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NUANCE

Tech Talks

...explore with us

Please join us for monthly user meetings!

Tech staff will:

- ✓showcase our state-of-the-art capabilities,
- ✓provide updates on the latest innovations,
- ✓discuss any topics you find interesting.

Bring your questions and suggestions!

March 20, 2019 — Dr. Reiner Bleher, Assistant Research Professor

Materials Science & Engineering Conference Room, Cook #2036
12 - 1 p.m.

Successful Electron Microscopy of Biological and Soft Matter Samples

Adequate sample preparation is a pre-requisite for optimal results in electron microscopy. The BioCryo Facility of NUANCE offers a comprehensive array of methods and techniques for processing and preparing samples before they can be observed and analyzed in the electron microscope. The choice of the most suitable technique depends on the data we want to extract from a given sample. The intention of this tech talk is to give the users an idea of the capabilities of the BioCryo Facility and the rationale behind different workflows available.

The *NUANCE* Center

www.nuance.northwestern.edu

NU and Regional Facilities/ Coordination

ANL-APS
ANL-CNM
ANL-EMC

(Cook, Tech, Silverman, Hogan – Northwestern University)

EPIC
Electron Probe Instrumentation Center

SEM, TEM, FIB, EDS, EELS, eBeam-Litho, Sample Prep

TEM: K. He, J. Wu
SEM: B. Myers, K. Hagglund, T. Abbott

BioCryo
Cryo- and Conventional Soft-Matter EM, Microanalysis, and Sample Prep

cryo/SEM, TEM, STEM, EDS…

R. Bleher, E.W. Roth, C. Wilke

Keck-II
Keck Interdisciplinary Surface Science

XPS, ToF-SIMS, FTIR, Confocal Raman ...

Xinqi Chen + PD

SPID
Scanned Probe Imaging & Development

AFM, DPN, NSOM, f-s Laser, Nano-Indenter

G. Shekhawat, + PDs

NUFAB
Micro/Nano Fabrication

Deposition, Photo-Lithography, Thermal processing, Wet processing, Etching-Ashing

N. Basit, J. Ciraldo, A. Dhote, Y. Jia, S. Lu, +PD

SHyNE Director
Ben Myers

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Chad Goeser

Outreach Coordinator
Kathryn Dean

Program Administrator
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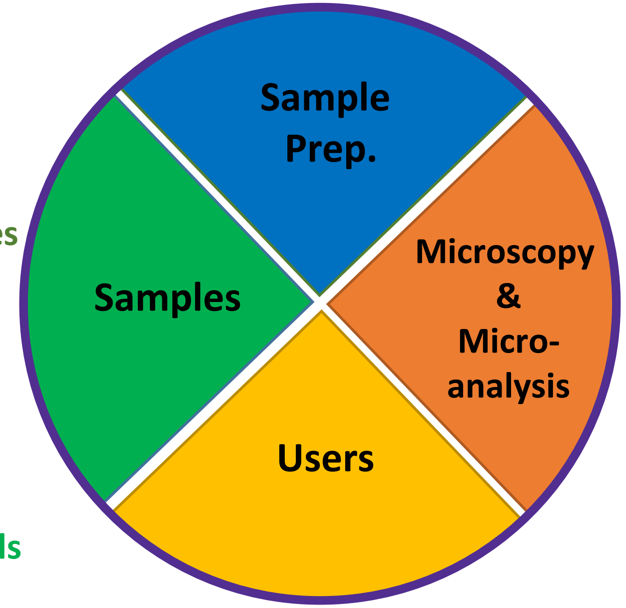
BioCryo Facility

- High-pressure freezing
- Plunge freezing
- Freeze fracture
- Cryo Ultramicrotomy

- Freeze Substitution
- Resin Embedding
- Ultramicrotomy
- Critical Point Drying

- Biological &
- Soft Matter
- Macromolecules
- Liposomes
- Cells, Tissues
- Hydrogels
- Polymers
- Katalysts
- MOFs
- Hybrid Materials

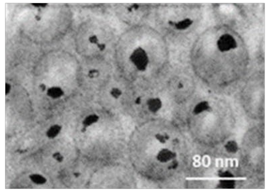


- Cryo S/TEM
- Cryo SEM
- TEM/SEM
- 3D reconstruction
- EDS
- EELS
- CLEM

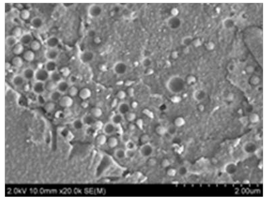
- Materials Science
- Life Sciences
- Interdisciplinary
- Industry

- Training
- Collaboration
- Service
- Consulting
- Outreach (Tours, Workshops)

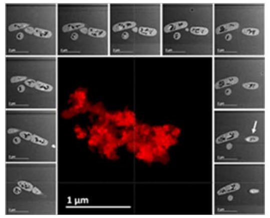
Cryo S/TEM



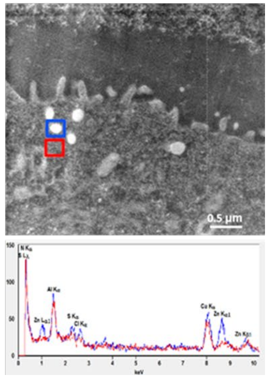
Cryo SEM



3D Reconstr.



STEM-EDS



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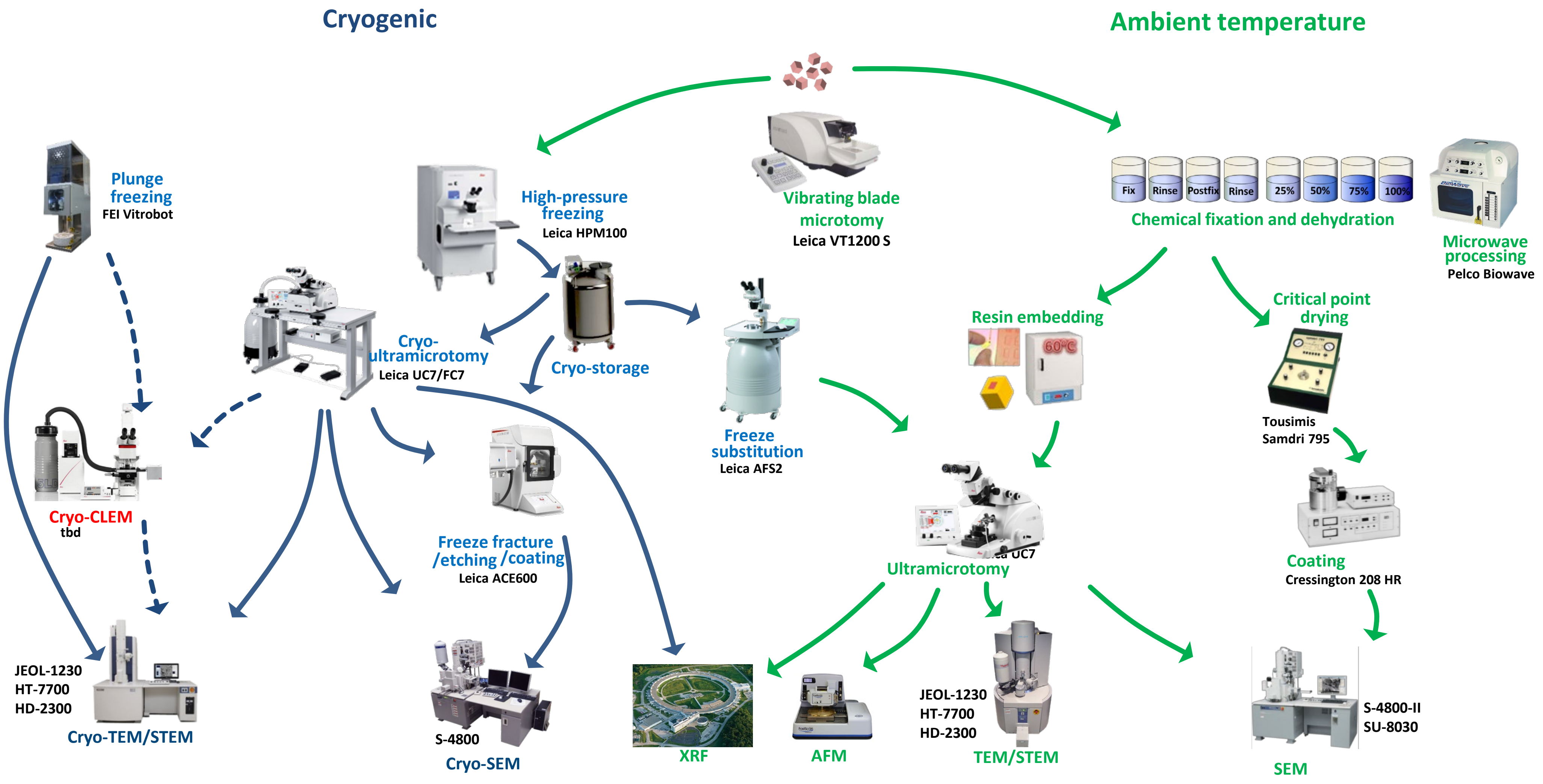
Basic workflows



TE, HAADF, Z-contrast, SE, BSE, Diffraction, EDS, EELS, WDS, LM, LA…

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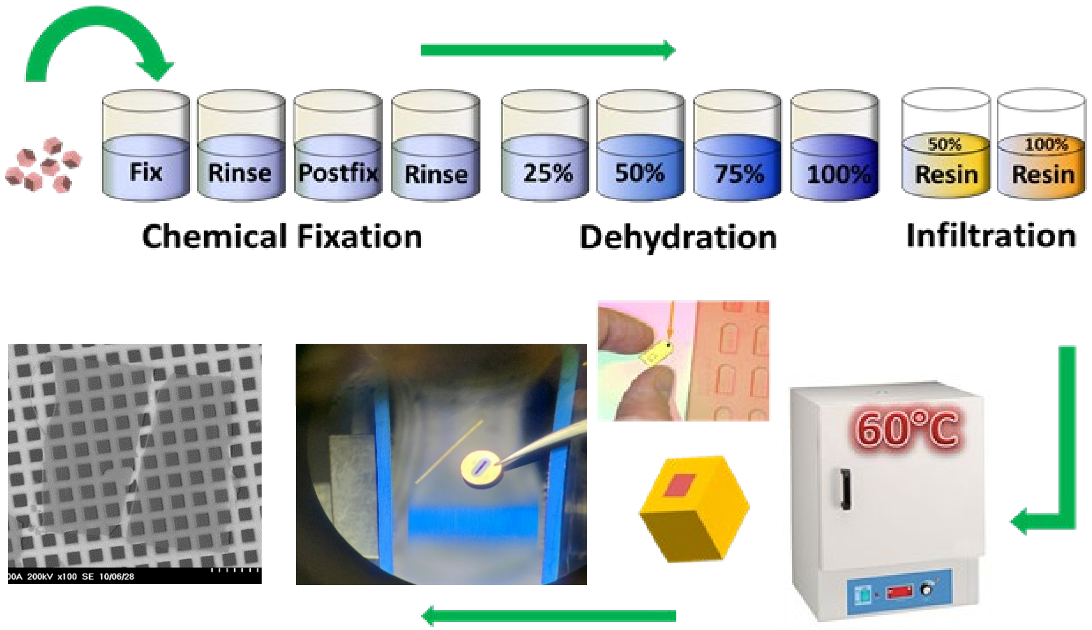
Conventional sample preparation for TEM



Fixation:
- crosslinking of proteins with glutaraldehyde and/or formaldehyde in buffer
- preservation of antigenicity for immunolabeling

Postfixation
- OsO_4 to stabilize and stain lipids (membranes)
- staining of charged sites with UA

Dehydration
- replacement of waterwith an as cending series of ethanol, acetone, or acetonitrile

Resin (epoxy or methacrylates):
- minimal shrinkage
- stability in the vacuum
- stable when exposed to the electron beam
- hardness/softness for ultramicrotomy
- stainability (LM and EM)
- preservation of antigenicity for immunolabeling

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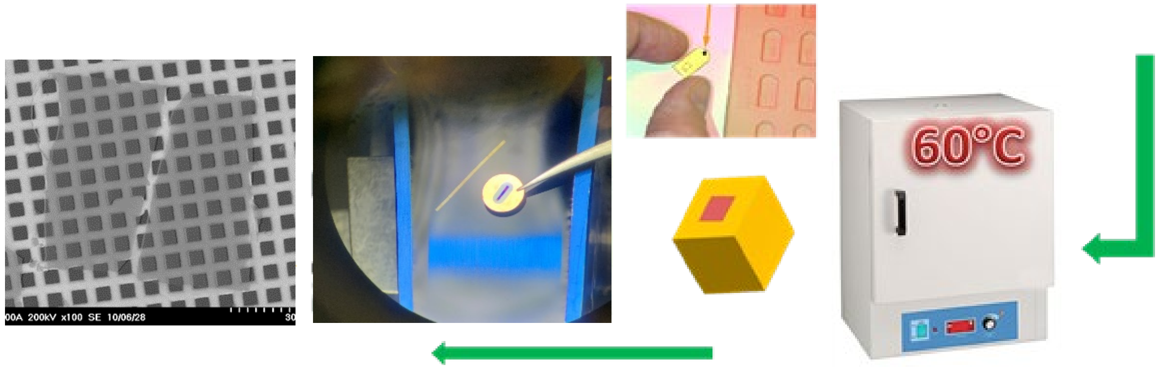
AUTOMATED conventional sample preparation for TEM

ASP-1000: Automated chemical fixation – dehydration – infiltration



- Time consuming
- Involves many steps
- Repeatability
- Reproducibility



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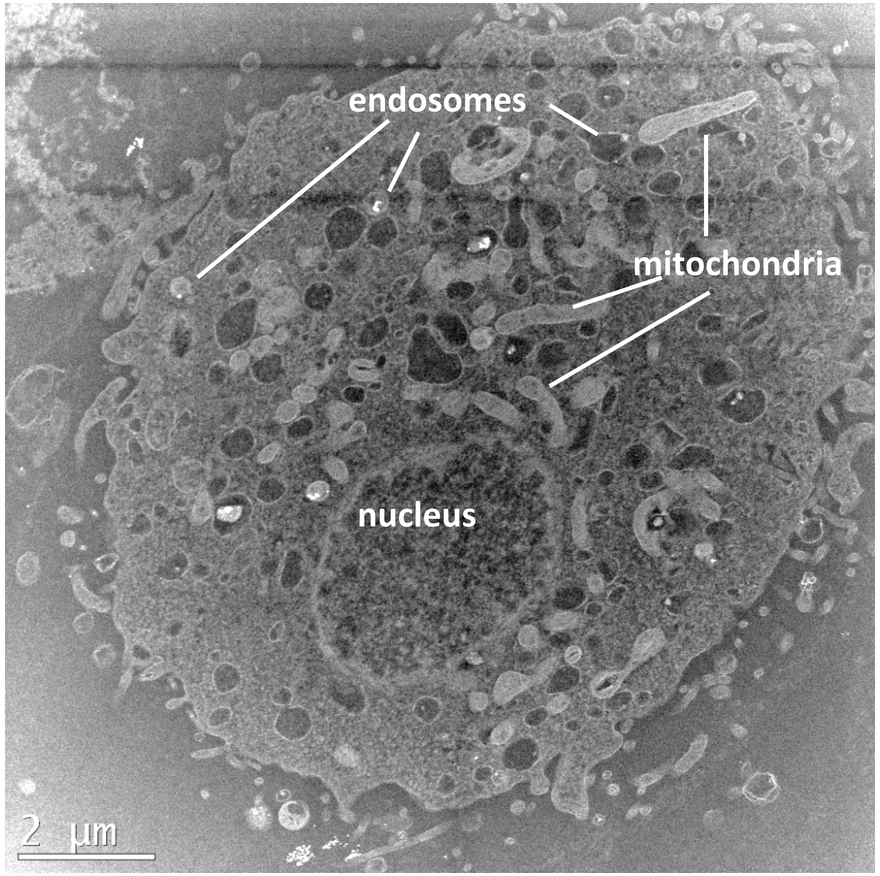
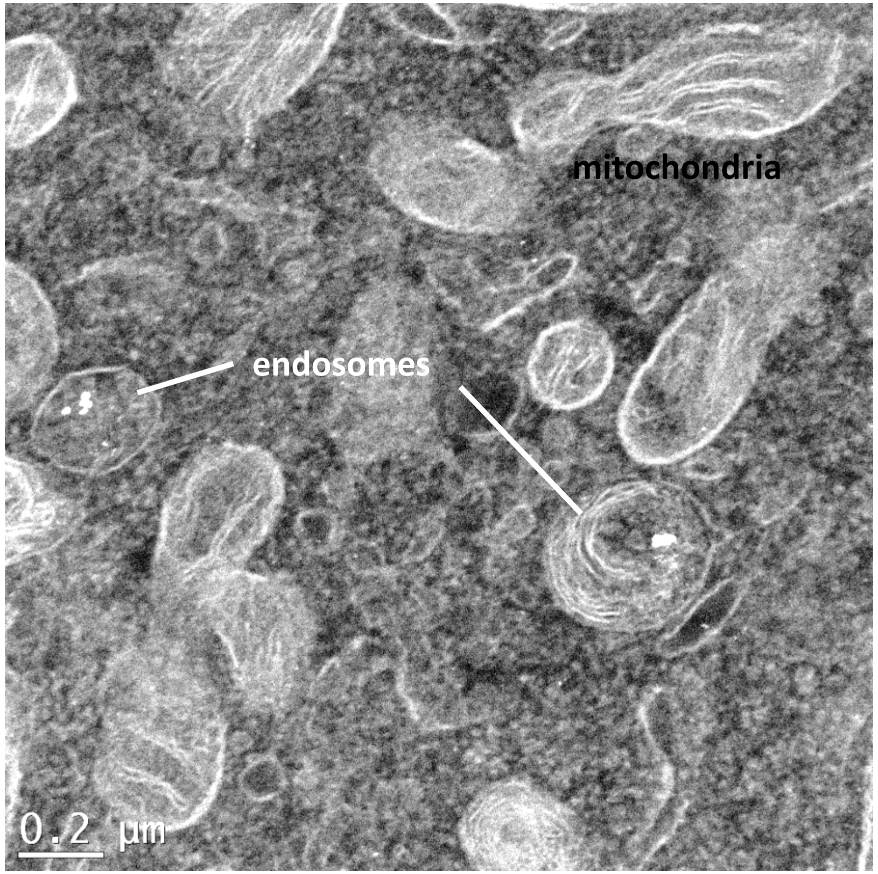
Uptake of nanoparticles by cancer cells





Samples were chemically fixed, dehydrated, and resin embedded. Sections of ca. 80 nm thickness were used.

(Cells were cultured by Naoyuki Shimazu, Mirkin Lab)

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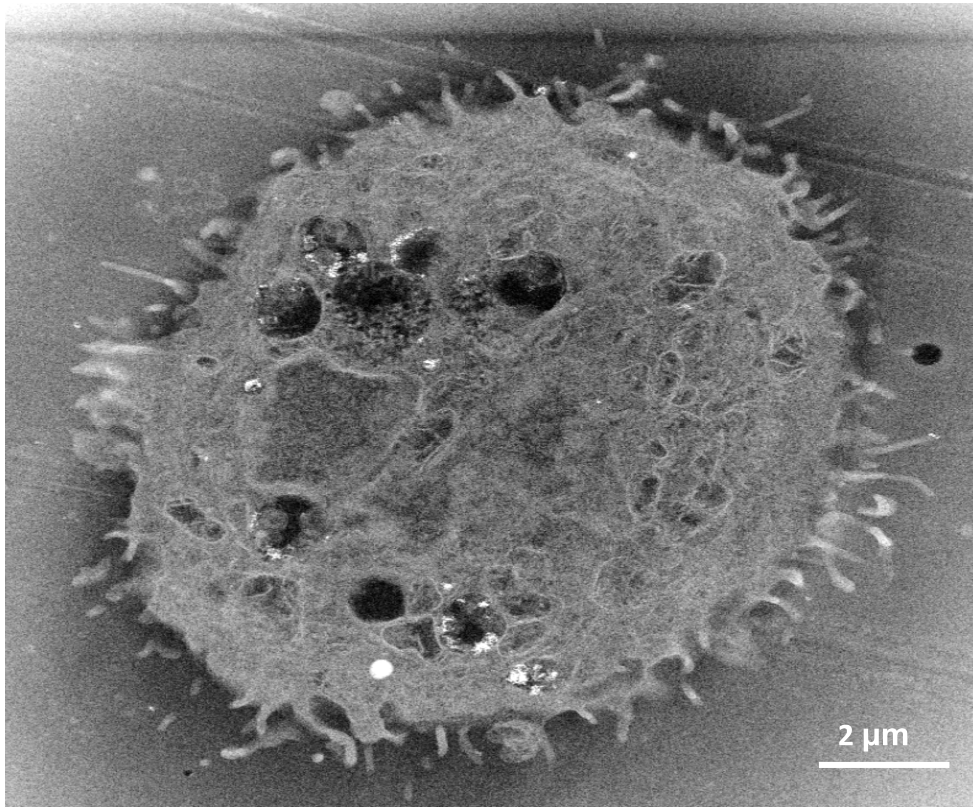
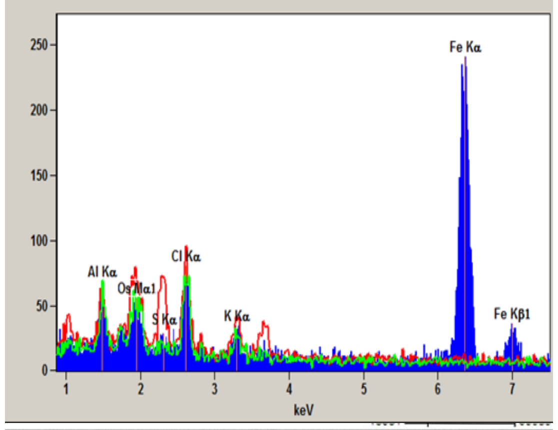
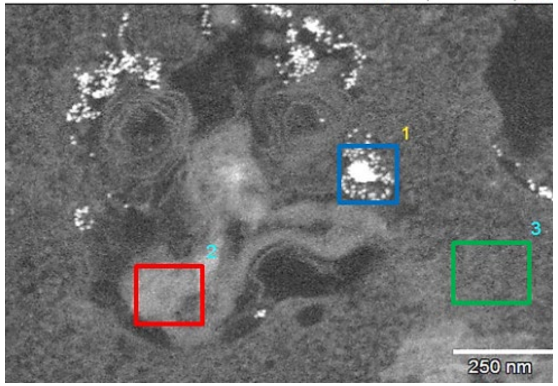
Detection and EDS analysis of nanoparticles inside of cancer cells with STEM







Project with DTC.

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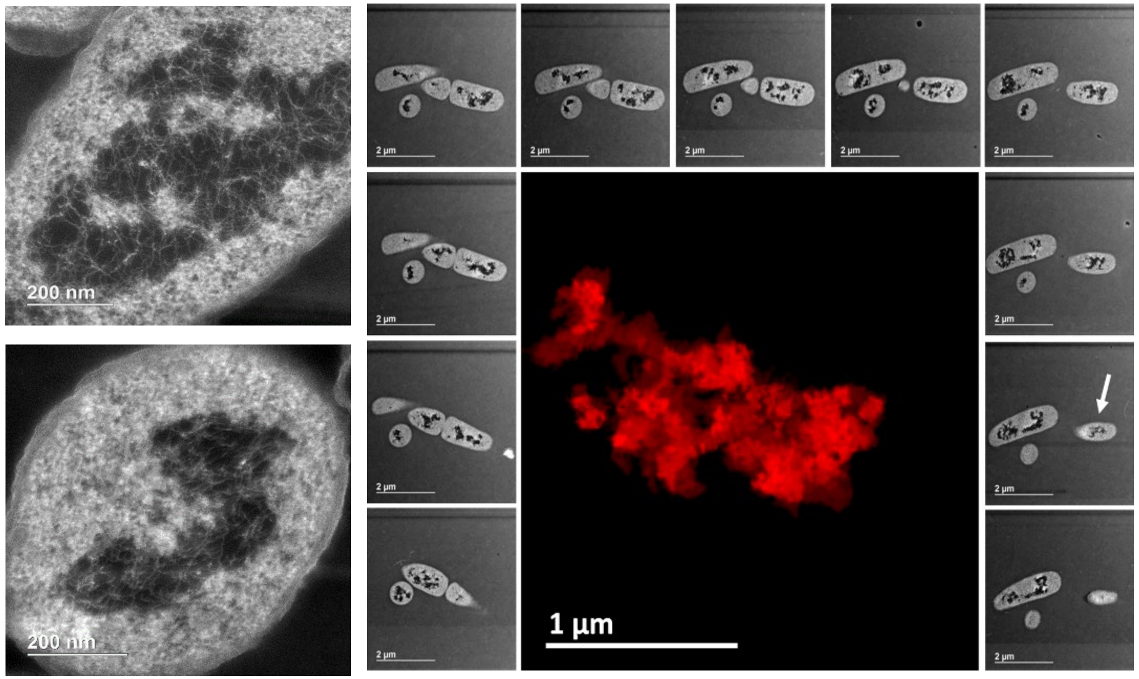
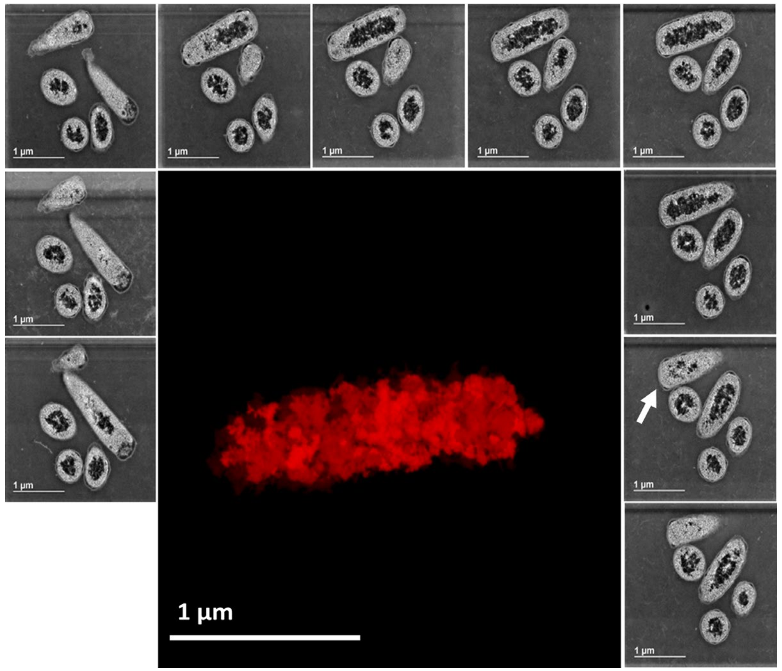
Serial resin sections for 3D-reconstruction of the nucleoid of E. coli





Images were processed with TrackEM2 ImageJ plugin

Reiner, Bleher, et al. **"Nucleoid Structure of Escherichia coli as Revealed by Scanning Transmission Electron Microscopy (STEM) and by 3D-Reconstruction of Z-Contrast Images of Serial Sections."** Microscopy and Microanalysis 19.S2 (2013): 144-145.

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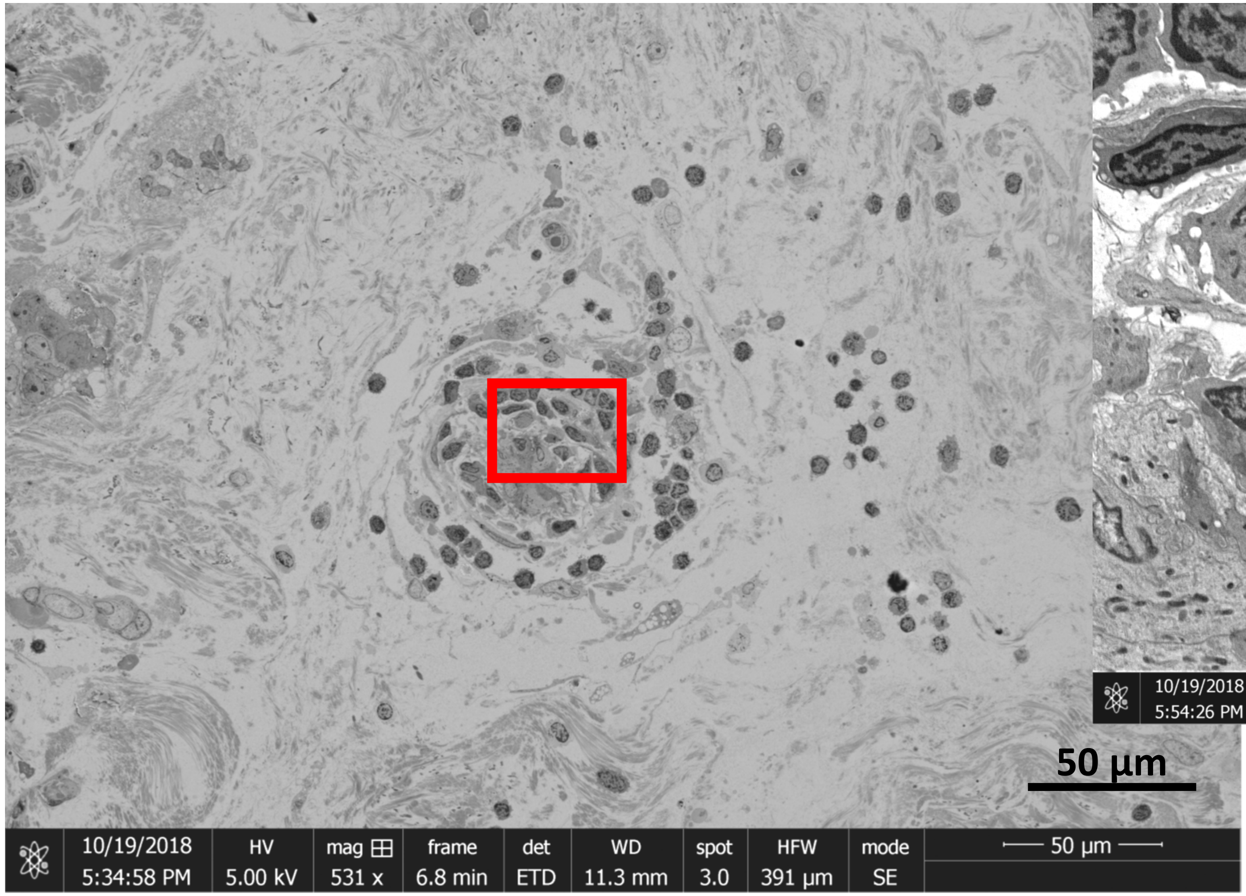
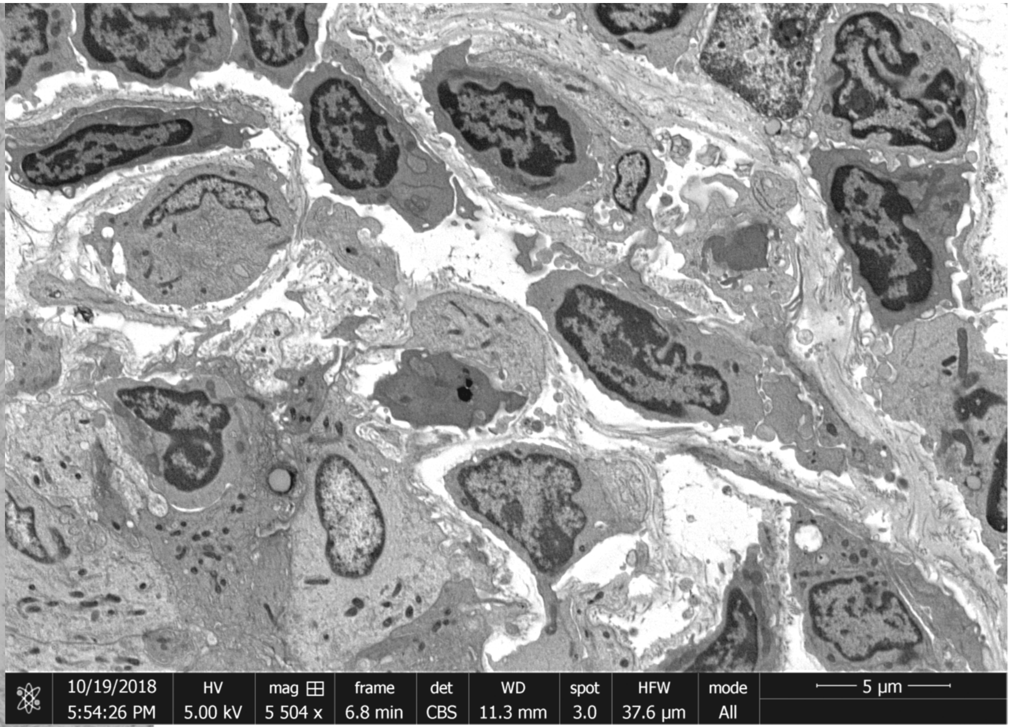
Imaging of a thick section of a resin-embedded breast tumor sample





Reiner Bleher, Project with Wenan Qiang, CLP

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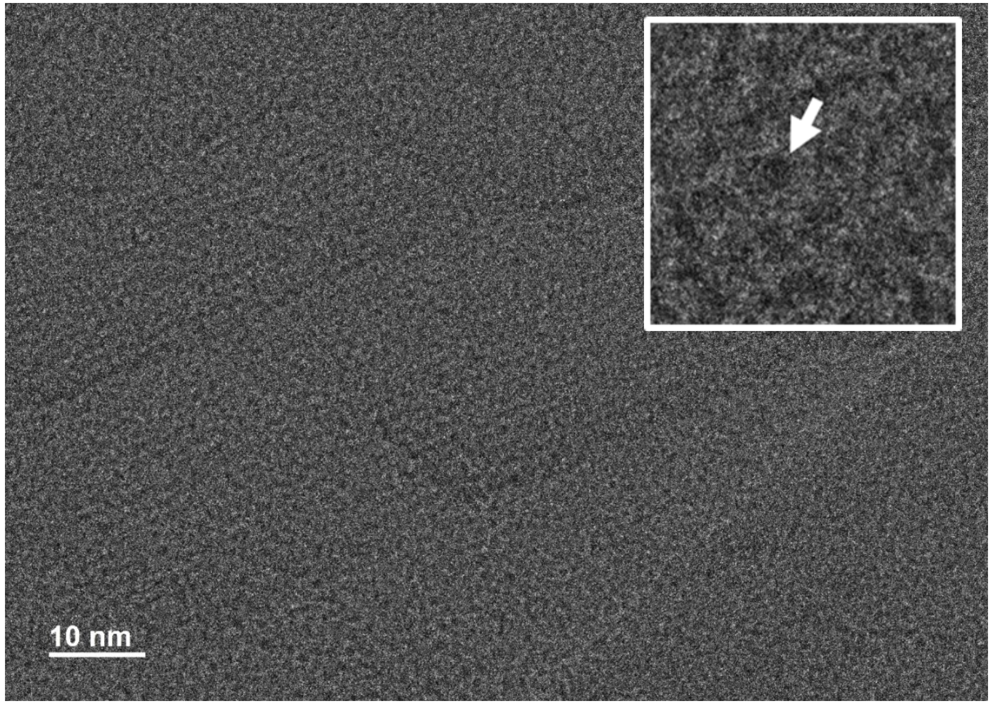
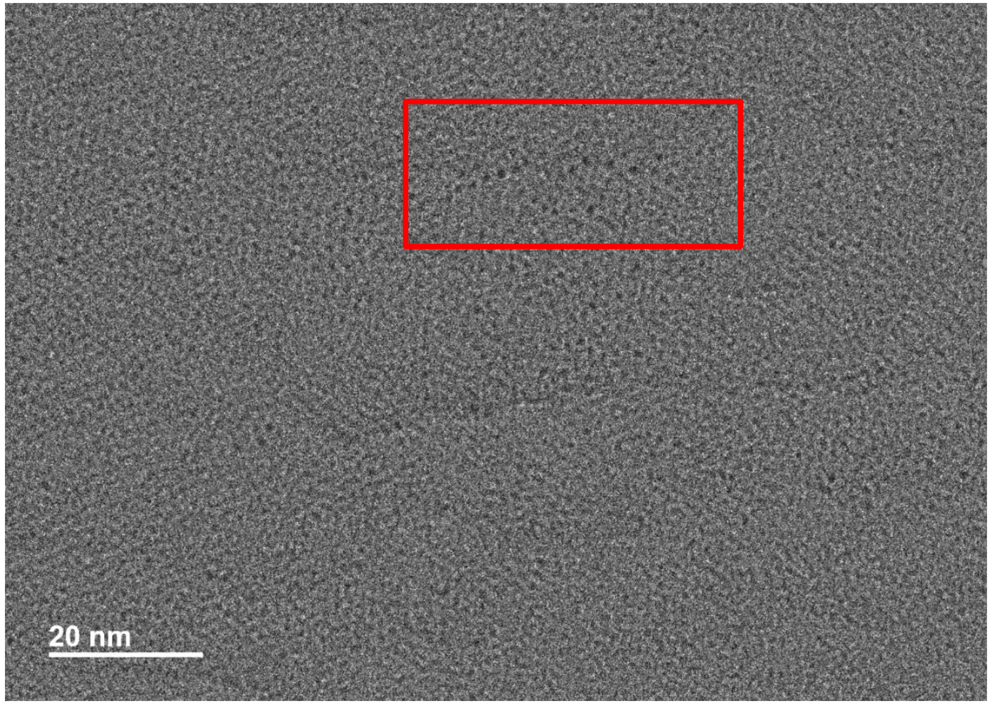
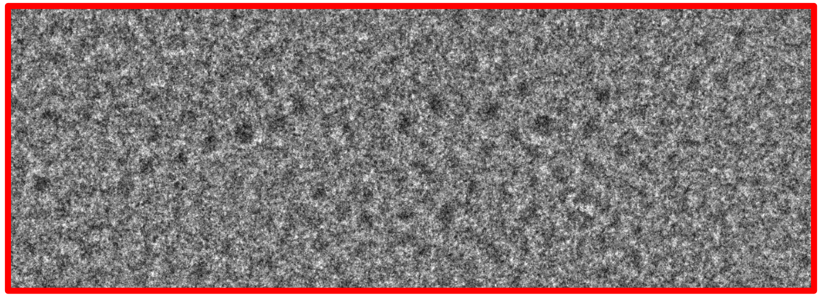
TEM of an ultrathin (~40nm) section of a resin embedded MOF sample







Sample: Xinyi Gong (Farha Group)
TEM: Roberto dos Reis (VPD Group)

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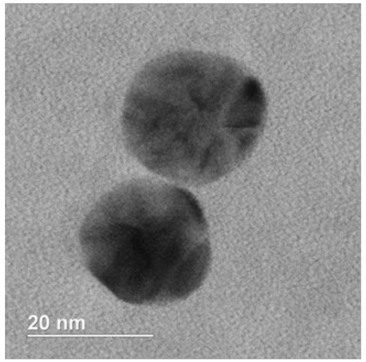
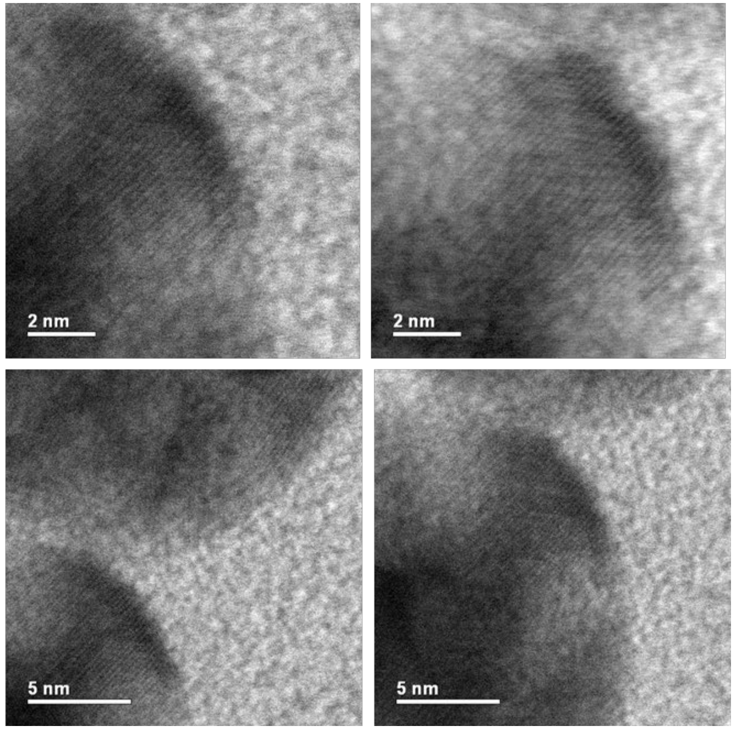
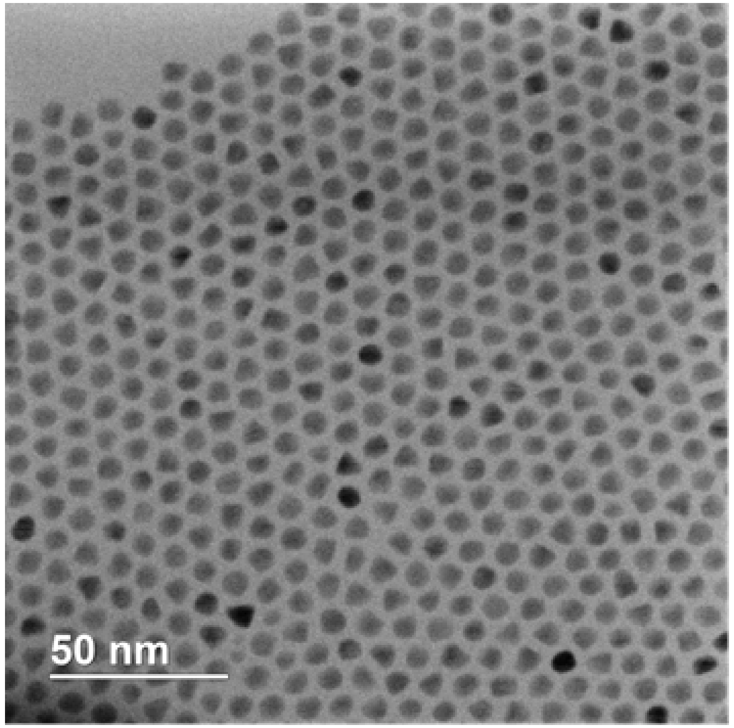
Imaging/analysis of NPs and QDs







Reiner Bleher, Project with T. Duncan, FDA

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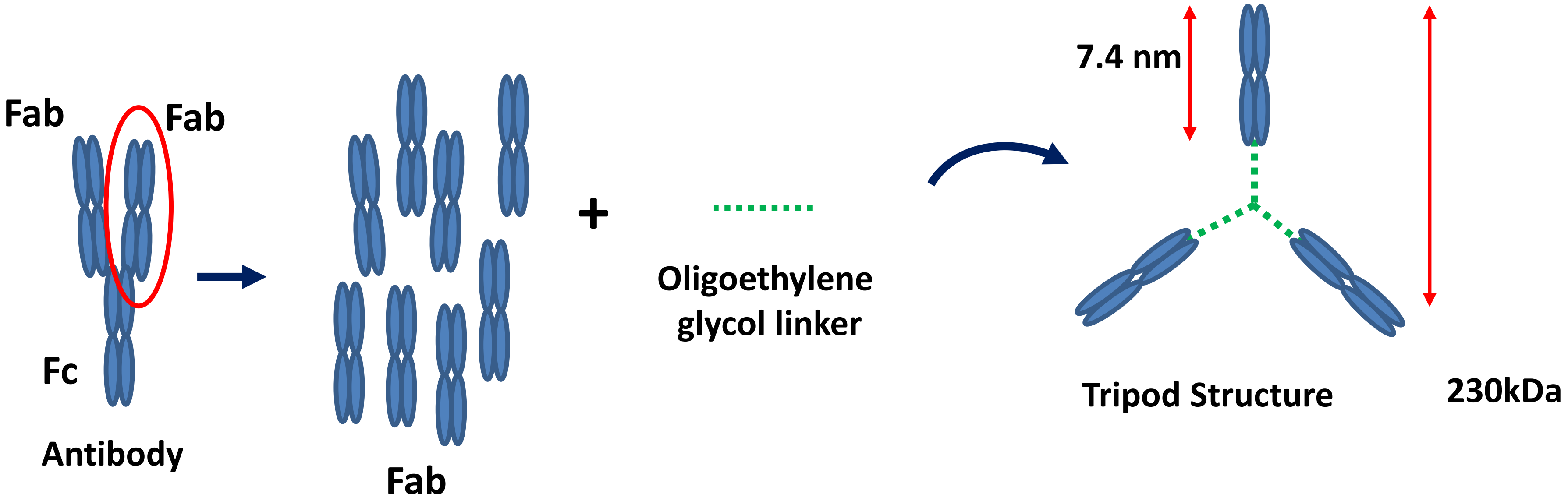
Negative staining

- Tripod kind of structure
- Each protein monomer in the structure is coming from antibody and then connected by a linker



Sample from Justin Modica (Milan group)

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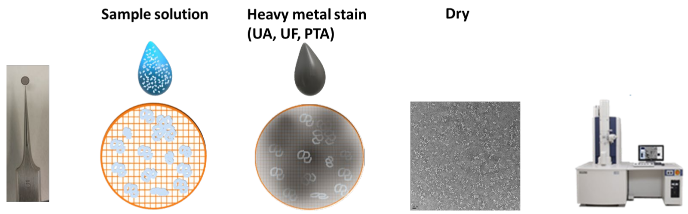
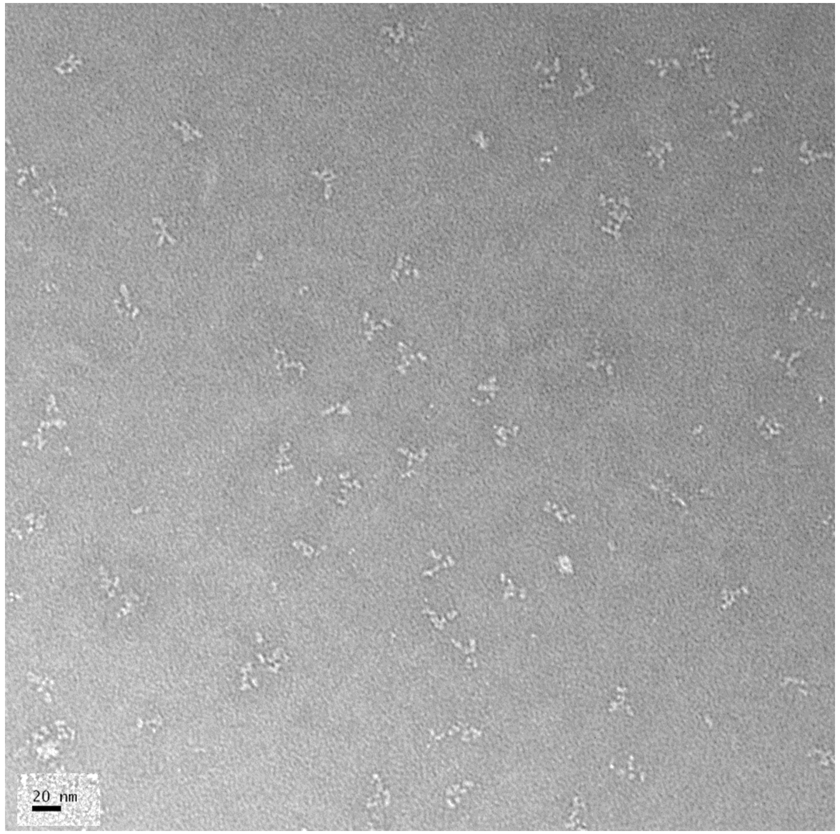
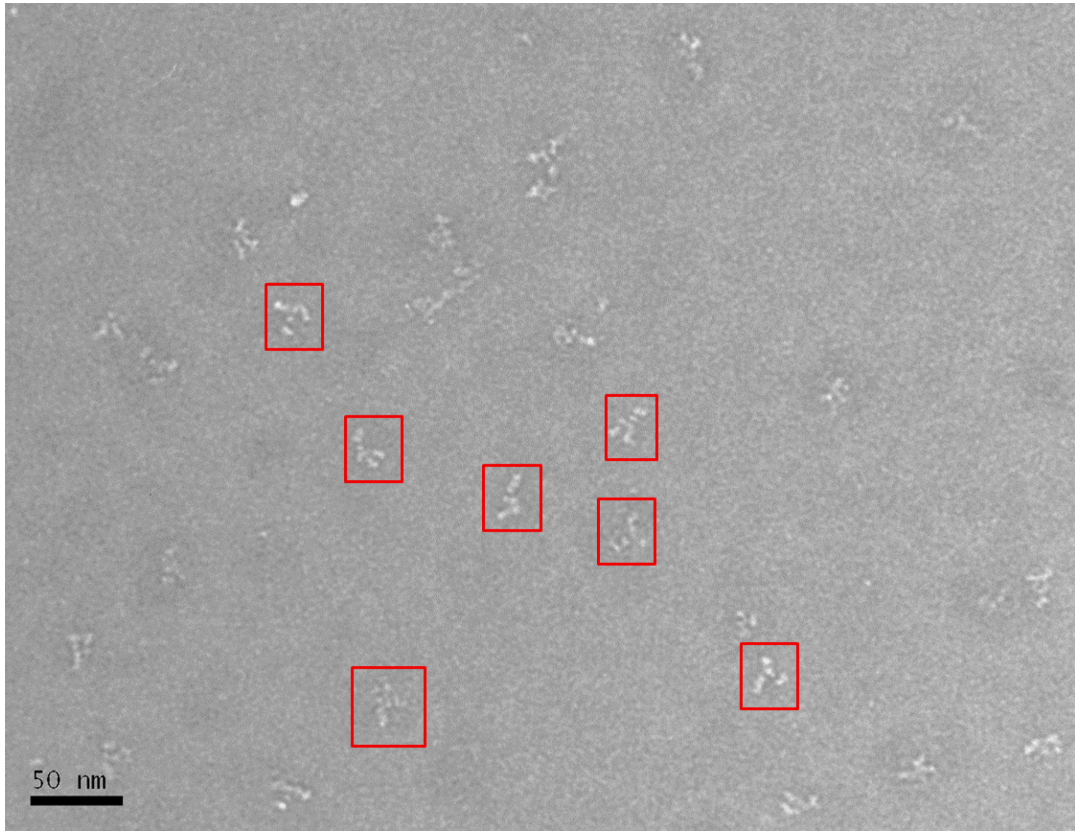
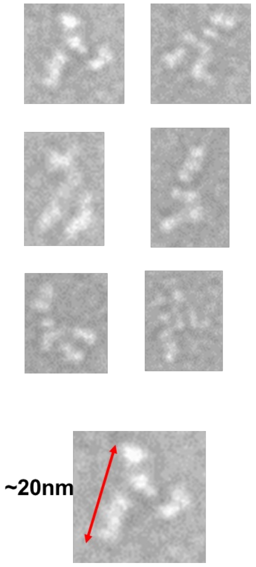
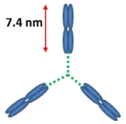
Negative staining











Sample: Justin Modica (Milan group)
TEM: Sonali Dhindval (VPD Group)

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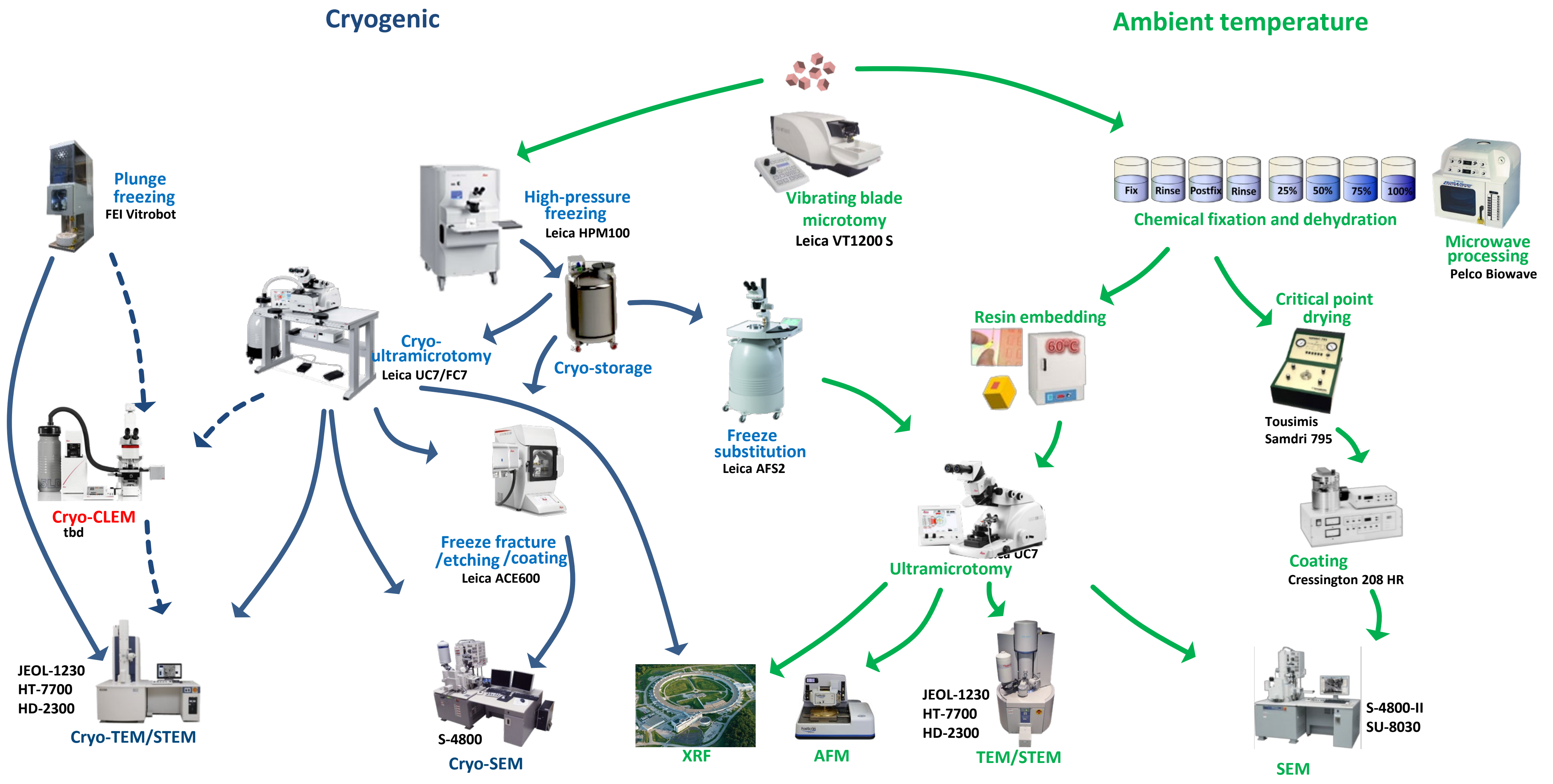
Basic workflows



TE, HAADF, Z-contrast, SE, BSE, Diffraction, EDS, EELS, WDS, LM, LA…

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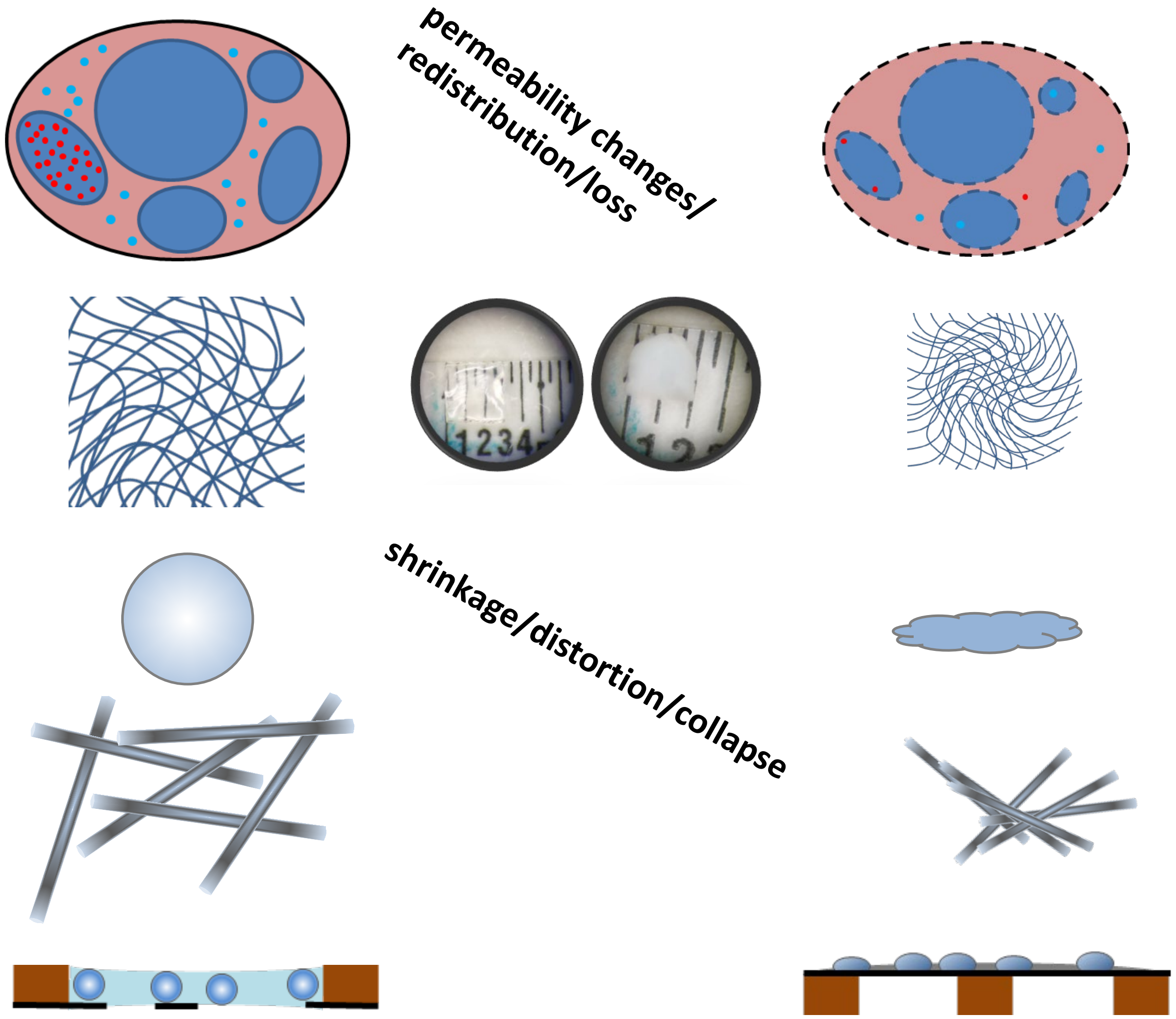
Cryo immobilization vs conventional processing



- Macromolecules
- Viral Particles
- Bacteria
- Cells
- Tissues
- Liposomes
- Micelles
- Hydrogels
- Microgels
- Nanofibers
- Nanotubes …

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Conventional sample processing vs. Cryogenic sample processing

Chemical Fixation
- Slow process
- Osmotic effects
- Change of membrane permeability
- Loss/re-distribution of diffusible ions and small molecules
- Conformational changes of proteins
- Masking of antigens

Postfixation
- OsO_4: Depolymerization of proteins

Dehydration (or CPD for SEM)
- Shrinkage
- Conformational changes of proteins
- Extraction of lipids
- Collapse of structures (e.g. hydrogels)

Resin Embedding
- Extraction of lipids
- Shrinkage during polymerization

S/TEM, SEM, EDS, EELS, XRF, LM…

Cryo fixation
- Rapid process
- Vitrified sample w/o artifacts

Processing for Observation
- Cryo-ultramicrotomy for cryo-TEM
- Freeze fracture for cryo-SEM)

Cryo-S/TEM, cryo-SEM, cryo-XRF, cryo-LM, FS…

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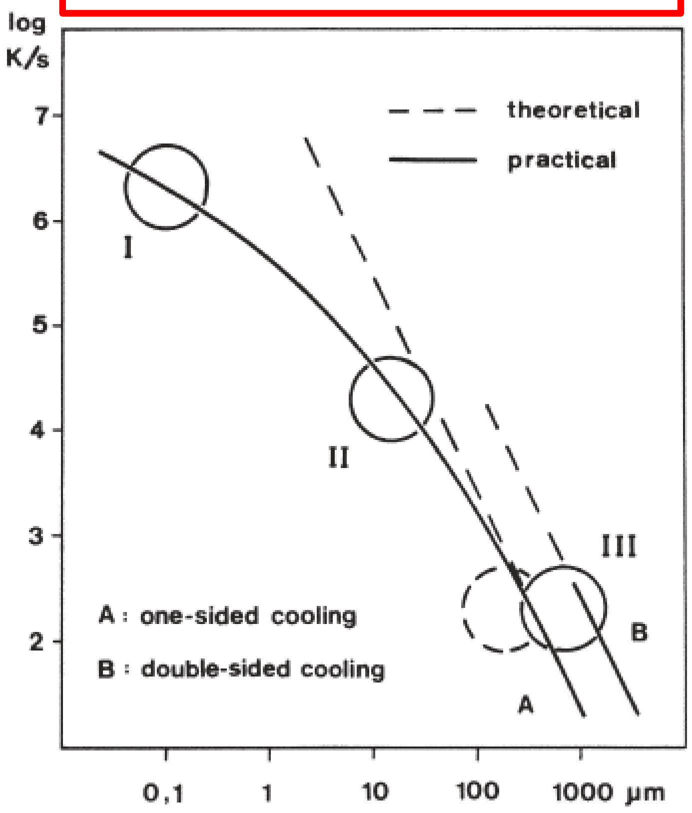
Cryofixation

Realizable Cooling Rates



I Range of vitrified pure water

II Range of vitrified animal cells and tissues

III Range of specimens vitrified with high pressure

Which cryogens are suitable?

Cryogen	Melting Pt. [°C]	Boiling Pt [°C]	Freezing Rate [°C/s]
Freon 13	-181	- 81	98000
Propane	-189	- 42	98000
Ethane	-183	- 89	97000
Isopentane	-160	28	45000
Nitrogen	-209	- 196	16000

Moor, Hans. "Theory and practice of high pressure freezing." In *Cryotechniques in biological electron microscopy*, pp. 175-191. Springer Berlin Heidelberg, 1987.

Achievable vitrified sample thickness

Device	Freezing depth (μm)
Plunge freezer	10-20
Spray freezer	10-20
Slam freezer	20-40
Propane jet	40
High-Pressure freezer	50-400

Moor, Hans. "Theory and practice of high pressure freezing." In *Cryotechniques in biological electron microscopy*, pp. 175-191. Springer Berlin Heidelberg, 1987.

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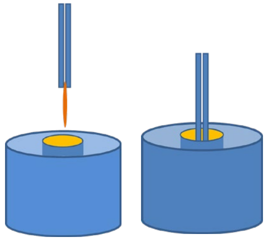
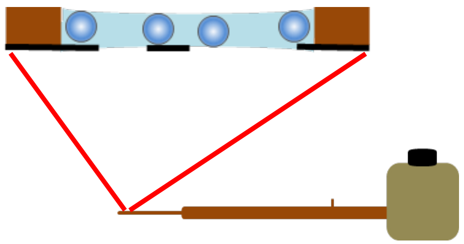
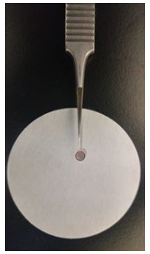
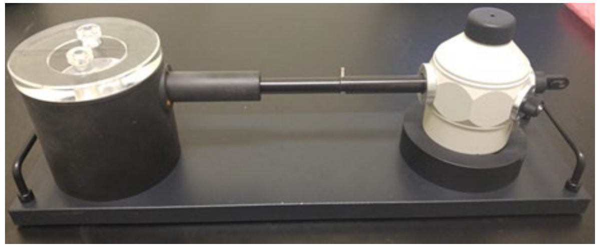
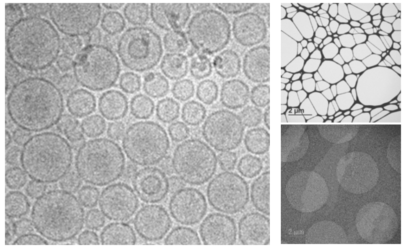
Plunge freezing











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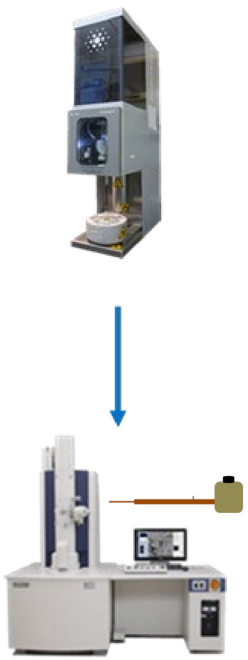
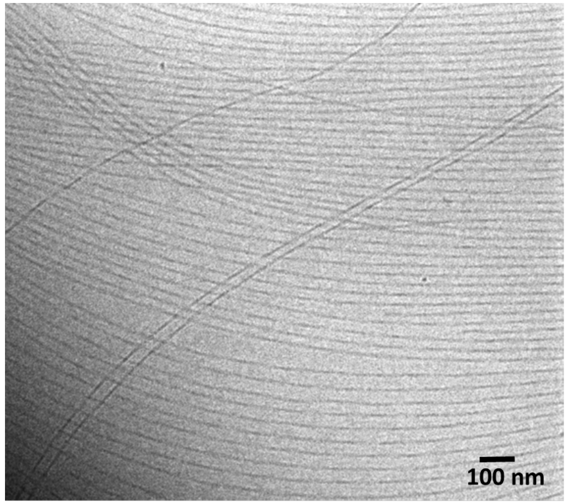
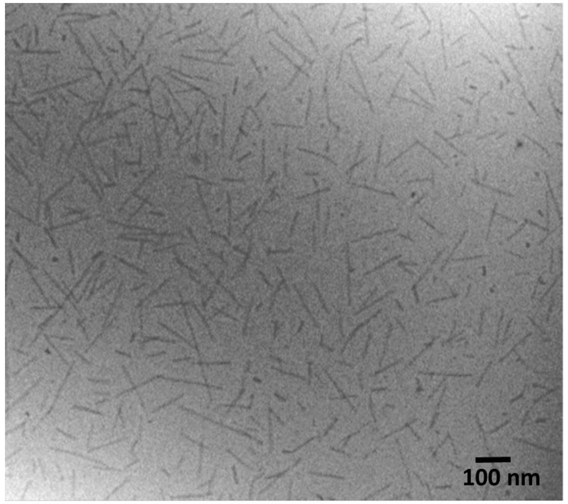
Peptide amphiphile nanofibers

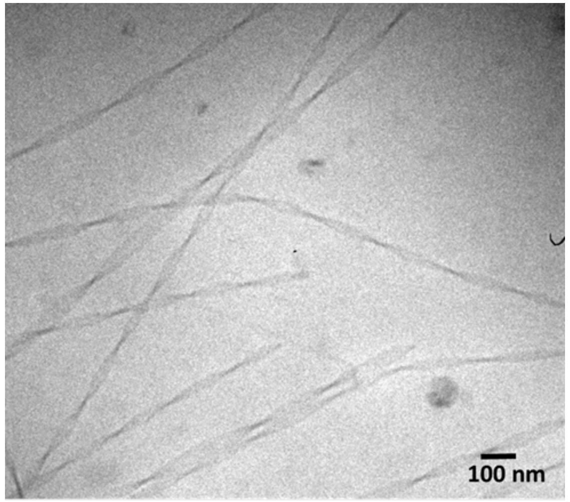




1: Peptide amphiphile molecules self-assemble into nanofibers in water. Used as an artificial extracellular matrix to promote cell growth.



2: The same peptide amphiphile molecules form shorter nanofibers through a different self-assembly process.



3: Peptide amphiphile molecule that self-assembles into twisted nanoribbons in water. This material facilitates neural regeneration.

Sato, Kohei, Mark P. Hendricks, Liam C. Palmer, and Samuel I. Stupp. "Peptide supramolecular materials for therapeutics." *Chemical Society Reviews* 47, no. 20 (2018): 7539-7551.

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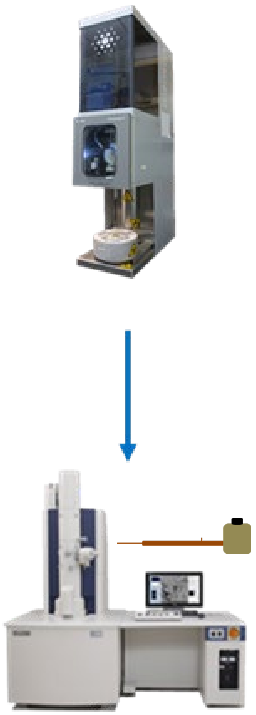
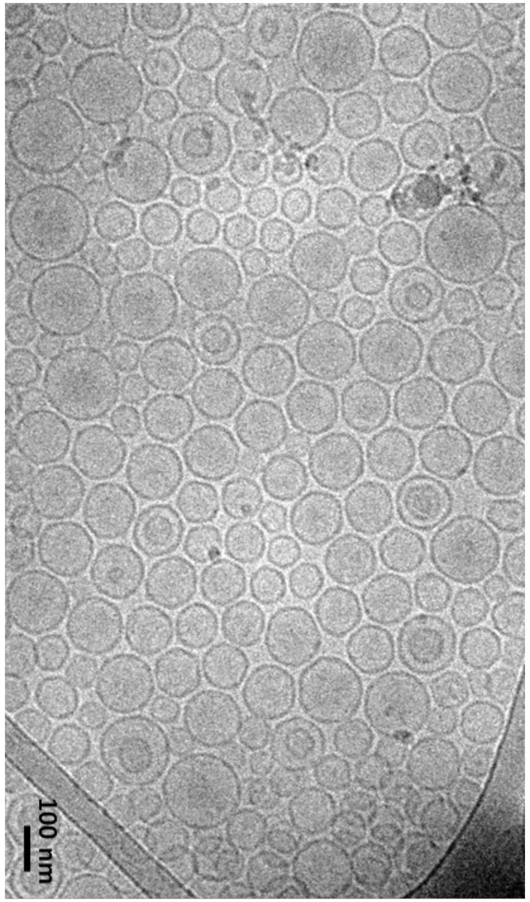
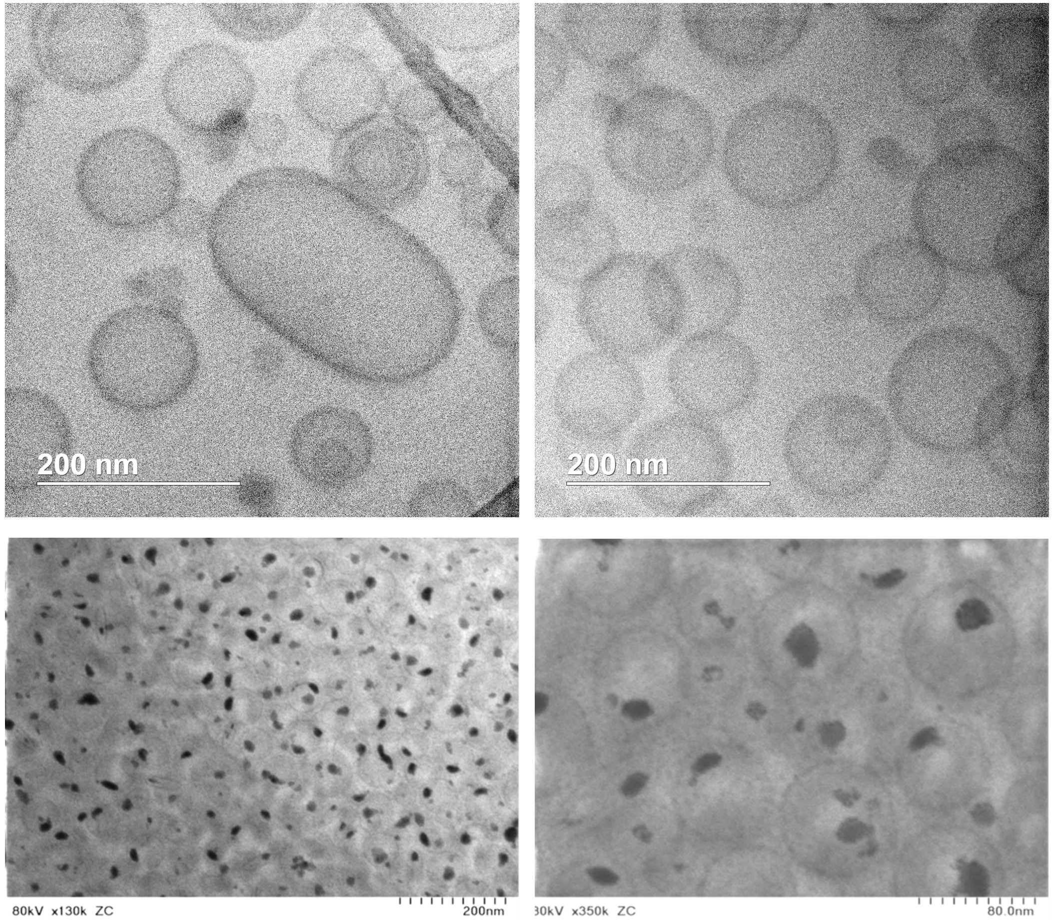
Plunge Freezing







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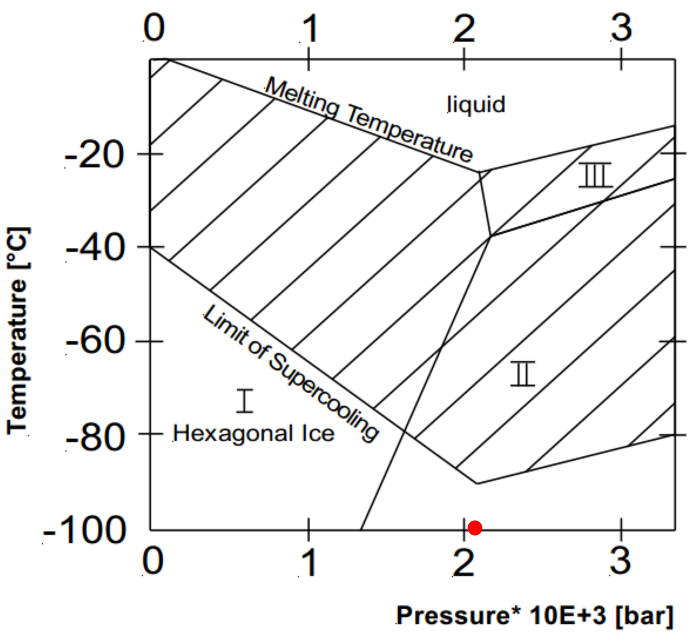
High-Pressure Freezing

When water freezes, its volume increases (Le Chatelier)

High pressure (~2050 bar) inhibits this expansion and reduces the critical freeze rate to a range between 100 and 500 °/s

How?

1) Lowering of the freezing point
2) Lowering the supercooling temp. limit
3) Reduction in the rate of ice crystal nucleation
4) Slowing the growth of ice crystals



H_2O Phase Diagram
1 Supercooling capability curve
2 Melting point curve

Dahl R, and Staehlin AL, Journal of electron microscopy technique, 1989 vol:13 iss:3 pg:165 -174.

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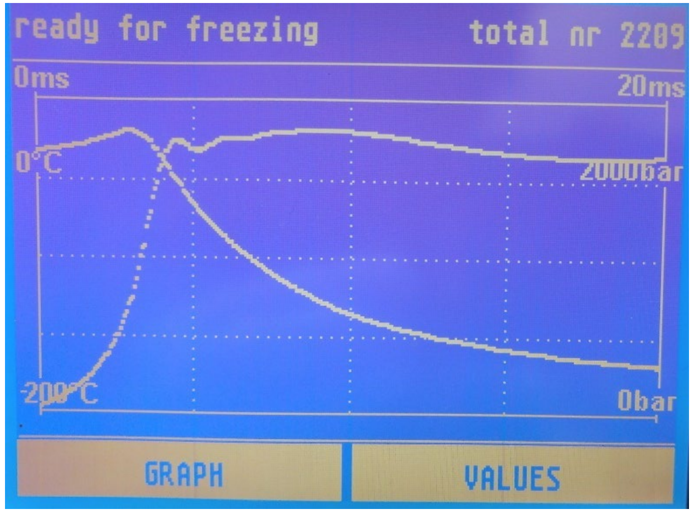
High-pressure freezing

EMPACT2

HPM100

Ice

HPF Compact02



HPM100 Temperature and Pressure during a freeze on 11/15/16.

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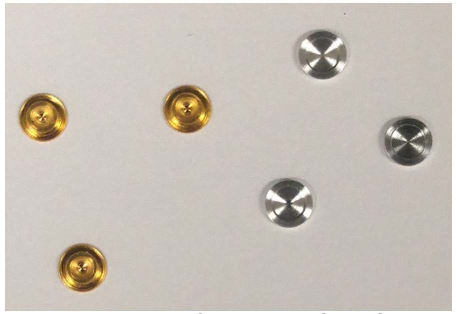
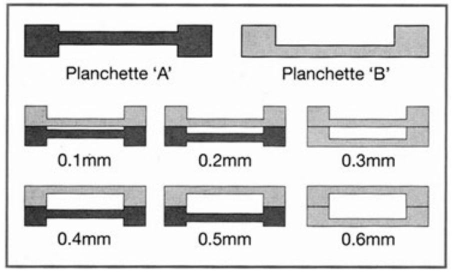
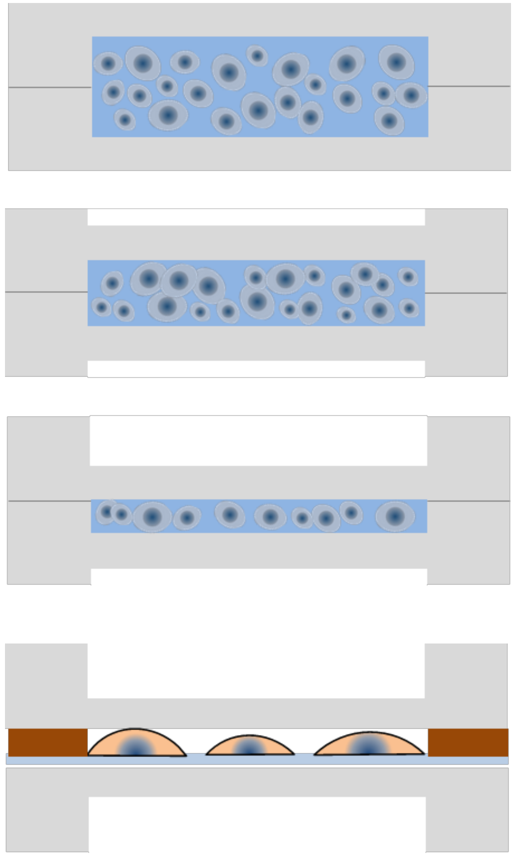
High-pressure freezing

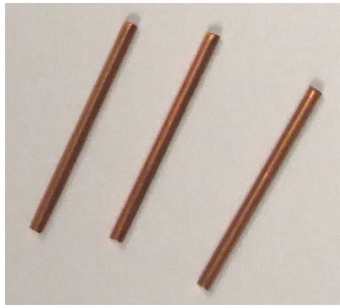
Sample Holders for HPM100



3 mm o.D. and 0.5 mm height







Cu Capillaries 0.65 mm o.D. and 0.3 mm i.D.

- Keep processing time as short as possible.
- Avoid air bubbles!
- Use space fillers, e.g.: hexadecene, dextrane, BSA, yeast paste.

- Suspensions (bacteria, cells, liposomes, micelles…)
- Tissues, hydrogels
- Can be used in combination with cellulose capillaries

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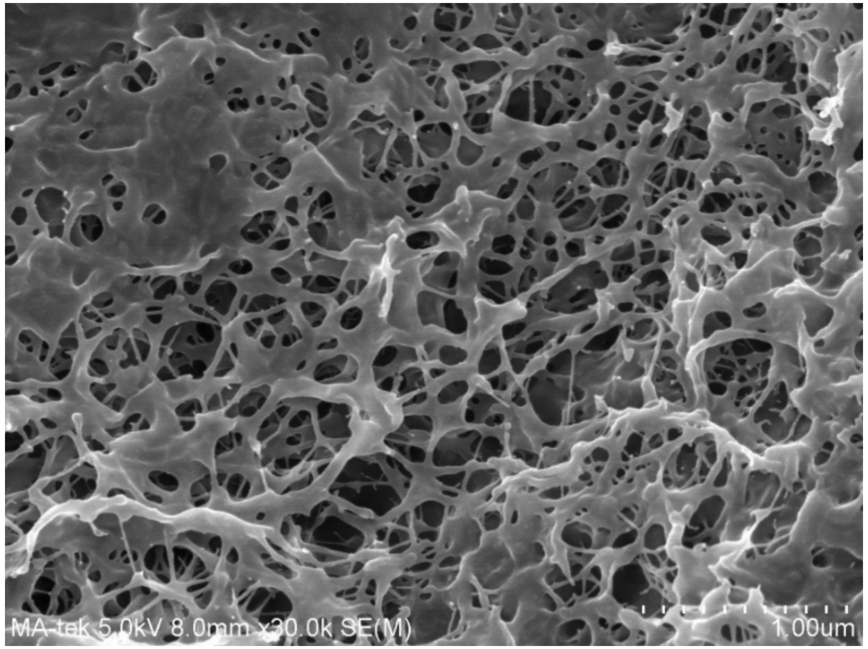
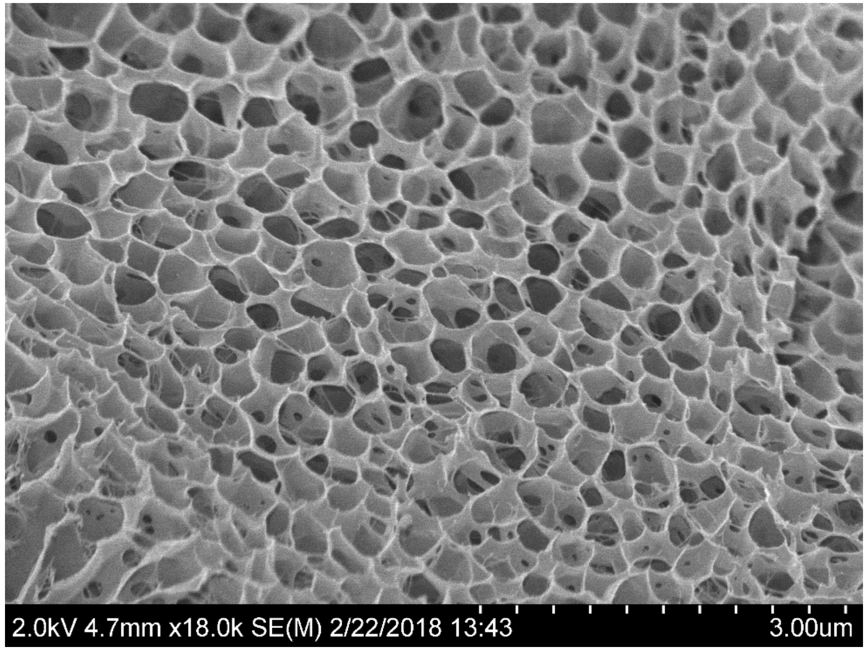
SEM and cryo-SEM of Hydrogels

SEM image of critical point dried hydrogel



Cryo-SEM image of a high-pressure frozen and freeze fractured hydrogel



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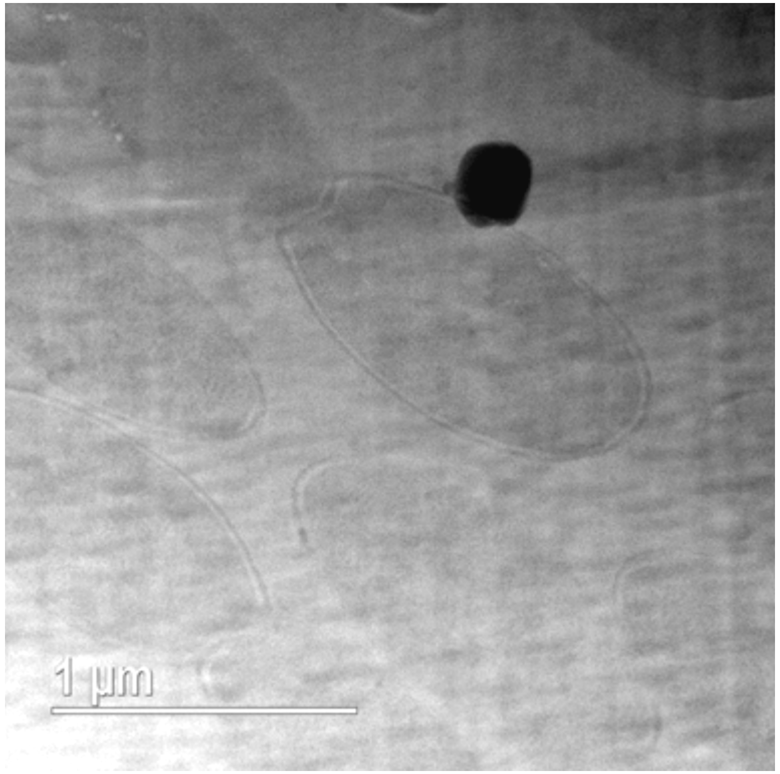
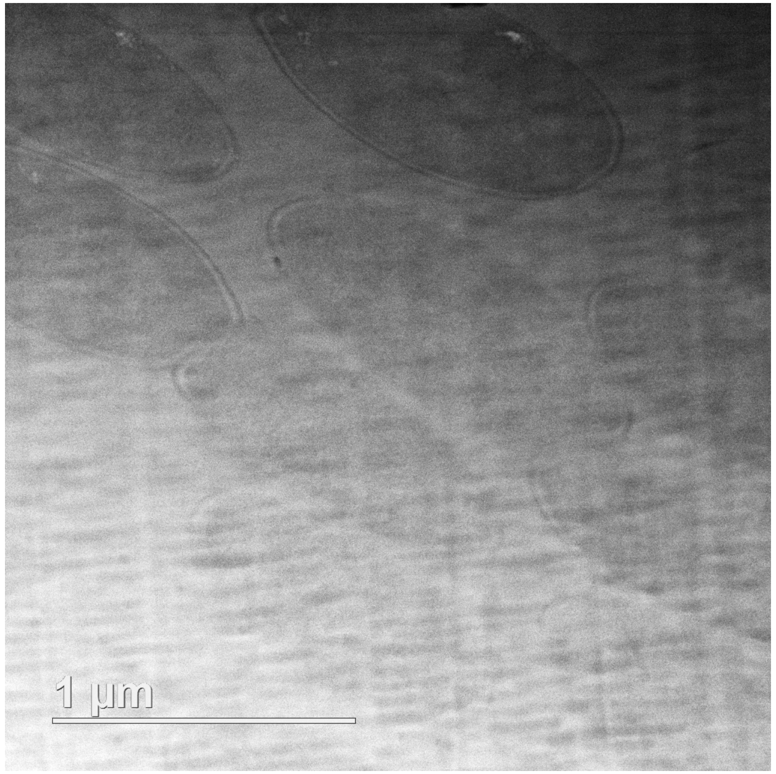
Cryo-STEM of cryosections of E. coli ΔcusR





Cells were high-pressure frozen and sectioned at -170° C. Images were recorded at -165 ° C. The nominal thickness of the section was 100 nm.

Reiner Bleher, 2015

Please note:
Samples that are too soft at RT can be cooled down and sectioned with the cryo-ultramicrotome, e.g. polymers, rubbers, chewing gum, etc..

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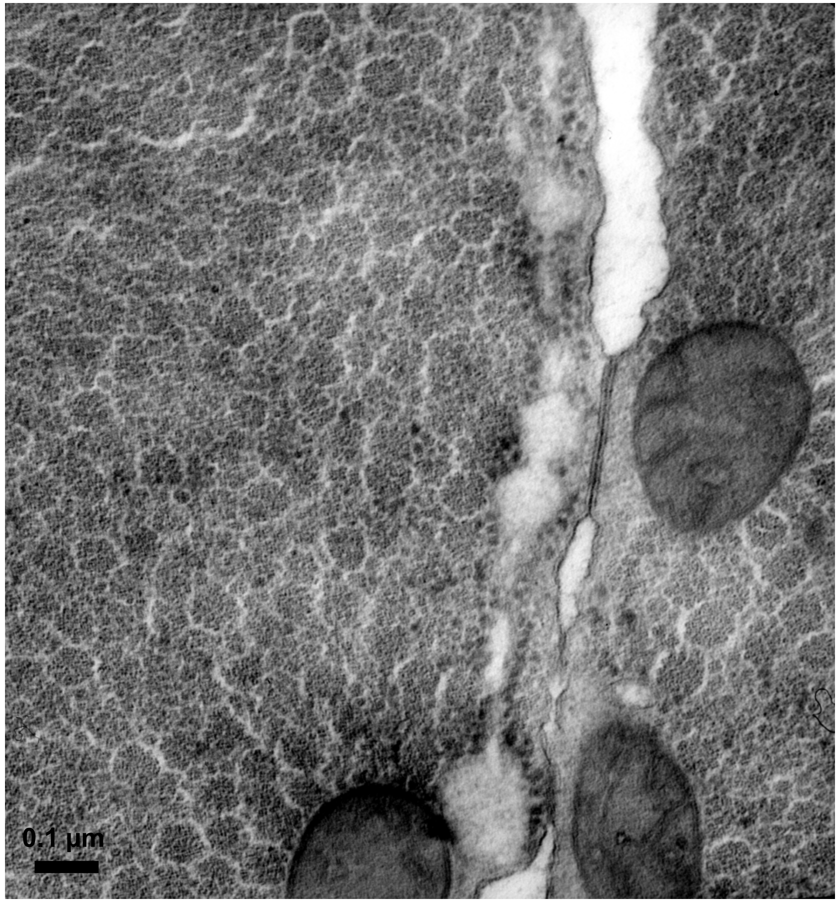
High-pressure freezing and freeze substitution

Glycogen Particles are well retained



HPF – FS:

- **Dissection and mounting**
- **High pressure freezing**
- **Transfer into freeze substitution medium (e.g. Acetone/OsO4) at low temp.**
- **Freeze substitution (-90 C to RT)**
- **Infiltration with resin**
- **Polymerization (can be at low temp. with UV)**
- **Ultramicrotomy**
- **(Immunolabeling)**
- **Contrasting**
- **Imaging/Analysis**

Bleher, Reiner, and Jorge Machado. "Paracellular pathway in the shell epithelium of Anodonta cygnea." *Journal of Experimental Zoology Part A: Comparative Experimental Biology* 301, no. 5 (2004): 419-427.

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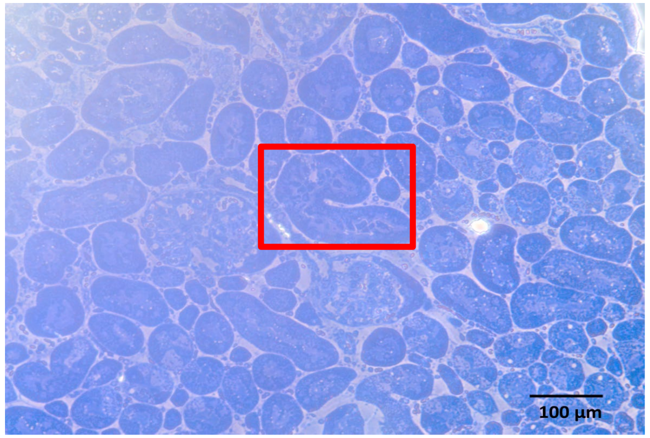
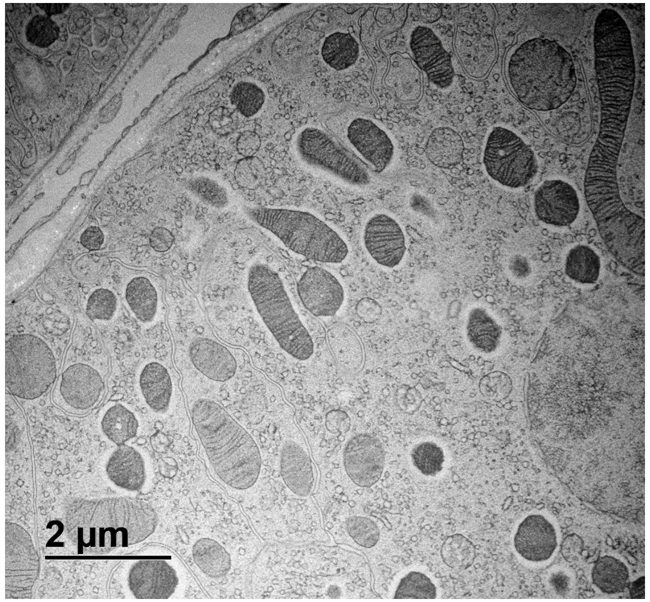
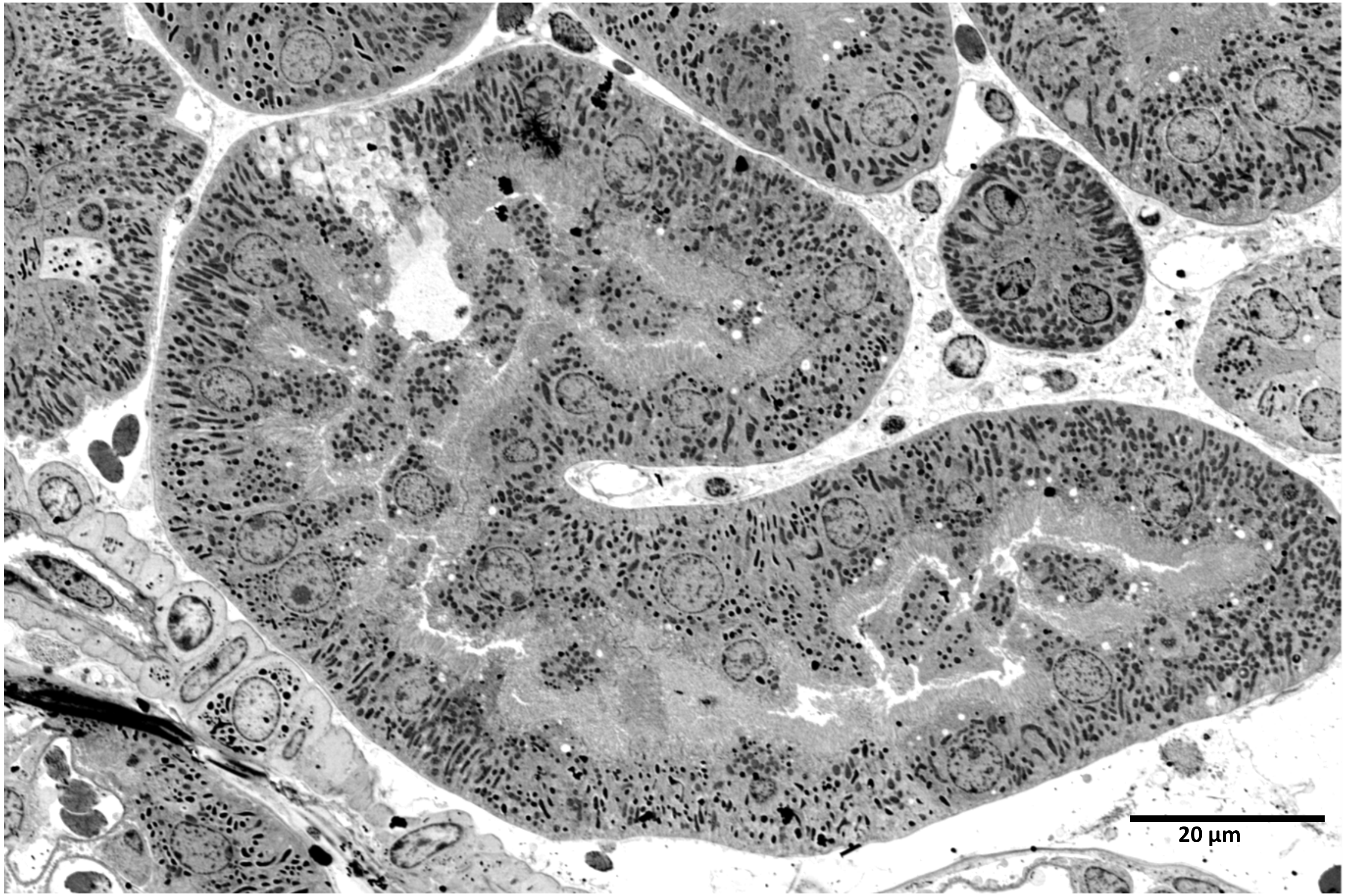
High-pressure frozen and freeze substituted mouse kidney







Reiner Bleher, Project with Hou lab, Washington University St Louis, 2018.

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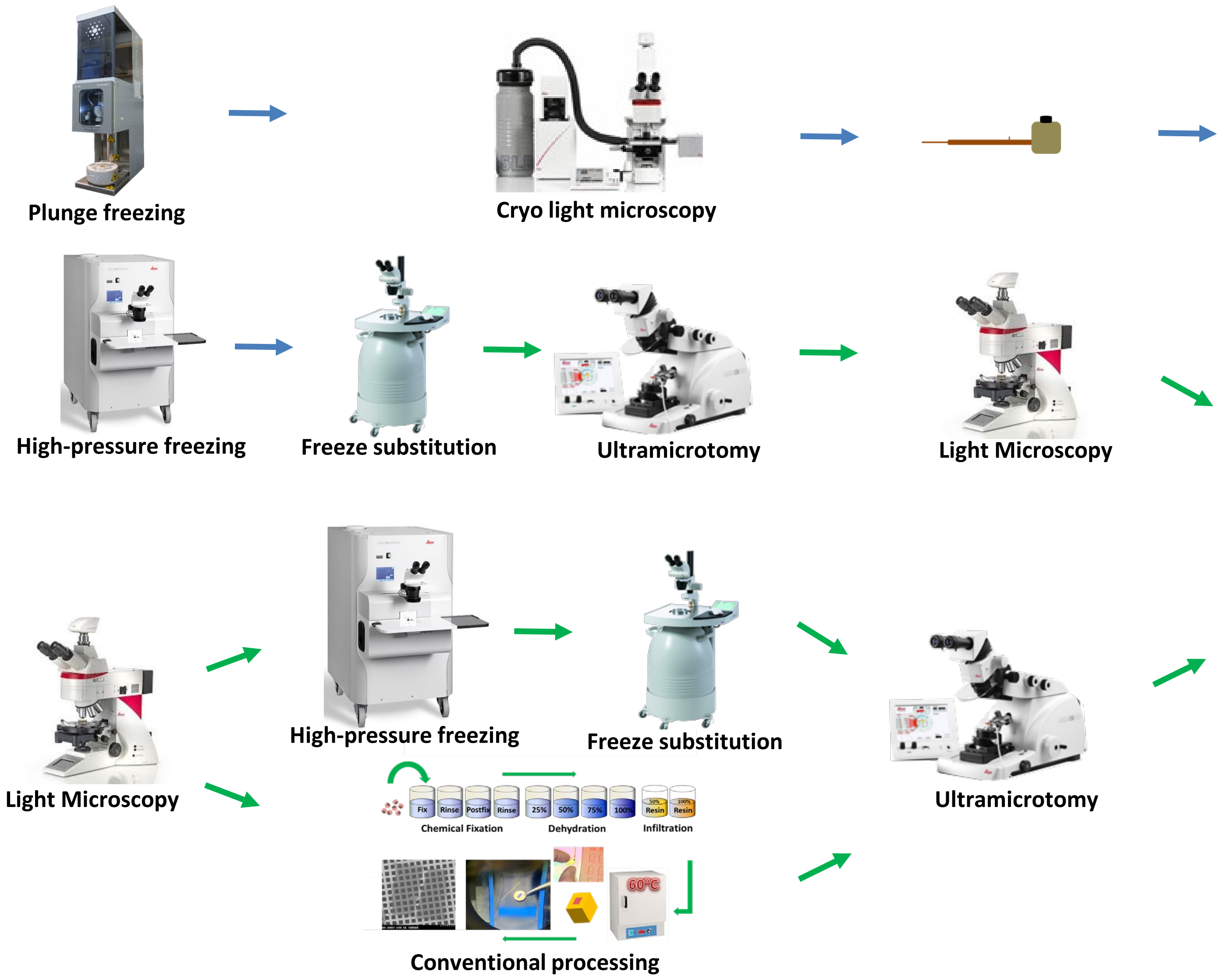
CLEM: Correlative Light and Electron Microscopy



Cryo- microscopy and microanalysis

For molecules/ particle suspensions, monolayers of bacteria or small cells

Needs particular FS medium and resin that retains fluorescence.

Microscopy and microanalysis

Mainly for cell monolayers, thin tissue slices

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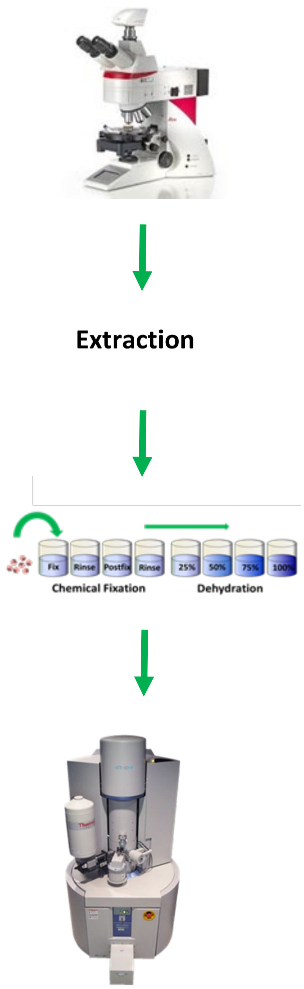
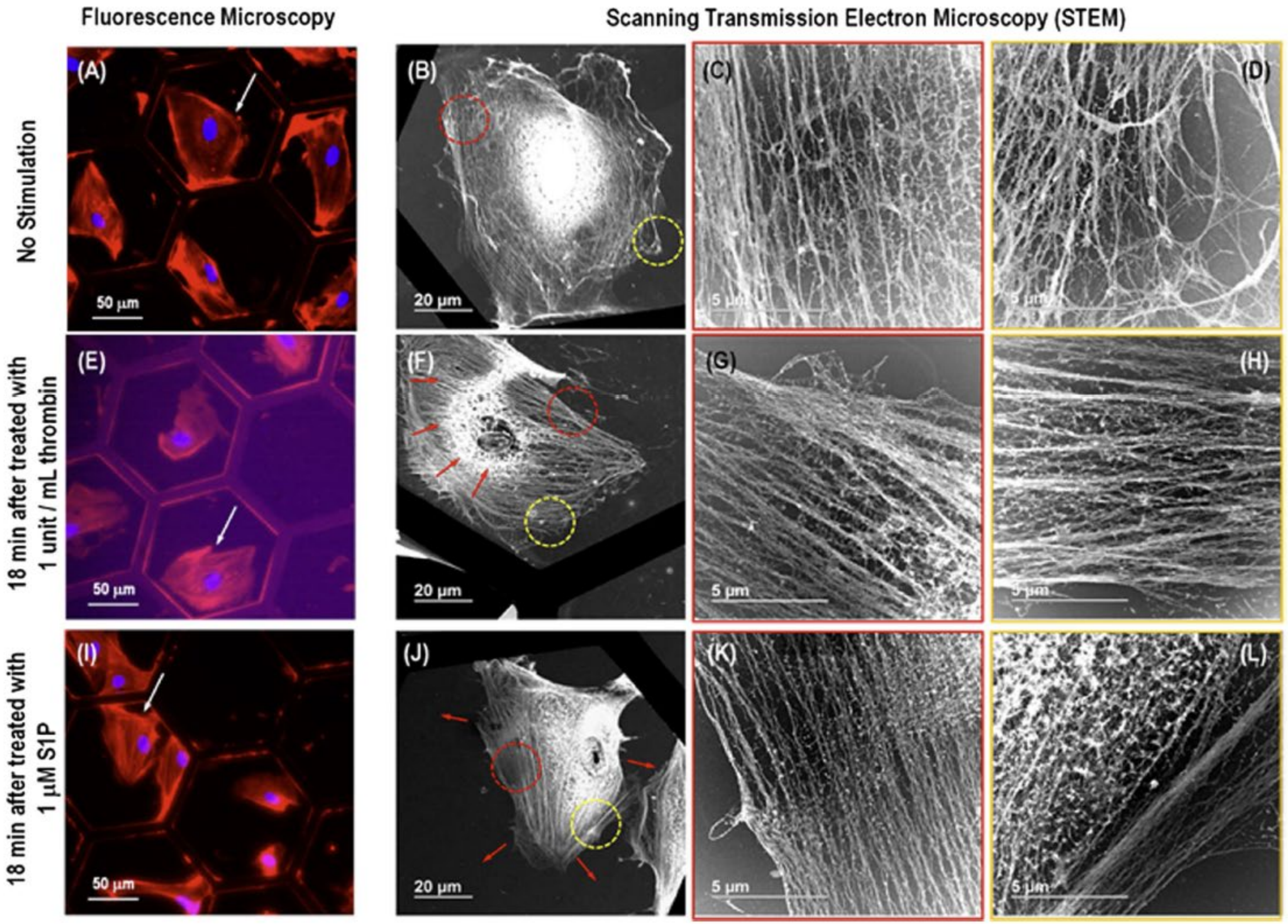
Correlative fluorescence microscopy and STEM of structural details of actin filaments.





Wang, Xin, Reiner Bleher, Mary E. Brown, Joe GN Garcia, Steven M. Dudek, Gajendra S. Shekhawat, and Vinayak P. Dravid. "Nano-biomechanical study of spatio-temporal cytoskeleton rearrangements that determine subcellular mechanical properties and endothelial permeability." *Scientific reports* 5 (2015): 11097.

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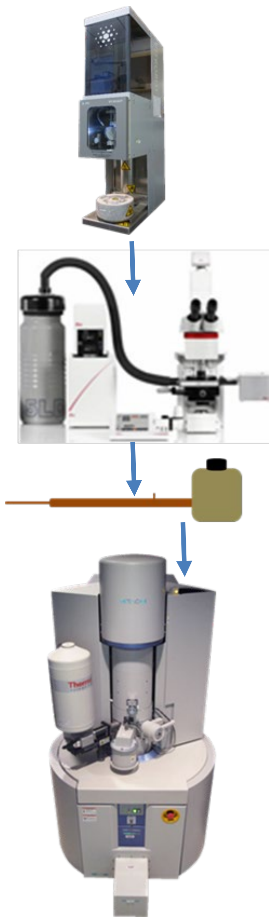
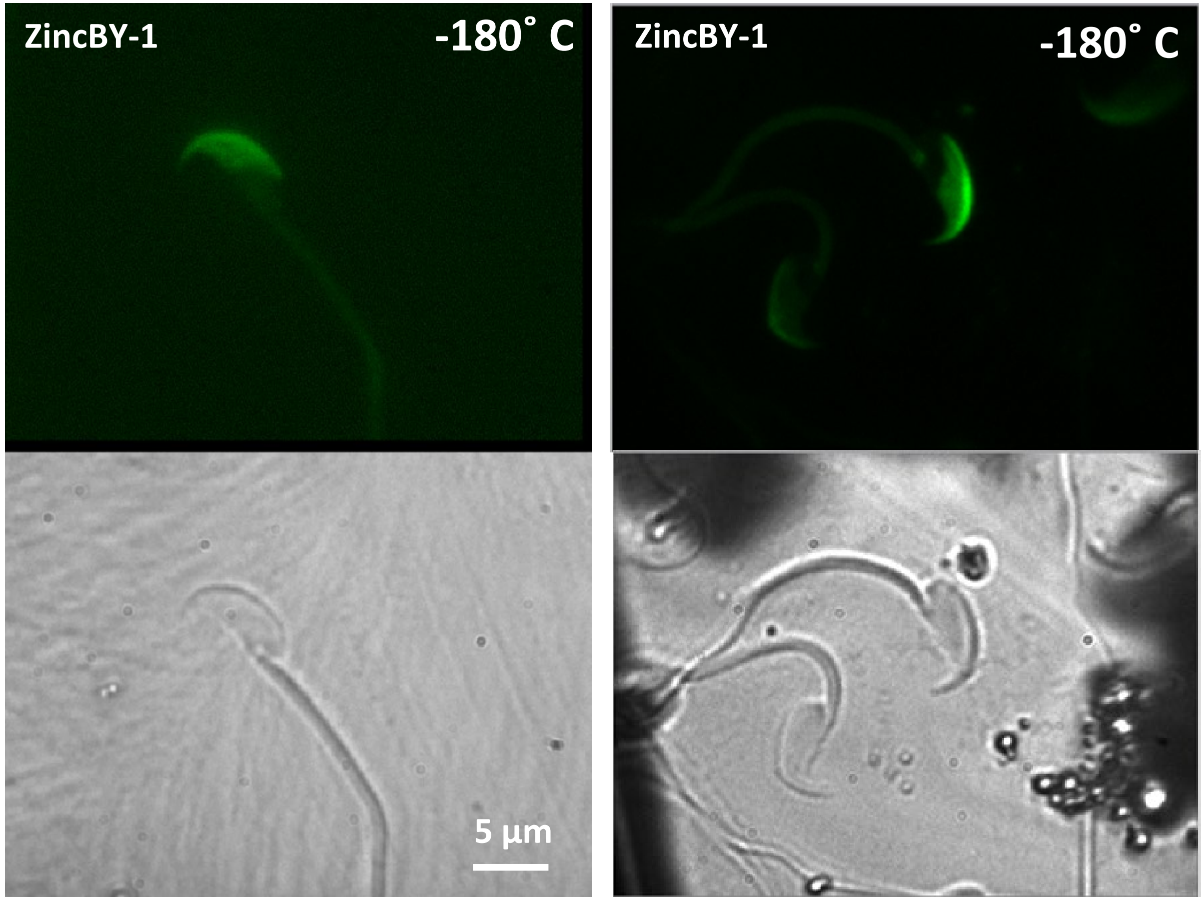
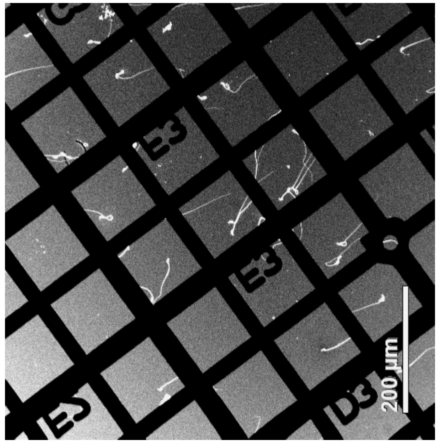
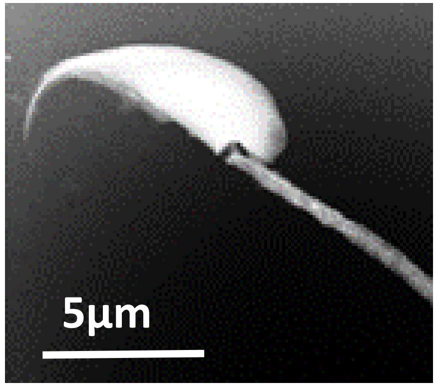
(Cryo) CLEM: Correlative Light and Electron Microscopy





Cryo fluorescence and bright field image of ZincBY-1-labelled sperm cells.





STEM image of a freeze dried sperm cell

Reiner Bleher, Project with Tom O'Halloran Lab

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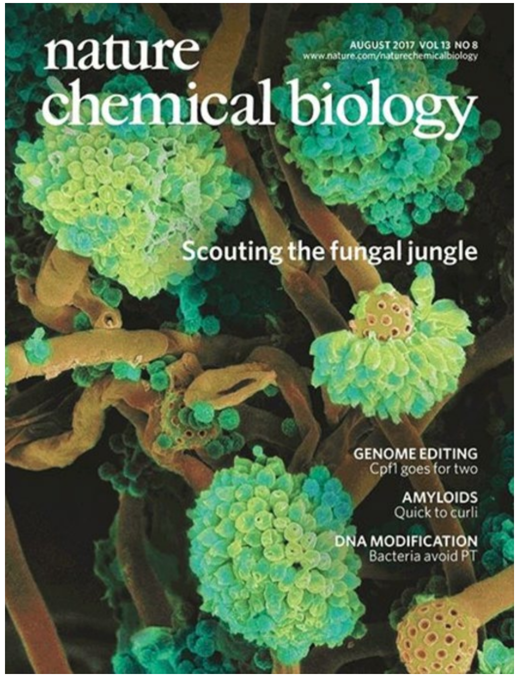

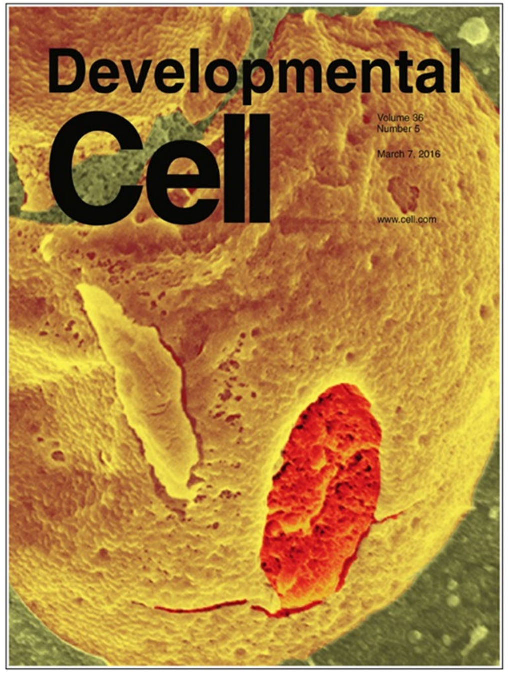

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Thank you for your Attention!

Questions?