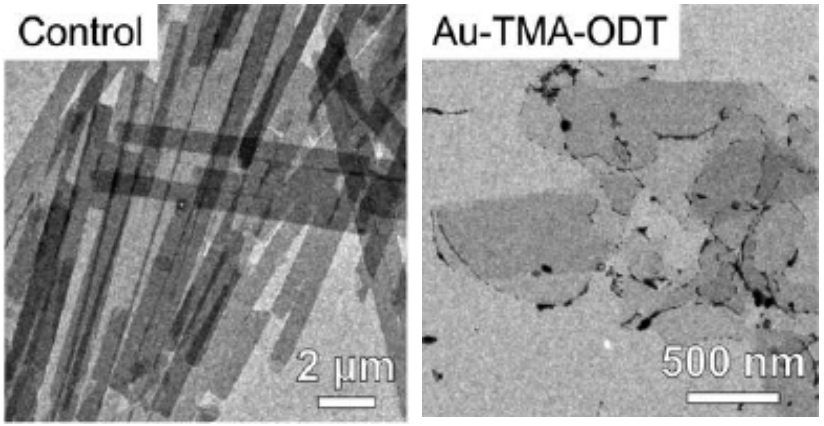
2) Inhibiting the formation of gallstone precursors

NP amphiphiles stabilizing transient assemblies

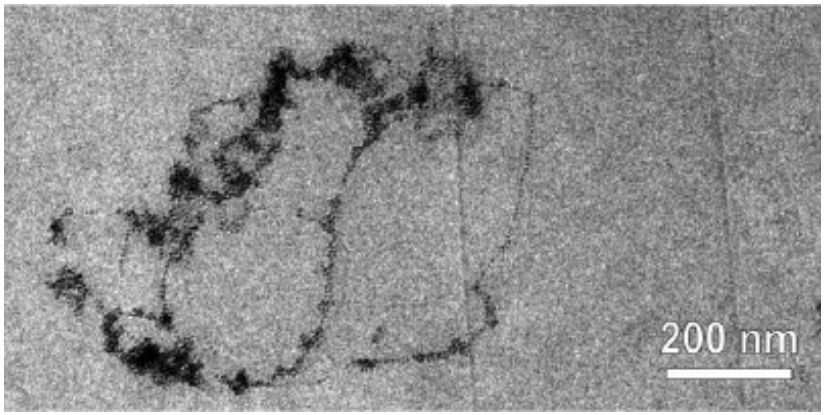


$DC_{8,9}PC$ lipid tubule

TEM

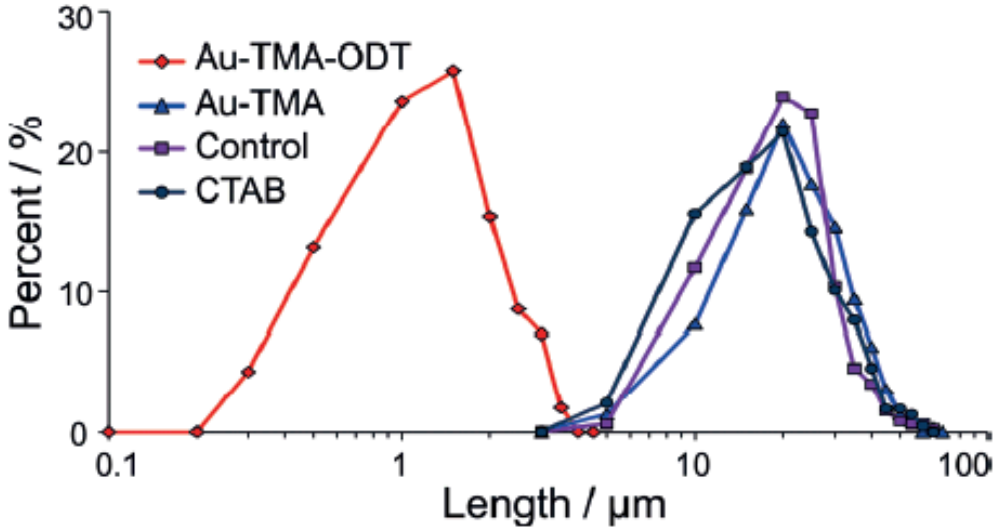


CryoEM

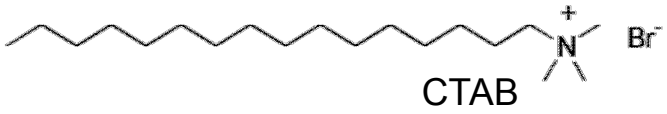


Shin et al. *Angew. Chem. Int. Ed.* 54, 10816 – 10820 (2015)

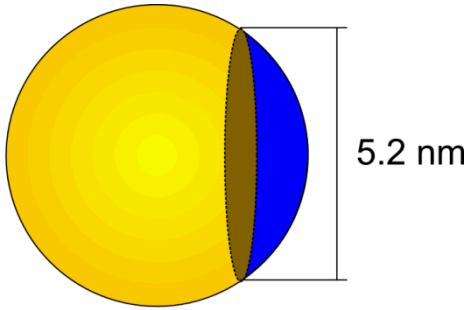
Hydrophobic interaction



- Nanoscopic size of NP amphiphiles

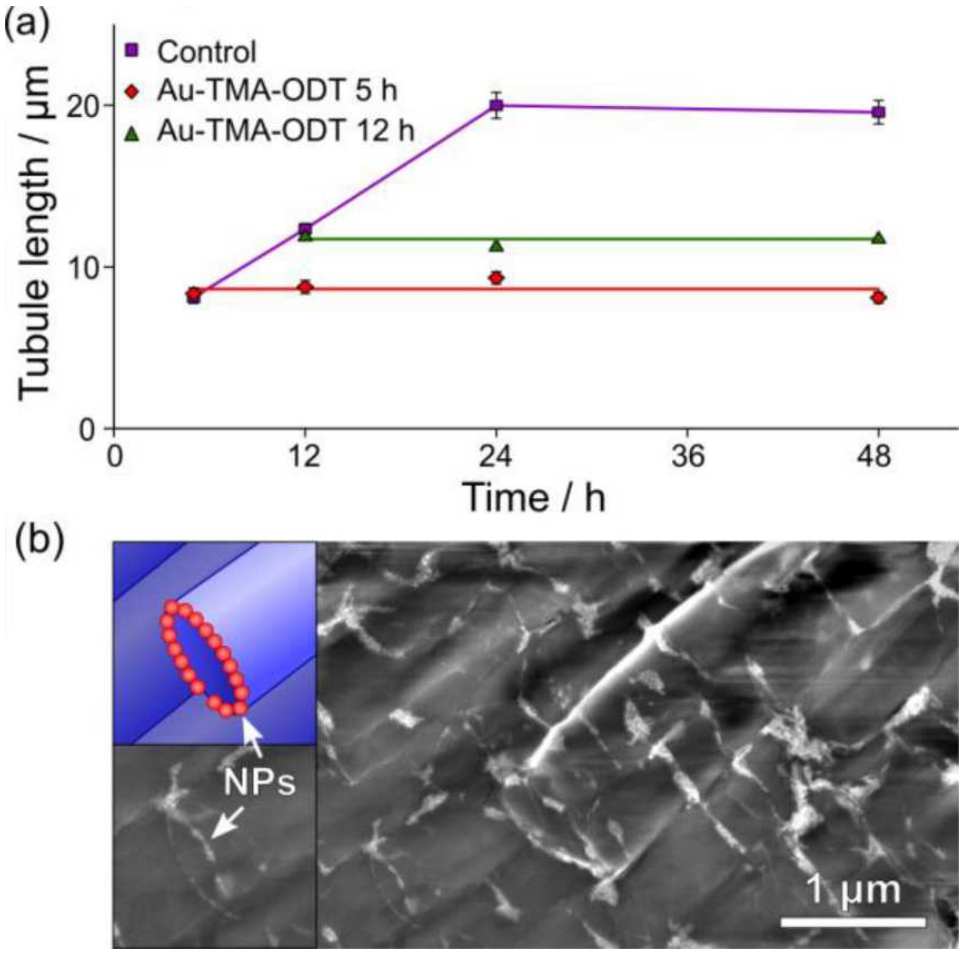


CTAB



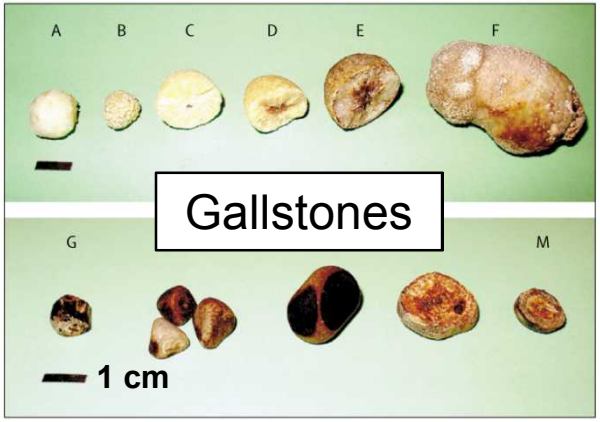
TMA : ODT = 77 : 23

Tubule of desired length

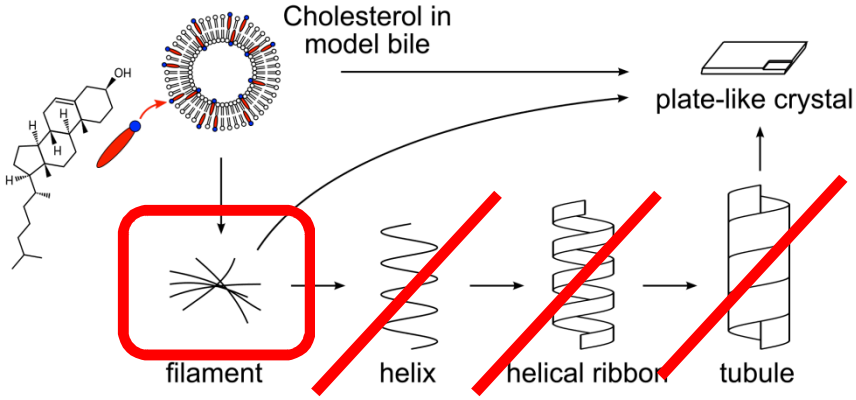


SEM

Inhibition of undesired assembly

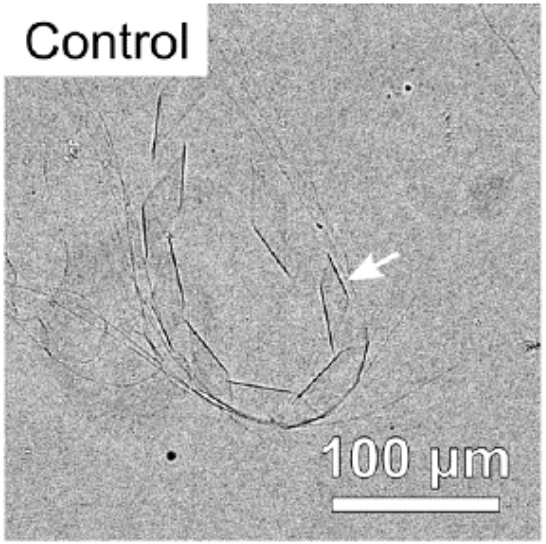


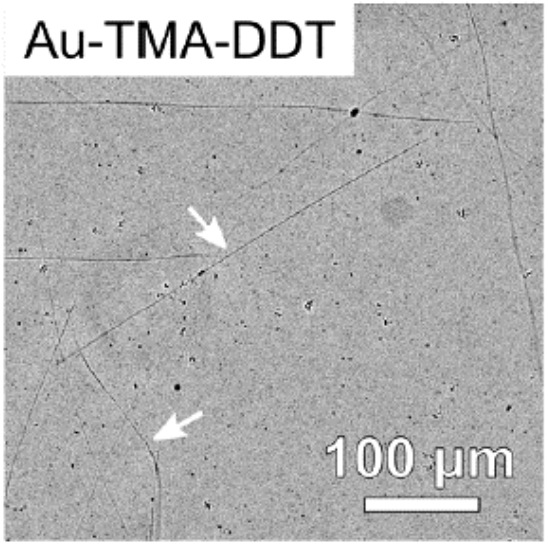
Lancet **368**, 230-239 (2012)



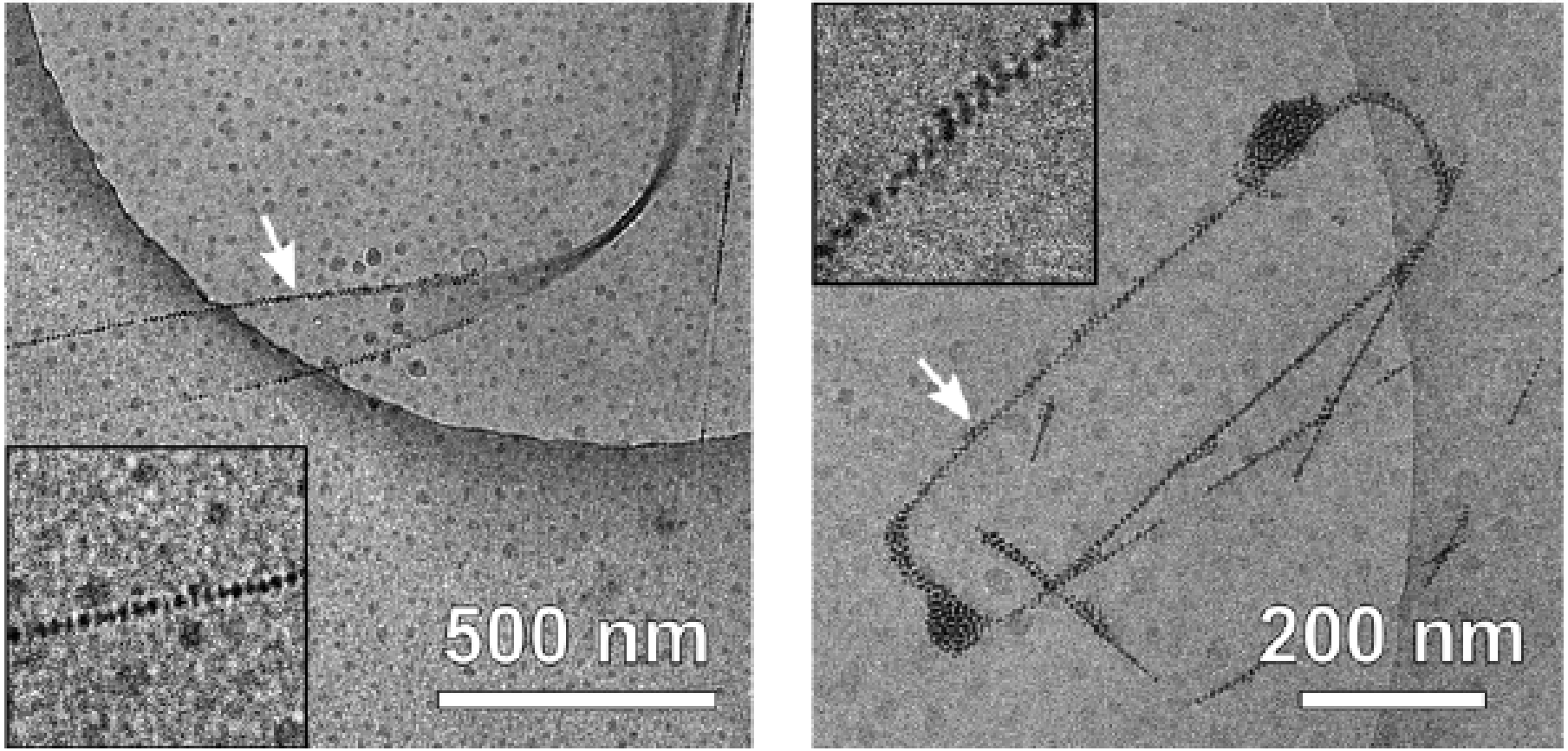
Cholesterol helical ribbon

OM





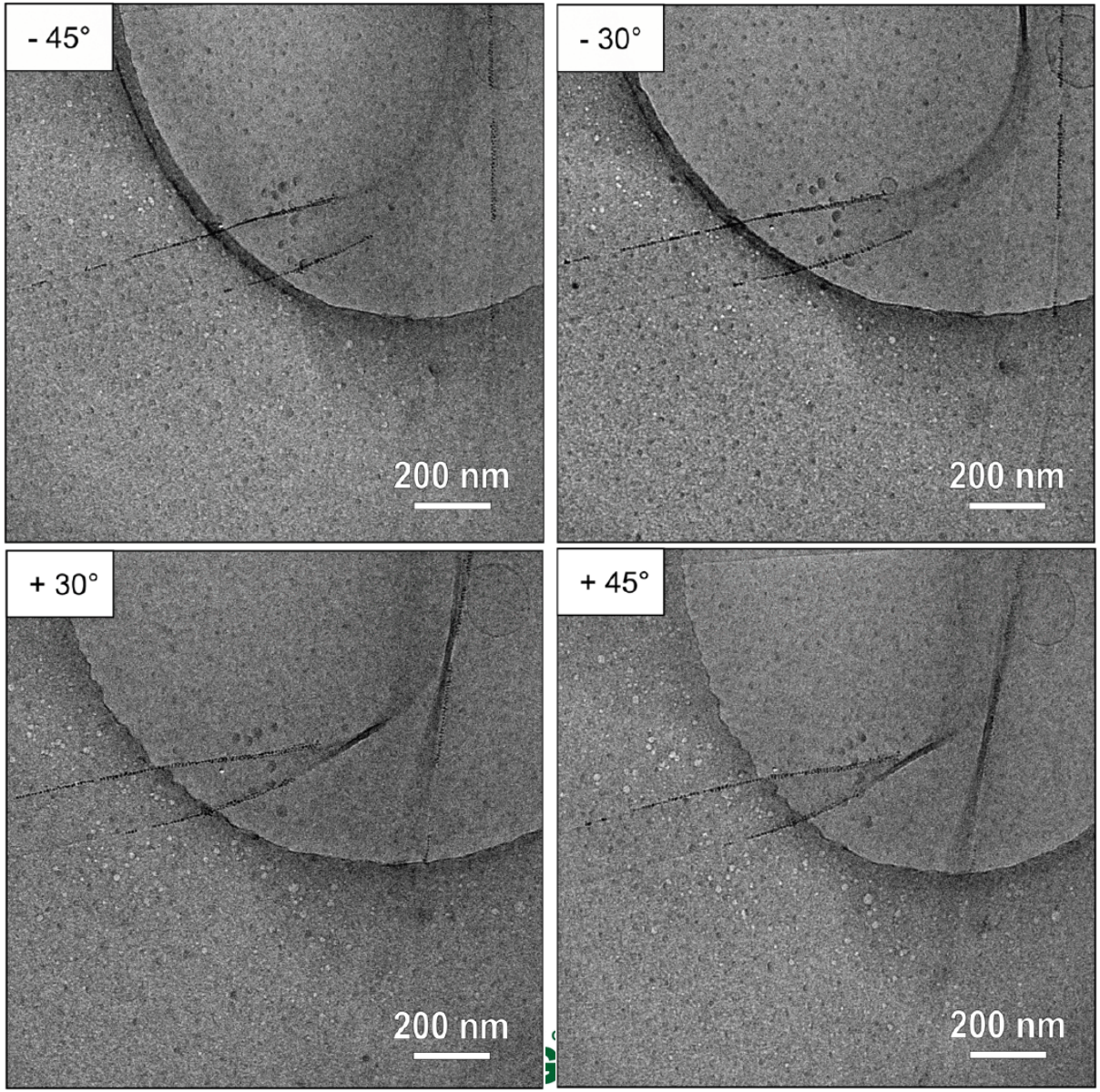
Selective binding of NPs to cholesterol filaments (1)



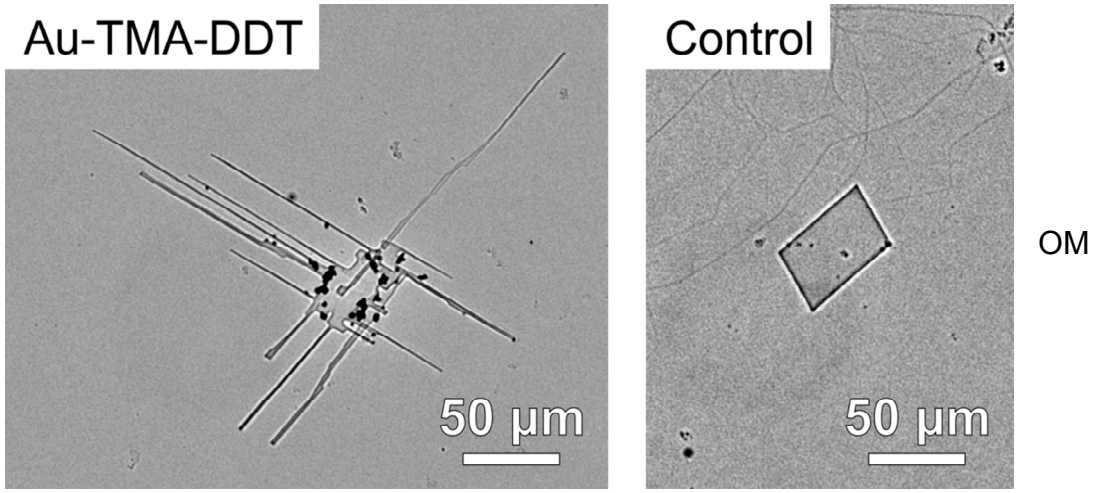
CryoEM

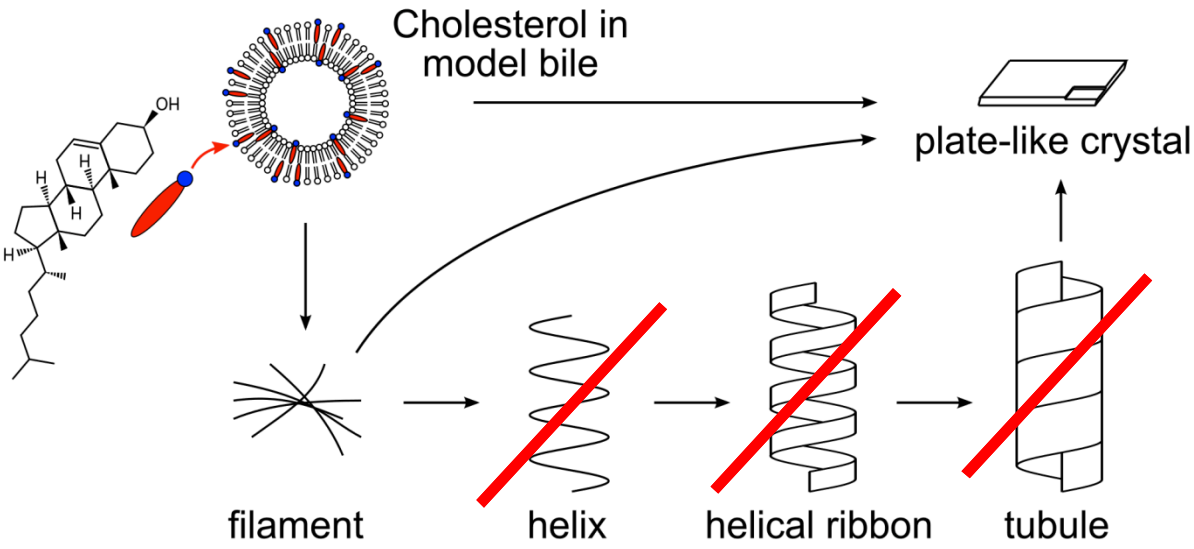
Selective binding of NPs to cholesterol filaments (2)

CryoEM with tilt angle



Incomplete inhibition of crystallization

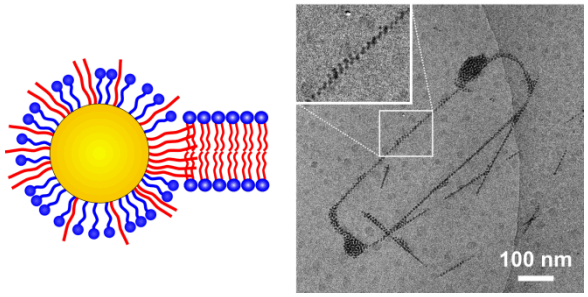




Conclusion

Part 2. Interaction between inorganic-organic materials

2.1. Selective binding of nanoparticles to open edges



- Stabilization of transient structures
- Inhibition of undesired assemblies

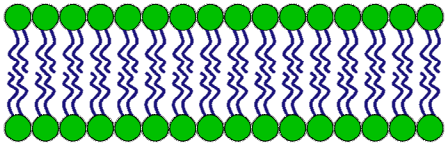
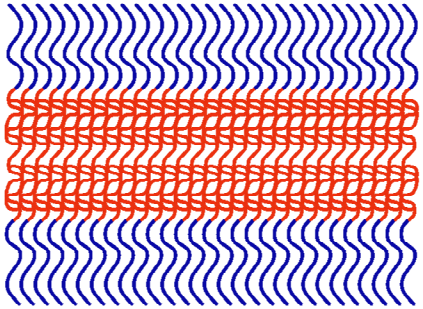
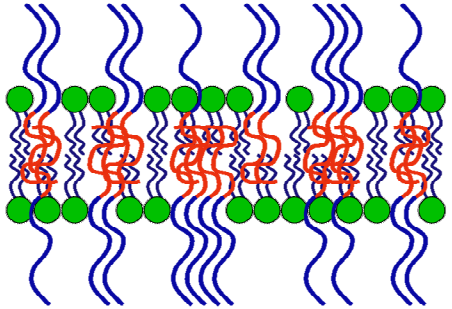
2.2. Formation of nanoparticle-supported bilayers

Smart delivery vehicles

Mesoporous silica nanoparticle (MSNP)

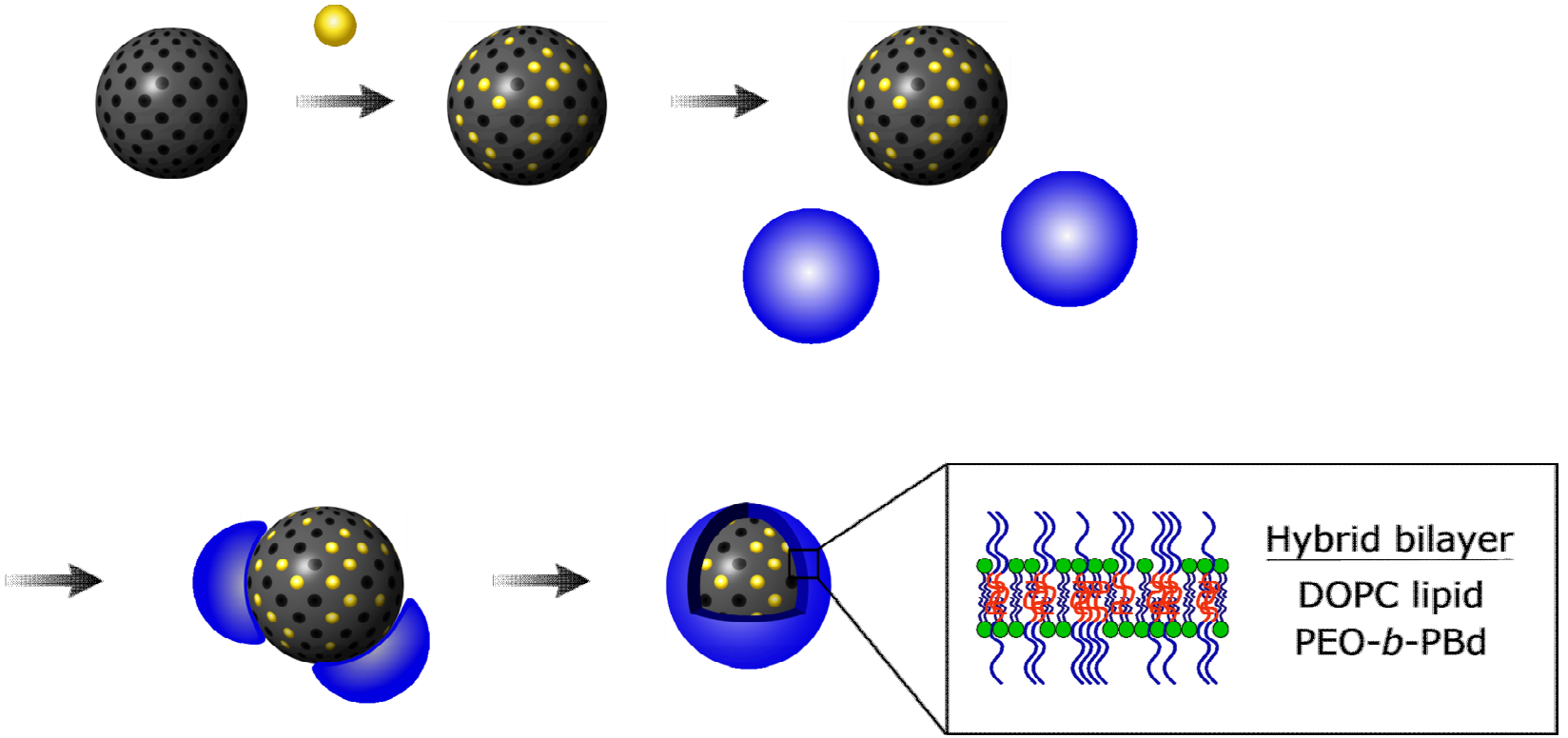
- High loading capacity
- Controllable size, shape, pore size, surface chemistry
- Biodegradable
- **Often require coatings**
  - Cargo encapsulation
  - Colloidal stability
  - Blood circulation time
  - Potential toxicity of unmodified silica

Hybrid bilayers

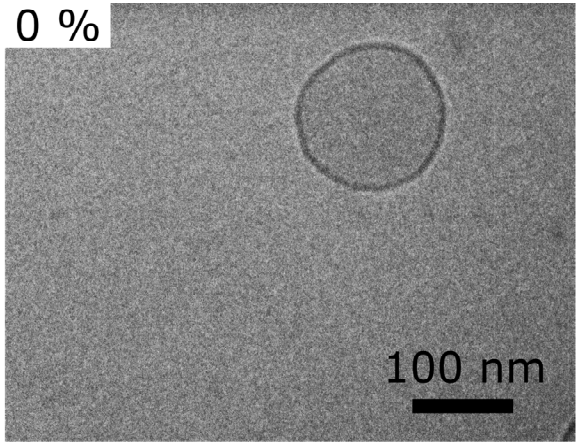
	Lipid	Polymer	Hybrid bilayer
			
+	Fluidity Biocompatibility	Stability Versatility	Synergistic membrane properties
−	Stability	Fluidity	

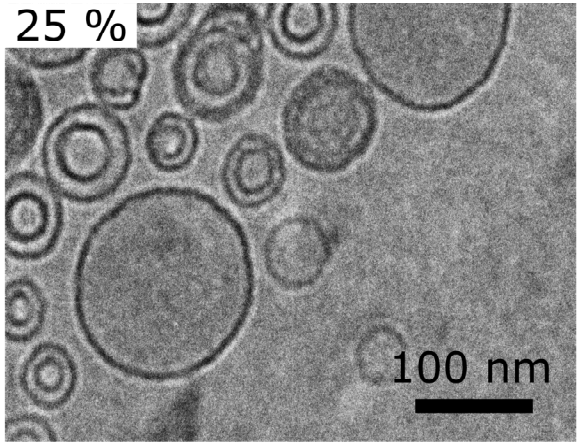
MSNP based delivery vehicles

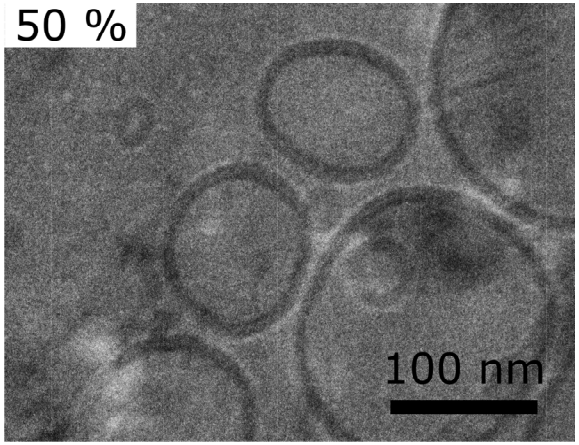
Formation of supported hybrid bilayer on MSNP by fusion

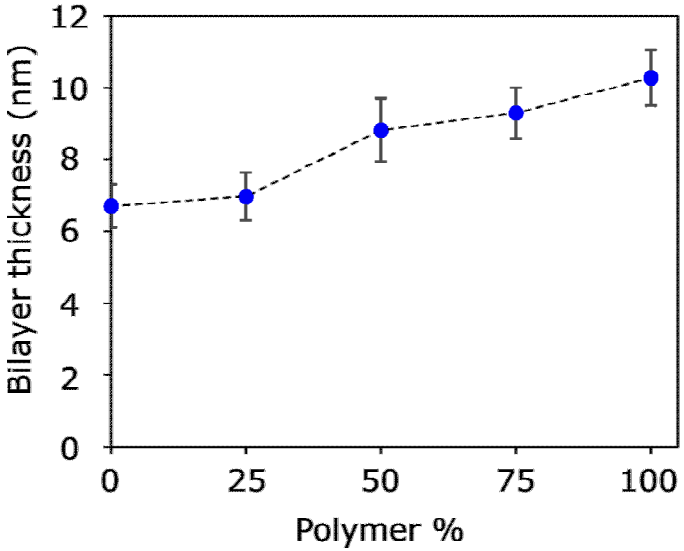


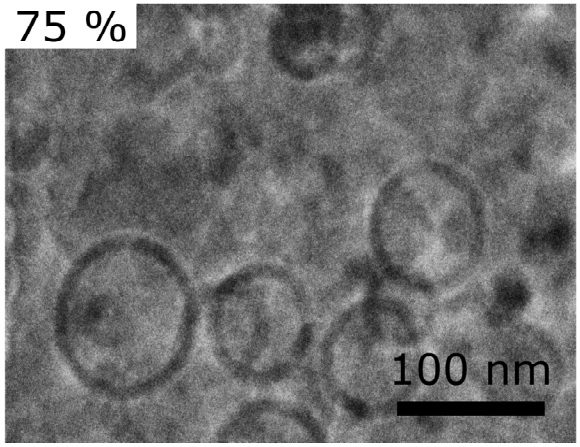
Characterization of hybrid bilayers

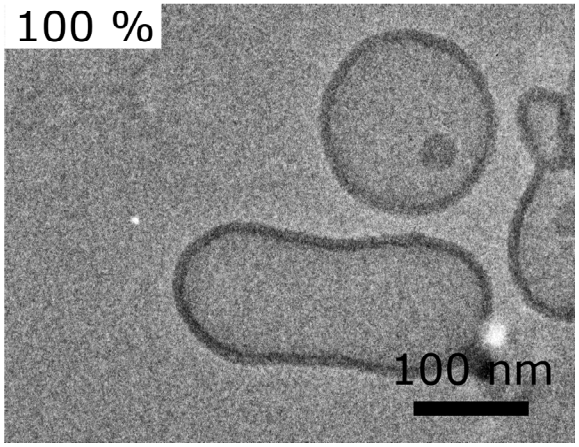






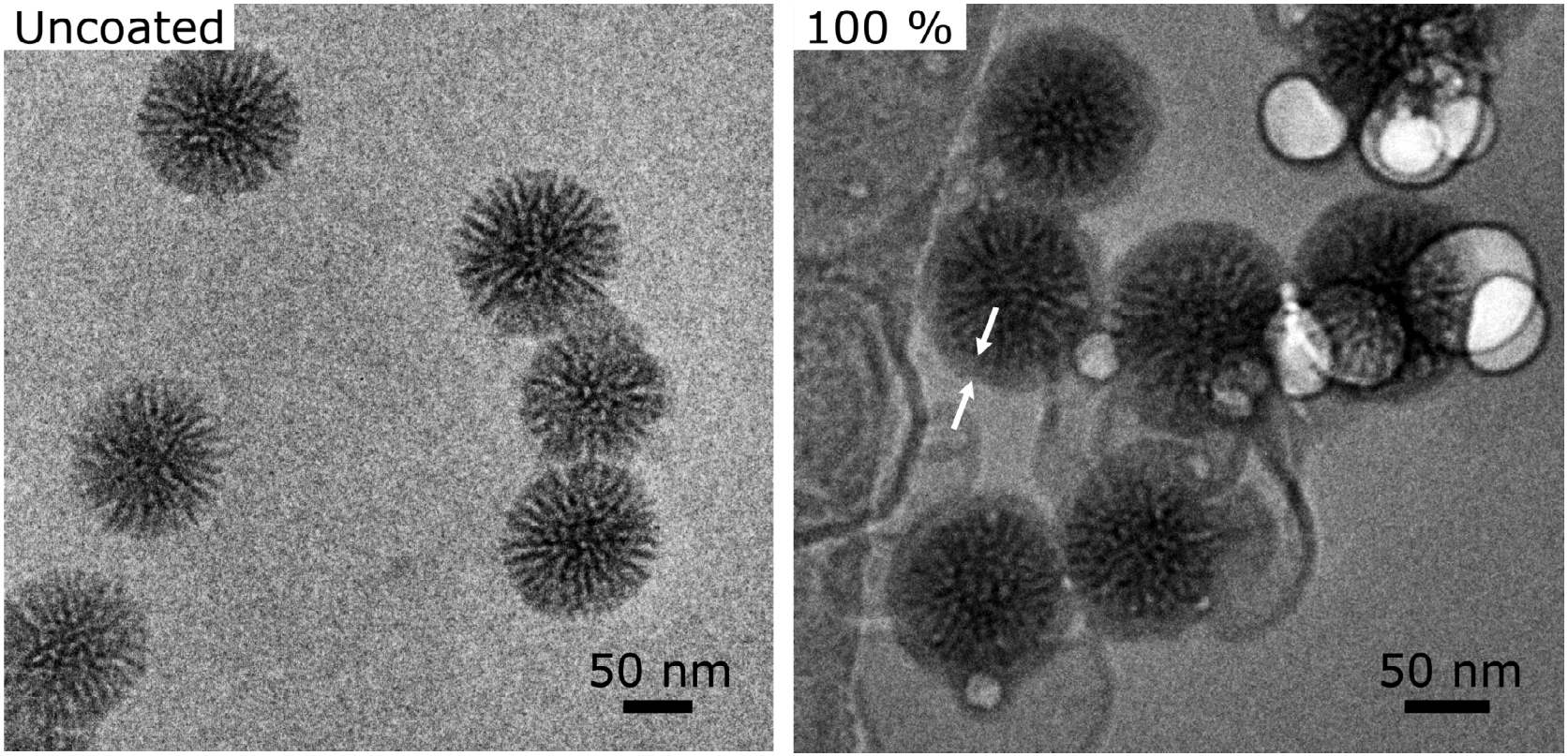






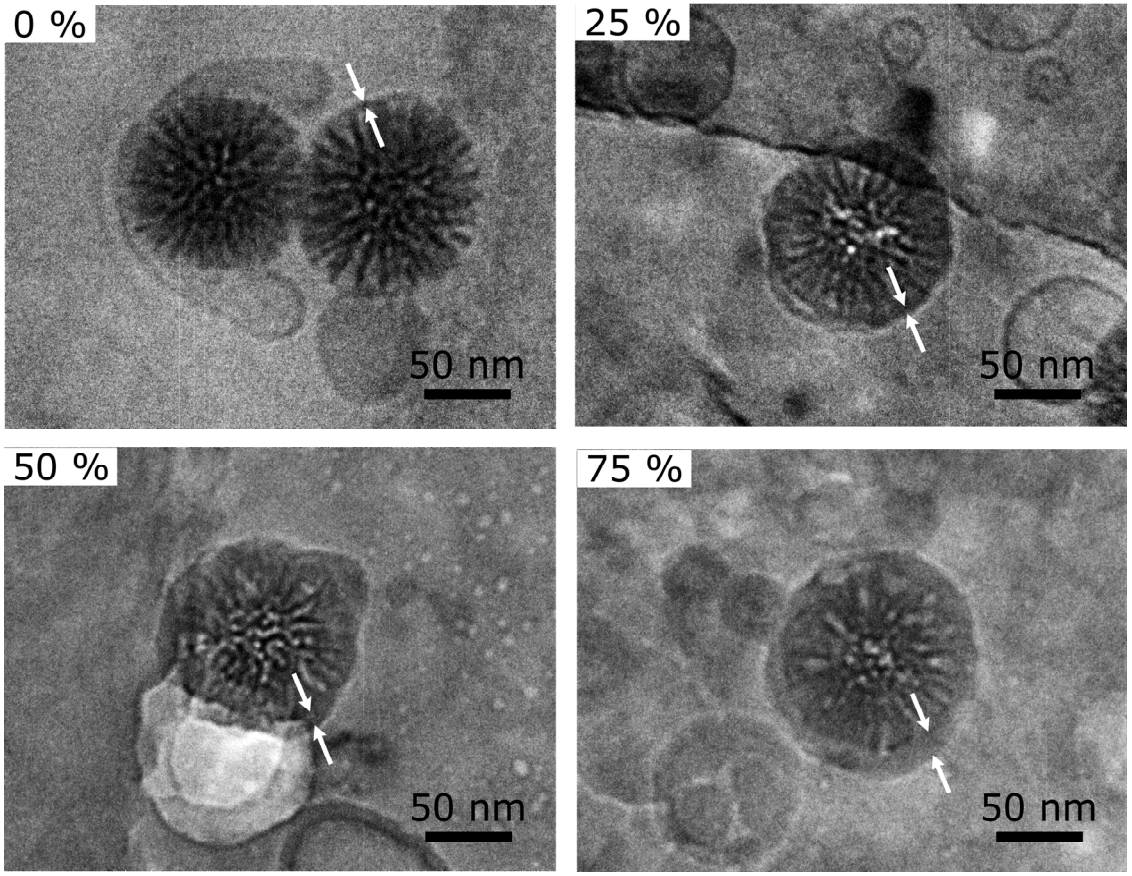
CryoEM

MSNP-supported hybrid bilayers

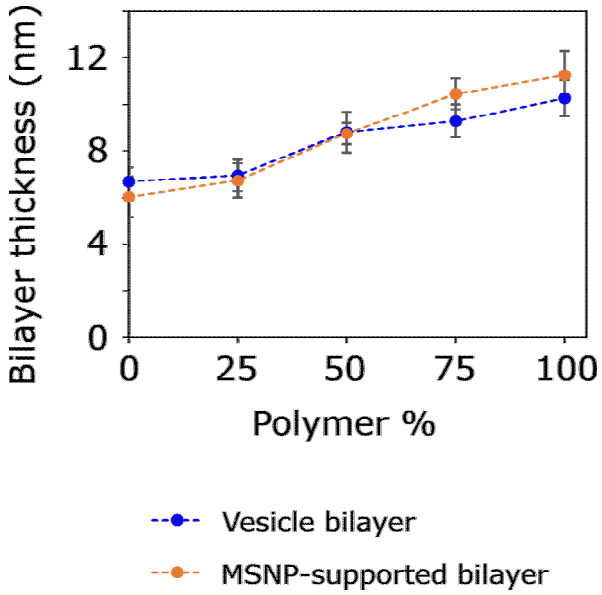


CryoEM

MSNP-supported hybrid bilayers

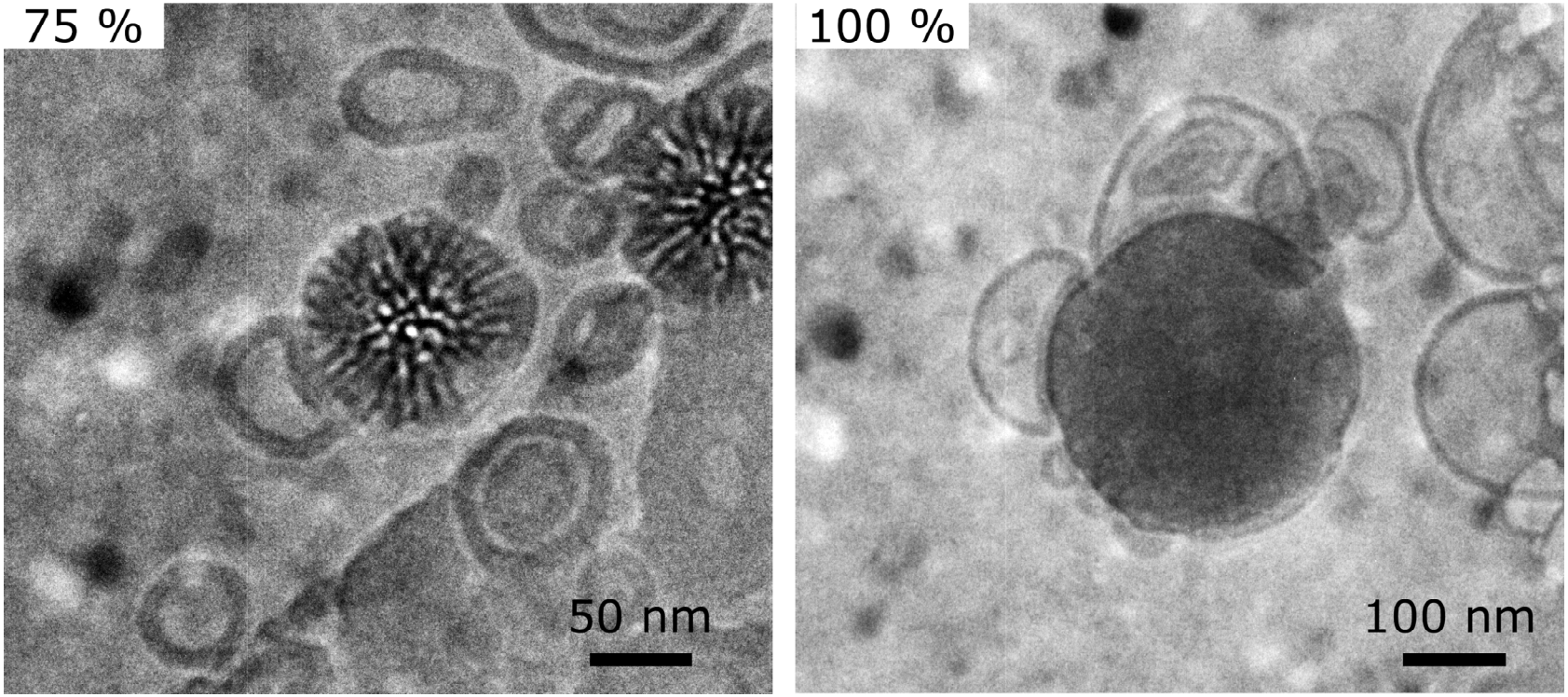


CryoEM



MSNP-supported hybrid bilayers

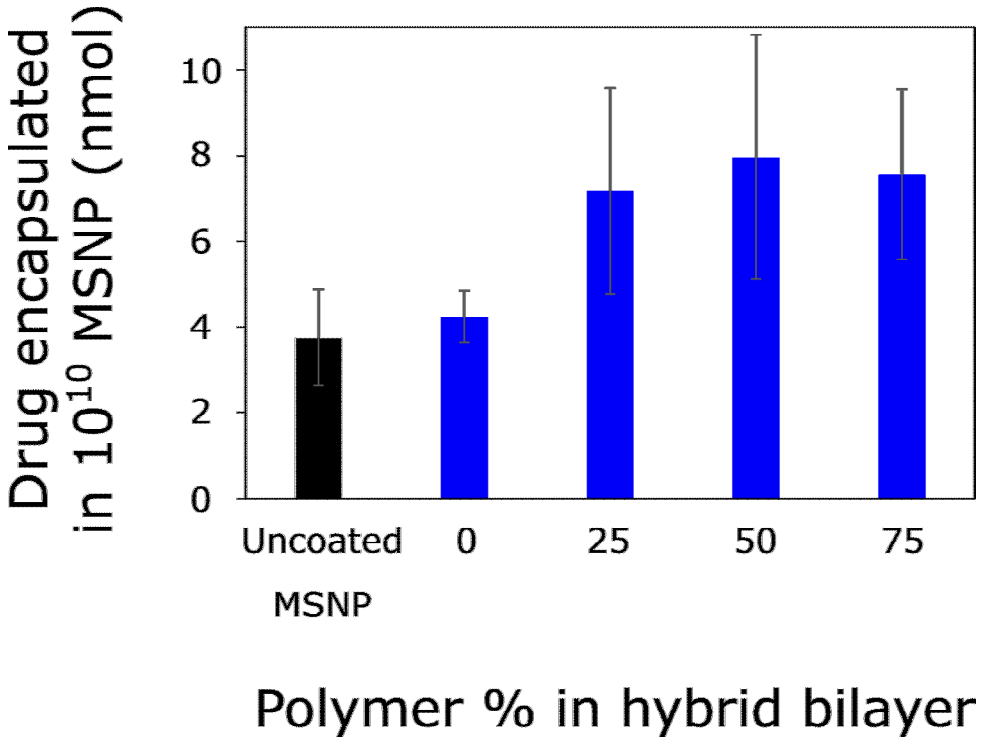
Adsorbed vesicles on MSNP due to robust membrane



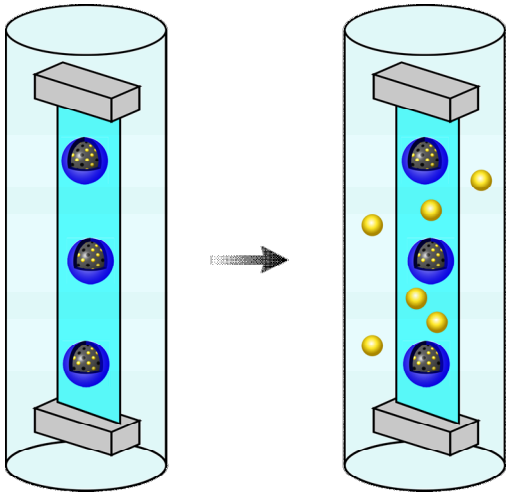
CryoEM

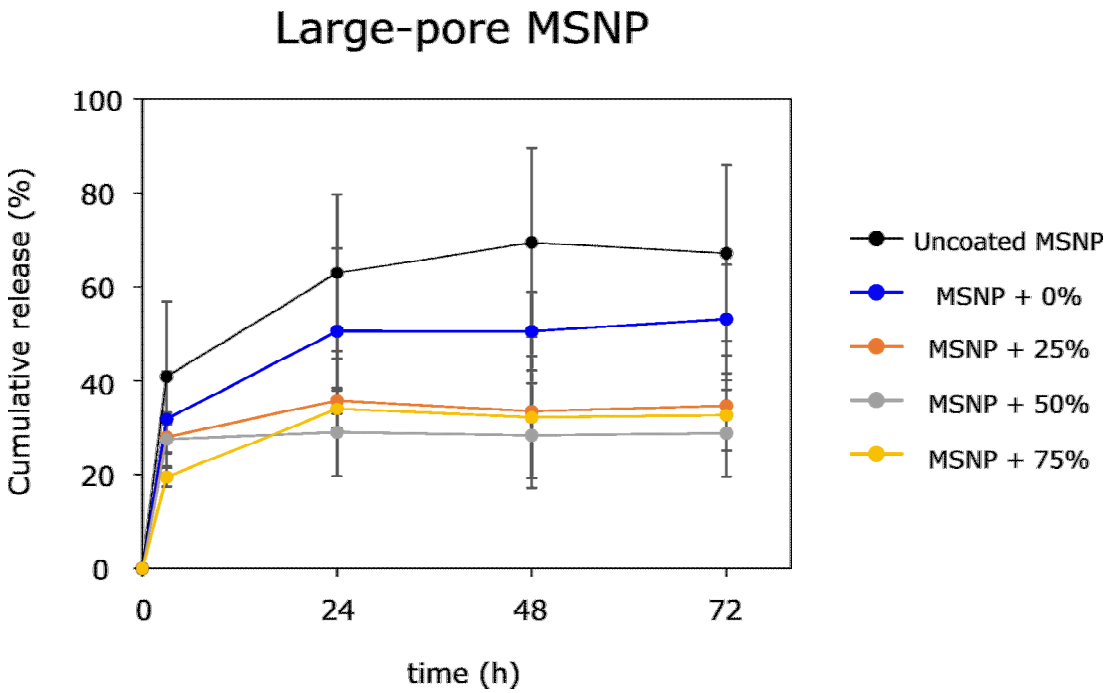
Encapsulation efficiency

MSNPs with hybrid bilayer coating (~7.5 nmol) encapsulate more drug than MSNPs with lipid bilayer coating (4.2 nmol) or uncoated MSNP (3.7 nmol)



In vitro drug release



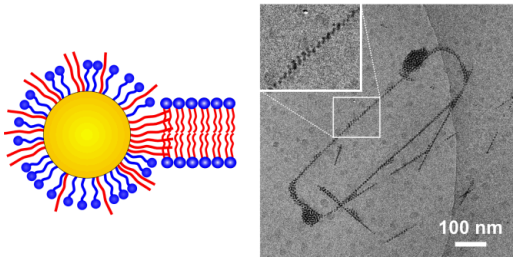


MSNPs with hybrid bilayer coating (32 %) release less drug less
MSNPs with lipid bilayer coating (53 %) or uncoated MSNP (67 %)

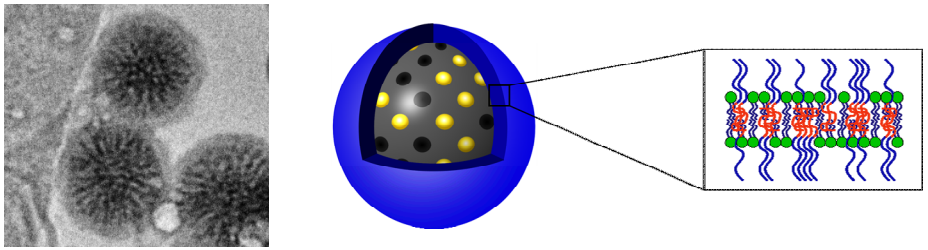
Conclusion

Part 2. Interaction between inorganic-organic materials

2.1. Selective binding of nanoparticles to open edges



2.2. Formation of nanoparticle-supported bilayers



- Supported hybrid bilayers by fusion
- Decreased permeability of hybrid bilayers

Part 1. Self-assembly of inorganic nanoparticles in solution

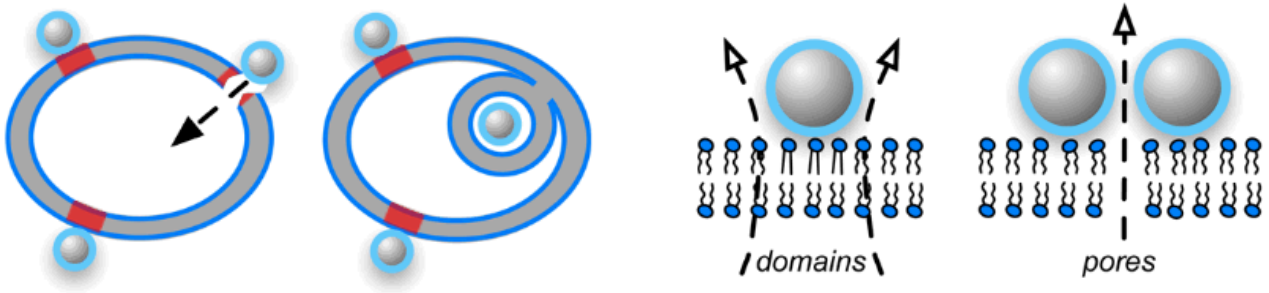
Part 2. Interaction between inorganic-organic materials

Part 3. Research plan

- 3.1. 3D visualization in synthetic architectures
- 3.2. Time-resolved characterization of phase transition
- 3.3. *In situ* characterization at a wide range of temperature

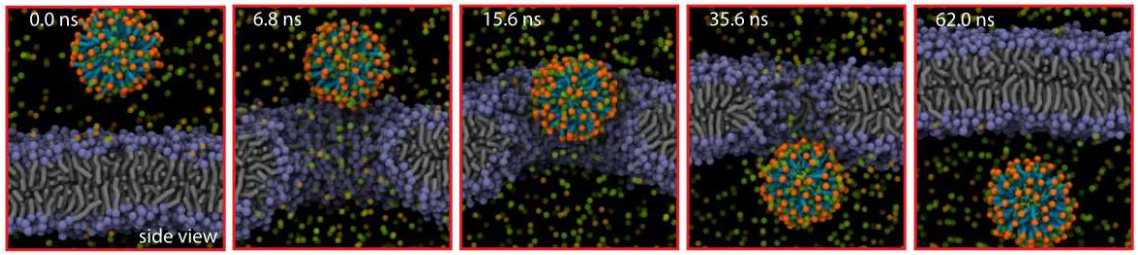
1. 3D visualization in synthetic architectures

Interaction of nanomaterials with biological interfaces



Environ.Sci.Technol. 48, 873-880 (2014)

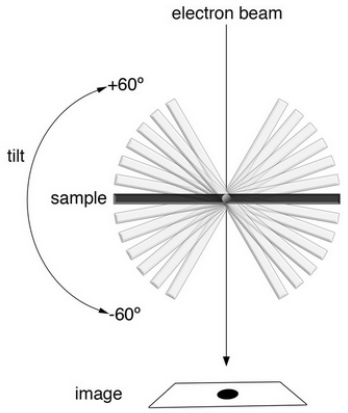
Theoretical and computational investigations of nanomaterial-biomembrane interaction

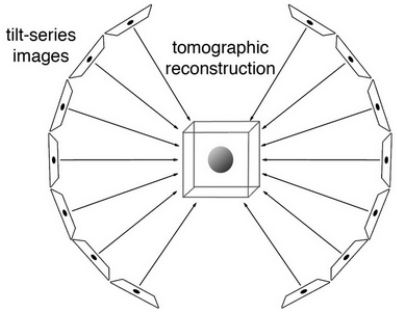


ACS Nano 7, 10799-10808 (2013)

1. 3D visualization in synthetic architectures

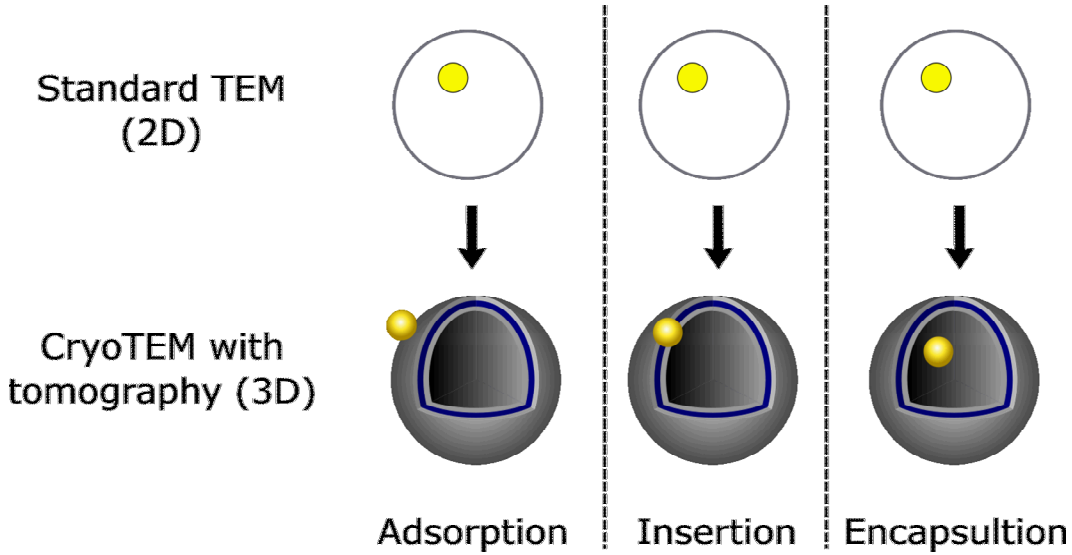
Cryo tomography

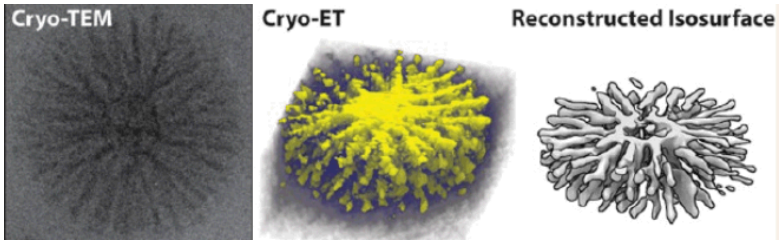




www.wikipedia.org

Hidden structural features revealed by tomography

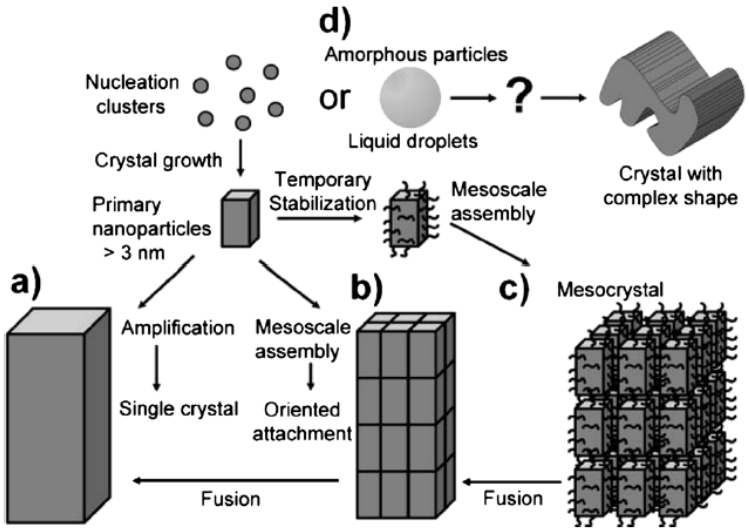




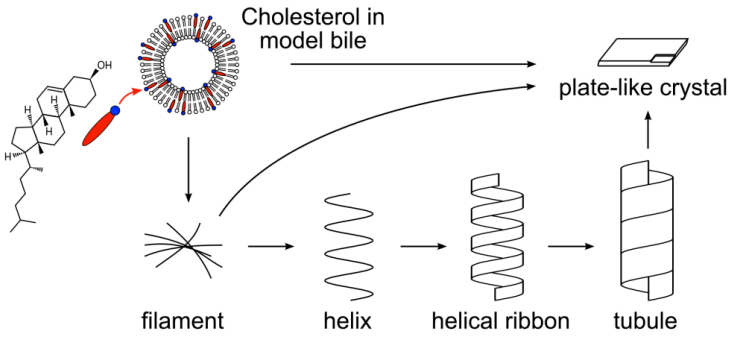
ACS Nano 8, 11330-11340 (2014)

2. Time-resolved characterization of dynamic phase transition

Metastable phases during nucleation and growth of crystals



Nanoscale 4, 54-65 (2012)



Angew. Chem. Int. Ed. 54, 10816–10820 (2015)

Limitation: disordered, transient, highly hydrated intermediates

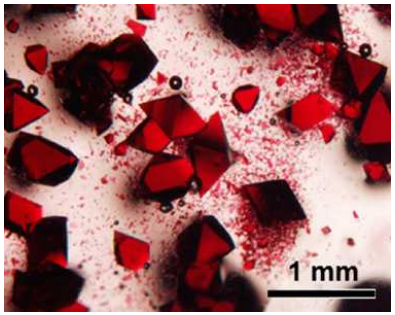
2. Time-resolved characterization of dynamic phase transition

- Detection of precursors in their native state during early stages of the precipitation/nucleation
- Size, shape, lifetime of metastable intermediates

→ Kinetic pathways of crystallization of organic/inorganic materials

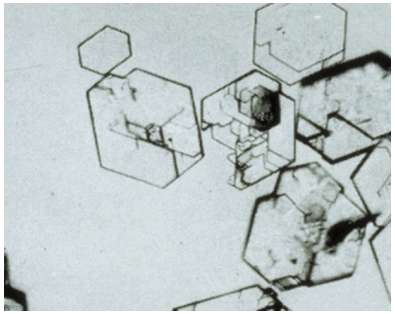
Protein crystallization

Hemoglobin



Crystals 7, 282 (2017)

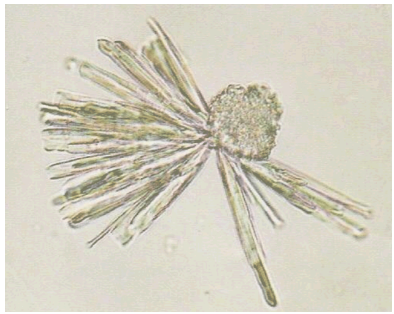
Cystine



www.laboratoryinfo.com

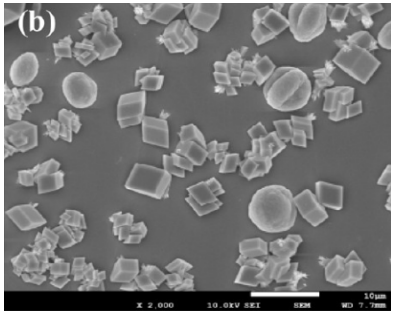
Biomineralization

Calcium phosphate



www.laboratoryinfo.com

Calcium carbonate

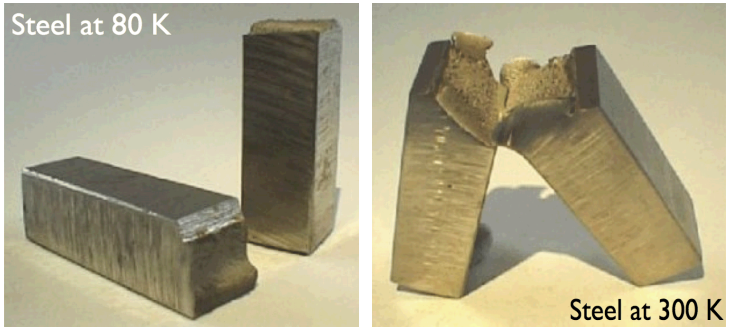


Cryst.Growth.Des. 18, 1710-1721 (2018)

3. *In situ* characterization at a wide range of temperature

Brittle Fracture

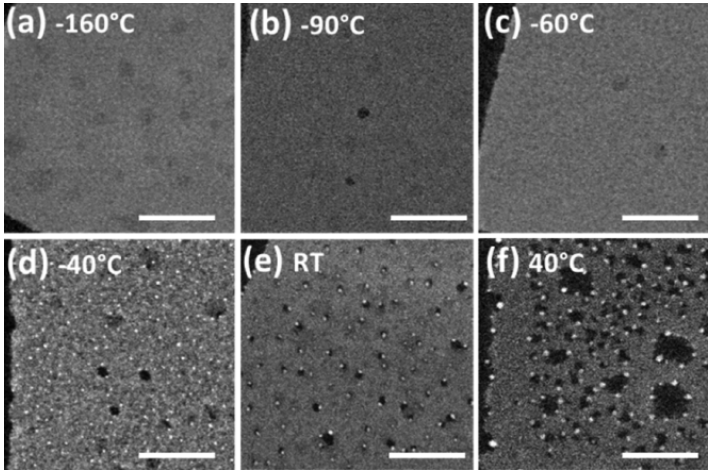
Ductile Fracture



www.civildigital.com

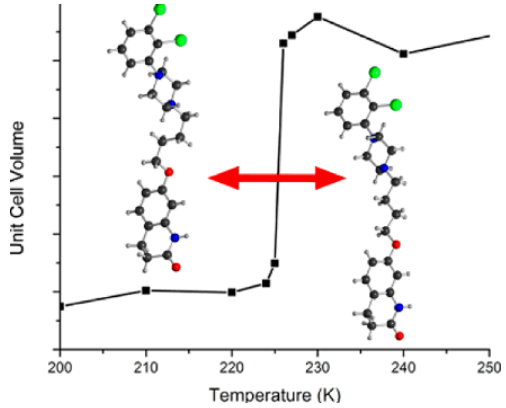
Low temperature phase transition in crystal

Nucleation of Pb NPs on $CsPbBr_3$ nanocrystal



ACS Nano 11, 2124-2132 (2017)

Aripiprazole Form VIII ⟷ Form II



Cryst.Growth.Des. 14, 5004-5010 (2014)

3. *In situ* characterization at a wide range of temperature

CryoEM/FIB + *in situ* TEM

- Accessible temperature range of -170 ºC to 300 ºC
- Low dose imaging technique

Temperature-dependent studies

- Interface between hard and soft matters
- Structural phase transition in crystals
- Electron beam sensitive perovskites and metal-organic frameworks
- *In situ* nanomechanical testing

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  Student Intern: Haley Monteith (SNL)

Paxton Monteith

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  Achraf Noureddine, Ph.D.; Jeffrey Brinker, Ph.D. (Univ. of New Mexico)

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Sandia National Laboratories

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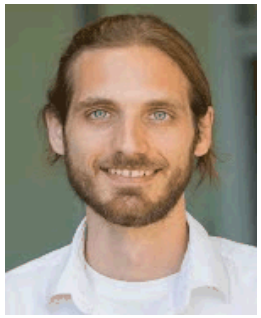
Hee-Young Lee, Ph.D. (currently at KIT)
Aaron Drews, Ph.D. (currently at UCSD)
Aaron Chirsan
Sean Lewis


Bishop


Lee


Drews

CBES Center for Bio-Inspired Energy Science

non-equilibrium eNErgy research center

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