CRYOEM 001 :
EM SAMPLE PREPARATION

NCCAT Embedded Training – Master Class series

September 2020

NATIONAL CENTER FOR
CRYOEM ACCESS & TRAINING

New York Structural
Biology Center

SIMONS ELECTRON
MICROSCOPY CENTER

CRYOEM 001 : SINGLE PARTICLE MASTERCLASS

Introduction to cryoEM: SPA

Building a cryoEM toolkit

EM compatible samples

EM support films and grids

Sample preparation

Tools of the trade:

- microscopes and detectors

Microscope operations

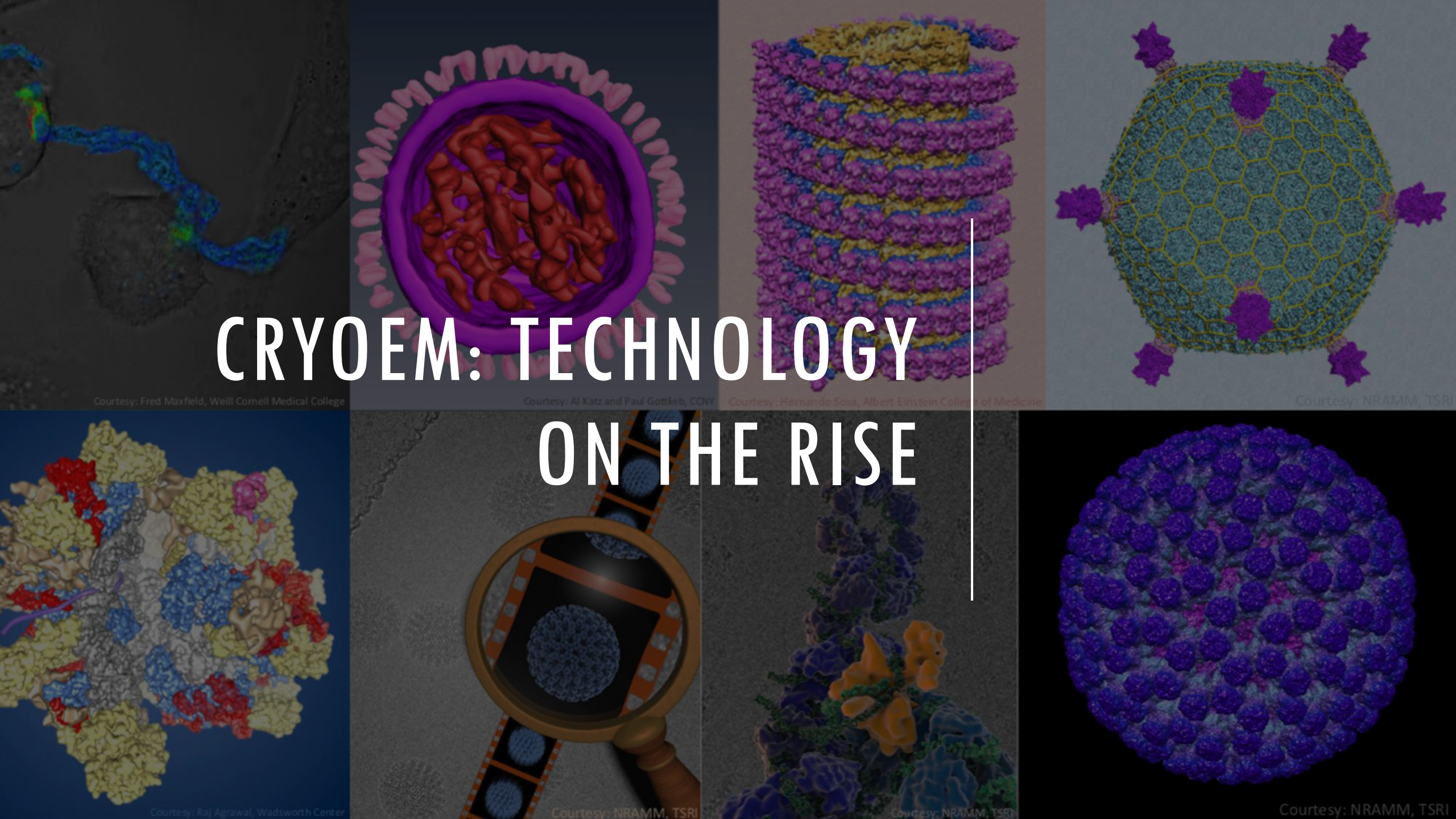
Data collection strategies

Data assessment & QC

Data processing:

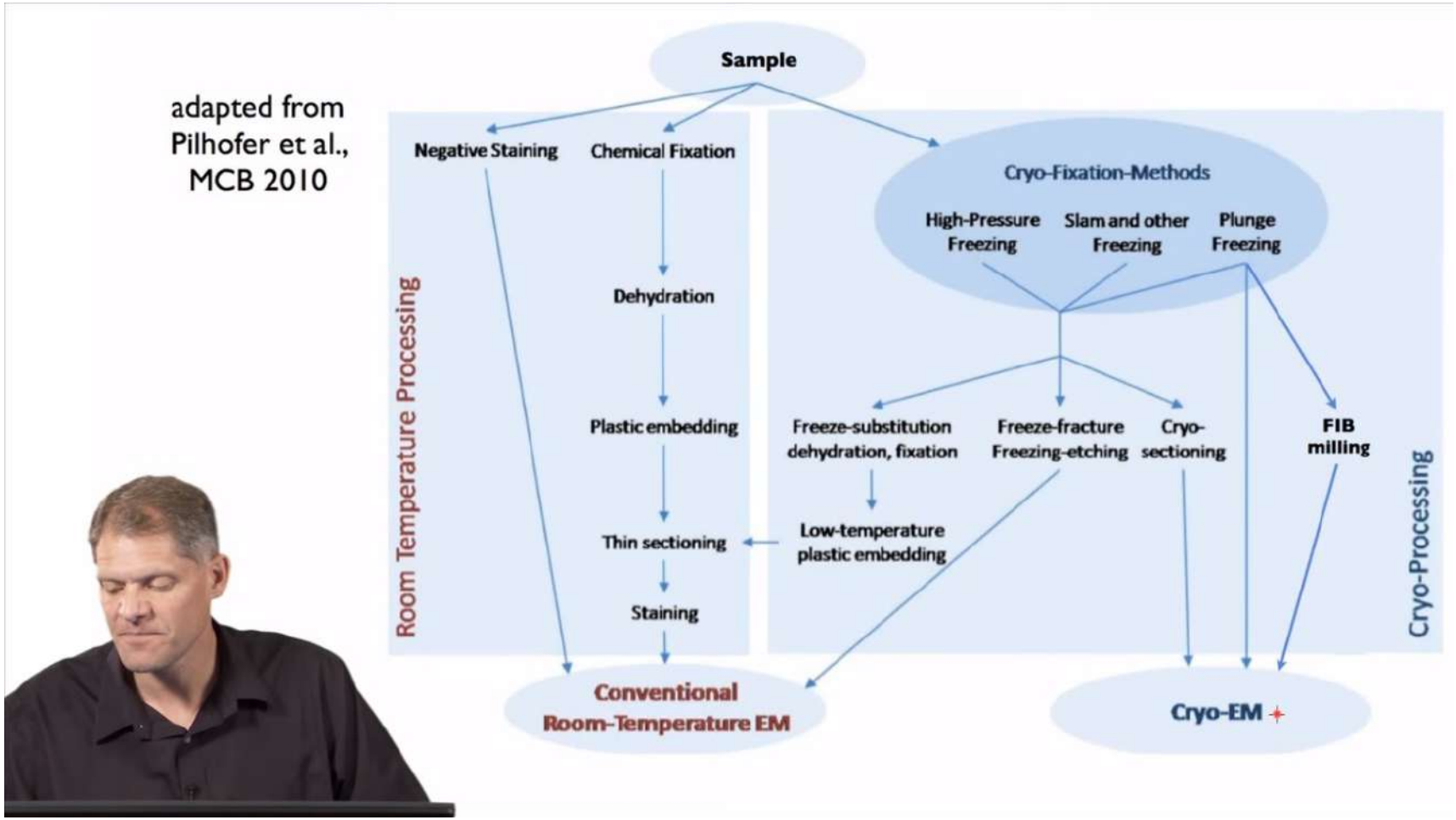
- cryoEM IT infrastructure
- On-the-fly feedback
- 3D Reconstruction

Visualization and validation

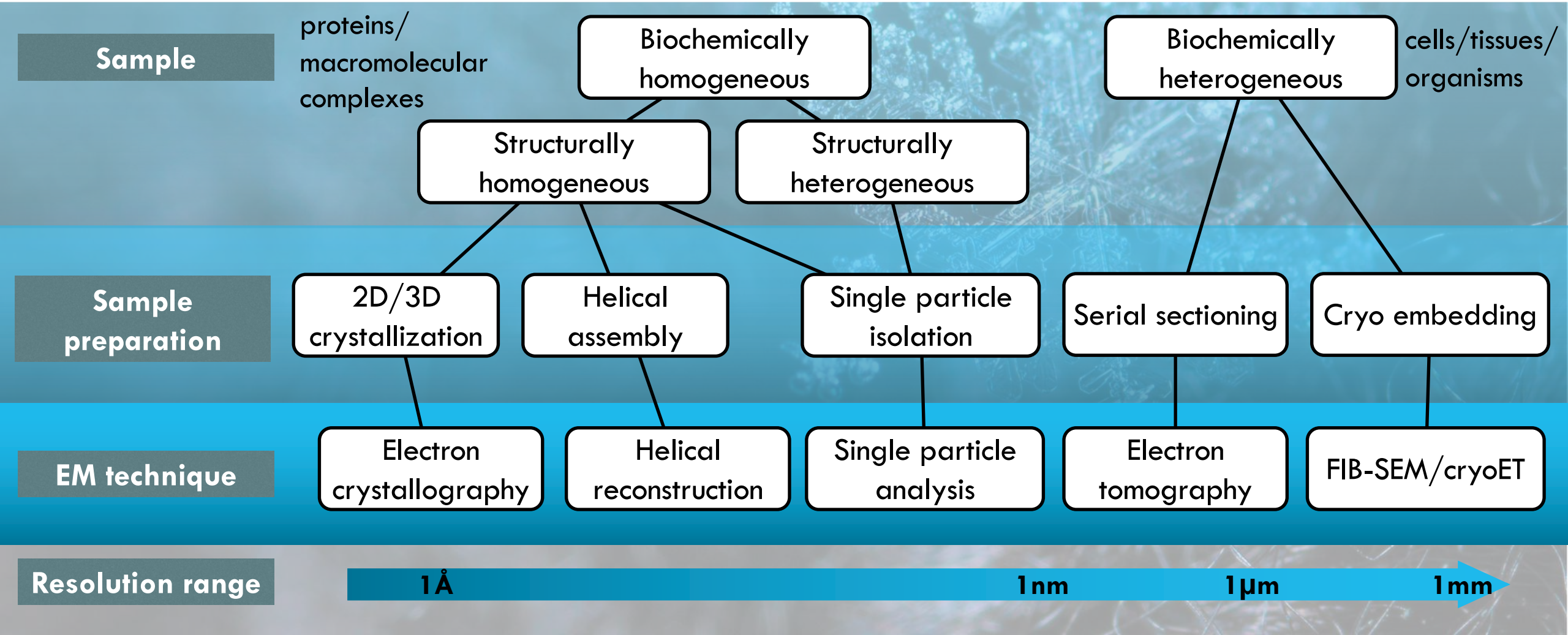


CRYOEM: TECHNOLOGY ON THE RISE

RT & CRYO SAMPLE PREP METHODS



HOW ARE SAMPLES PREPARED?



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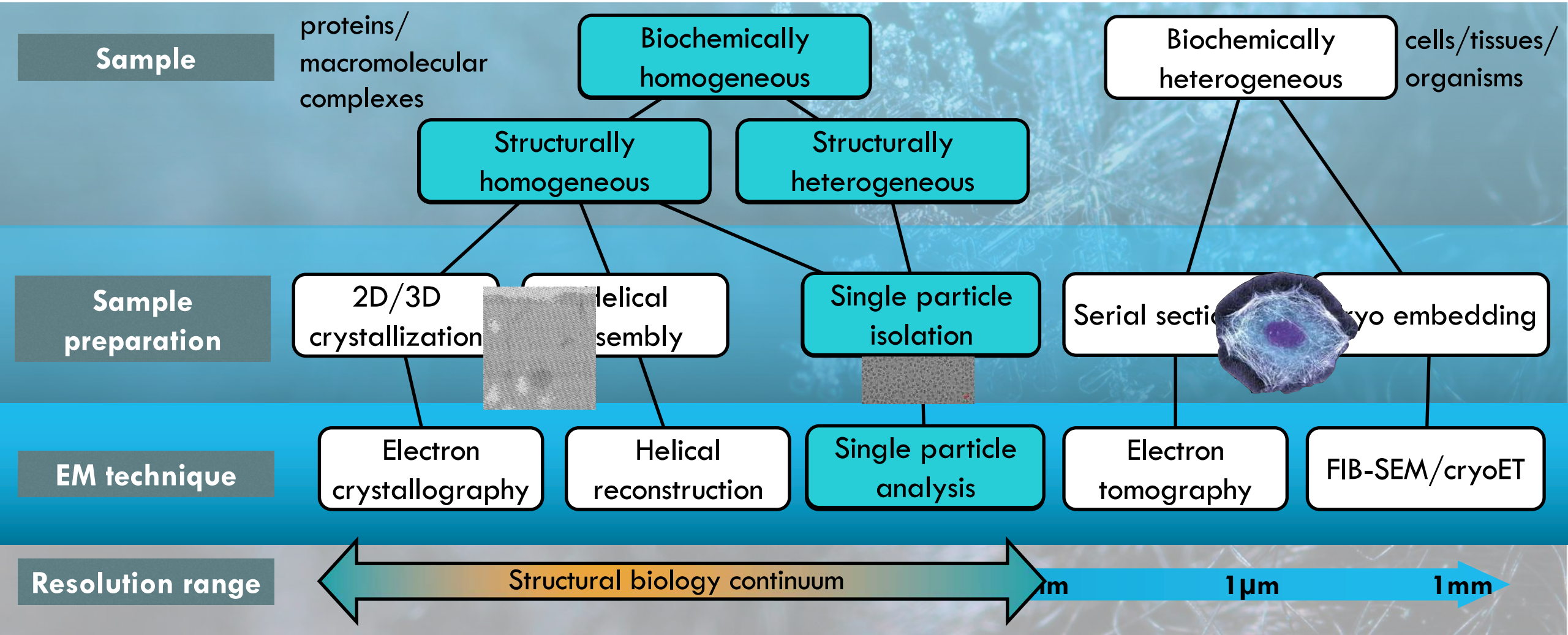
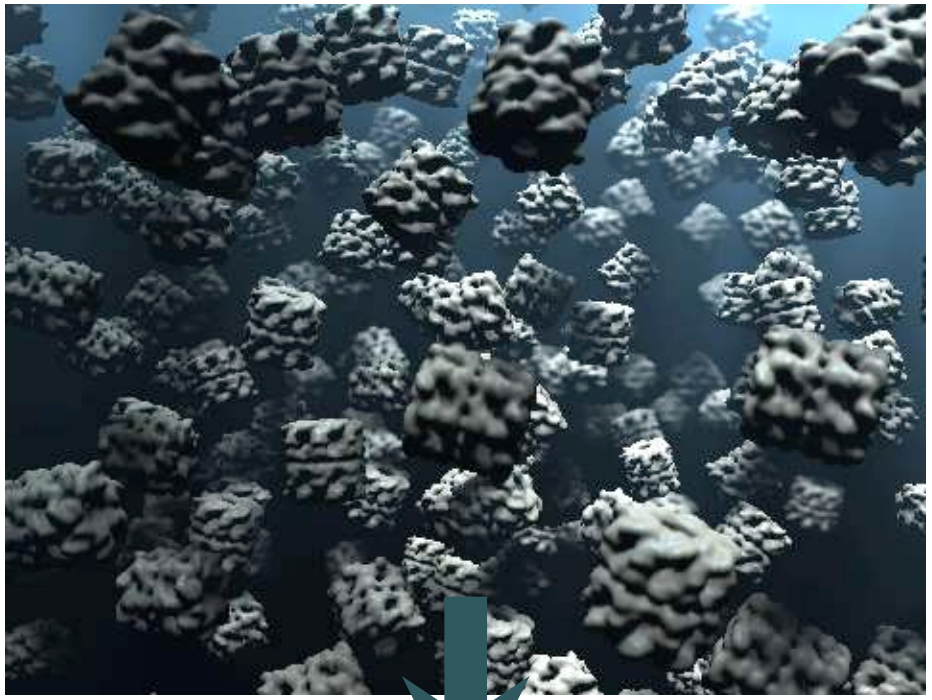
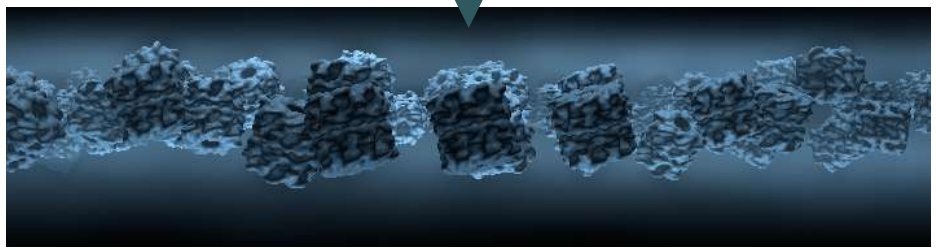
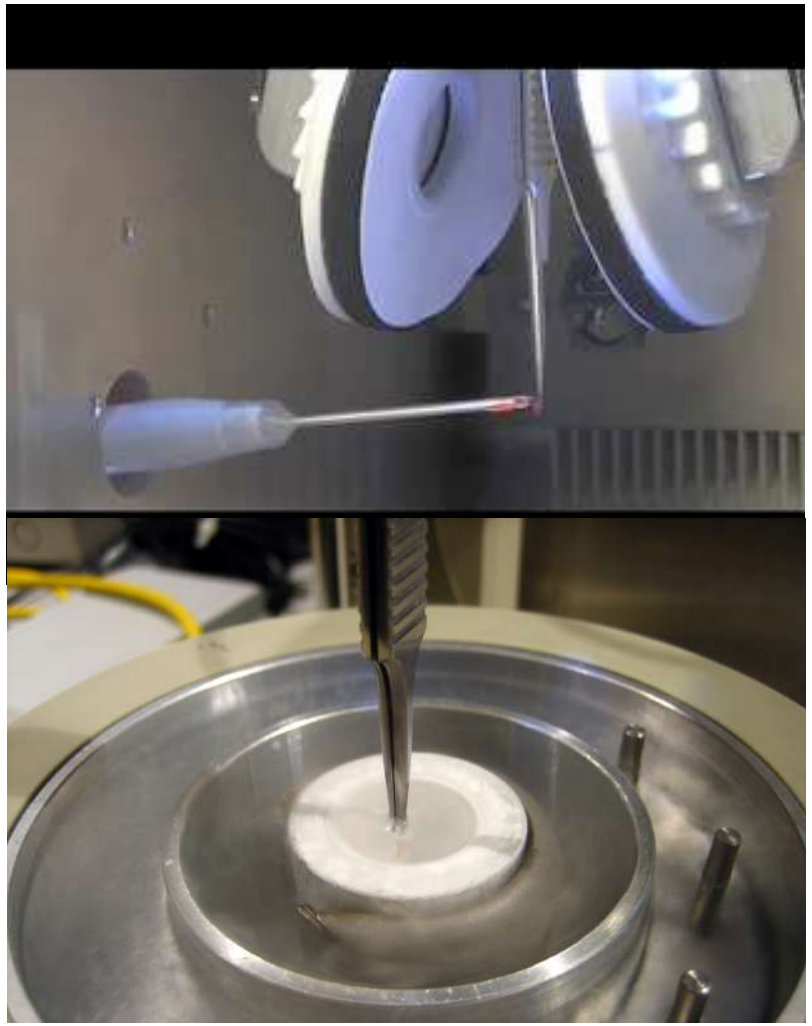


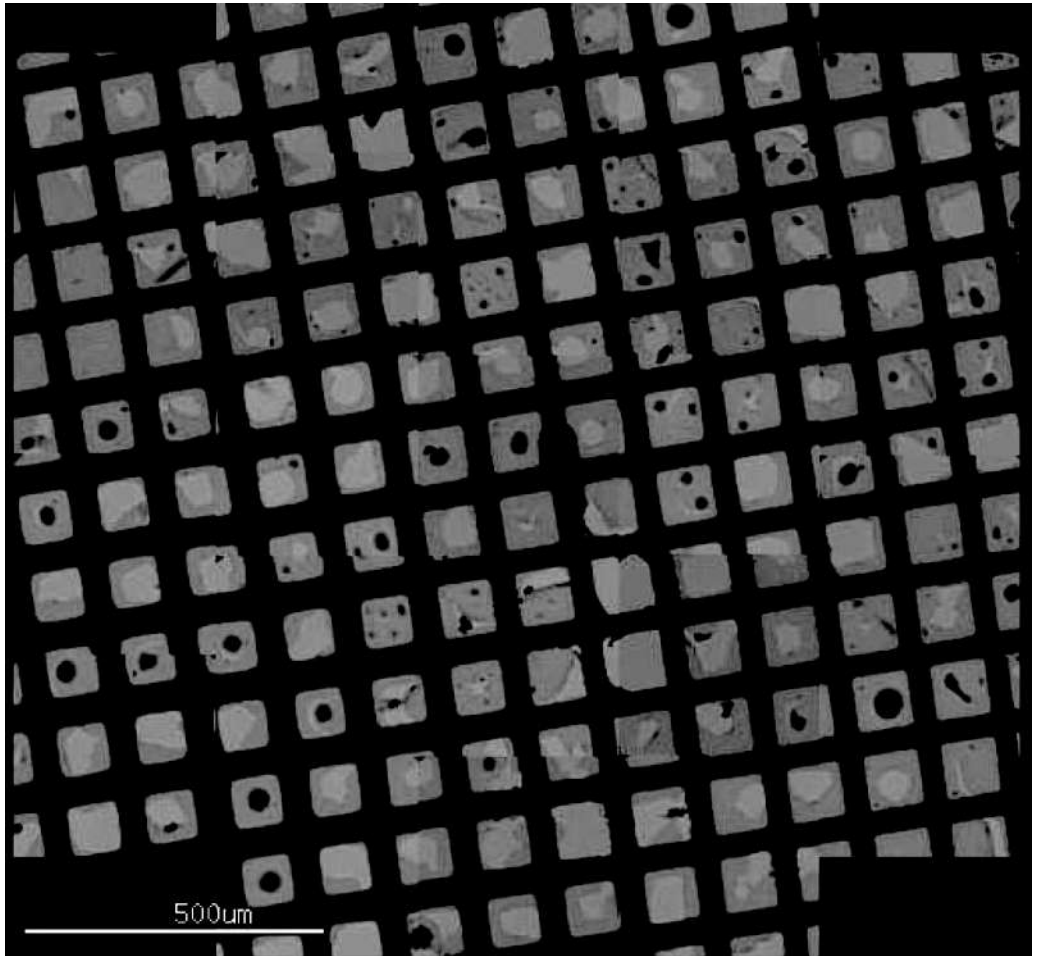
PLUNGE FREEZING

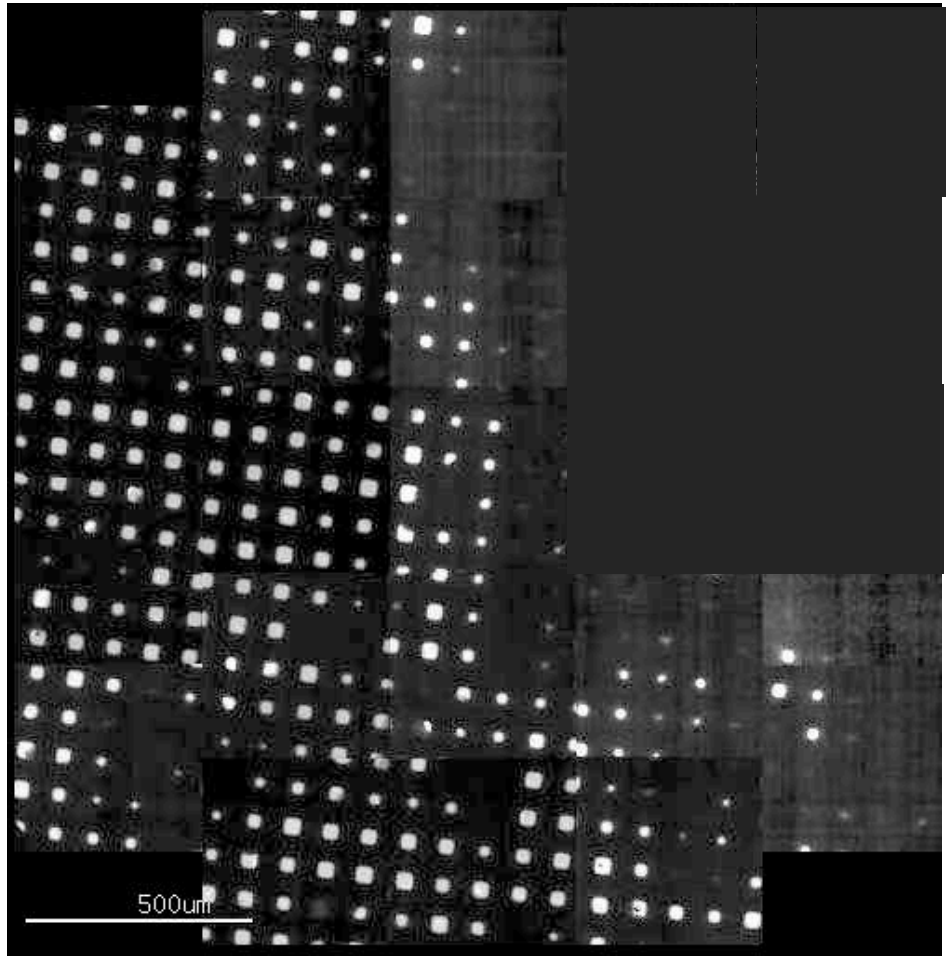




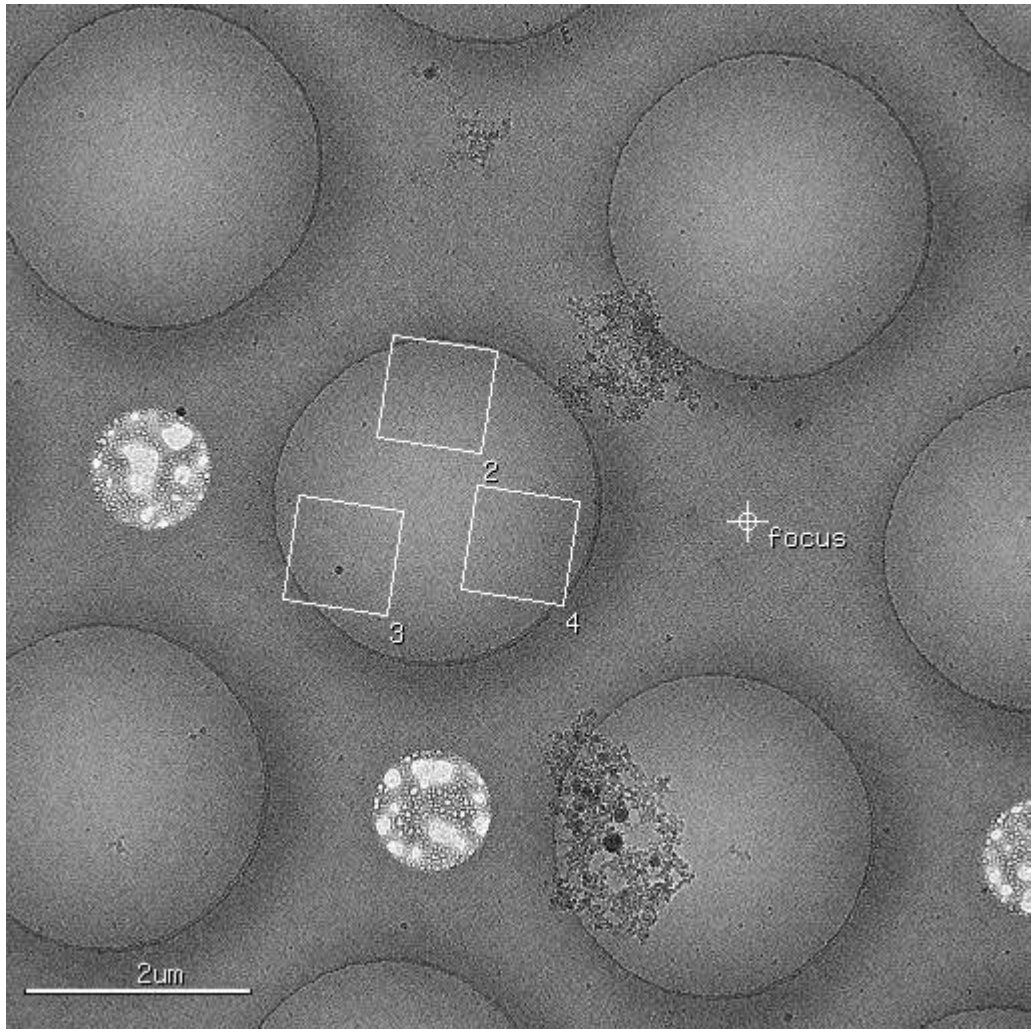


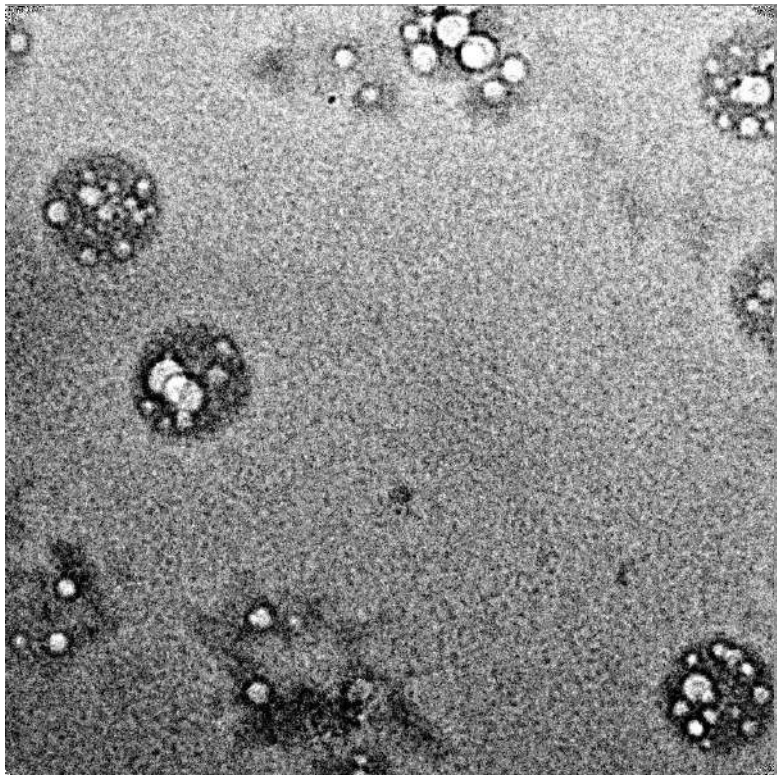
WHAT DO GRIDS LOOK LIKE?



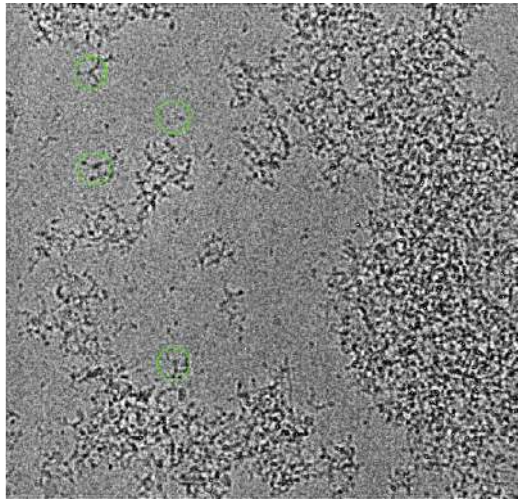


LOW DOSE IMAGING

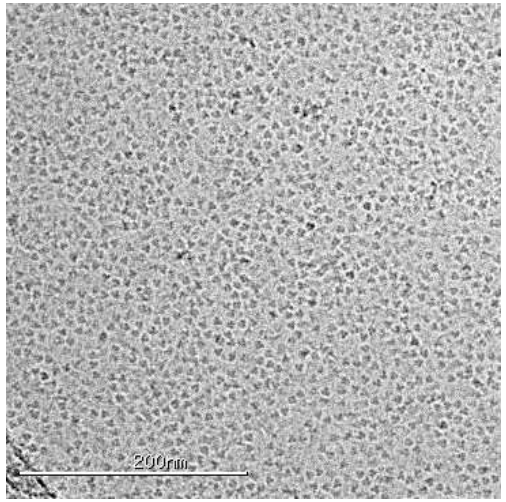




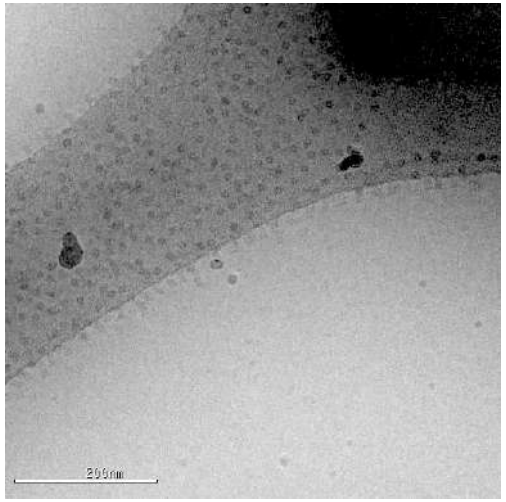
WHAT ISSUES ARISE?



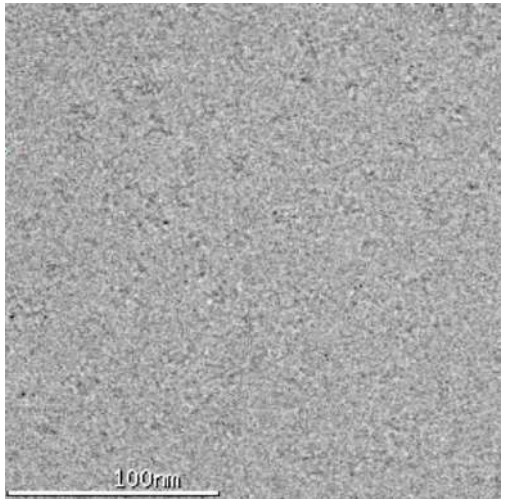
Aggregating in ice



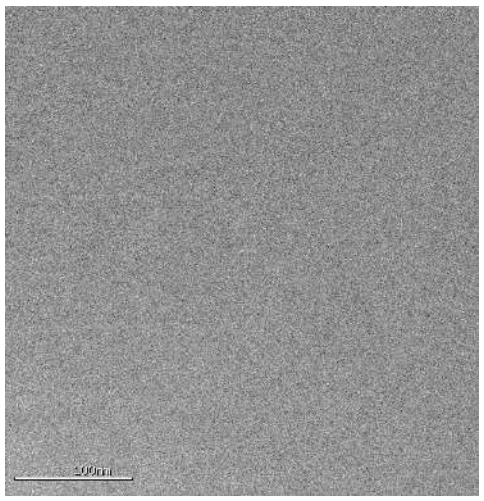
Preferred orientation



Particles not going into holes



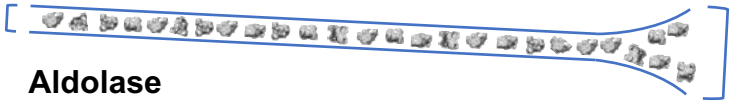
Rejecting 90% of particles



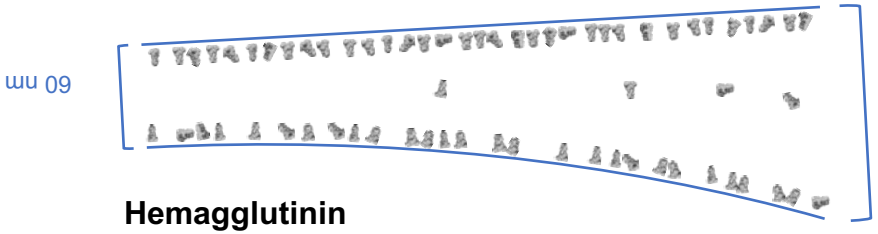
Particles disappearing in ice

WHAT ISSUES ARISE?

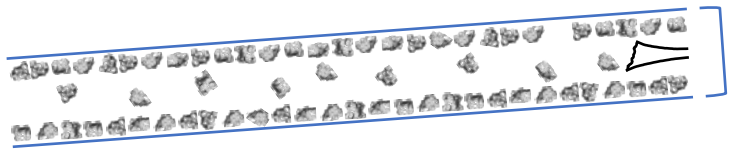
Hemagglutinin



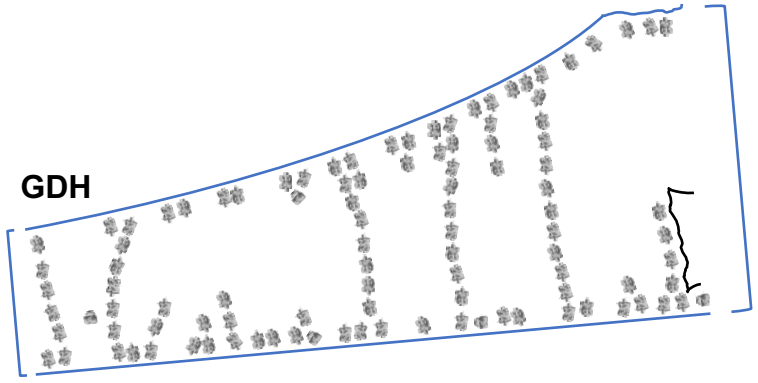
Aldolase

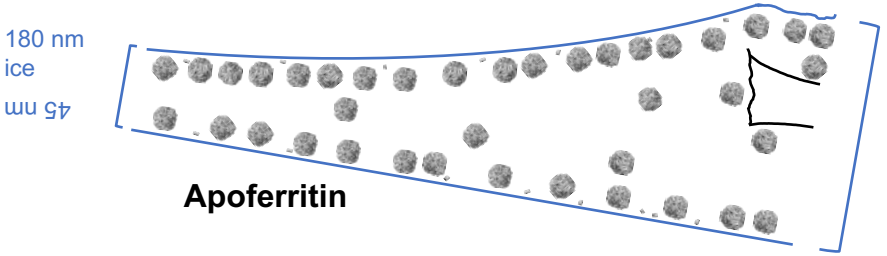


Hemagglutinin

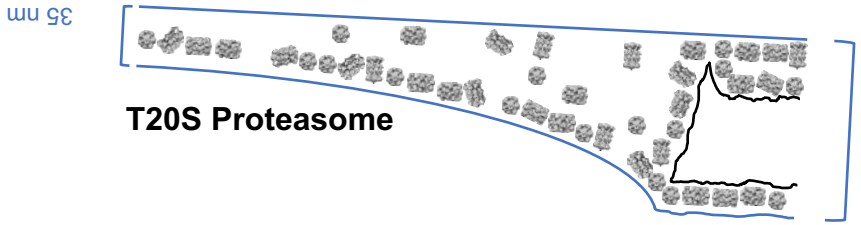


Aldolase

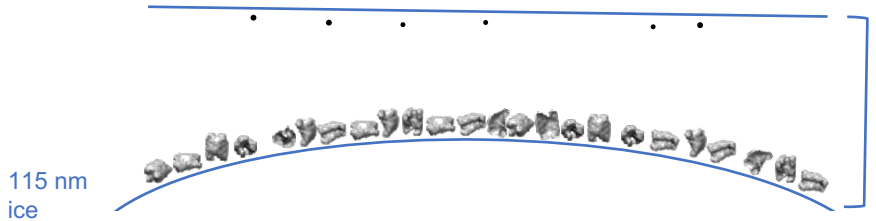




Apoferritin



T20S Proteasome

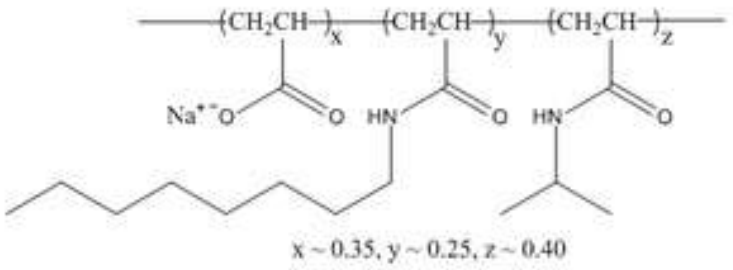


DNAB Helices

Noble AJ, et al. Routine single particle CryoEM sample and grid characterization by tomography. Elife. 2018;7.

Alex Noble

REAGENTS FOR IMPROVING VITRIFICATION OF CRYO-EM GRIDS USED IN SINGLE PARTICLE ANALYSIS.



Molecular Formula:
(C6.2H10.3O1.35N0.65Na0.35)35

Molecular Weight:
approx. 8 kDa

CAS#: 1423685-21-5

Amphipol A8-35

A short amphipathic polymer that is specifically designed for membrane protein stabilization. The surfactant possesses a very high affinity for the transmembrane surfaces and allows to solubilize membrane proteins in a detergent-free aqueous solution

REAGENTS FOR IMPROVING VITRIFICATION OF CRYO-EM GRIDS USED IN SINGLE PARTICLE ANALYSIS.

Surfactants and Cryoprotectants	Amount	Conc.	CMC	Class
Fluorinated Octyl Maltoside (FOM)	100 µl	0.41% (w/v)	0.07% (w/v)	non-ionic detergent
Hexadecyl-trimethyl-ammonium Bromide (CTAB)	100 µl	0.34% (w/v)	0.03% (w/v)	cationic detergent
n-Decyl-ß-D-Maltoside (DM)	100 µl	0.87% (w/v)	0.09% (w/v)	non-ionic detergent
n-Decyl-α-D-Maltoside (DαM)	100 µl	0.46% (w/v)	0.08% (w/v)	non-ionic detergent
n-Dodecyl-ß-D-Maltoside (DDM)	100 µl	0.09% (w/v)	0.01% (w/v)	non-ionic detergent
Sodium Deoxycholate	100 µl	1.66% (w/v)	0.17% (w/v)	anionic detergent
Triton X-100	100 µl	0.15% (w/v)	0.01% (w/v)	non-ionic detergent
Tween 20	100 µl	1% (w/v)	0.01% (w/v)	non-ionic detergent
CHAPSO	100 µl	2.5% (w/v)	0.5% (w/v)	zwitterionic detergent
Amphipol A8-35	100 µl	5% (w/v)		anionic surfactant
Glycerol	1 ml	30% (w/v)		cryoprotectant

https://www.mitegen.com/product/cryo-em-vitrification-starter-kit/

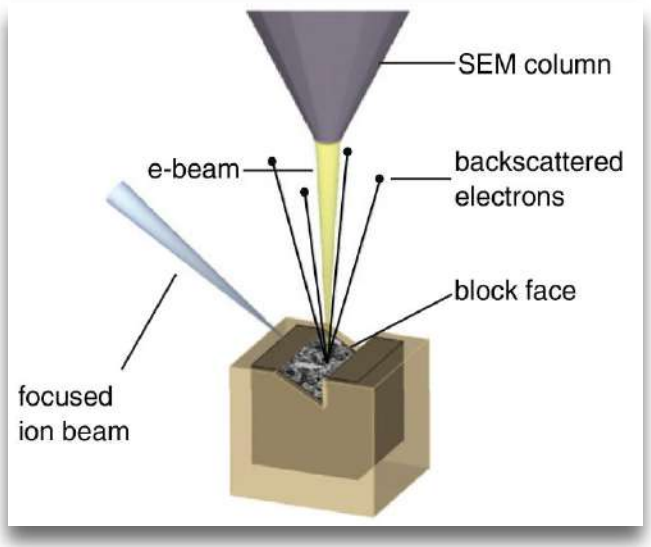
[1] Noble *et al.* (2018) Routine Single Particle CryoEM Sample and Grid Characterization by Tomography. DOI: 10.7554/eLife.34257.
[2] Thonghin *et al.* (2018) Cryo-electron microscopy of membrane proteins. *Methods* **147**:176.
[3] Drulyte *et al.* (2018) Approaches to altering particle distributions in cryo-electron microscopy sample preparation. *Acta Cryst. D* **74**:560.
[4] Glaeser *et al.* (2017) Opinion: hazards faced by macromolecules when confined to thin aqueous films. *Biophys Rep* **3**:1.
[5] Gatsogiannis *et al.* (2016). Membrane insertion of a Tc toxin in near-atomic detail. *Nat. Struct. Mol. Biol.* **23**:884.
[6] Efremov *et al.* (2015) Architecture and conformational switch mechanism of the ryanodine receptor. *Nature* **517**:39.

FIB/SEM VS THIN SECTION SAMPLE PREP

- Chemical fixation
- Staining
  En bloc, enhanced contrast and electrical conductivity
- Dehydration
- Embedding

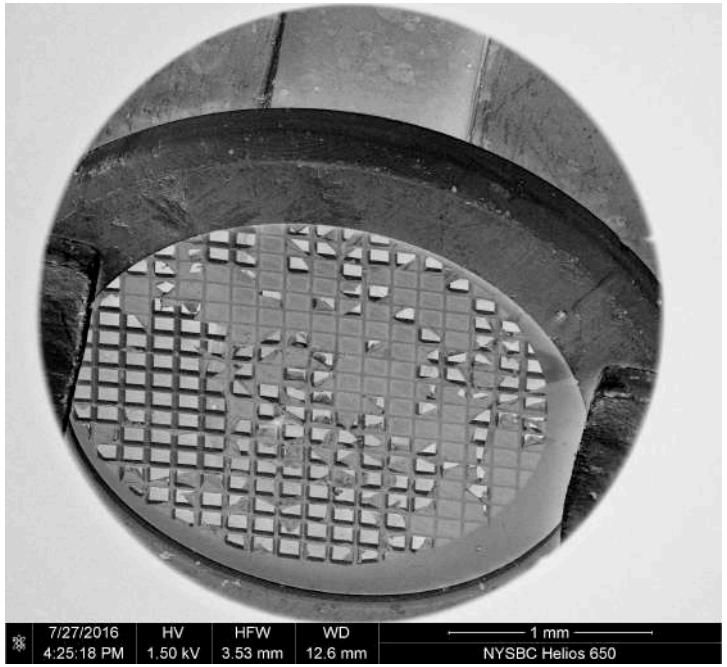
- Au/Pd coat
  Conductivity

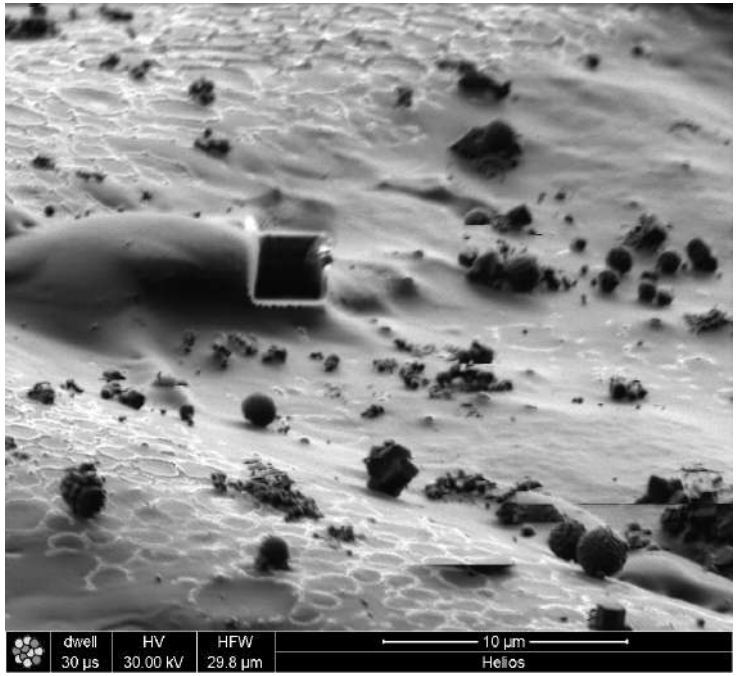
Cryofixation: High pressure freezing
Dehydration: Freeze substitution



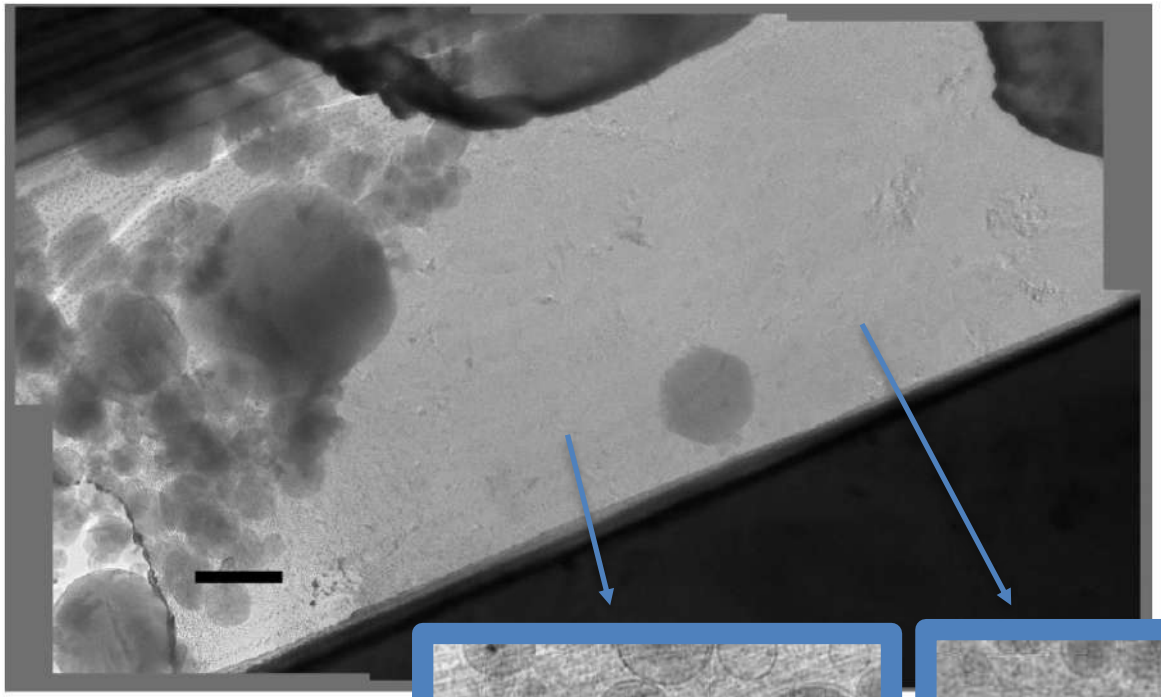
- Chemical fixation
- Dehydration
- Embedding
- Sectioning
- Staining

CRYO FIB MILLING

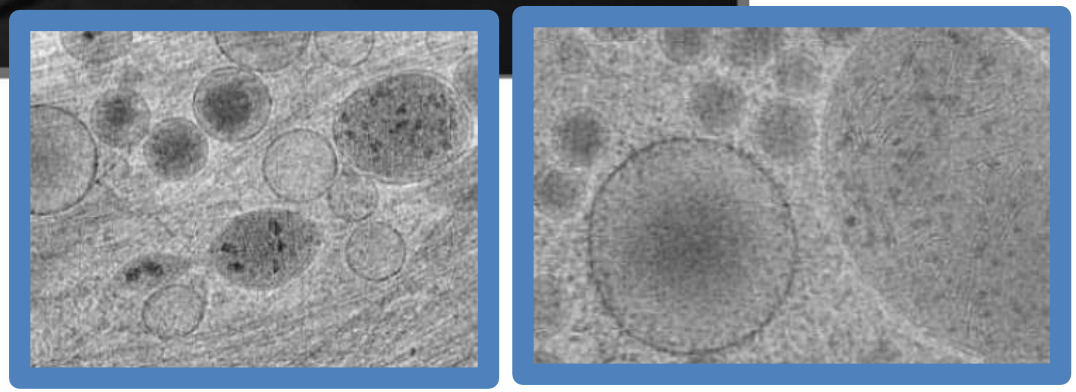


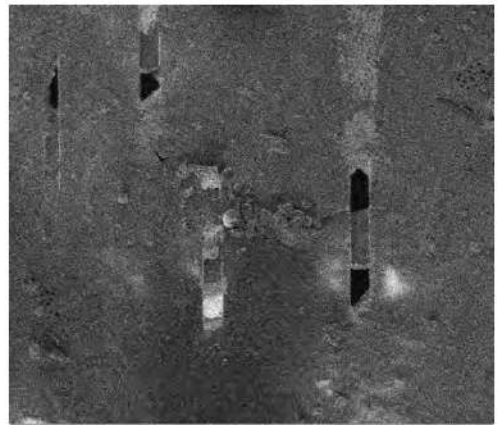


CRYO FIB MILLING

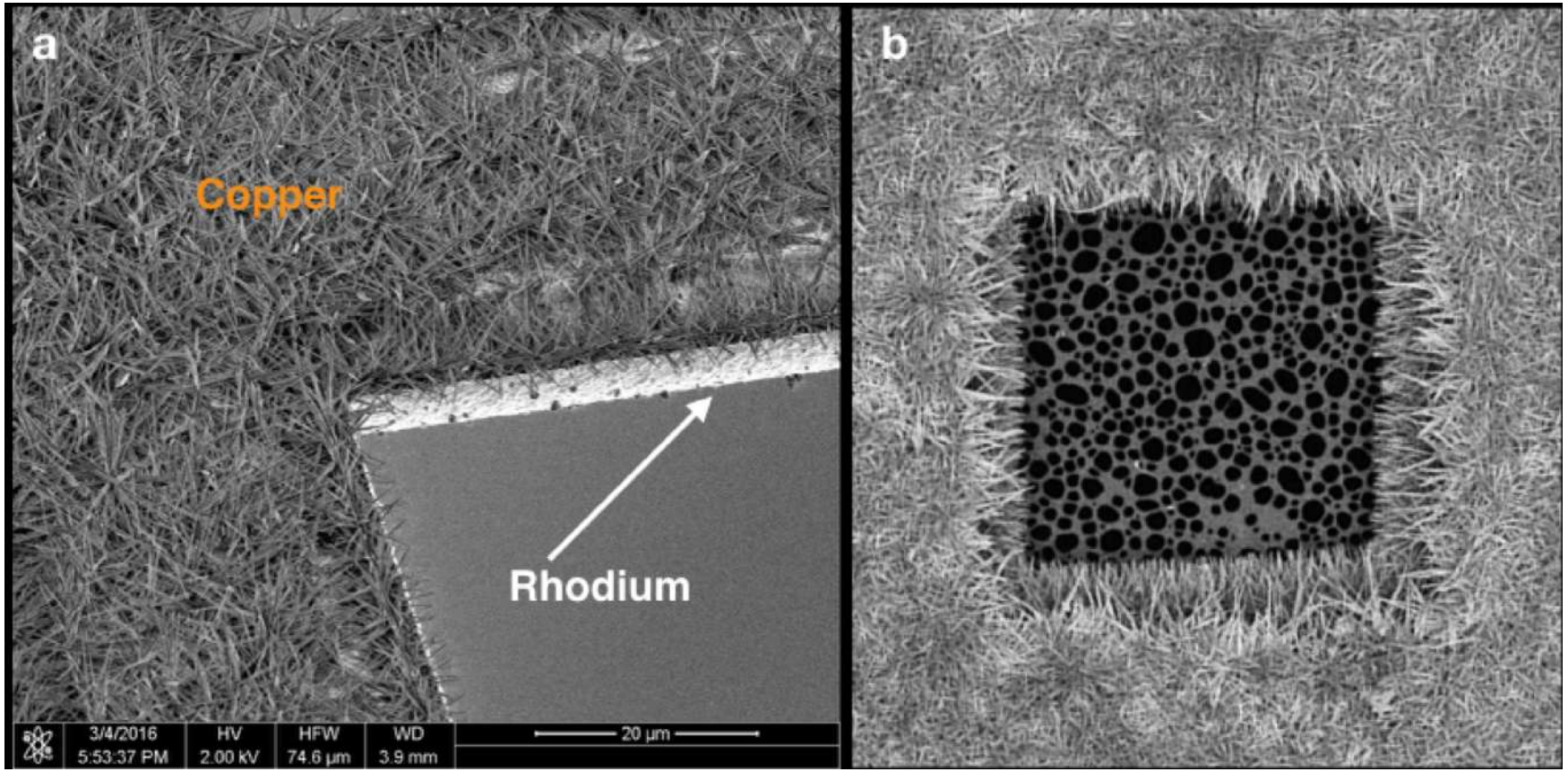


Scale bar: 1 µm





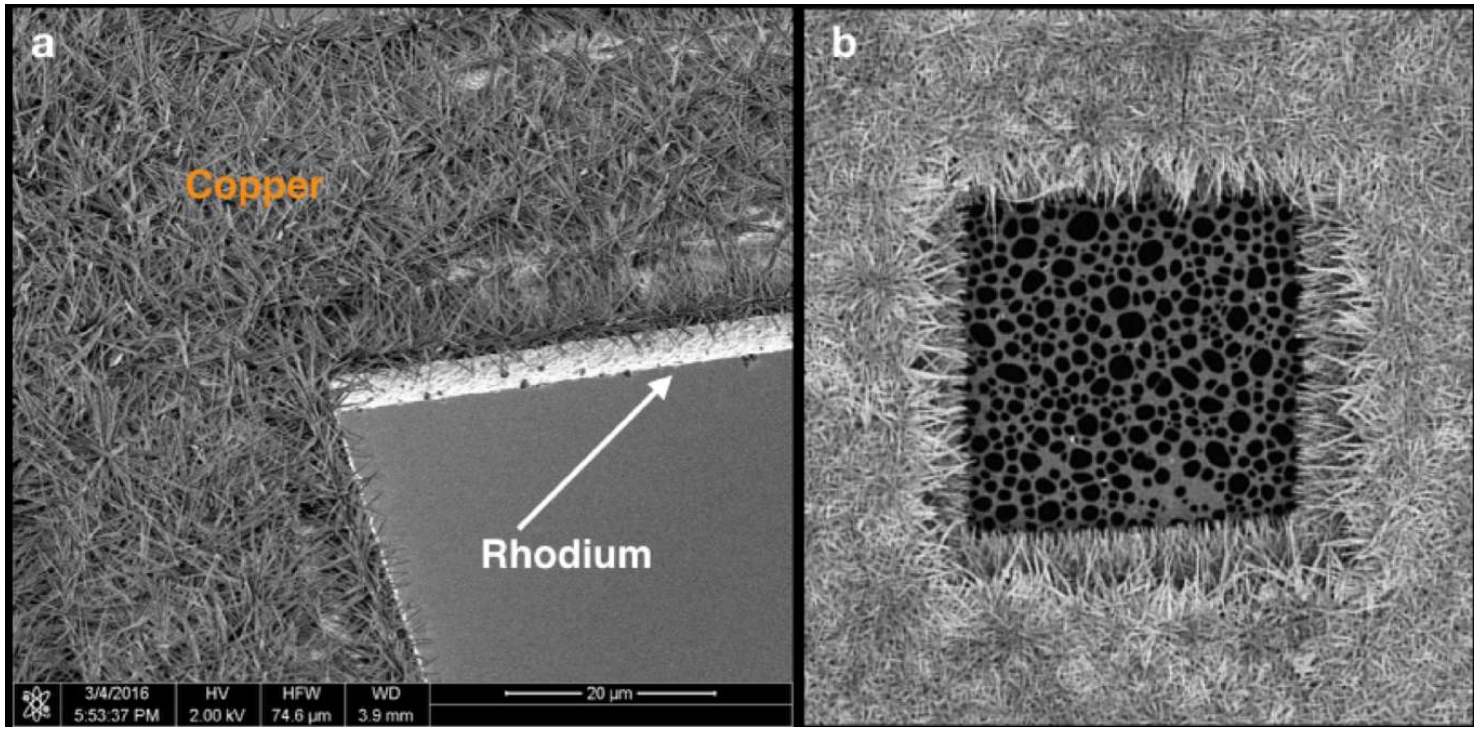
BLOT FREE VITRIFICATION



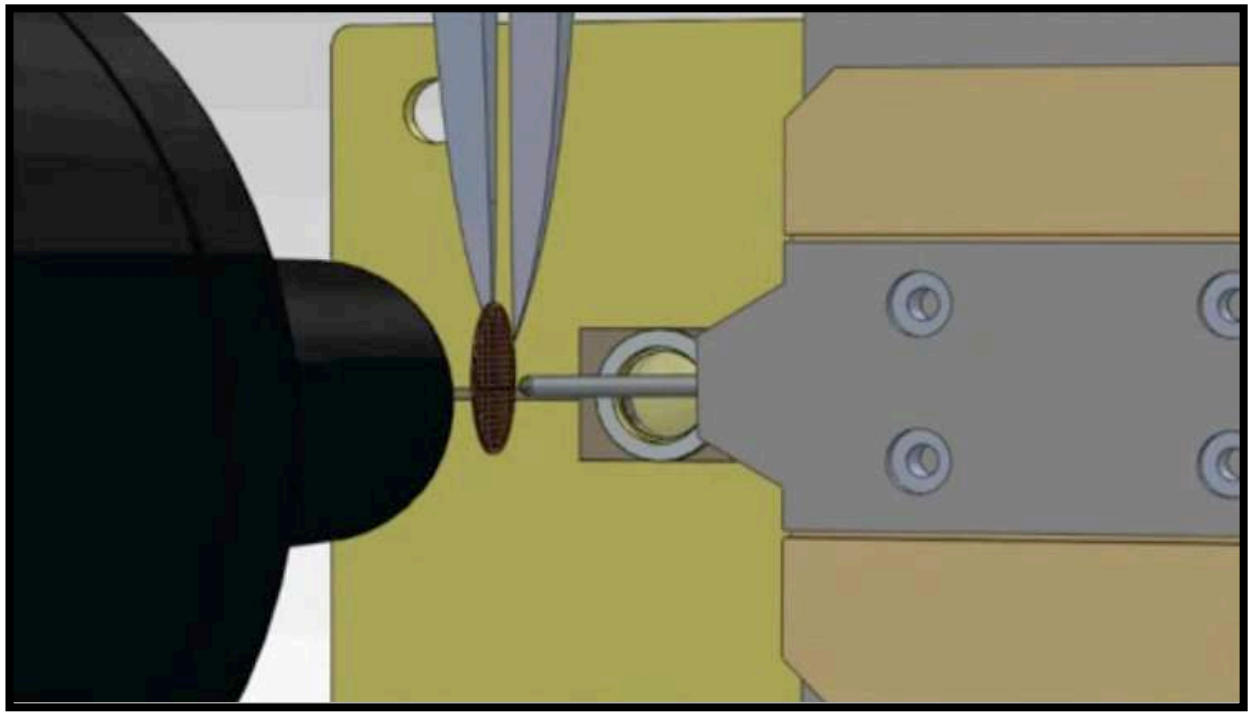
nramm.nysbc.org

SPOTITON | CHAMELEON

Spotiton



SPOTITON | CHAMELEON



WHAT NEXT?

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