

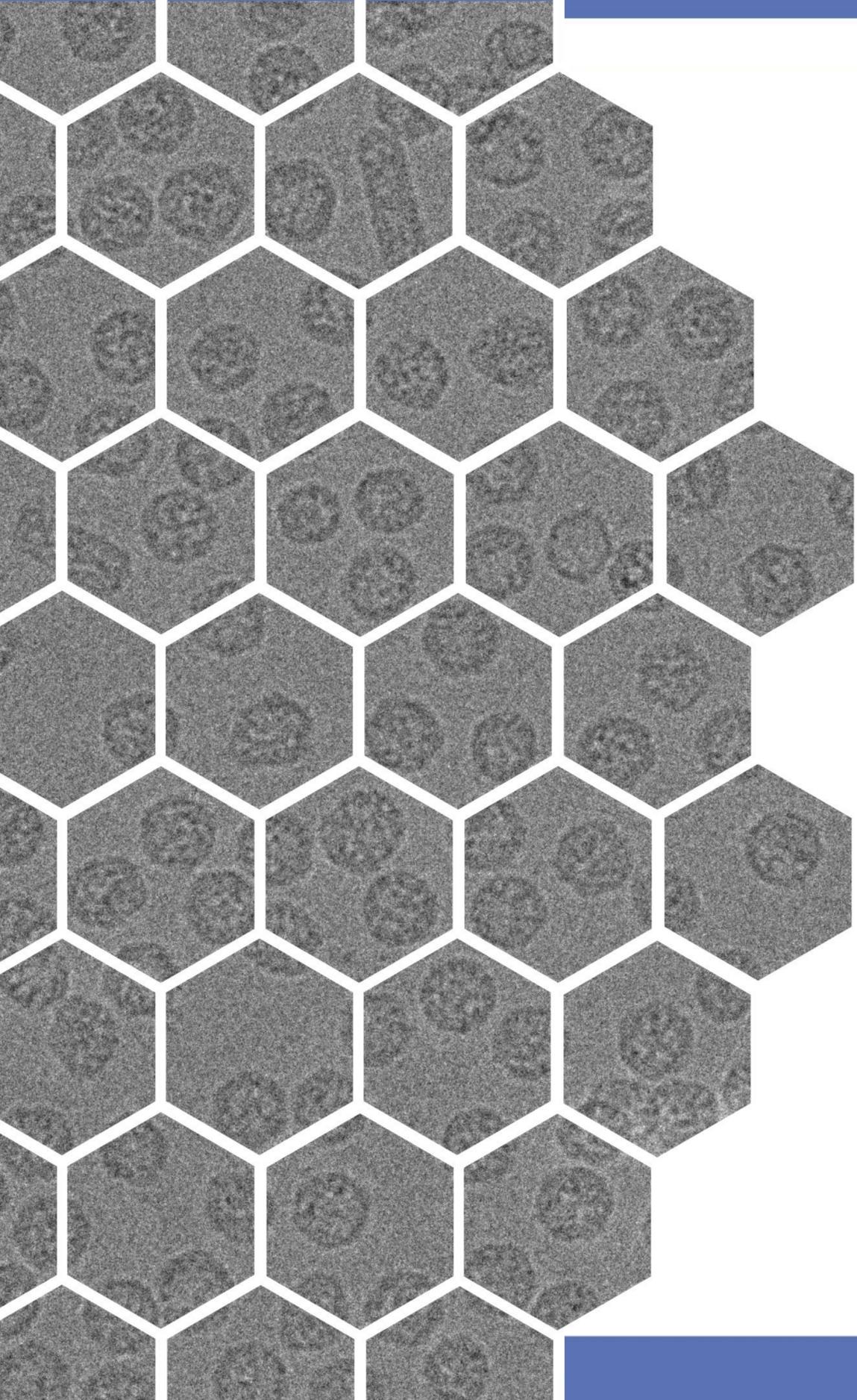
2026 cryoEM course

Considerations for biological cryoEM

March 16, 2026



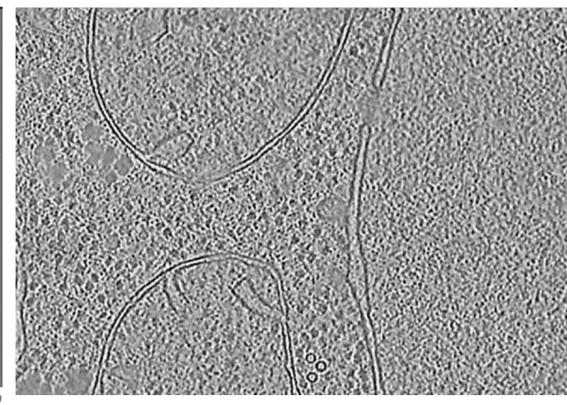
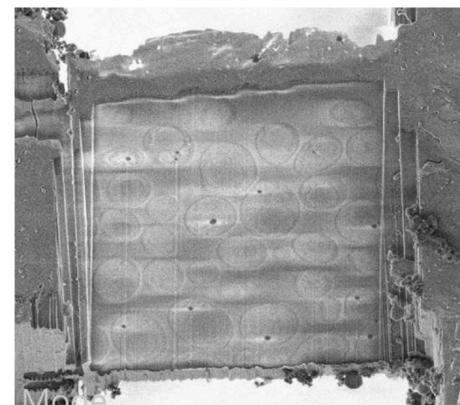
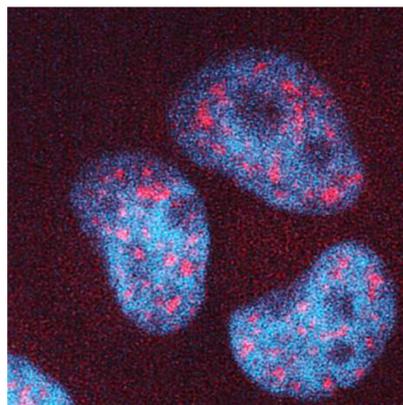
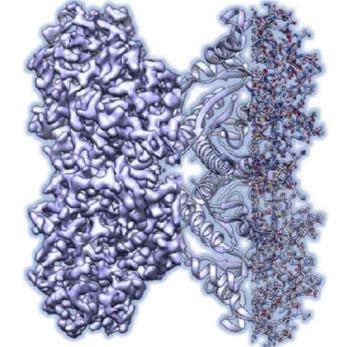
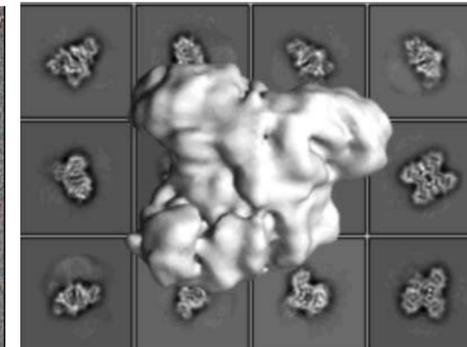
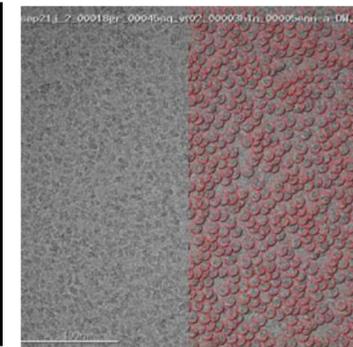
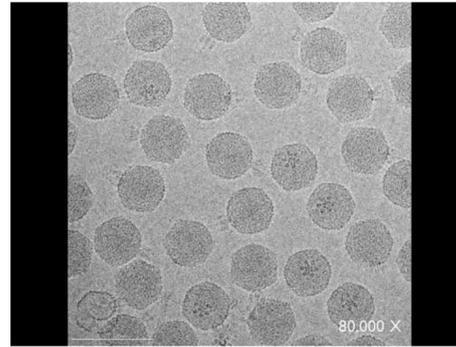
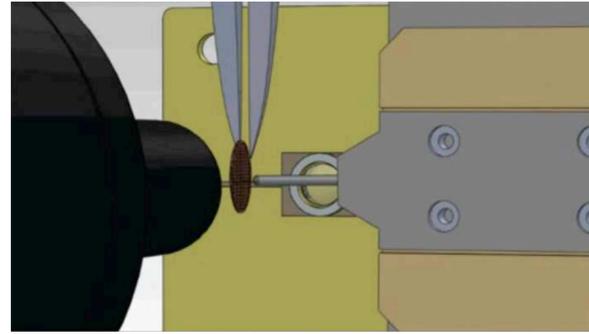
NYSBC SEMC



- ◆ Considerations for biological cryoEM
- ◆ Grids (our sample slide)
- ◆ What happens to a sample during vitrification
- ◆ Newer methods

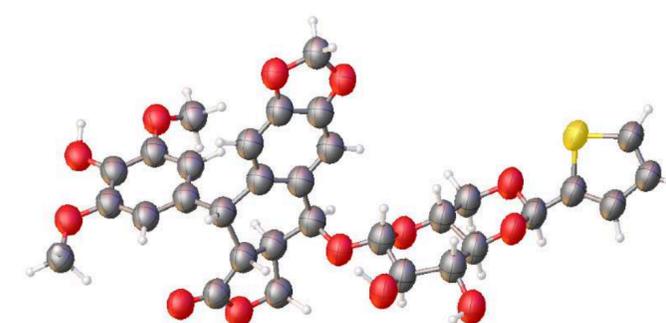
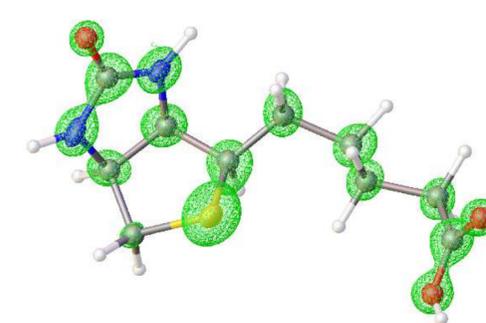
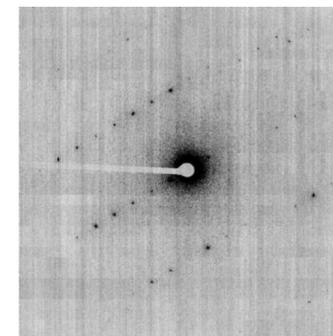
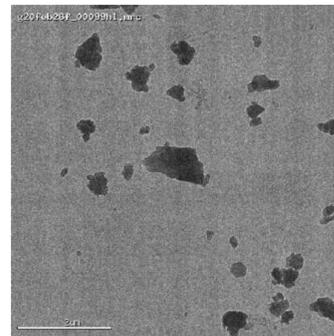
cryoEM: a technology on the rise

Single particle cryoEM

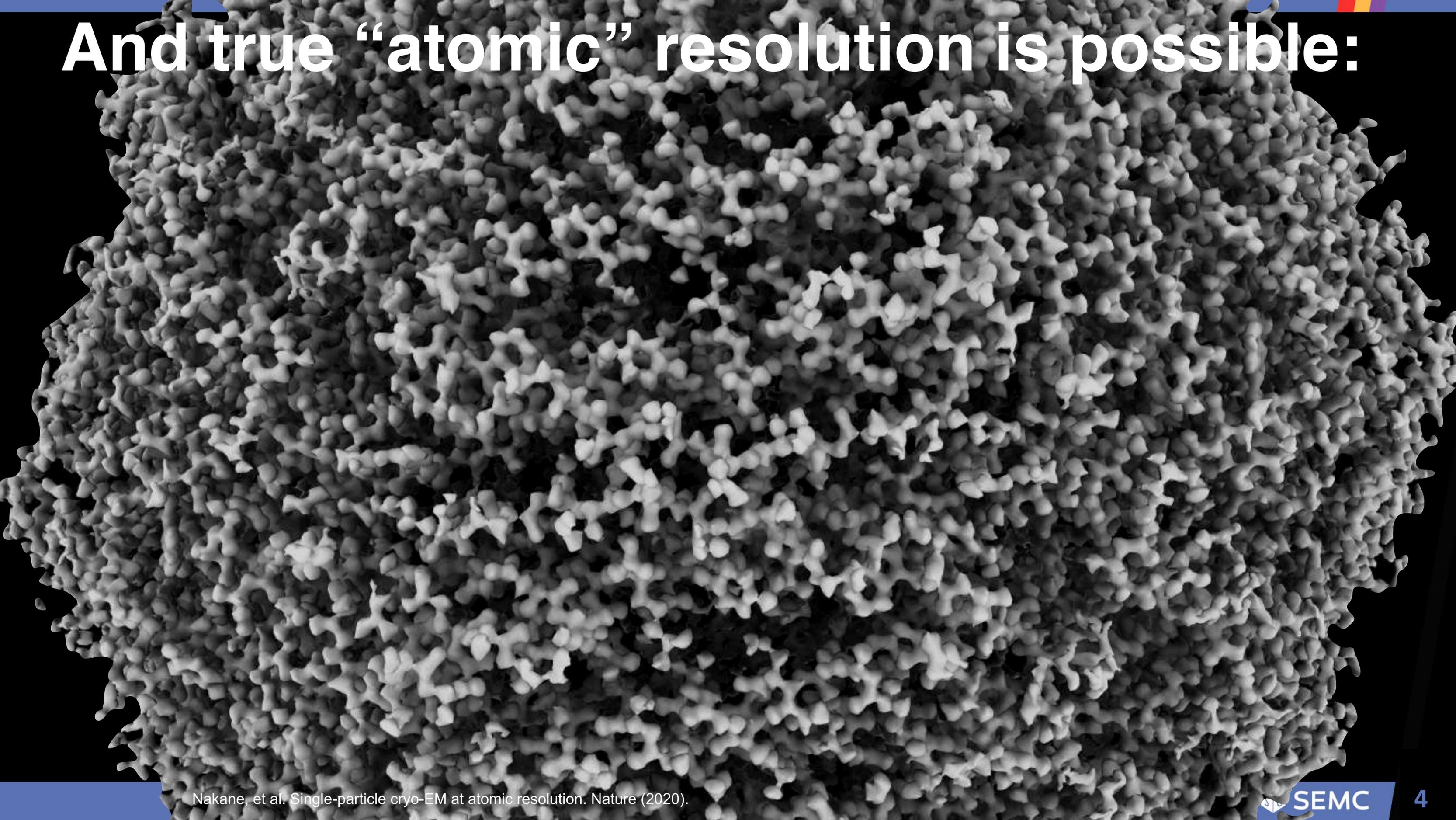


Cryo Electron Tomography (cryoET)

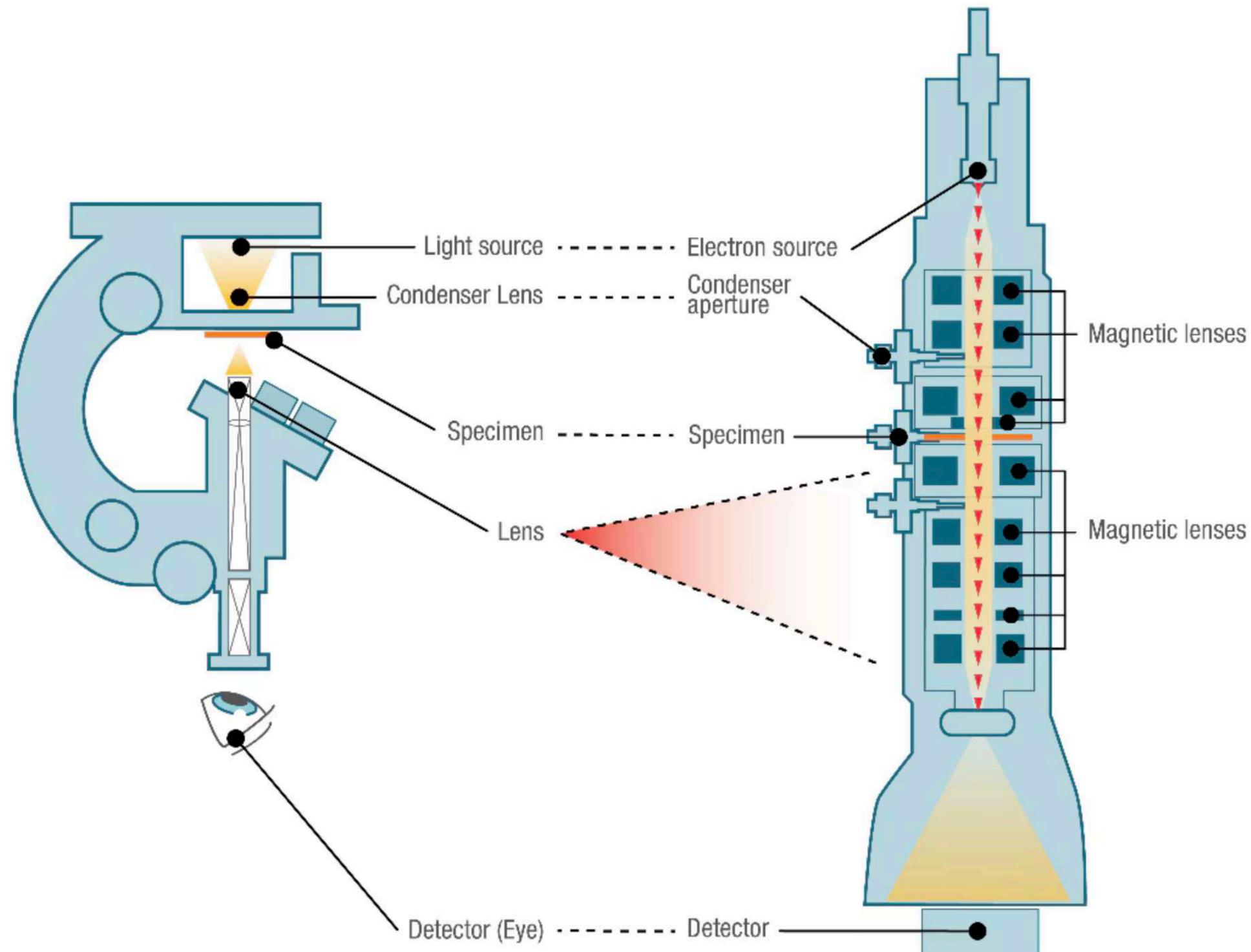
Micro crystal electron diffraction (microED)



And true “atomic” resolution is possible:

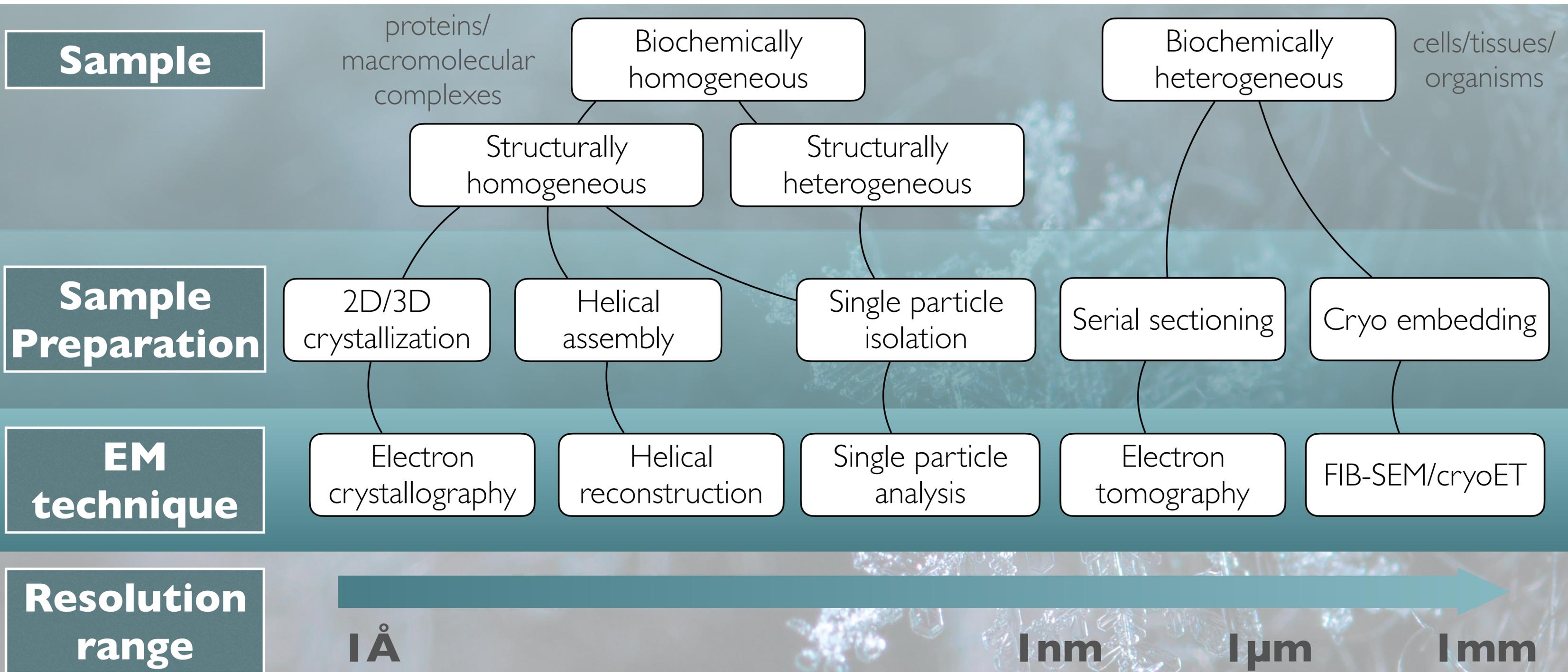


The electron microscope



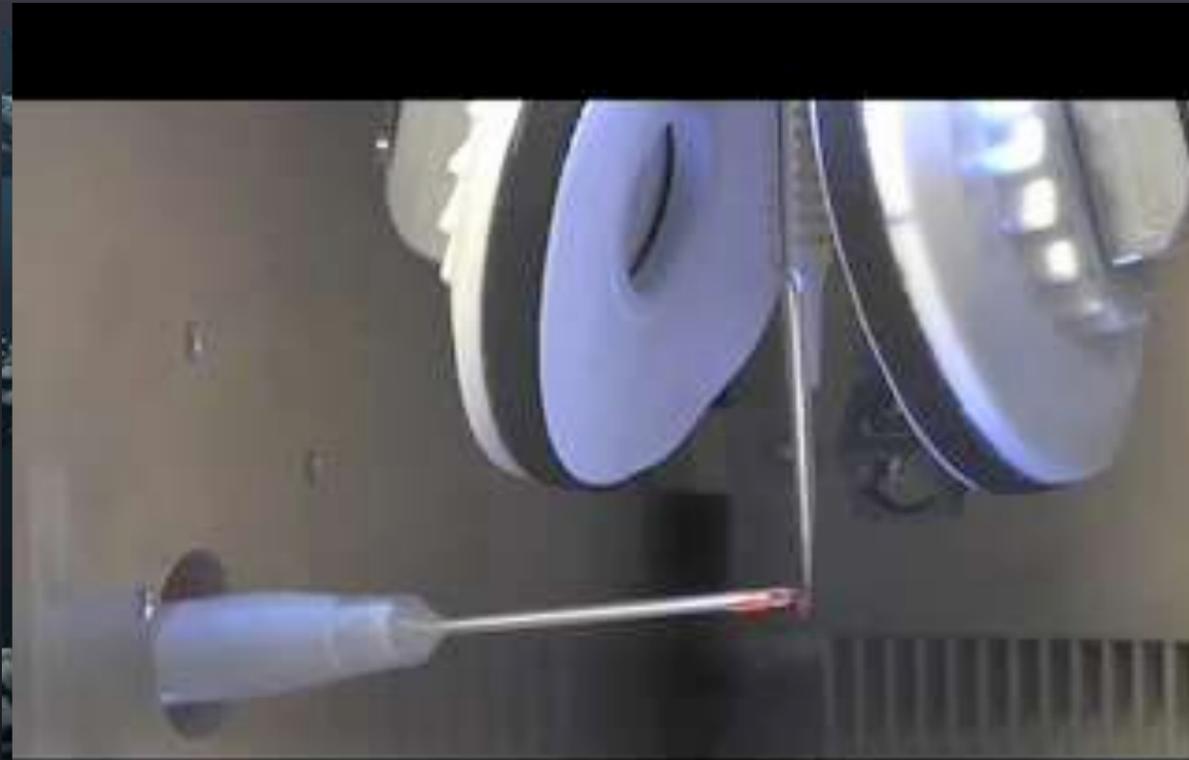
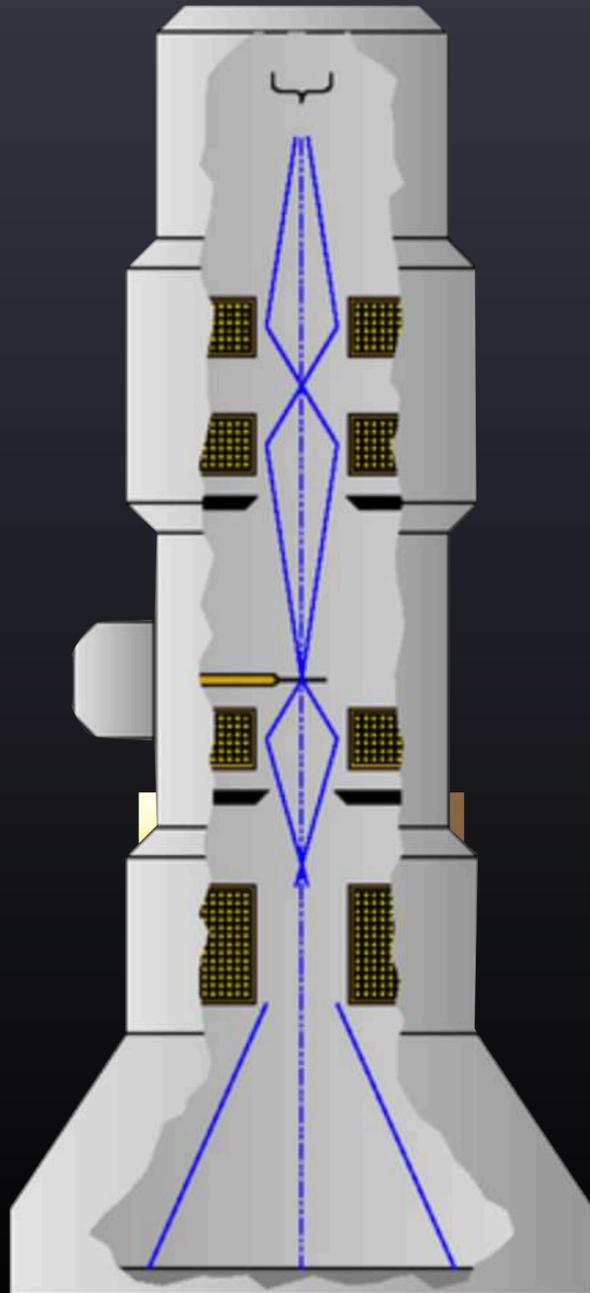
- Sample requirements:
- Thin (~100 nm)
 - Stable under vacuum

How are samples prepared for cryoEM?



How are samples prepared for cryoEM?

Vitrifying a biological sample



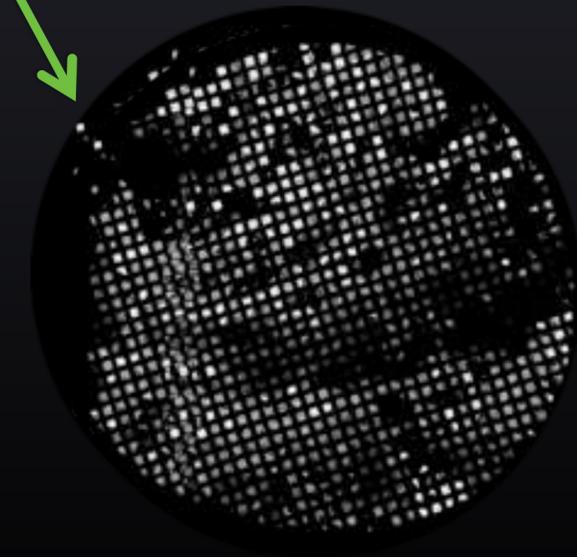
>99.999%



<0.001%

~3 μ l

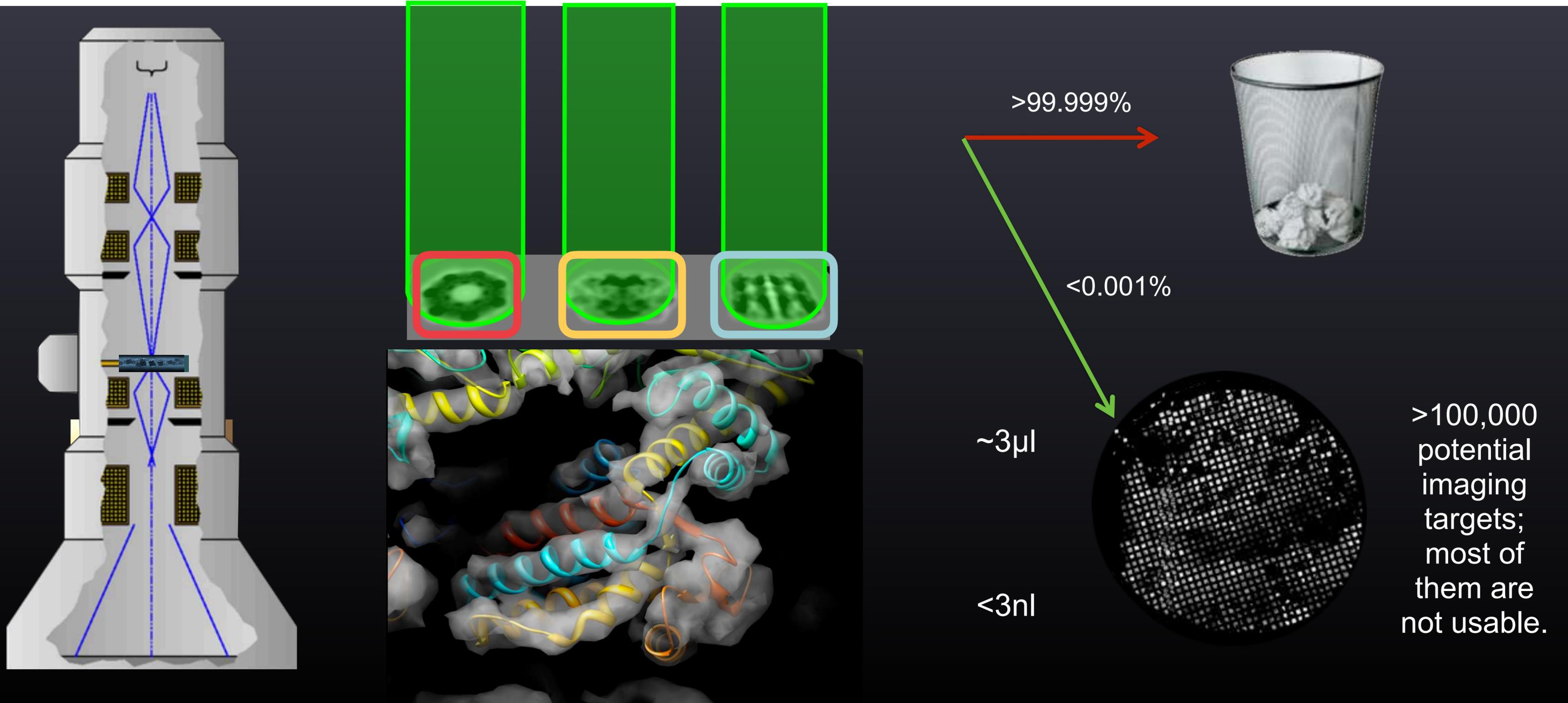
<3nl



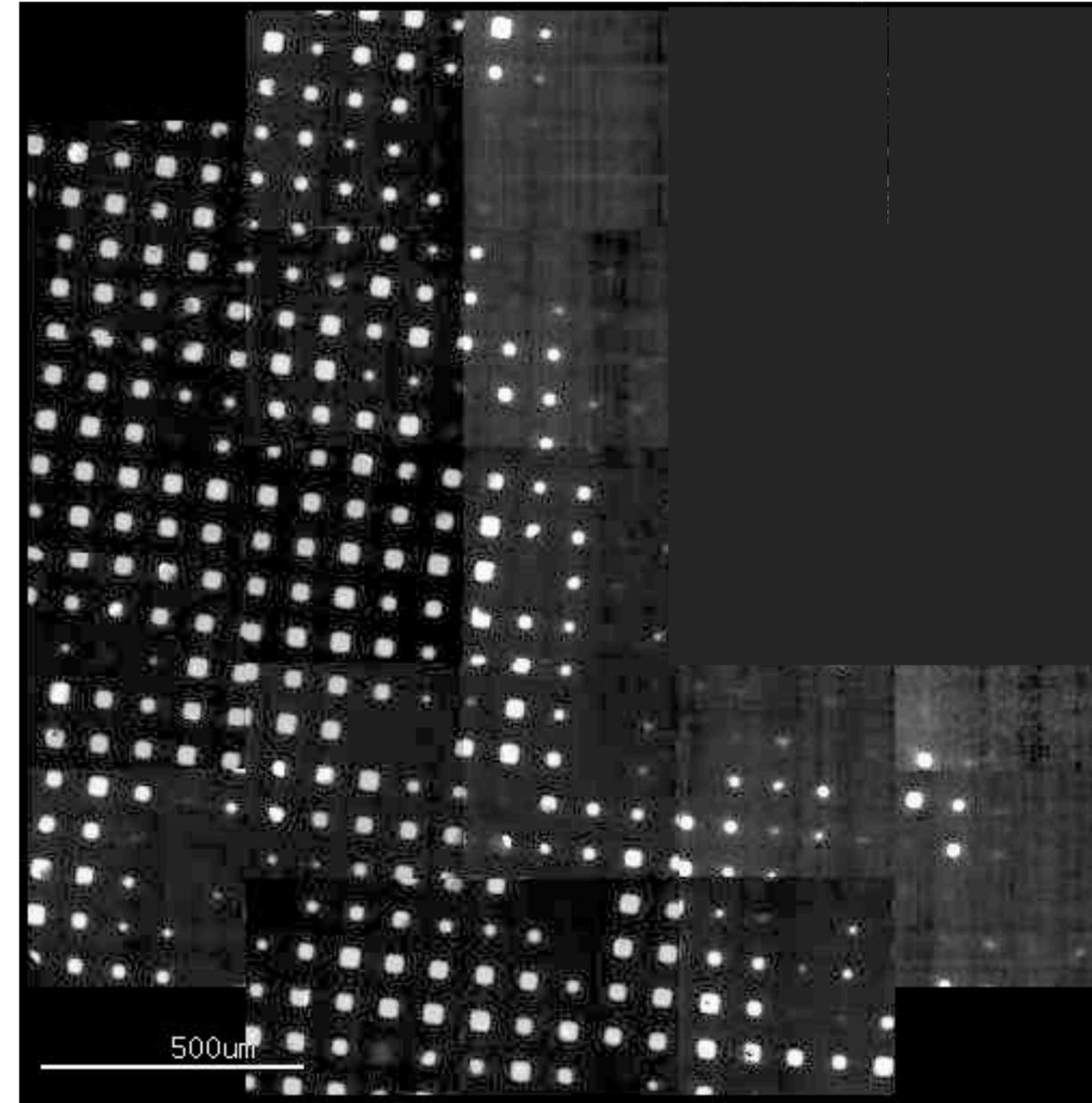
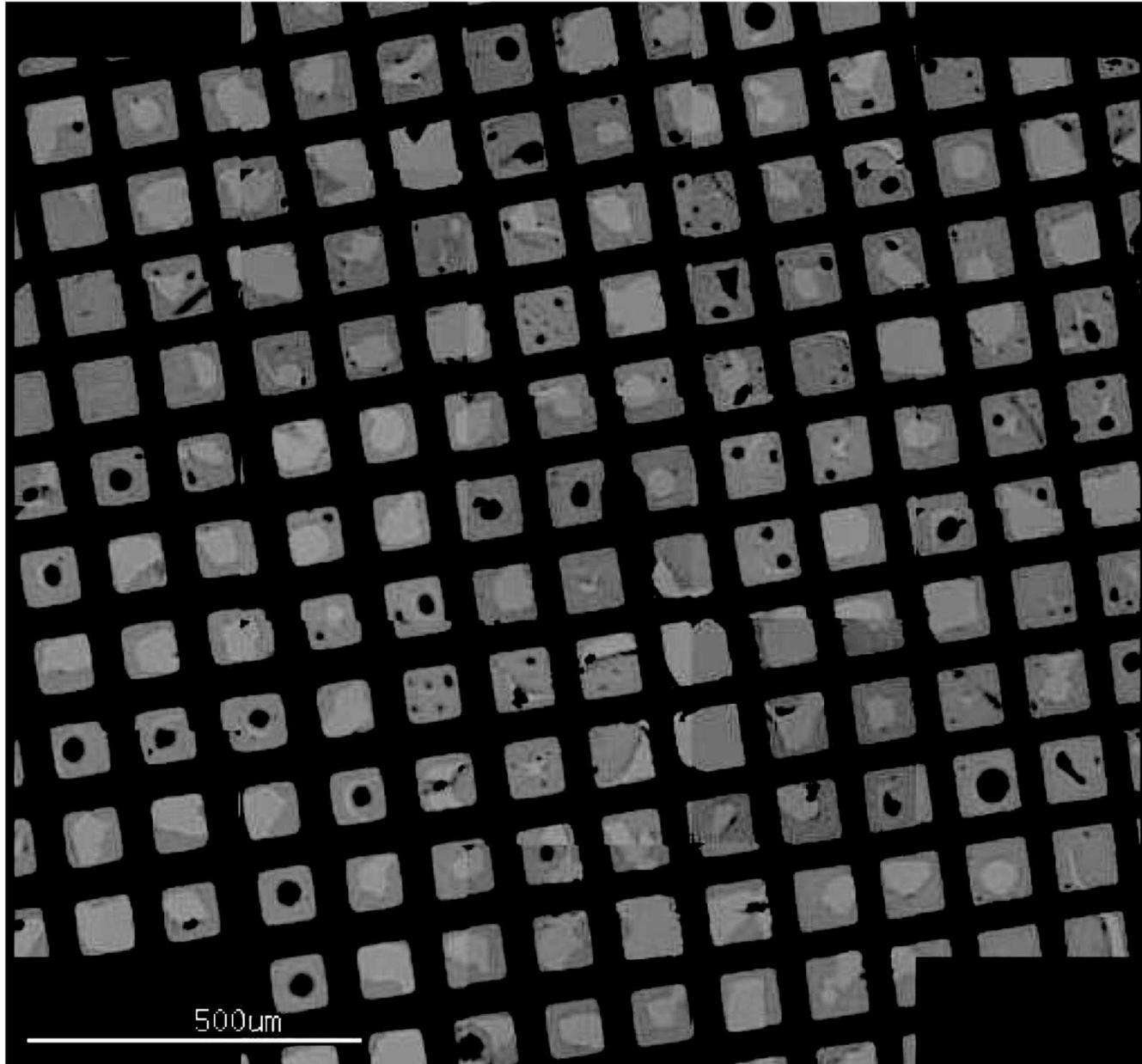
>100,000 potential imaging targets; most of them are not usable.

How are samples prepared for cryoEM?

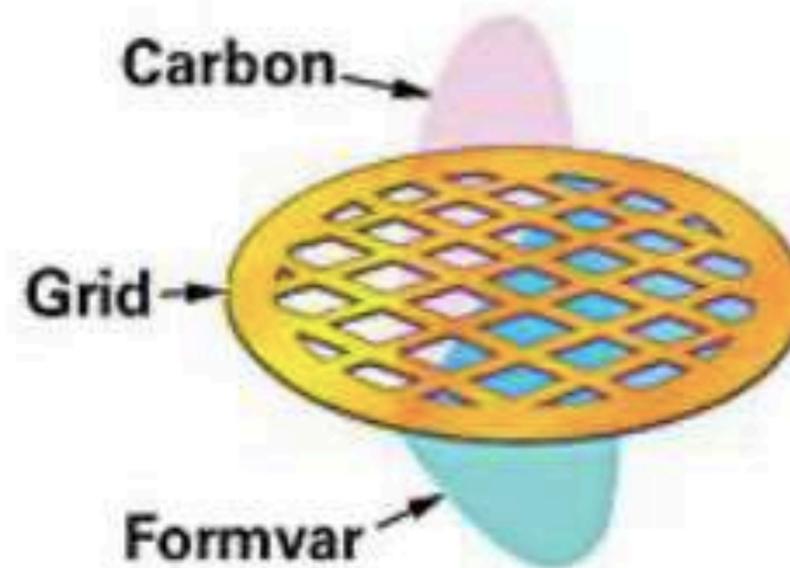
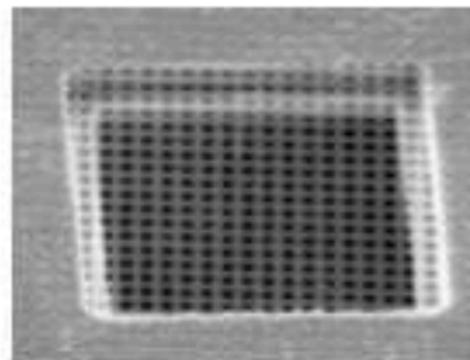
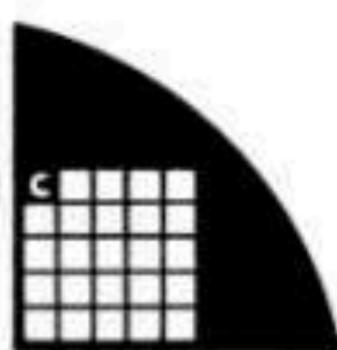
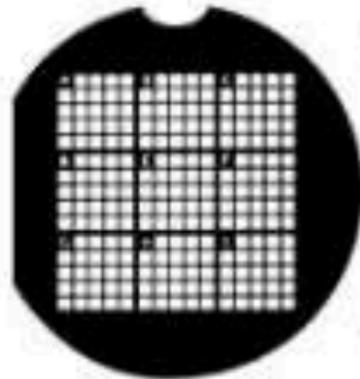
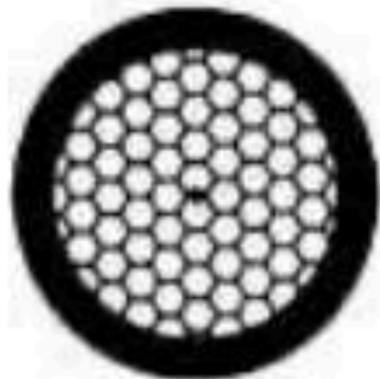
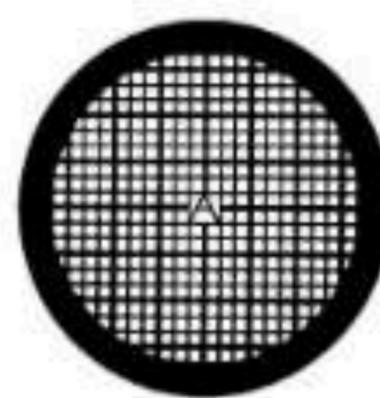
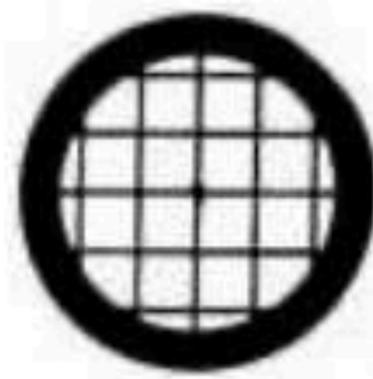
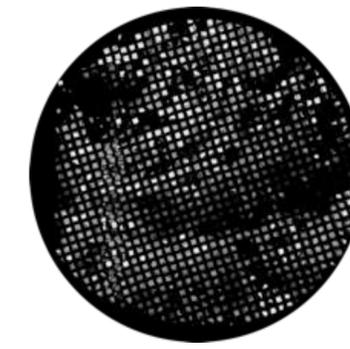
Vitrifying a biological sample



What do EM grids look like?



What do EM grids look like?



Common Materials

Copper

Nickel

Gold

Aluminum

Molybdenum

Titanium

Stainless Steel

https://www.tedpella.com/grids_html/

What do EM grids look like?

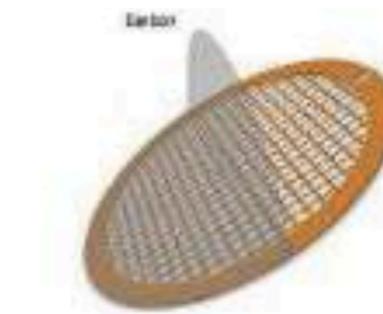
TERMINOLOGY

Grid (Cu, Au, Mo, etc...)

- mesh

Foil (C, Au, etc...)

- Continuous
- lacy
- holey (hole size and spacing)



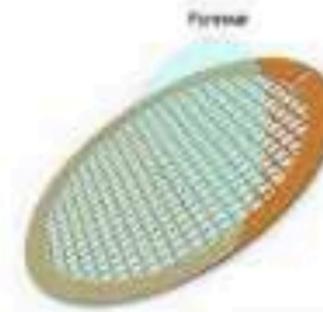
CARBON ONLY SUPPORT FILMS



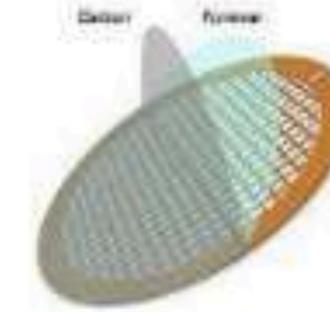
HOLEY CARBON SUPPORT FILMS



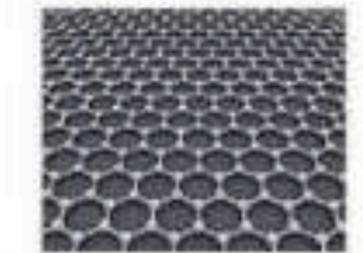
LACEY CARBON SUPPORT FILMS



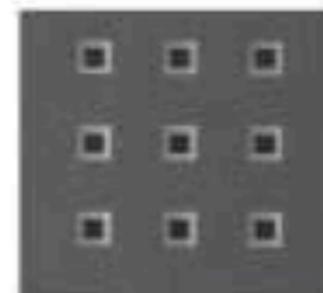
FORMVAR ONLY SUPPORT FILMS



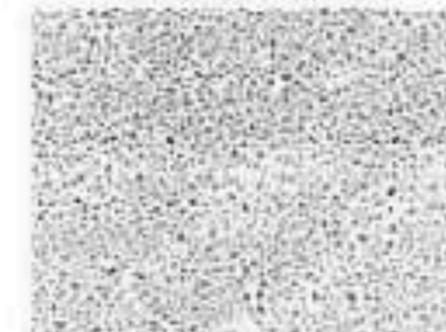
FORMVAR / CARBON
SUPPORT FILMS



EM-TEC GRAPHENE SUPPORT FILMS



EM-TEC SILICON NITRIDE
SUPPORT FILMS



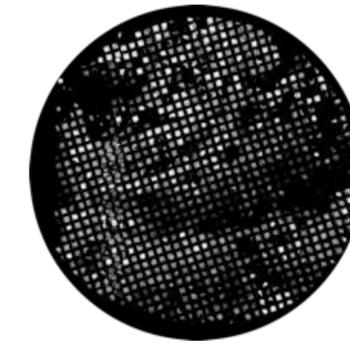
TEM CALIBRATION & TEST STANDARDS



TEM GRID STORAGE BOXES

<https://edgescientific.com/product-category/tem-supplies/tem-support-films/>

What do EM grids look like?



Rough grid parameters

Rim Width: 350-400 μ m.

Thickness: approximately 25 μ m thick.

Diameter: 3.0 to 3.05mm

Pitch: Is 1"/mesh or 25.4mm/mesh

Example 200 mesh pitch = $25.4/200 = 127\mu$ m

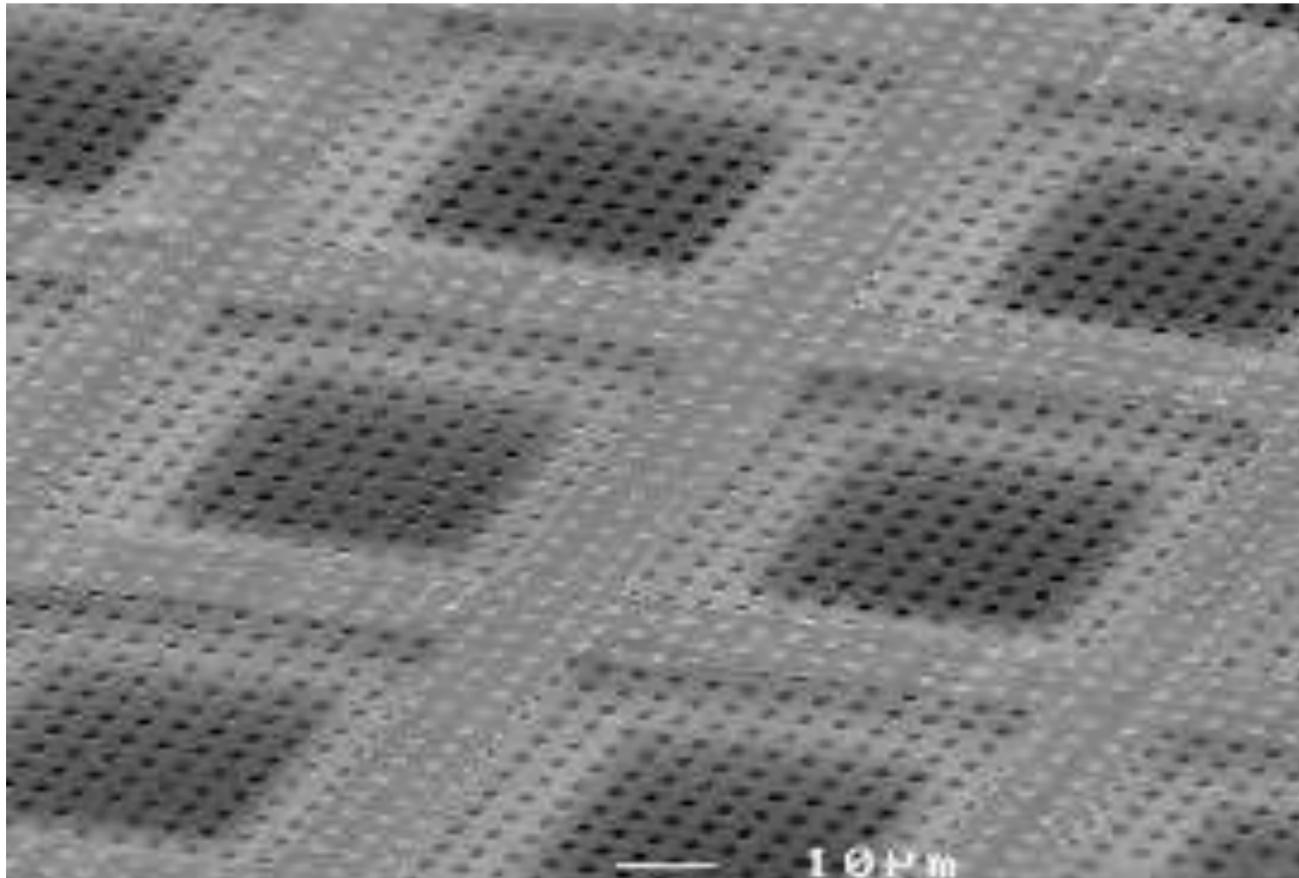
PELCO® Grid Size

Square Mesh	Pitch μ m	Hole μ m	Bar μ m	% Trans-mission
50	508	425	83	70
75	339	284	55	70
100	254	204	50	65
150	169	125	44	60
200	127	90	37	50
300	85	54	31	40
400	64	38	26	35
500	51	28	23	30

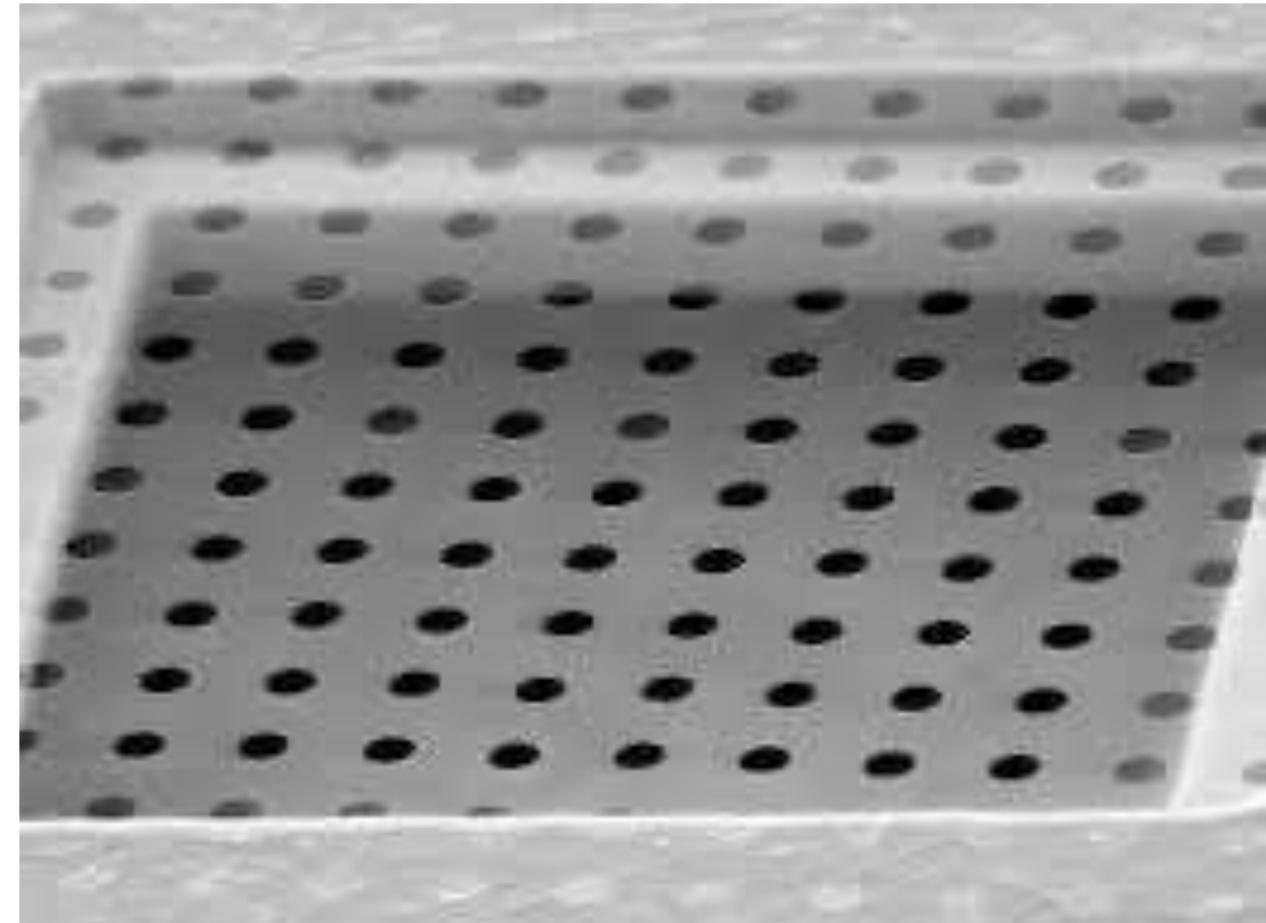
What do EM grids look like?



TERMINOLOGY

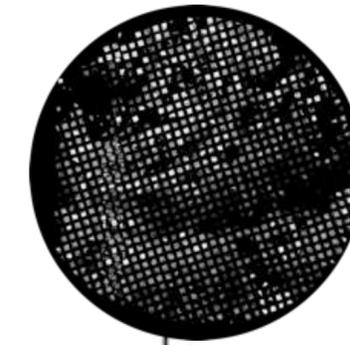


- Protochips.com



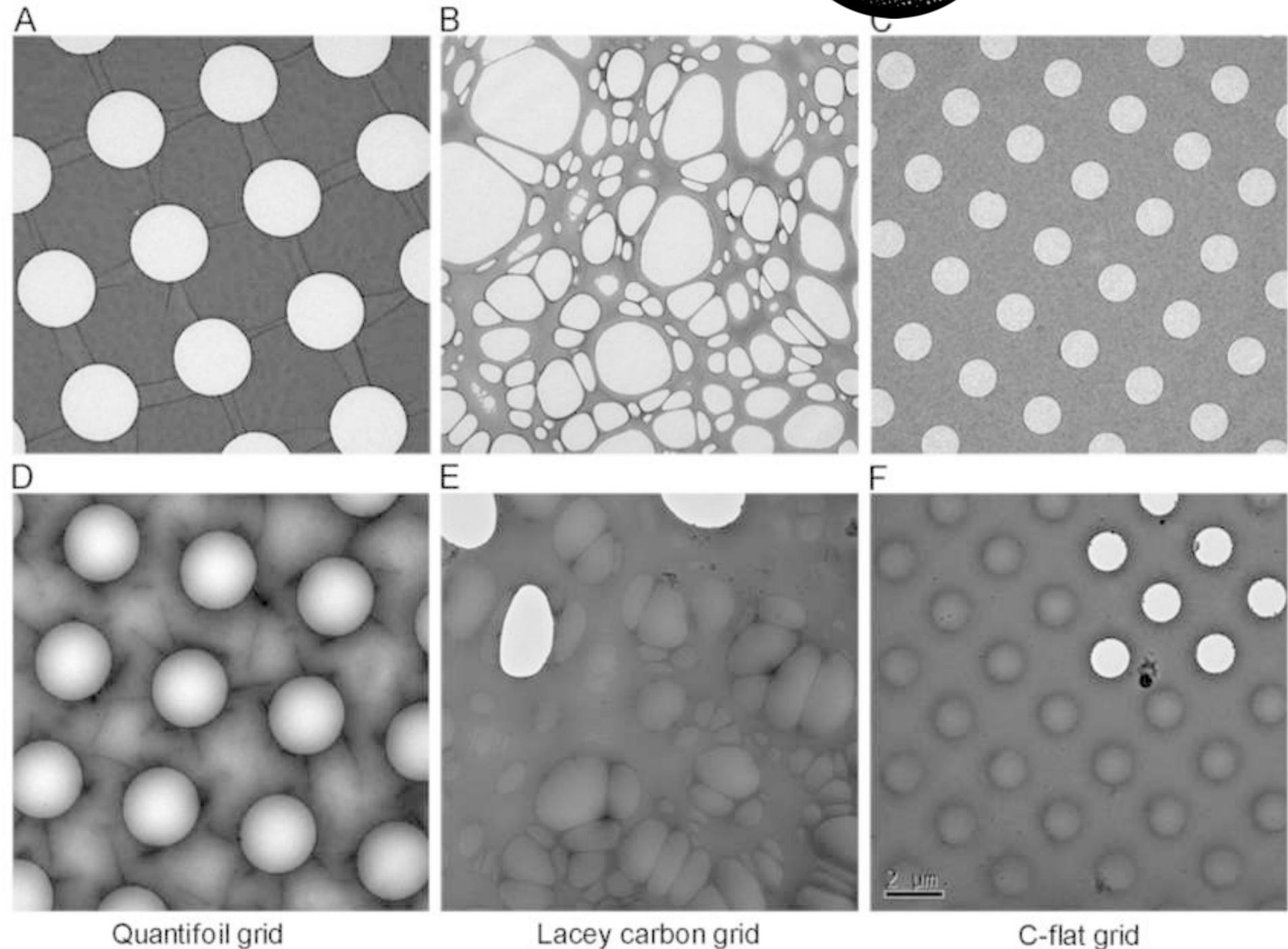
- Quantifoil.com

What do EM grids look like?



TERMINOLOGY

Cho, Hye-Jin & Hyun, Jae-Kyung & Kim, Jin-Gyu & Jeong, Hyeong & Park, Hyo & You, Dong-Ju & Jung, Hyun. (2013). Measurement of ice thickness on vitreous ice embedded cryo-EM grids: investigation of optimizing condition for visualizing macromolecules. *Journal of Analytical Science and Technology*. 4. 10.1186/2093-3371-4-7.



What do EM grids look like?

TERMINOLOGY

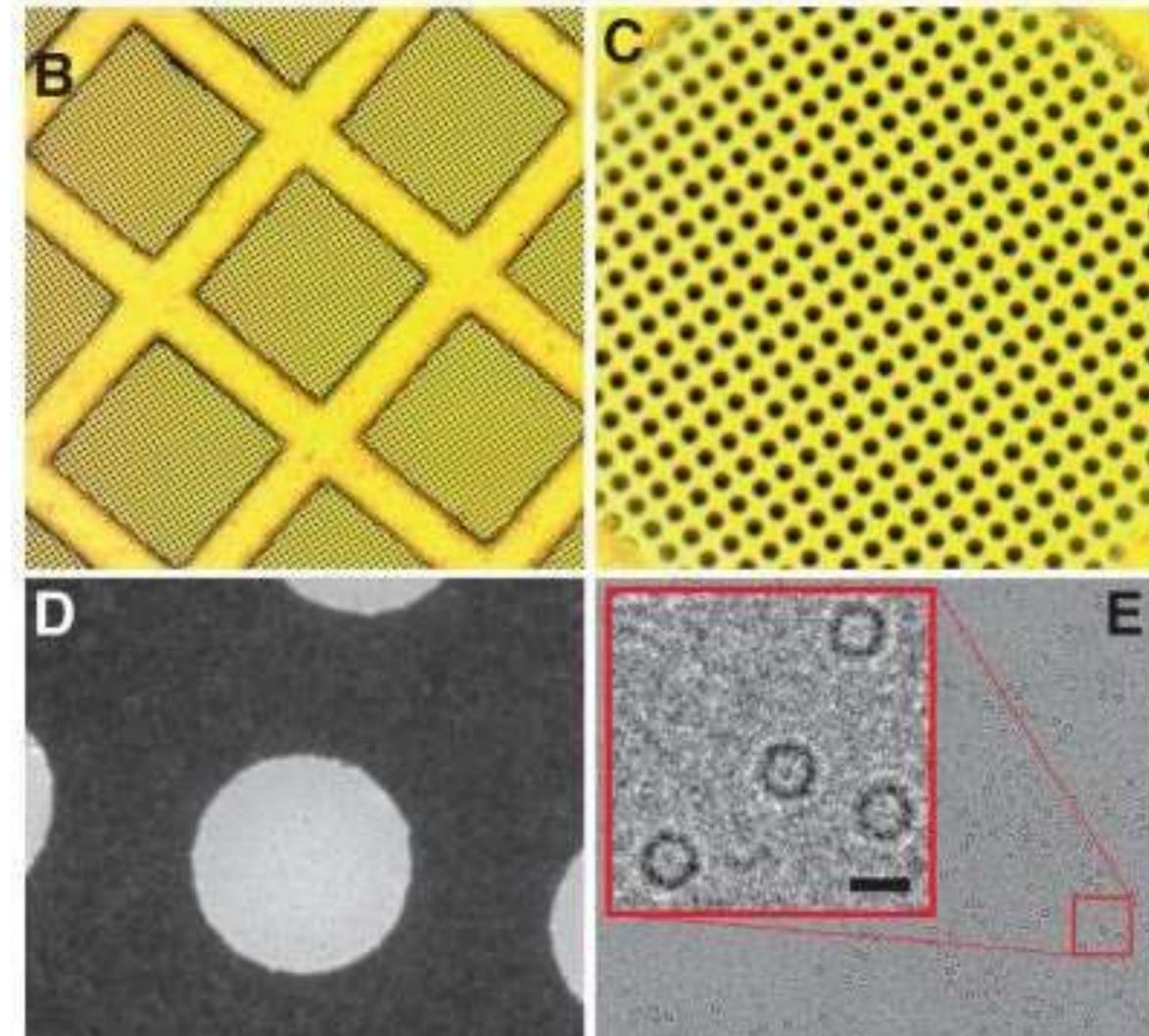
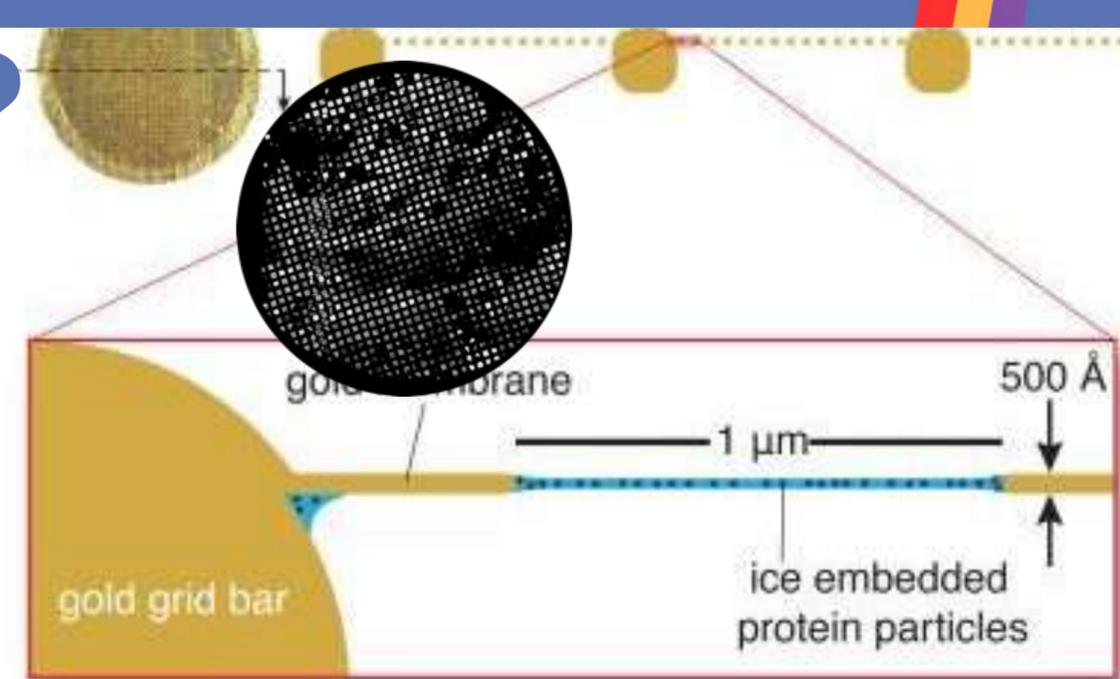
- Holey gold foil on gold mesh grid

Advantages:

- Prevents differential thermal contraction when freezing
- Reduces beam-induced specimen movement
- Combined with direct detector technology allows for near atomic resolution

Disadvantages:

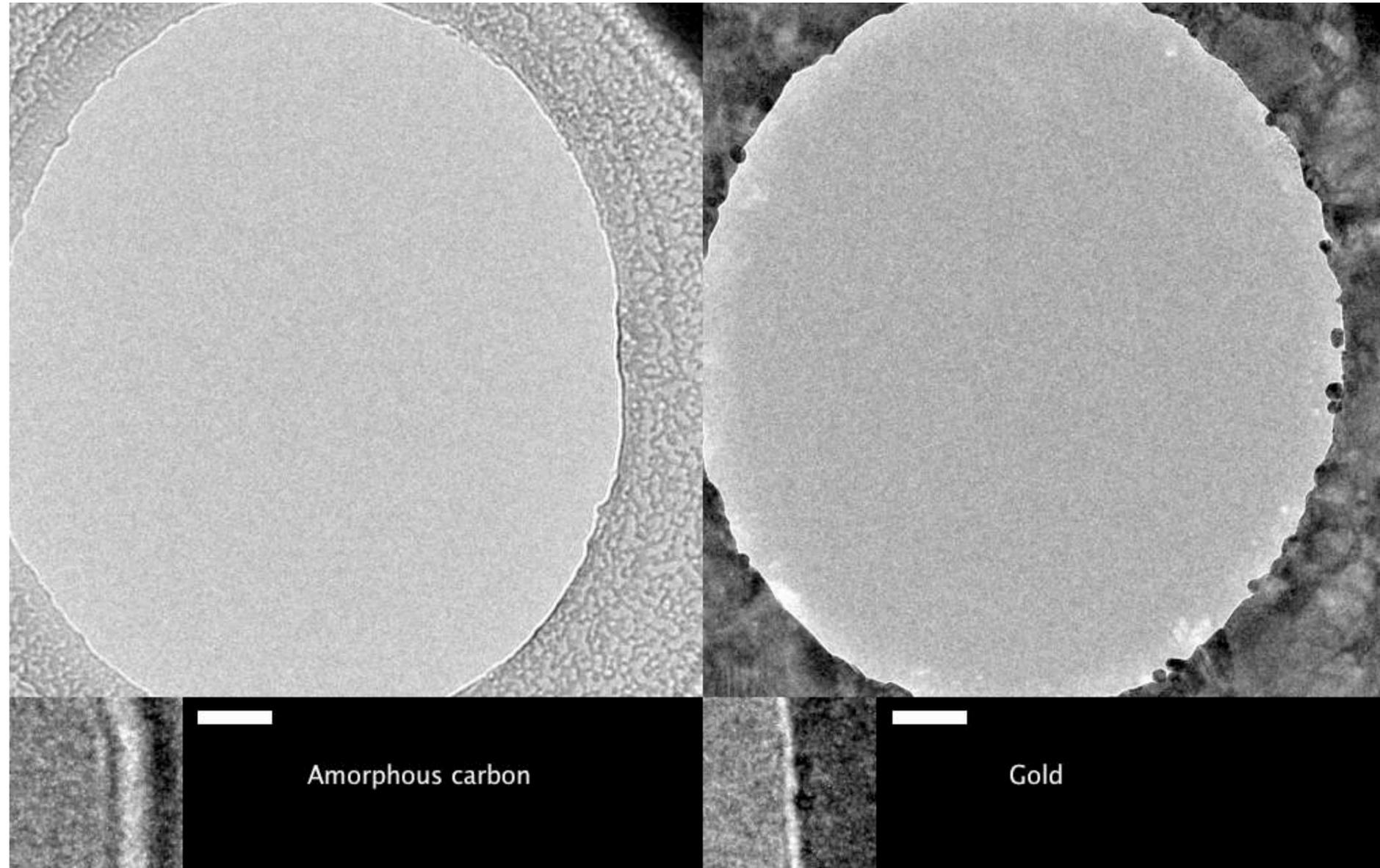
- Difficult to find focus due to lack of amorphous substrate



What do EM grids look like?



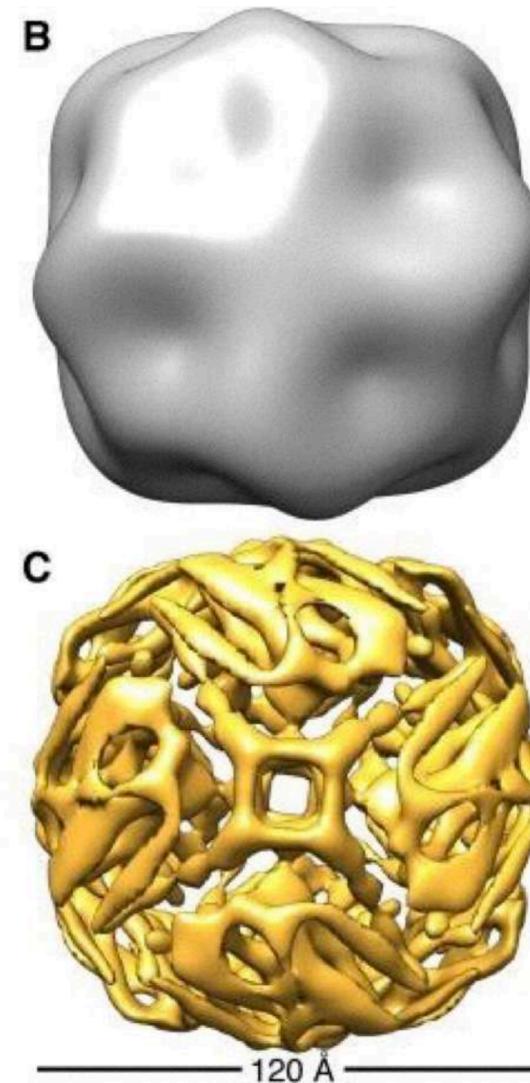
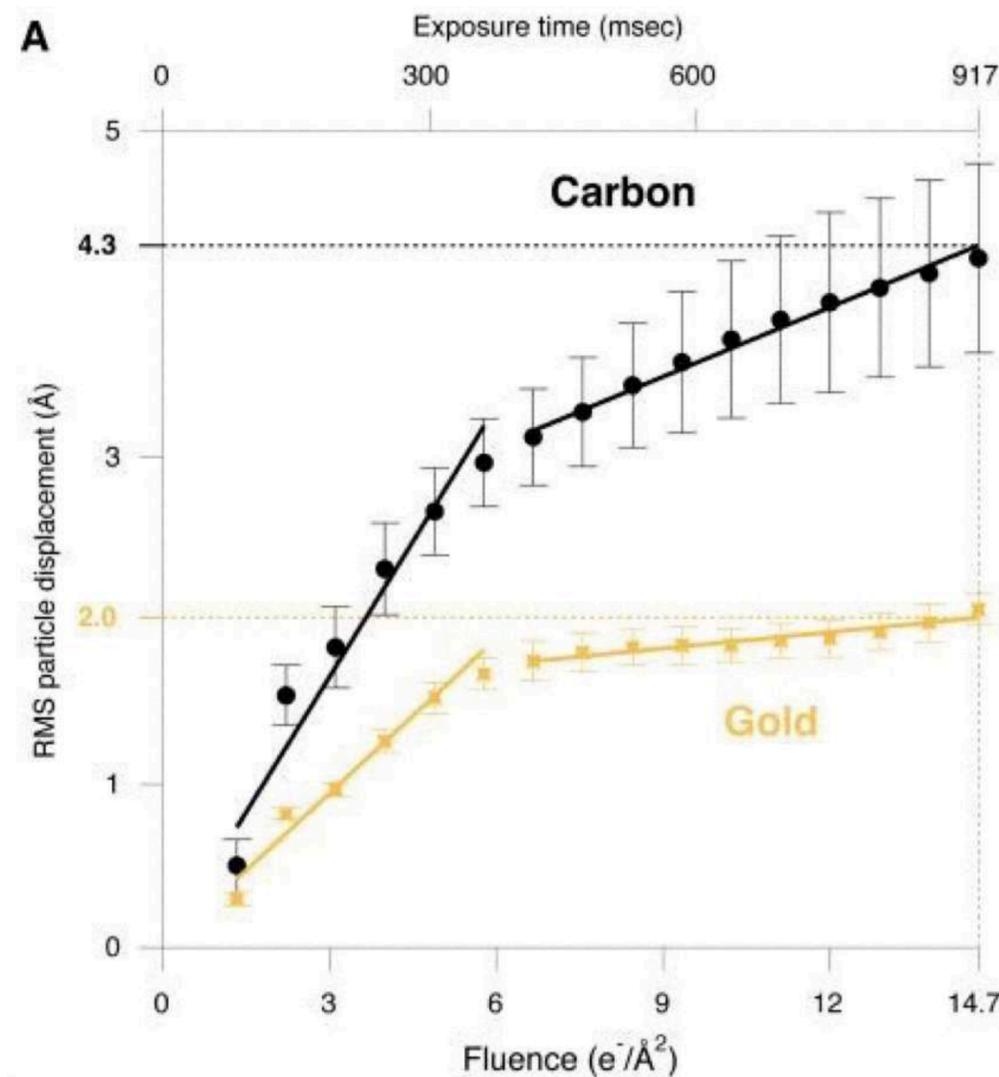
Gold grids



What do EM grids look like?

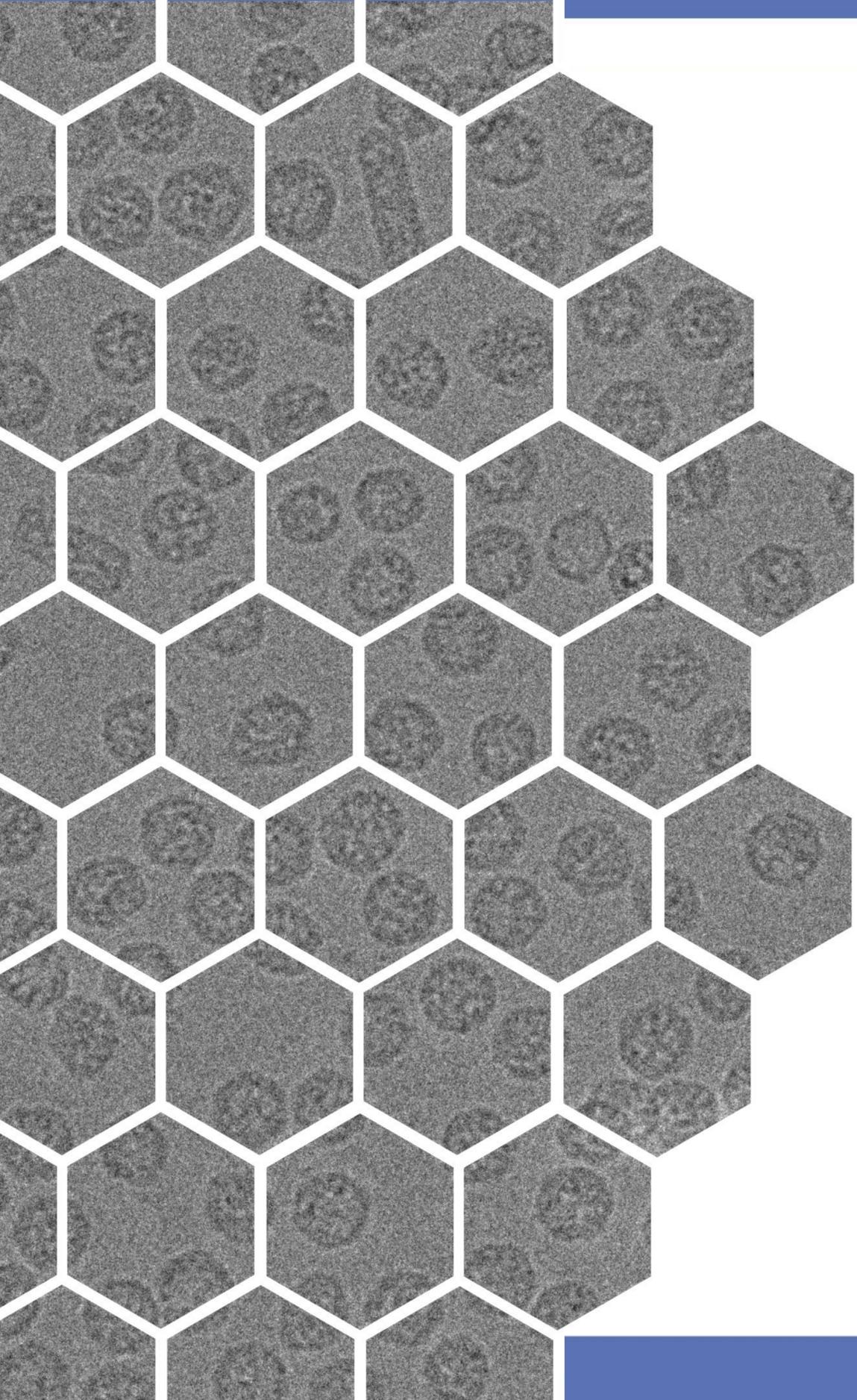


Gold grids



A. 80S ribosome movement during irradiation supported by amorphous carbon and gold using same imaging conditions.

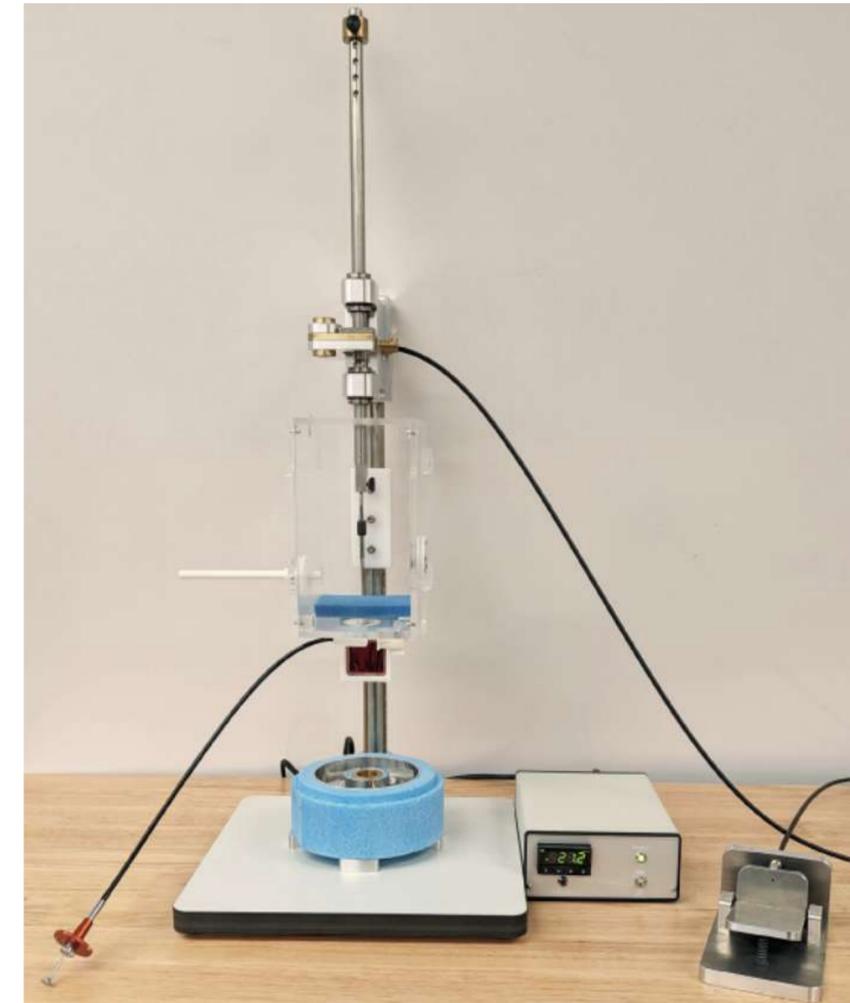
Apo ferritin density maps using same imaging conditions and identical processing for **B.** carbon and **C.** gold substrates. **B.** is at 25 Å and **C.** 8 Å resolution.



- ◆ Considerations for biological cryoEM
- ◆ Grids (our sample slide)
- ◆ What happens to a sample during vitrification
- ◆ Newer methods

How are samples prepared for cryoEM?

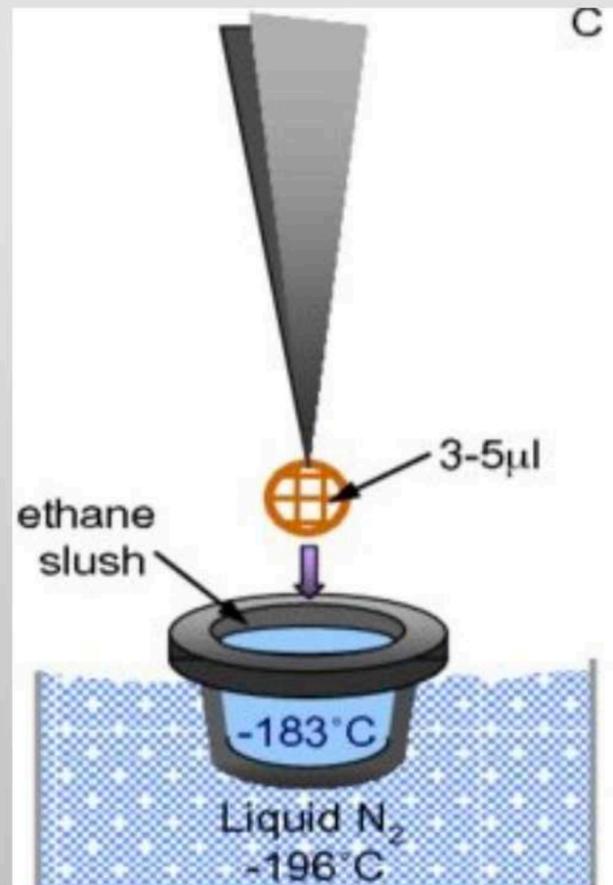
Vitrification process



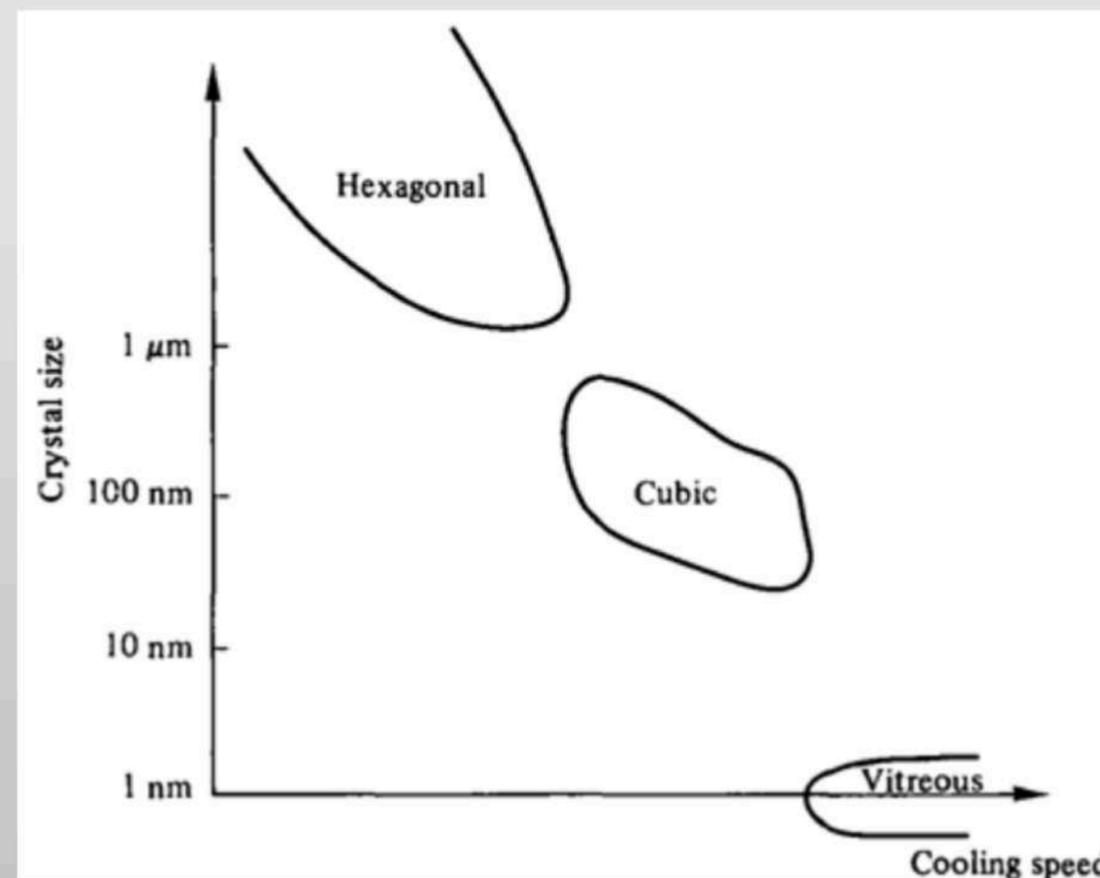
How are samples prepared for cryoEM?

Vitrification process

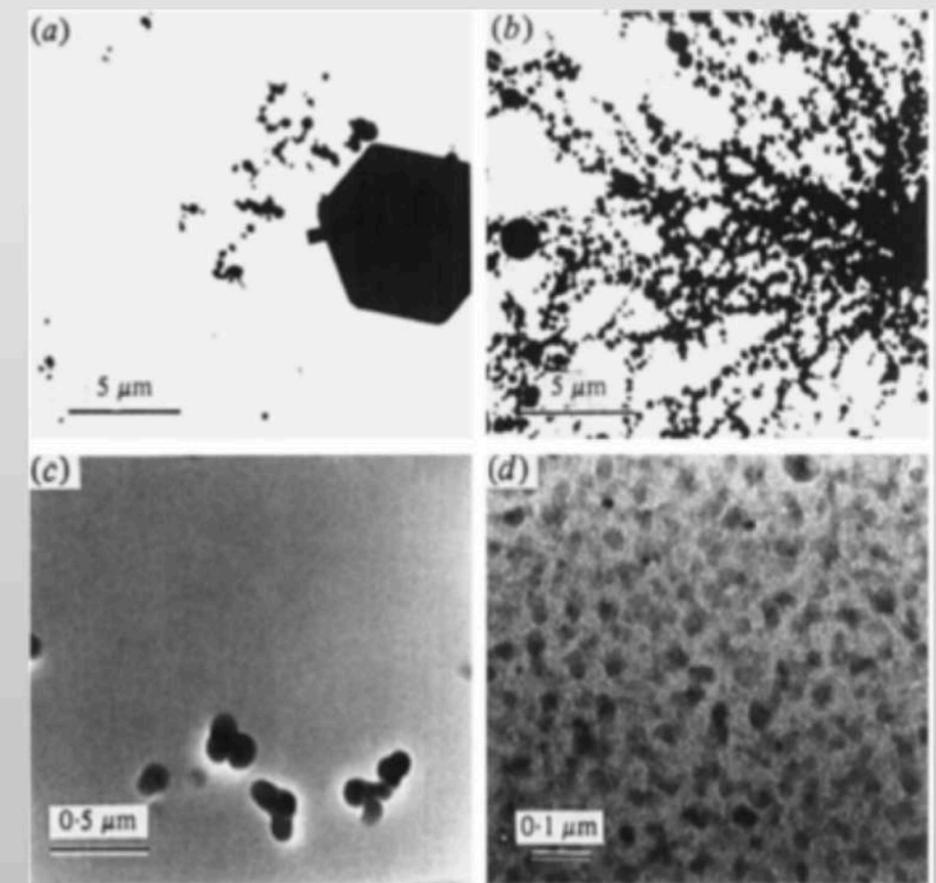
- Liquid ethane is a suitable coolant.
- Liquid nitrogen boils on contact, which makes it a poor coolant for cryo-EM.
- Cooling speed faster than 10^5 - 10^6 K/s ensure the formation of vitrified ice.



Setup of liquid ethane
(Image from Wen Jiang)



Cooling speed &
forms of ice

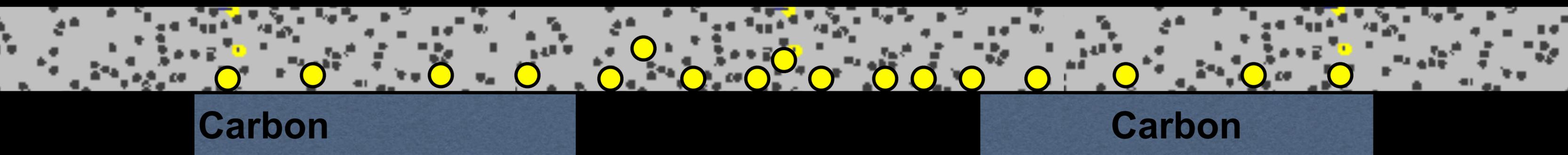


Different forms of ice contamination

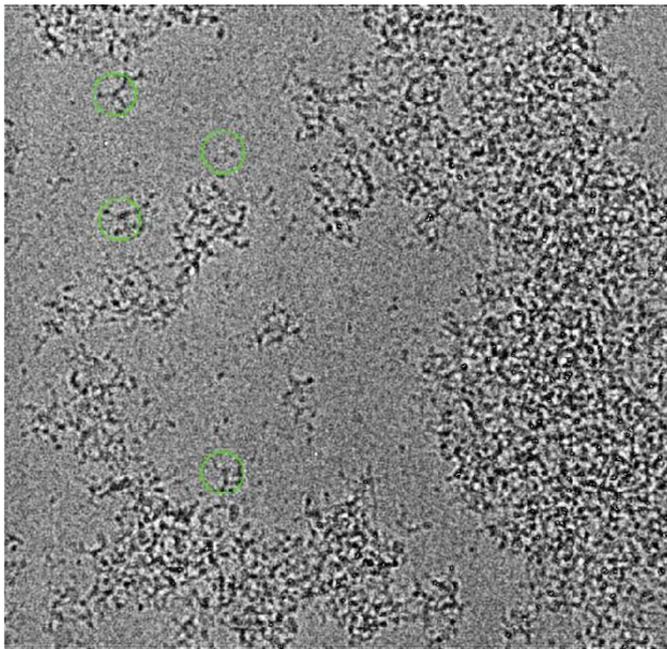
Jacques Dubochet et al., 1988

What happens to samples during vitrification?

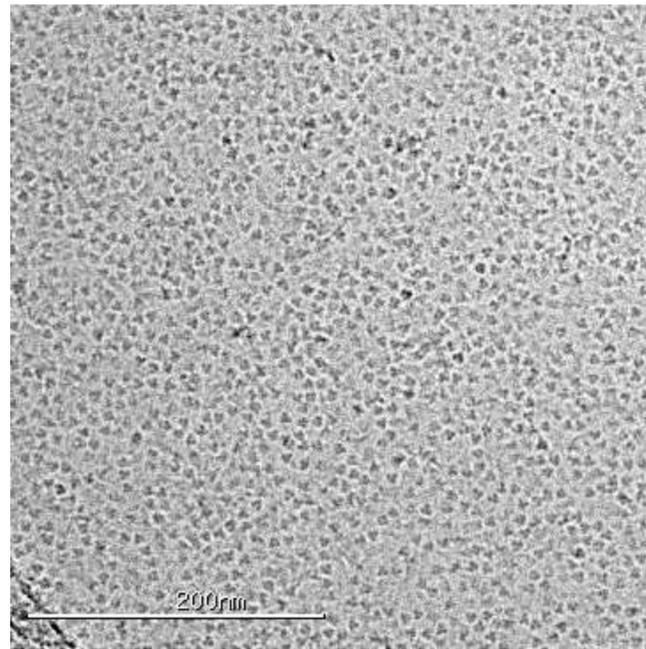
A hypothetical scenario during cryoEM grid preparation



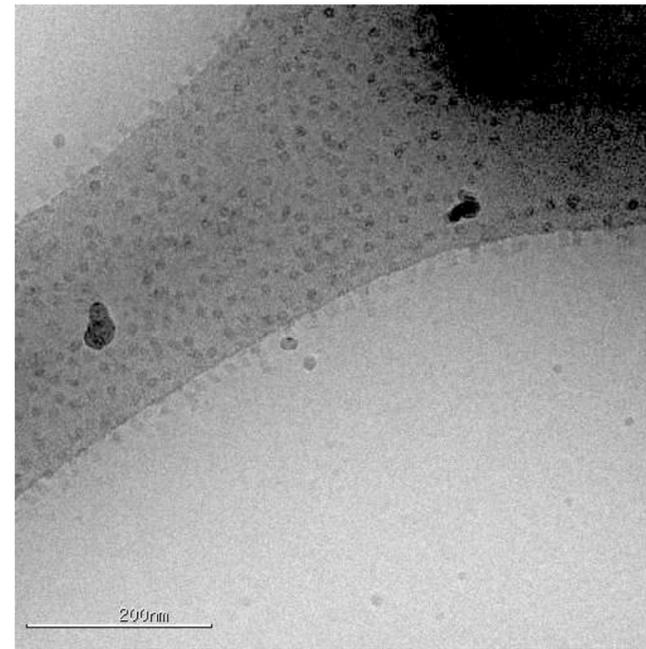
What issues arise?



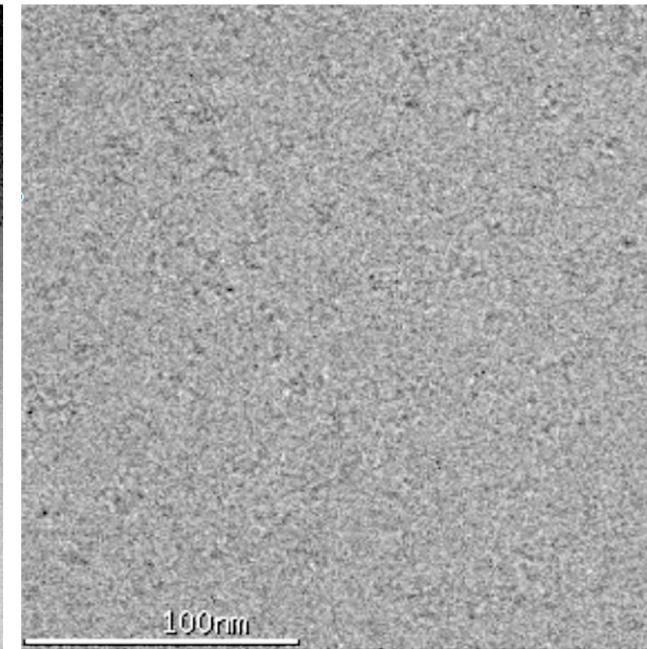
Aggregating in ice



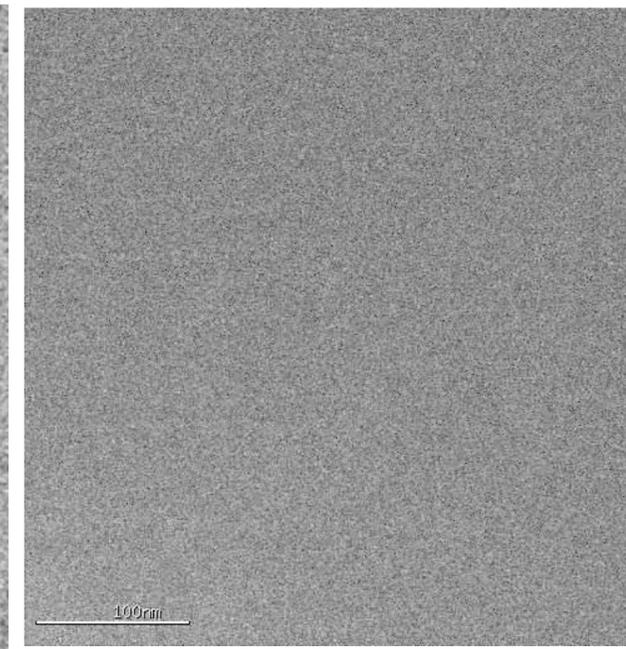
Preferred orientation



Particles not going into holes

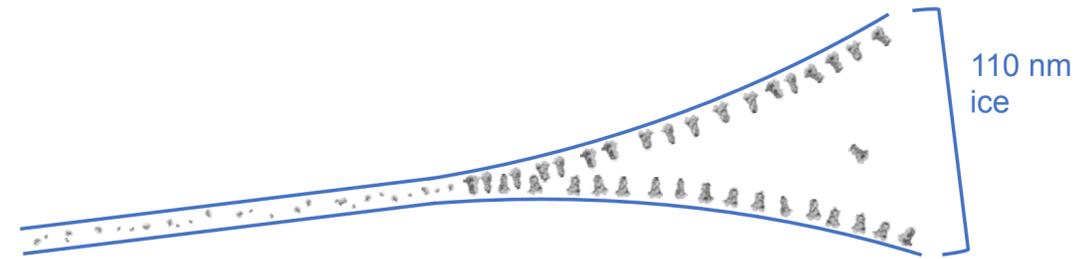


Rejecting 90% of particles



Particles disappearing in ice

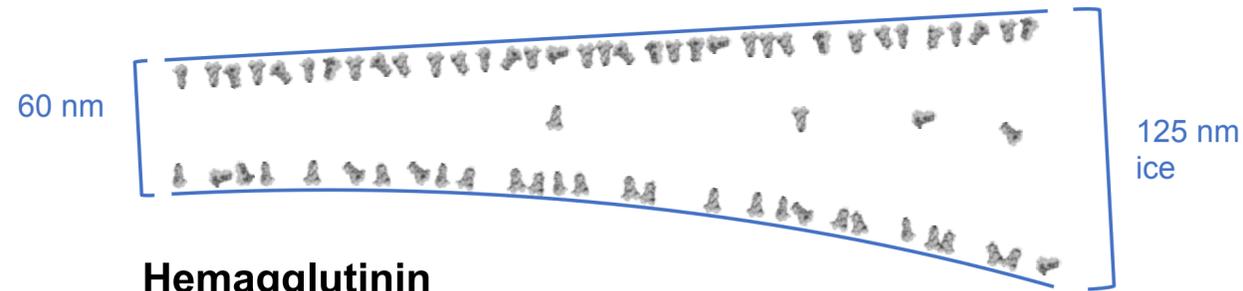
What issues arise?



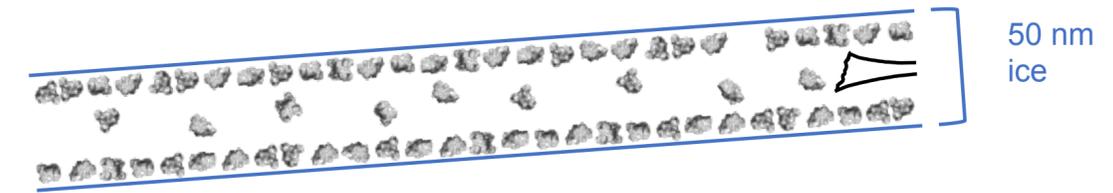
Hemagglutinin



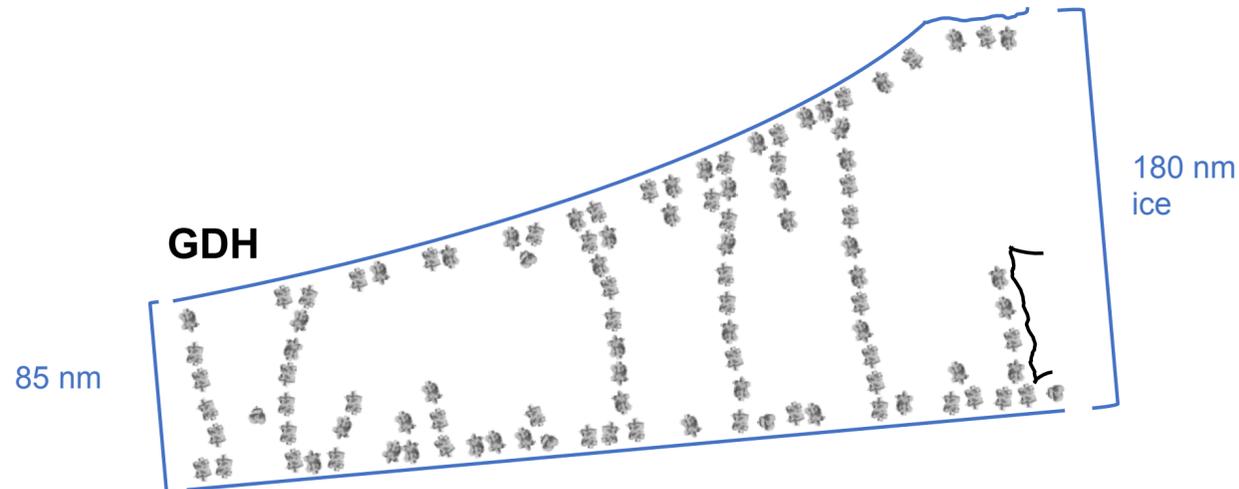
Aldolase



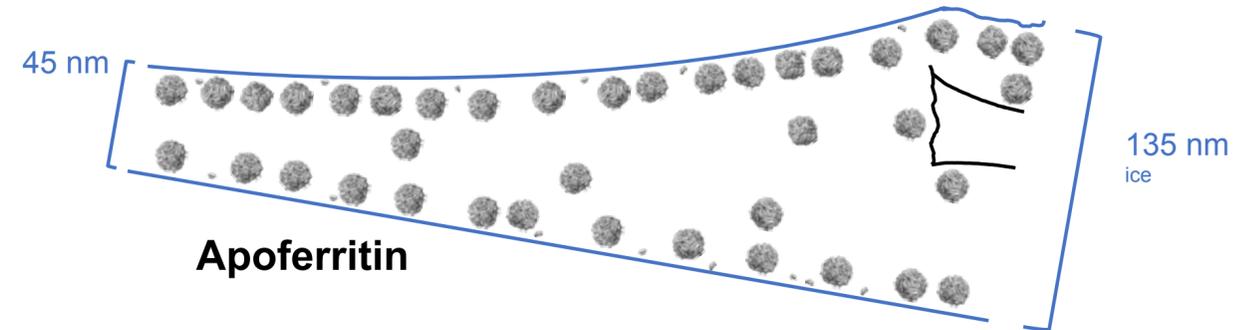
Hemagglutinin



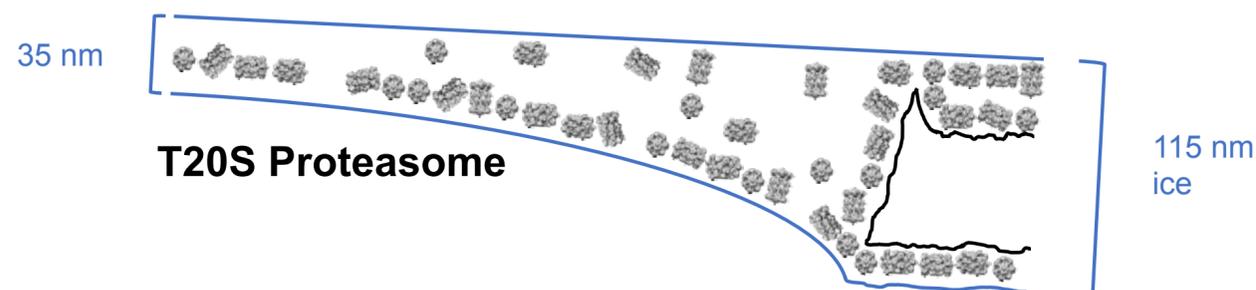
Aldolase



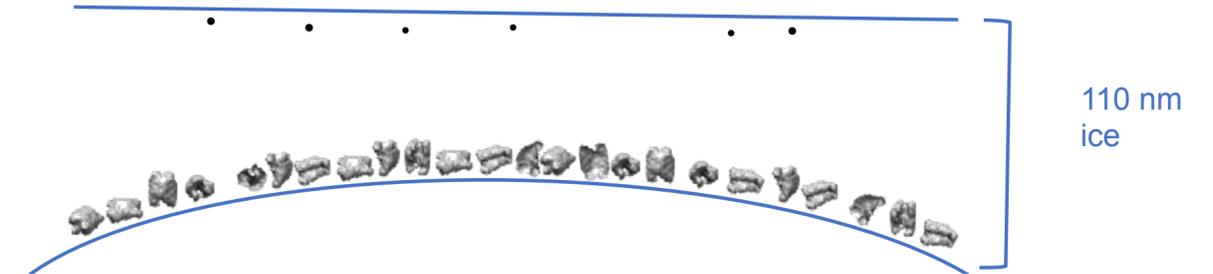
GDH



Apoferritin



T20S Proteasome



DNAB Helices

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



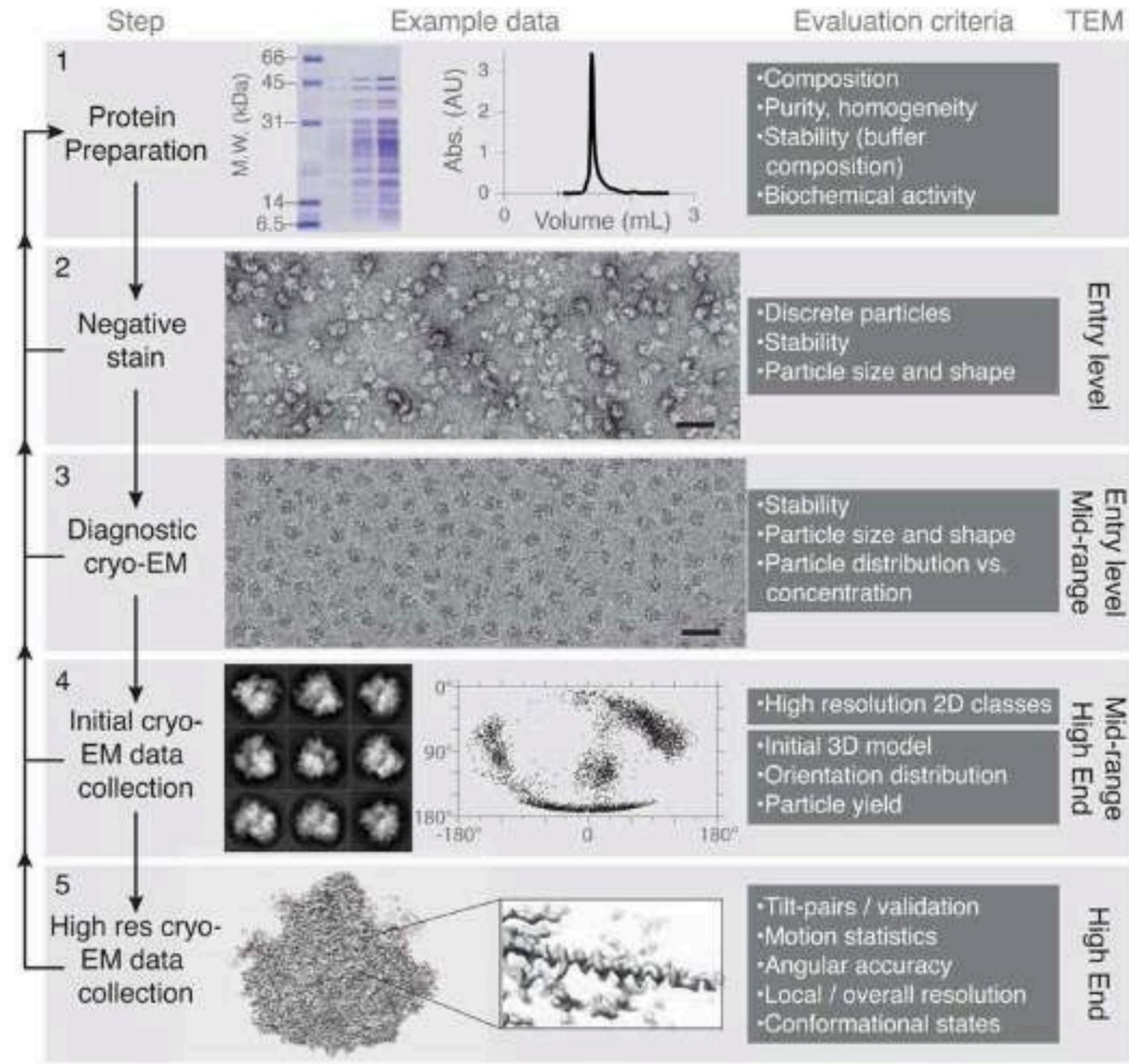
Alex Noble

Preparing EM ready samples

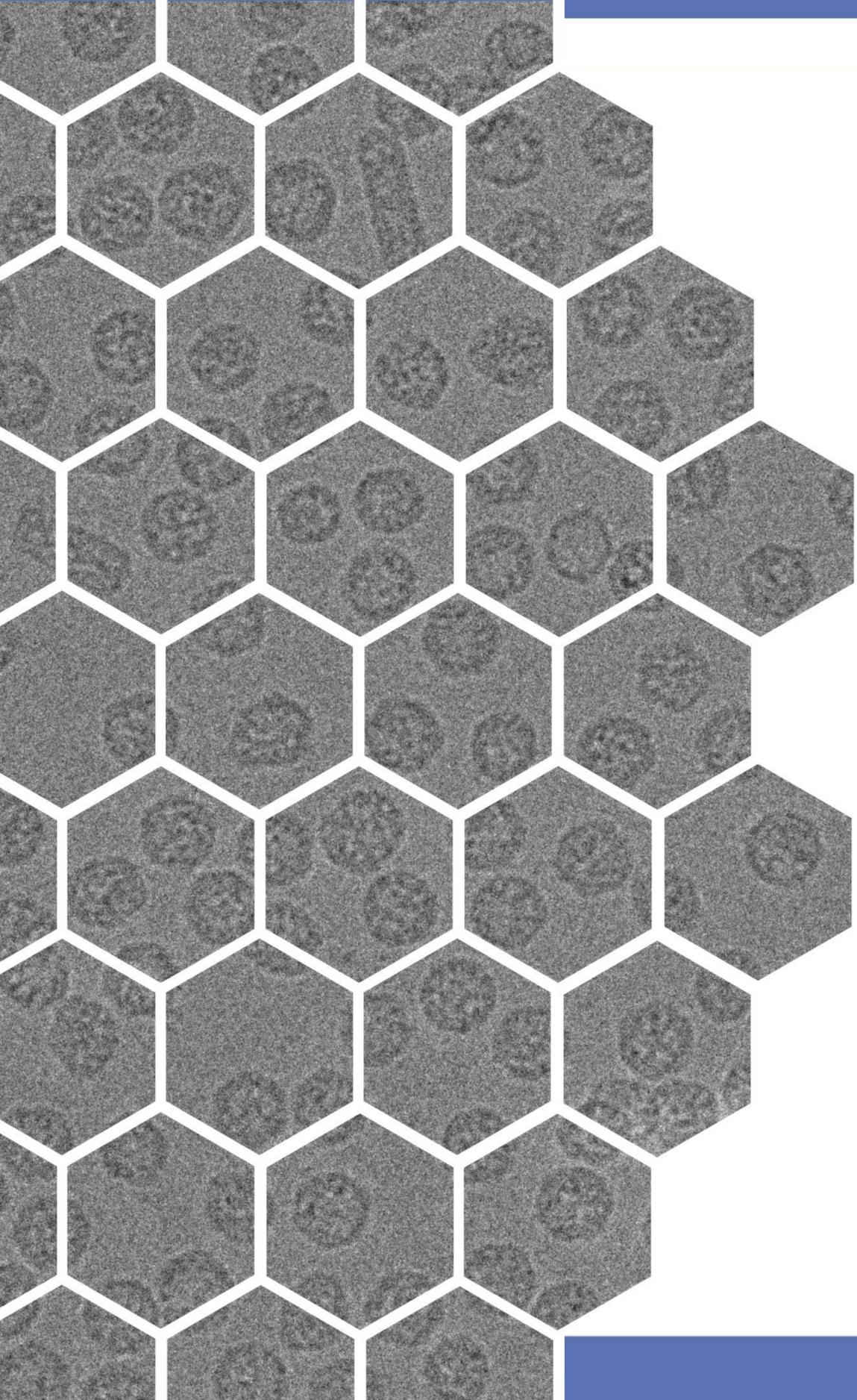
THE OPTIMIZATION WORKFLOW

Structure determination by cryo-EM.

A systematic approach to 3D structure determination is shown. In the left column, the major steps are listed. Each step should be performed successively and only after one has been completed successfully should the scientist move onto the next step. In the second column, example data are shown for ribosomes (details in text). Scale bars on the micrographs are 500 Å. Each step should be evaluated with the criteria listed in the third column, returning to earlier steps for troubleshooting.



<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5140023/>



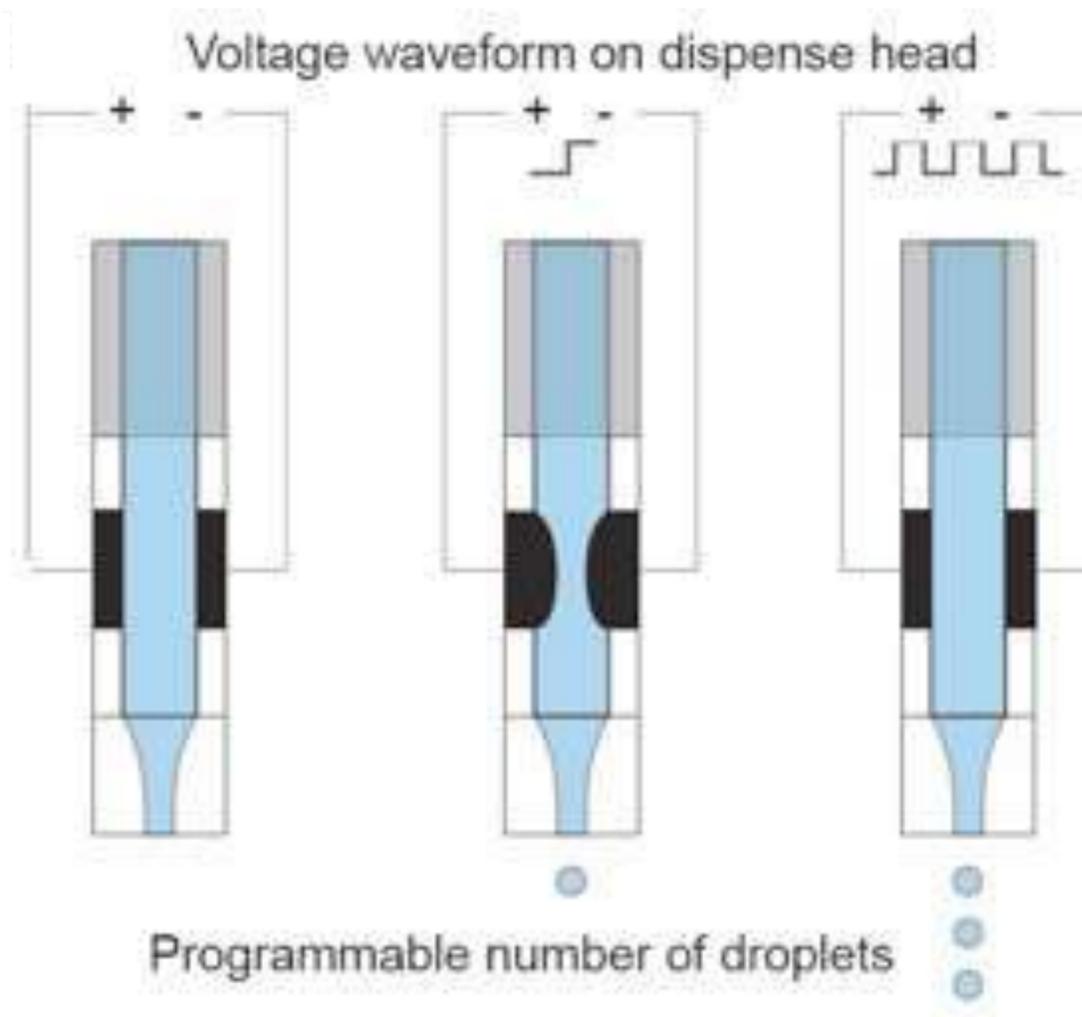
- ◆ Considerations for biological cryoEM
- ◆ Grids (our sample slide)
- ◆ What happens to a sample during vitrification
- ◆ Newer methods

Other methods

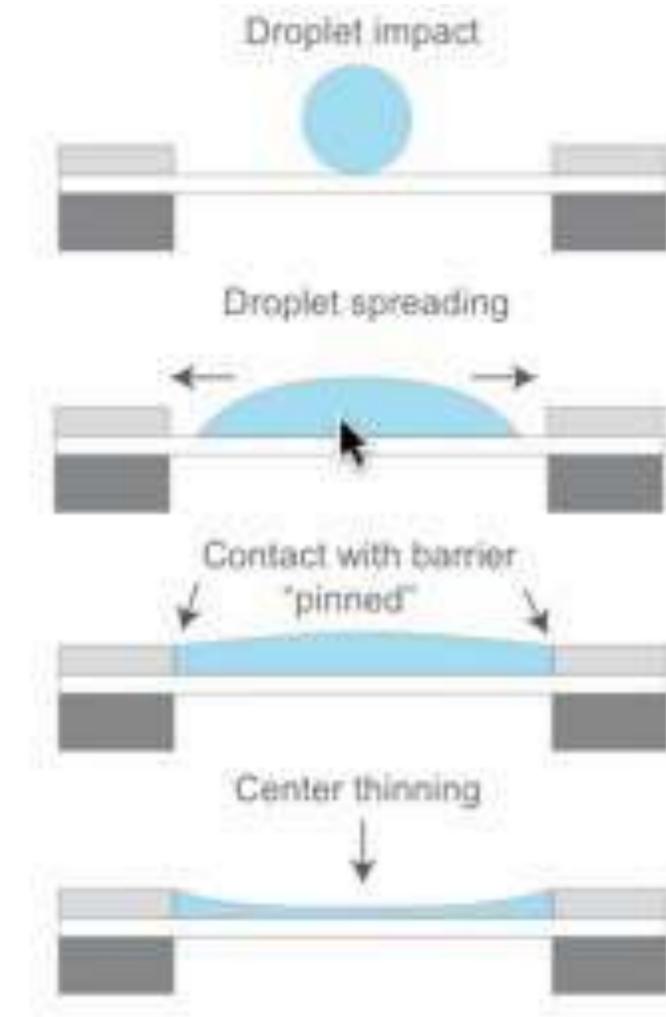


Improving Current CryoTEM Grid Preparation Methods

Accurate pL dispensing



Thin films without blotting



Dandey VP, Wei H,
Zhang Z, Tan YZ,
Acharya P, Eng ET,
Rice WJ, Kahn PA,
Potter CS, Carragher
B. Spotiton: New
features and
applications. Journal
of structural biology.
2018;202(2):161-9



Venkat Dandey



Hui Wei

Other methods



Improving Current CryoTEM Grid Preparation Methods

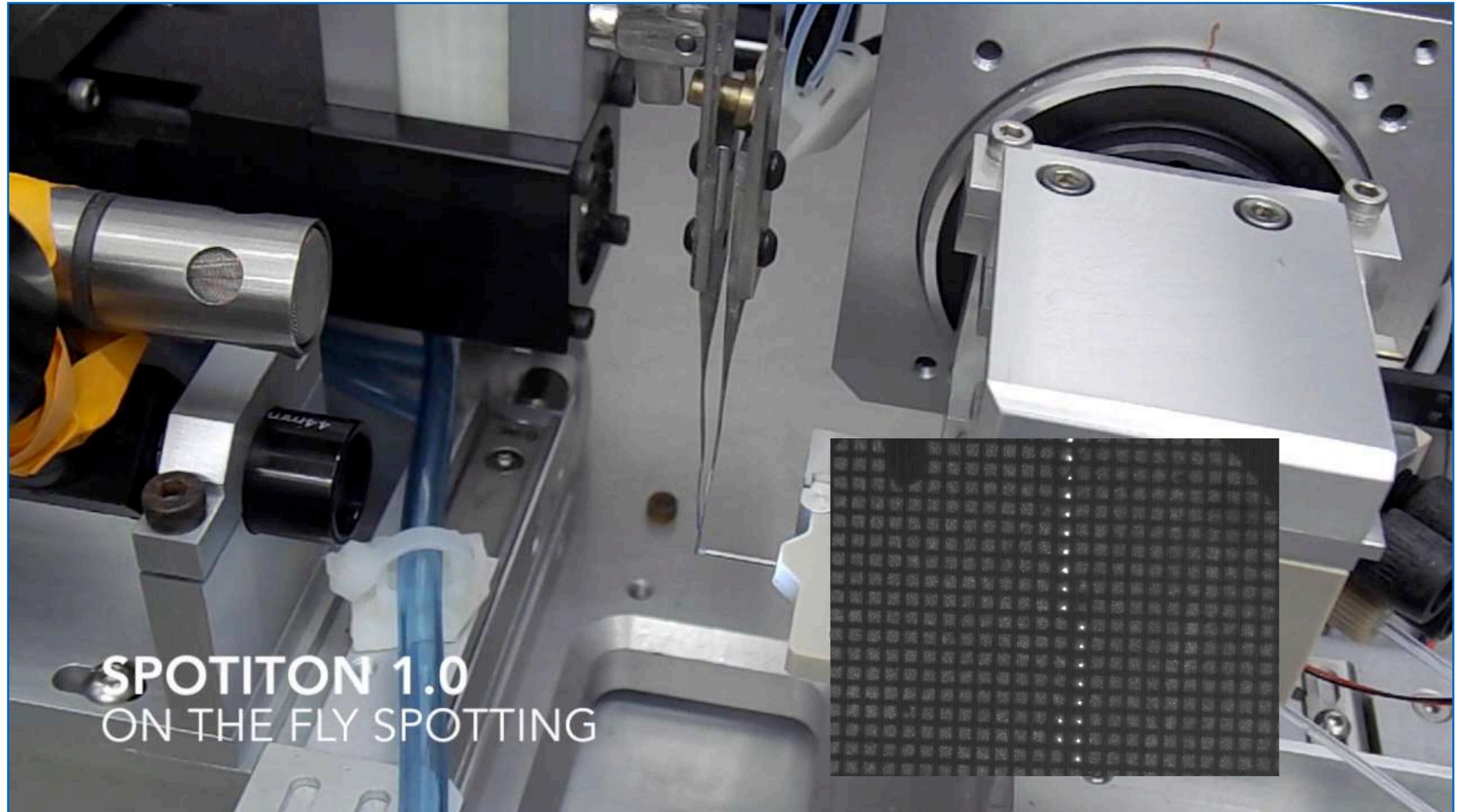
Dandey VP, Wei H,
Zhang Z, Tan YZ,
Acharya P, Eng ET,
Rice WJ, Kahn PA,
Potter CS, Carragher
B. Spotiton: New
features and
applications. Journal
of structural biology.
2018;202(2):161-9



Venkat Dandey



Hui Wei



Other methods



Improving Current CryoTEM Grid Preparation Methods

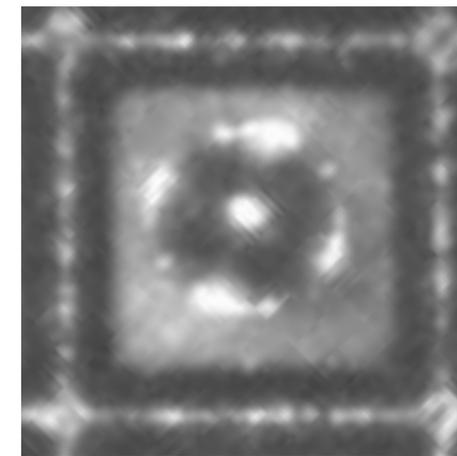
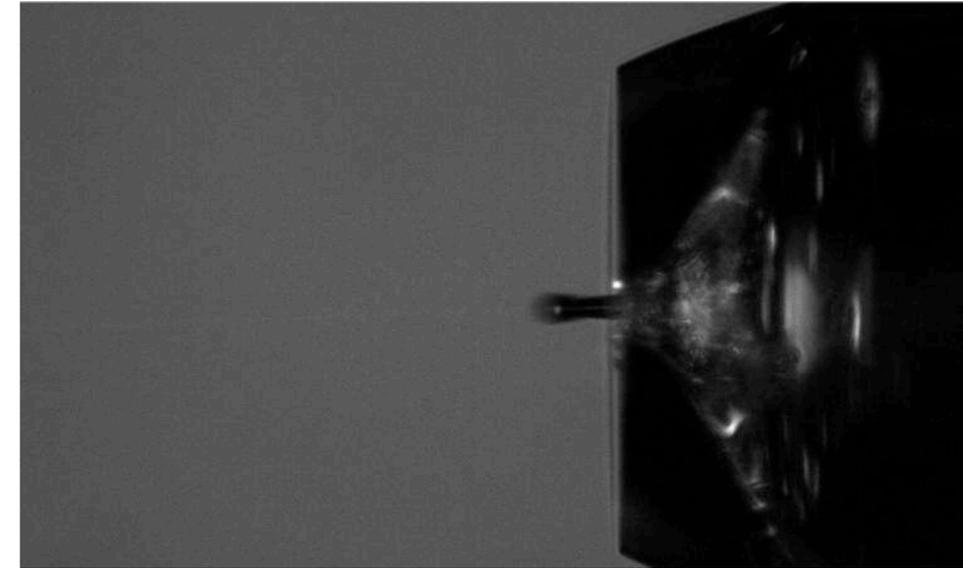
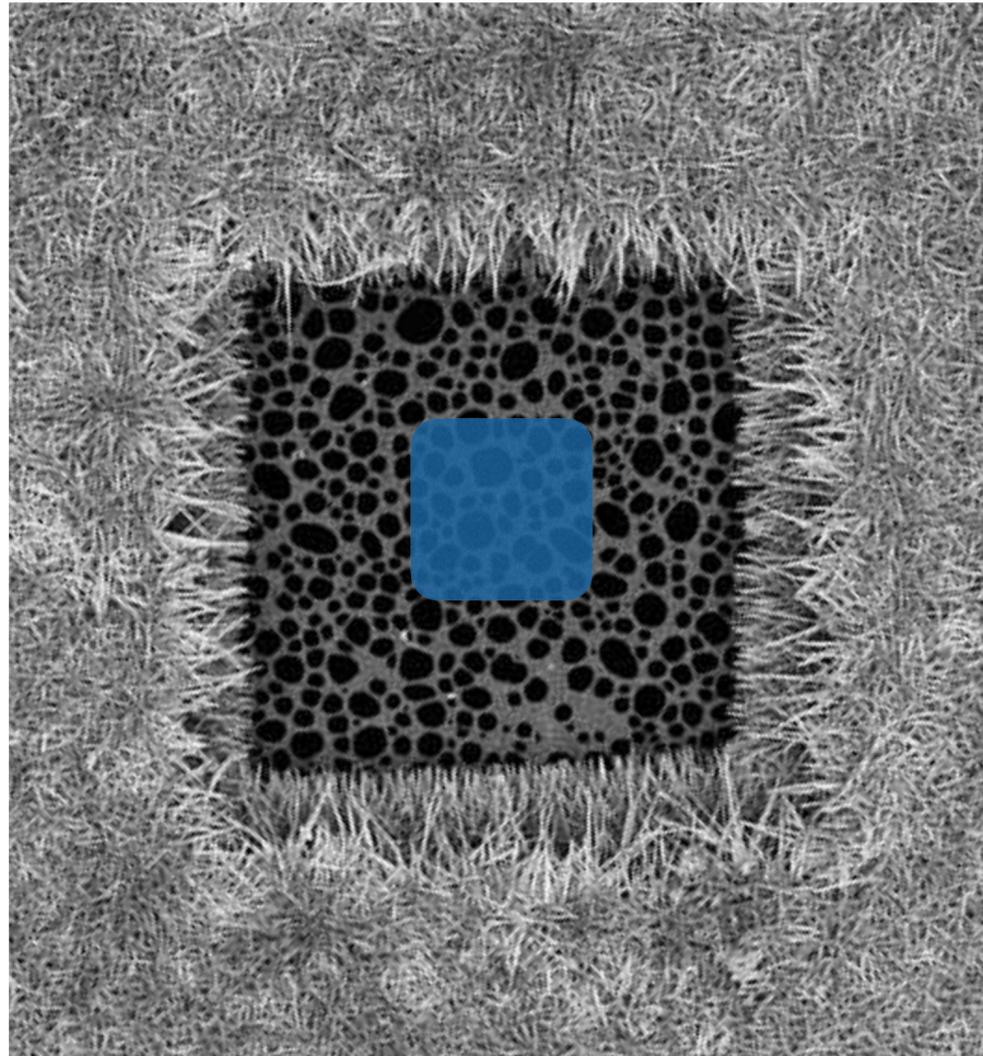
Wei H, Dandey VP,
Zhang Z, Raczkowski
A, Rice WJ,
Carragher B, Potter
CS. Optimizing "self-
wicking" nanowire
grids. J Struct Biol.
2018;202(2):170-4.



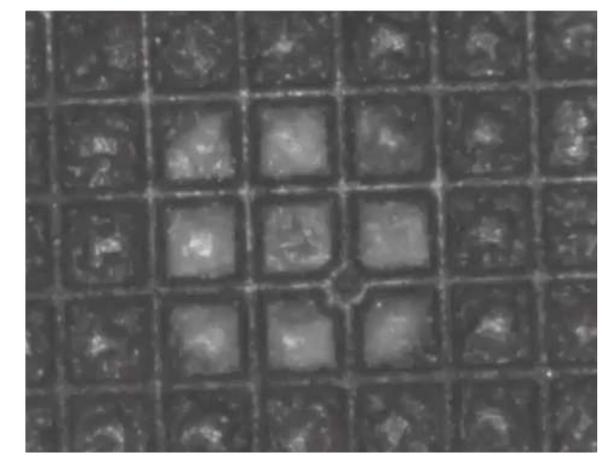
Venkat Dandey



Hui Wei



Single frame from loop



Video loop

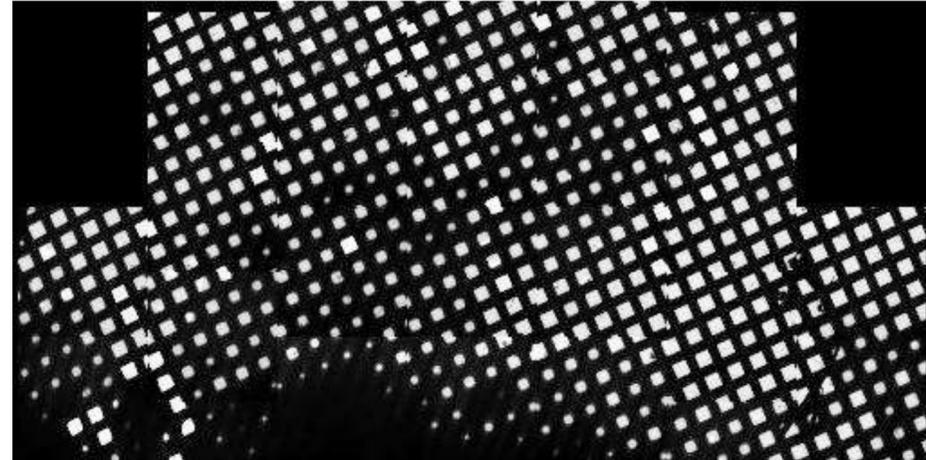
Other methods



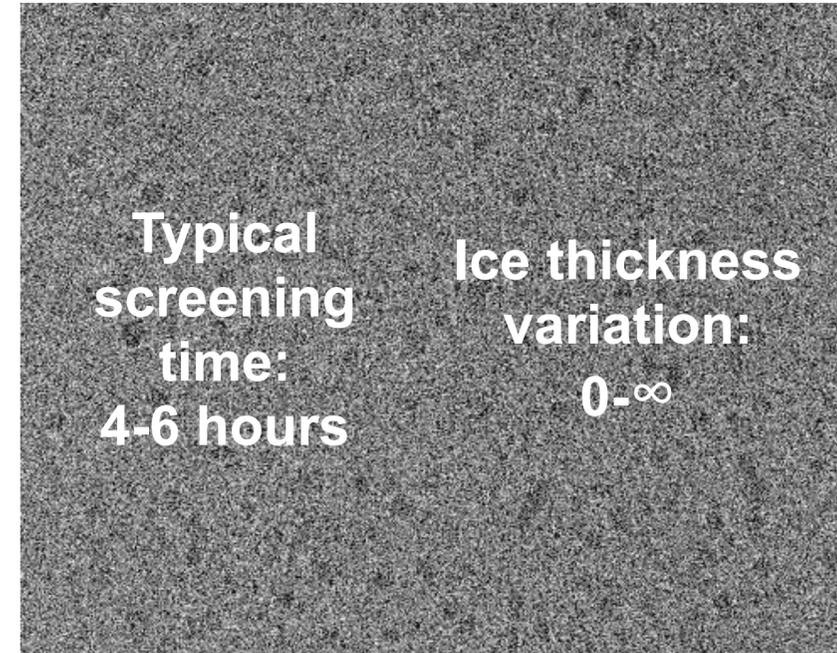
Improving Current CryoTEM Grid Preparation Methods

Vitrobot

3 uL of sample required for each grid; ~2nL on grid

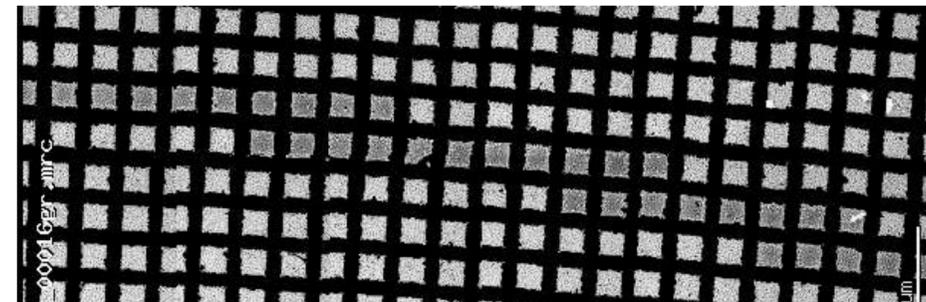


Usable area: ~0-10%

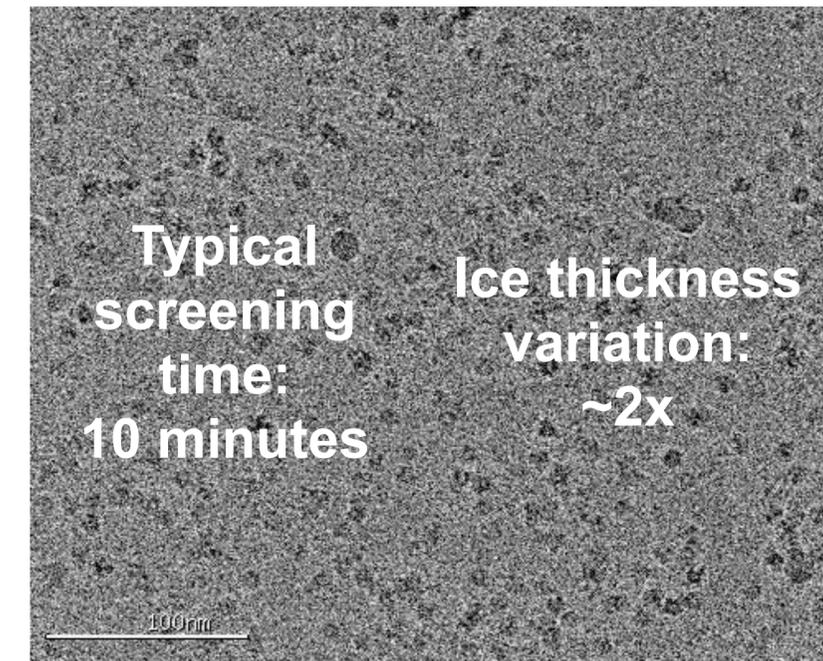


Spotiton

3 uL of sample enough for >100 grids; ~500pL on grid



Usable area: ~100%



Wei H, Dandey VP, Zhang Z, Raczkowski A, Rice WJ, Carragher B, Potter CS. Optimizing "self-wicking" nanowire grids. J Struct Biol. 2018;202(2):170-4.



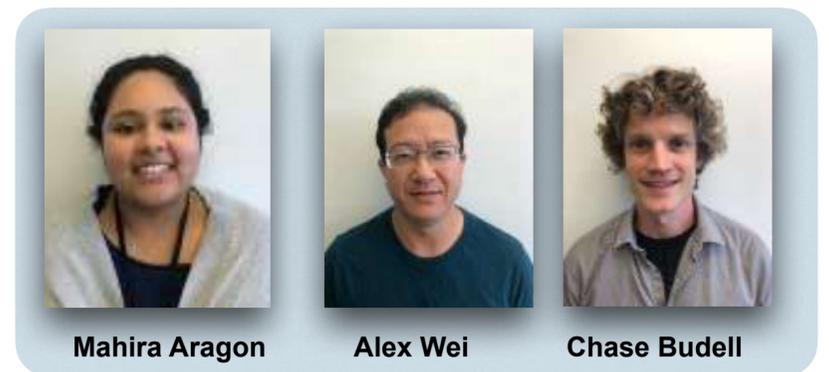
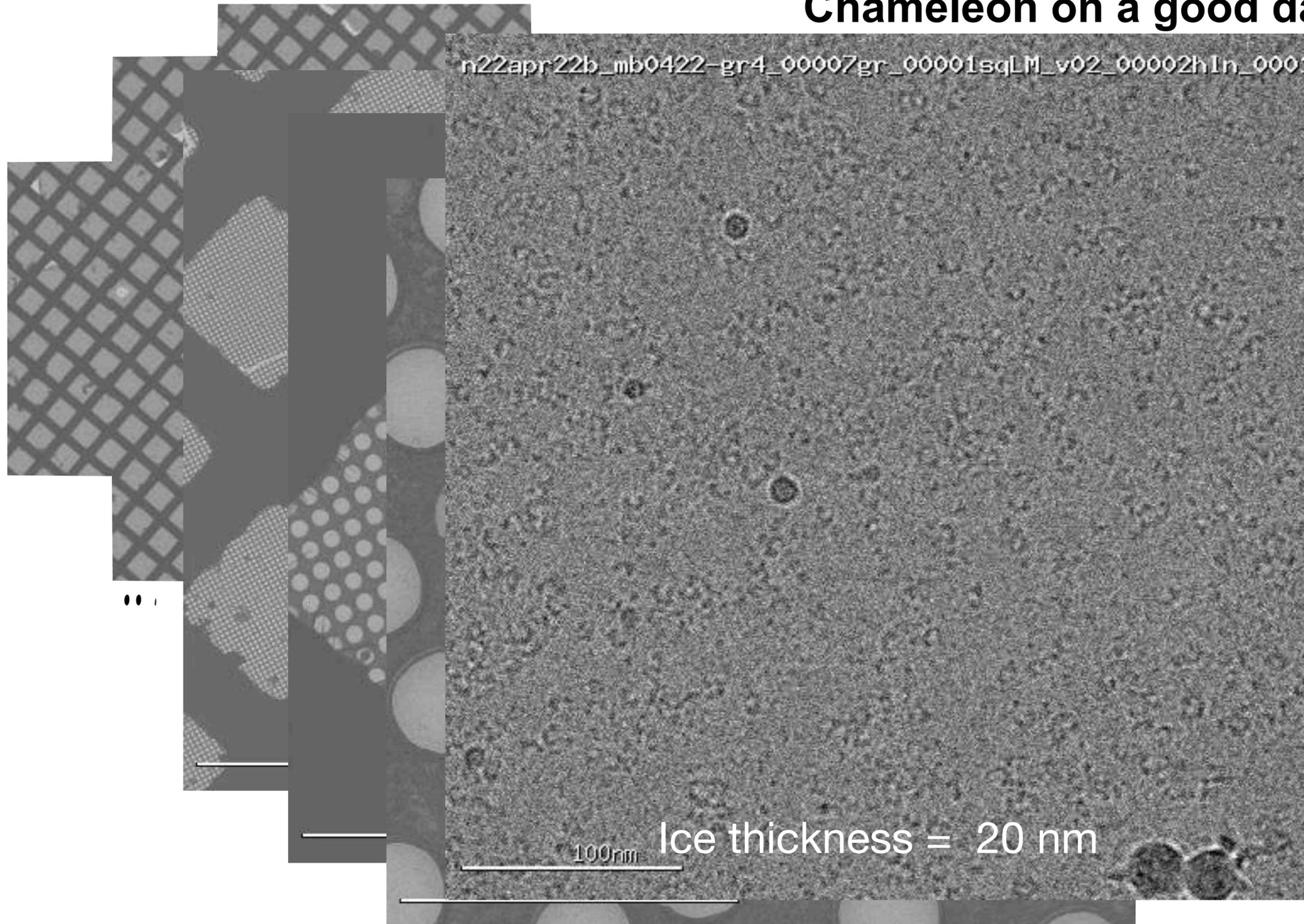
Venkat Dandey



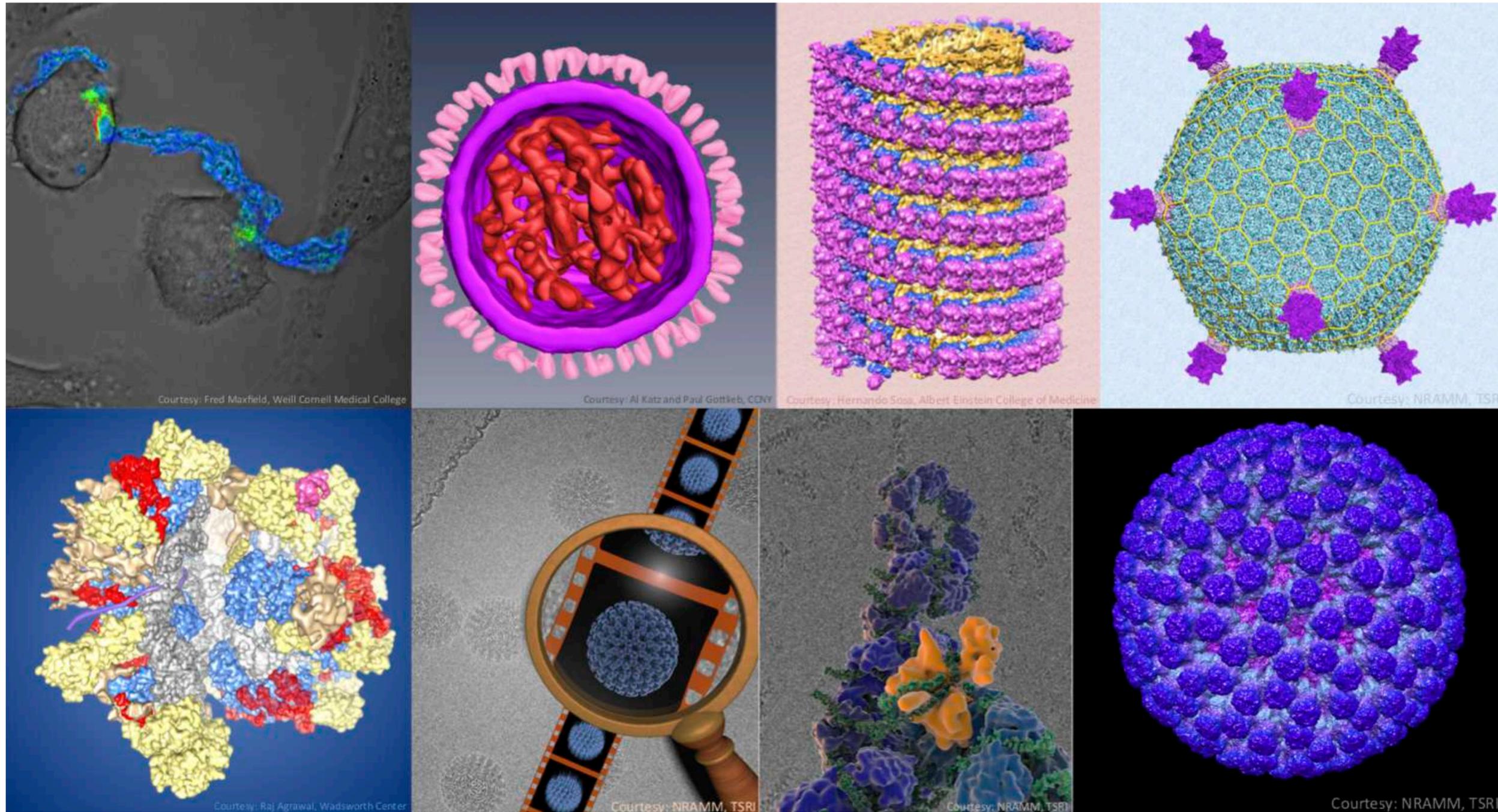
Hui Wei

What is chameleon?

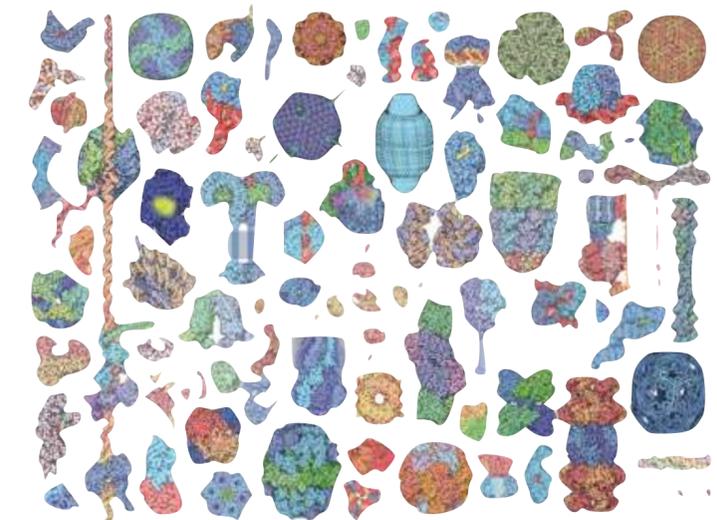
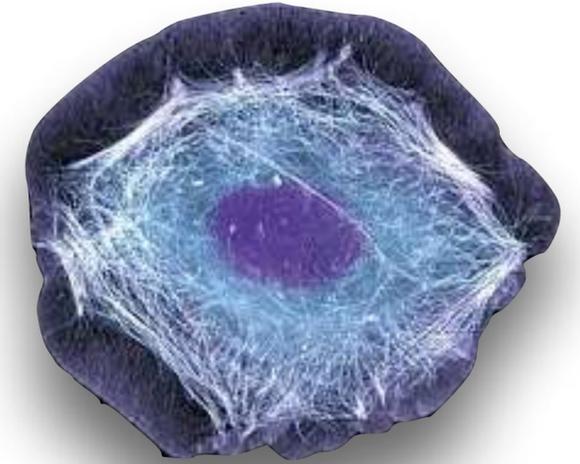
Chameleon on a good day



How are samples prepared for cryoEM?



What about thicker samples?



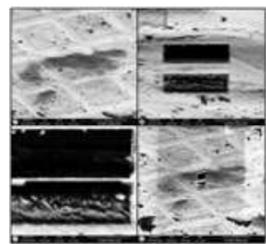
...

How are samples prepared for cryoEM?

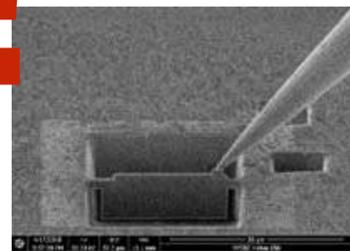
Towards Automation for
In Situ CryoEM



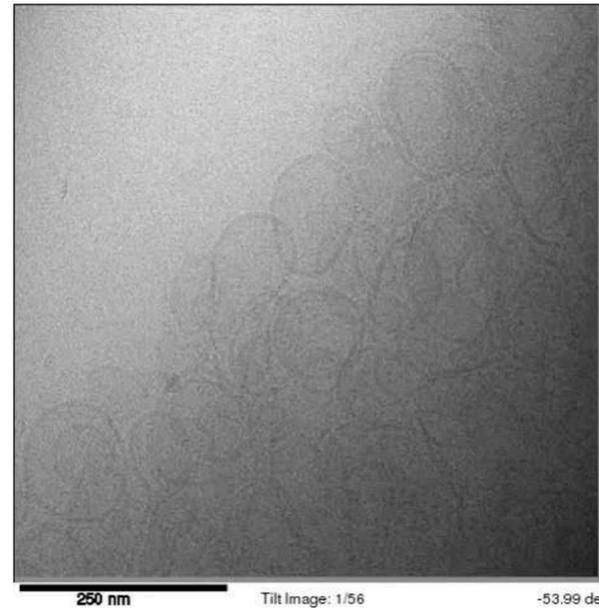
Sample



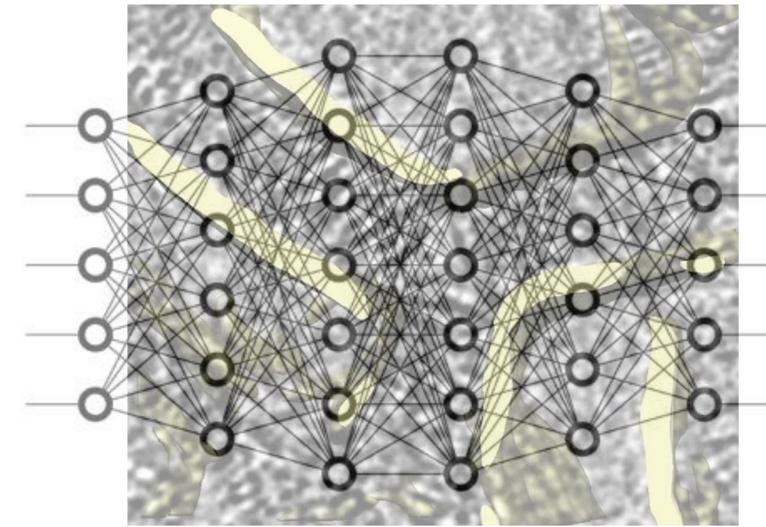
Milling
Grid preparation



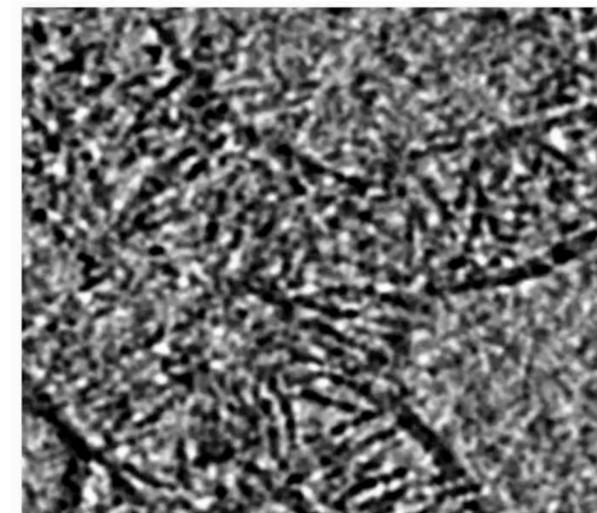
Lift out



Automated Data Collection
(Leginon, etc.)



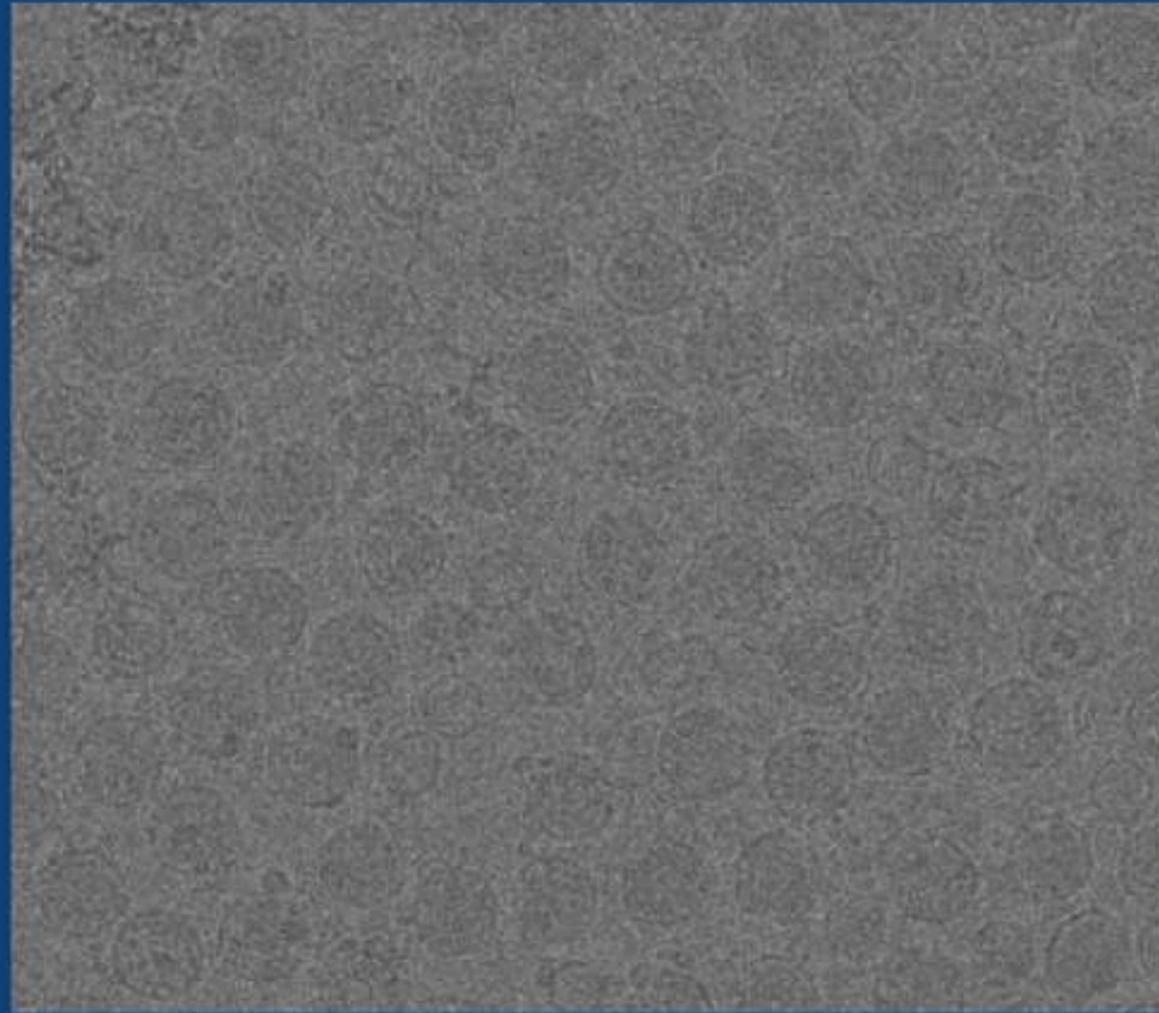
Deep learning?



Streamlined Processing
(Appion Protomo)

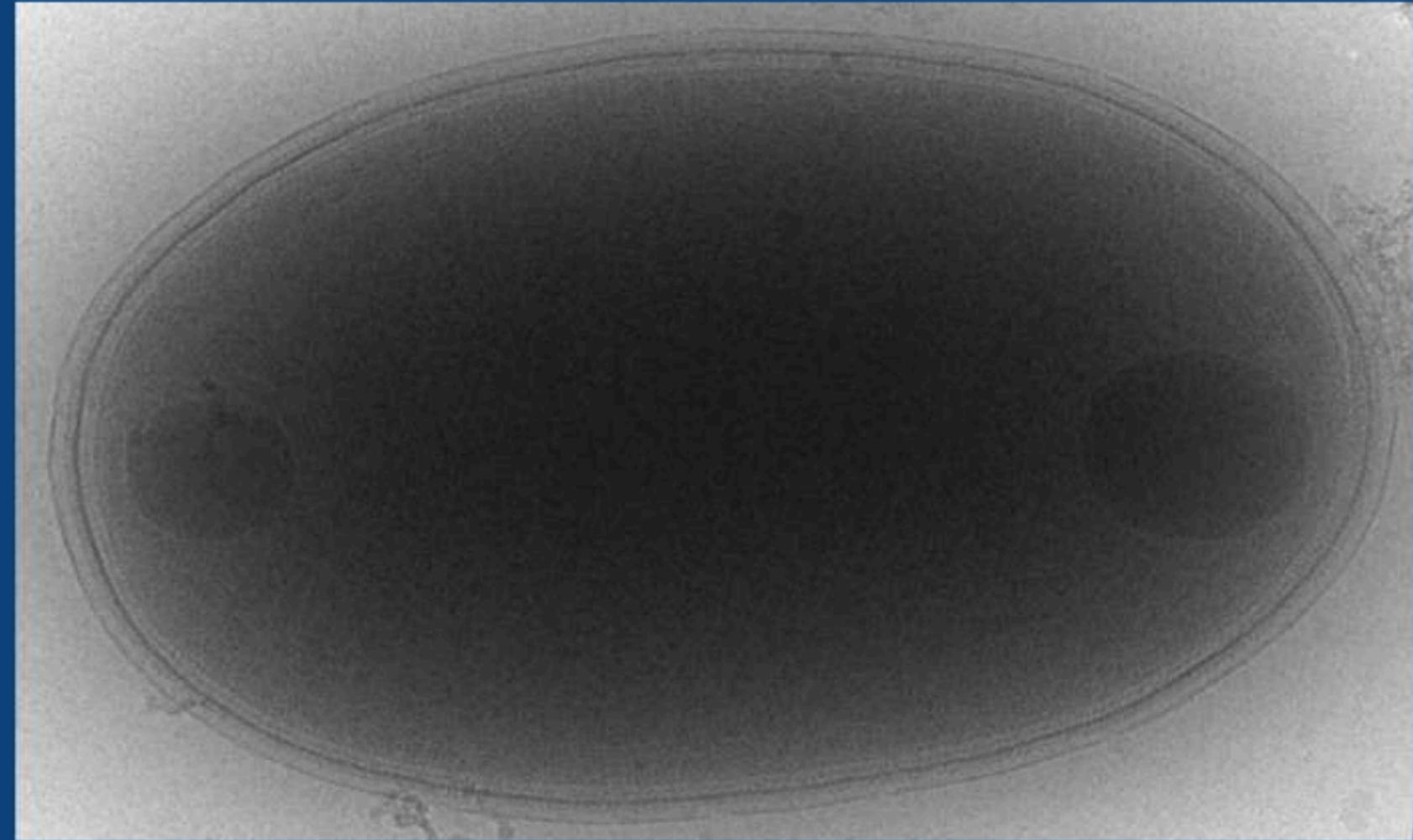
How are samples prepared for cryoEM?

HOW THIN DOES THE SAMPLE NEED TO BE?



50 nm

Bacteriophage (ϕ 12)

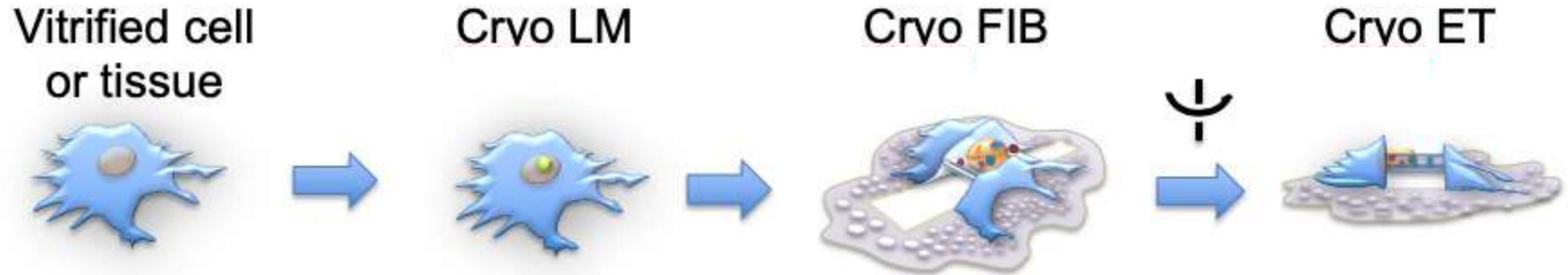


750 nm thick

E. coli, *Salmonella*, Cyanobacteria

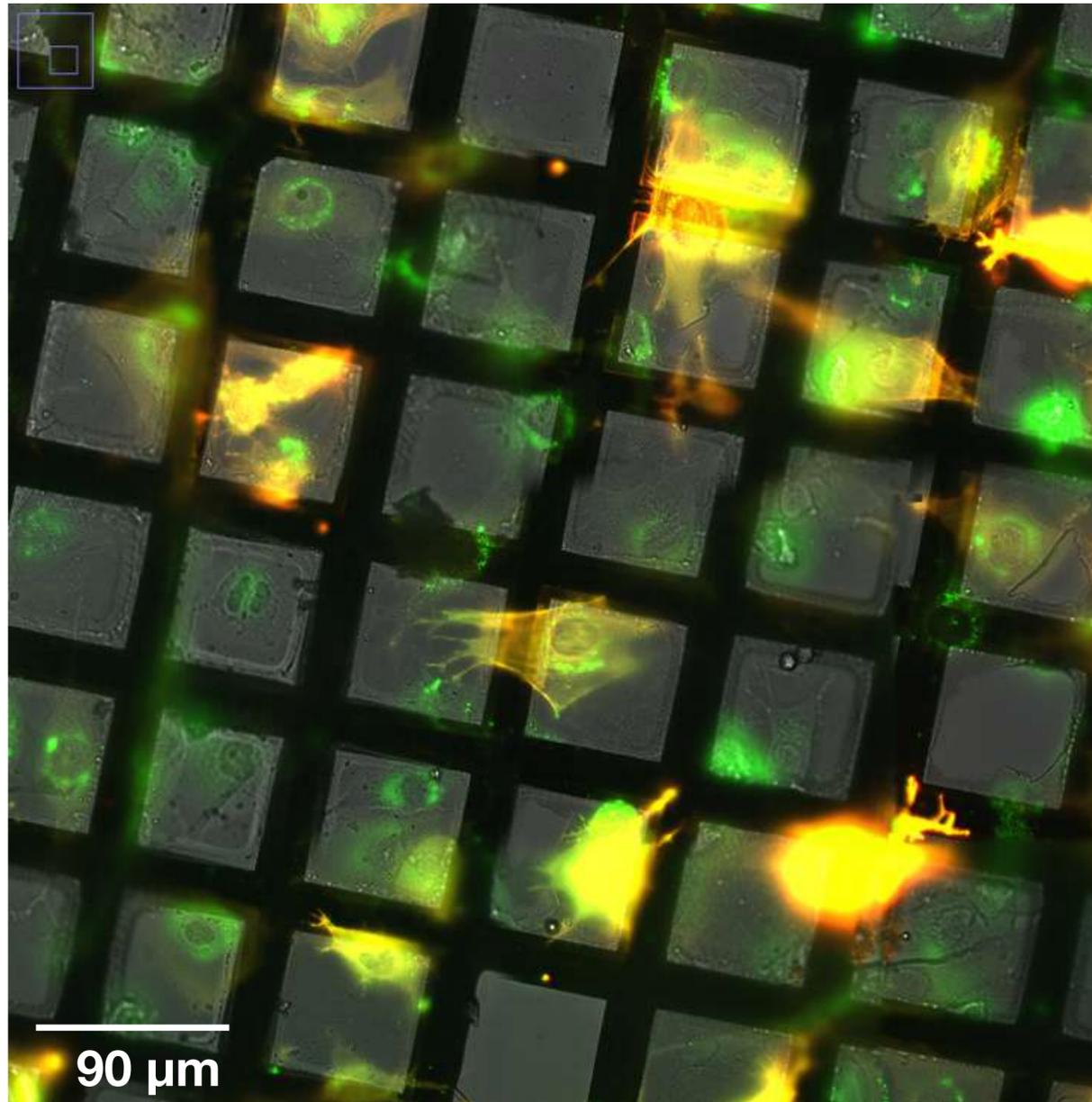
How are samples prepared for cryoEM?

CLEM workflow



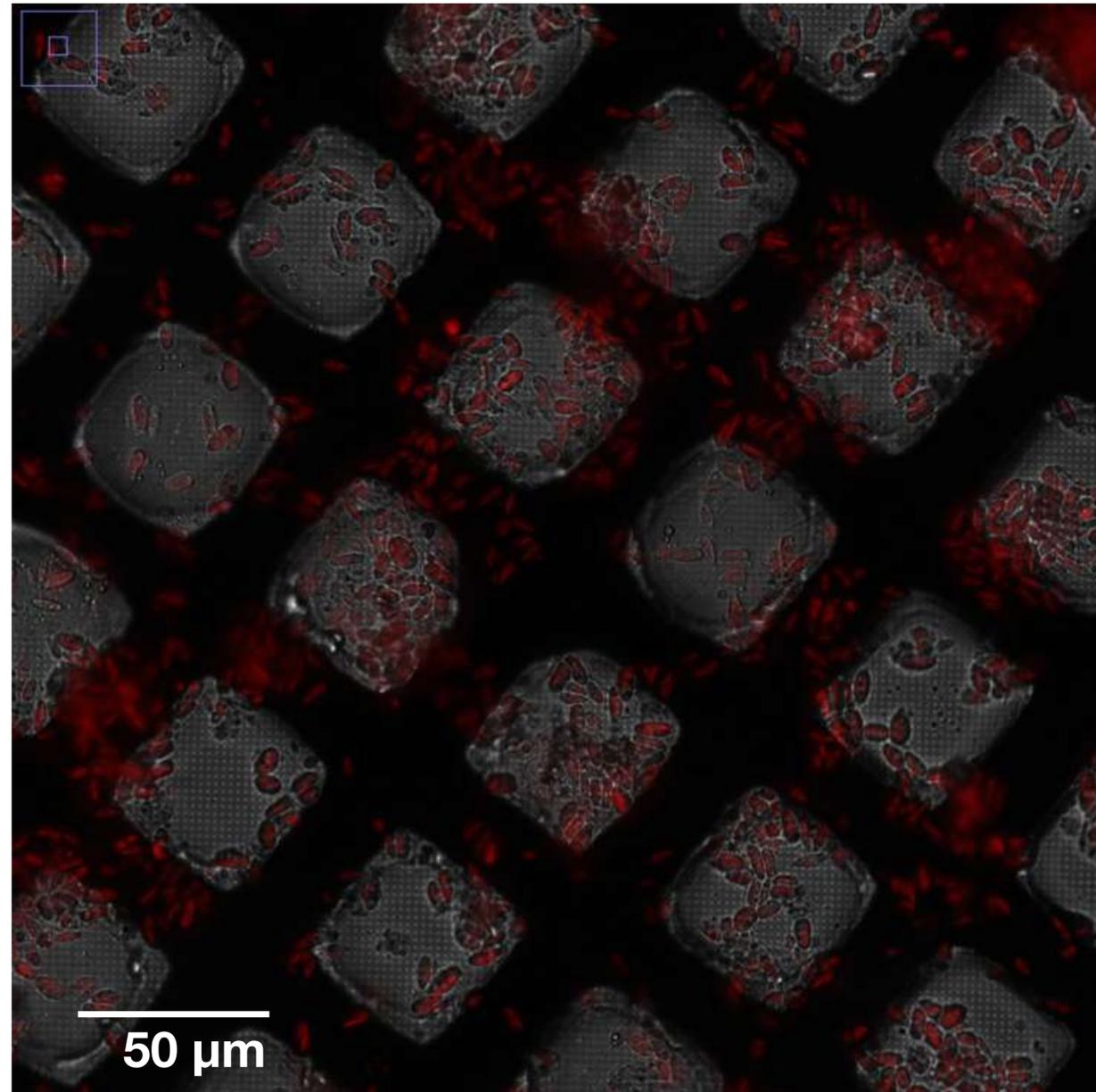
How are samples prepared for cryoEM?

Mouse fibroblasts



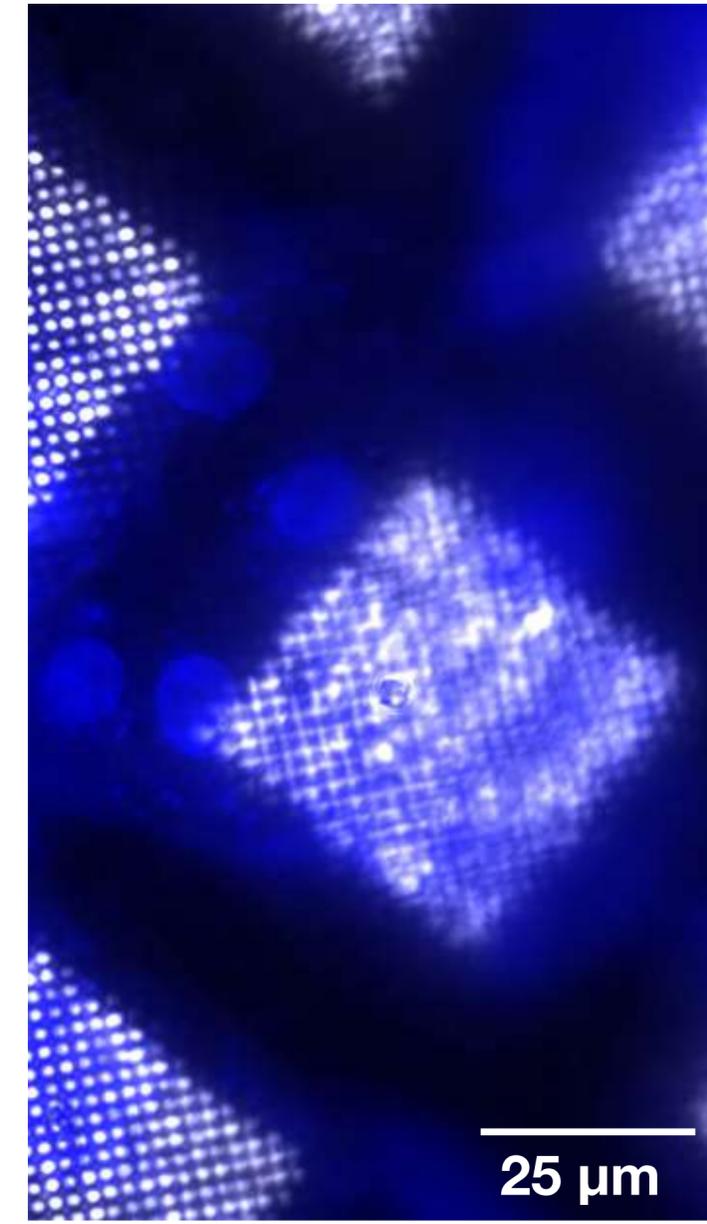
Transfected adhesion signaling protein tagged with GFP (green) and F-actin marker with mApple (red).
Greg Alushin (RU)

Diatoms



Auto-fluorescence.

Microsporidia



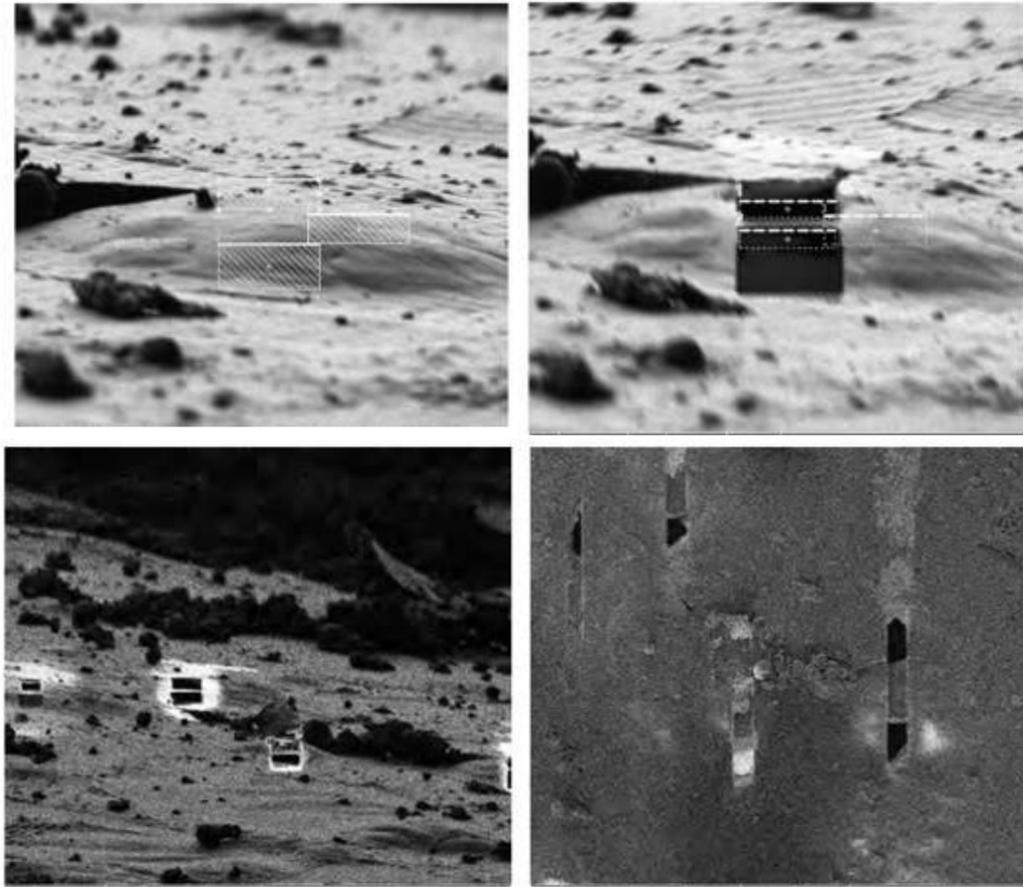
DAPI.

Wei Dai (Rutgers)

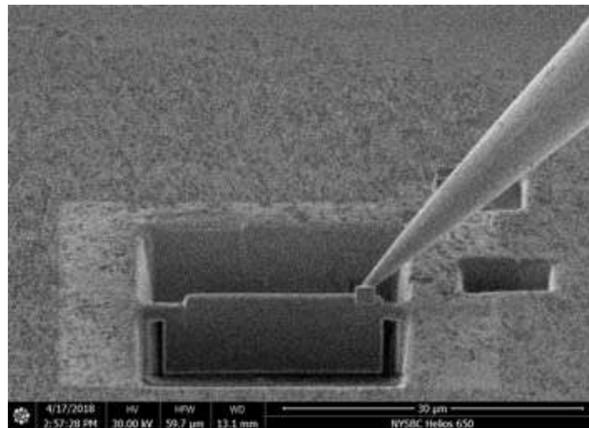
Gira Bhabha (NYU)

How are samples prepared for cryoEM?

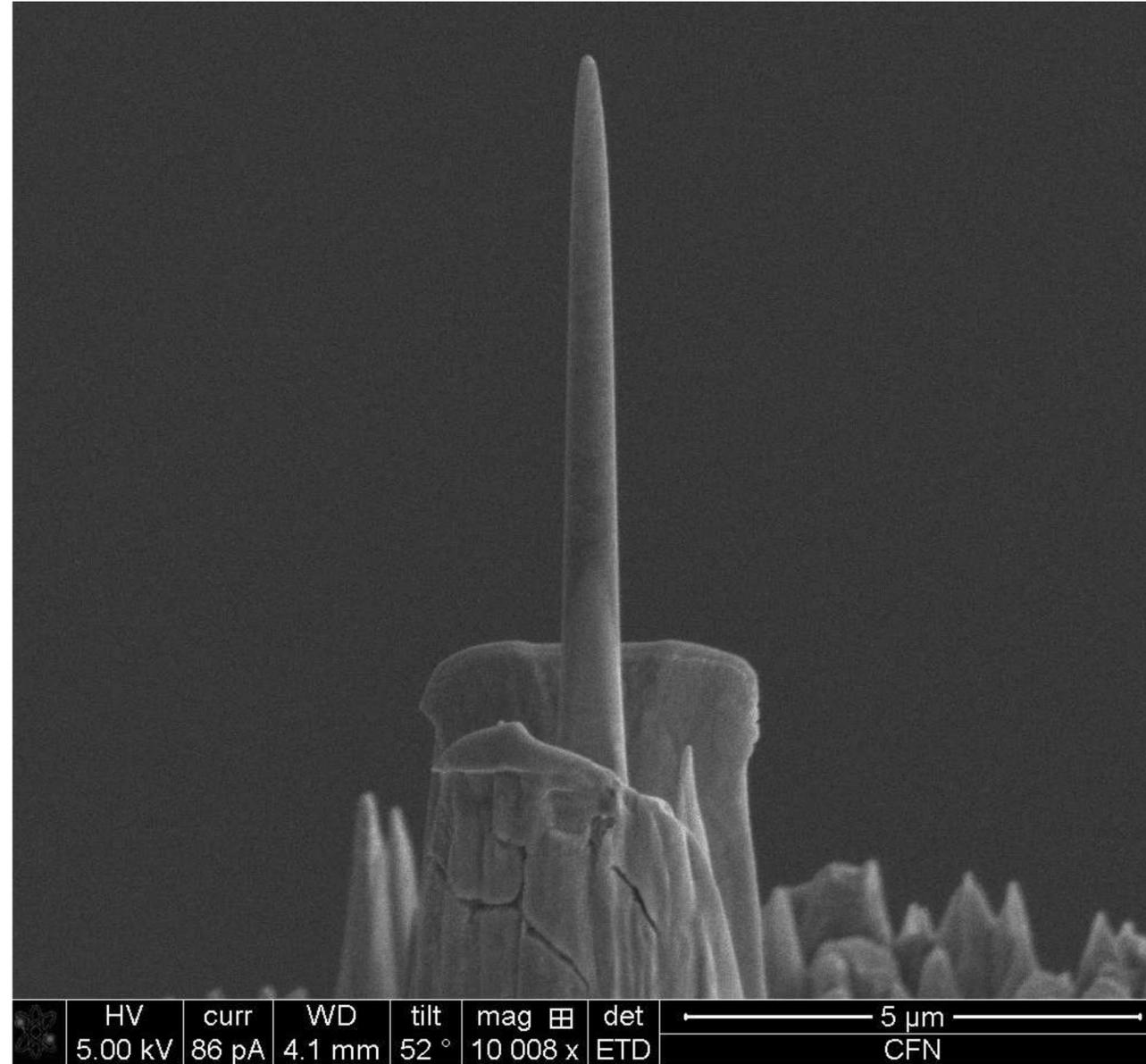
Lamella



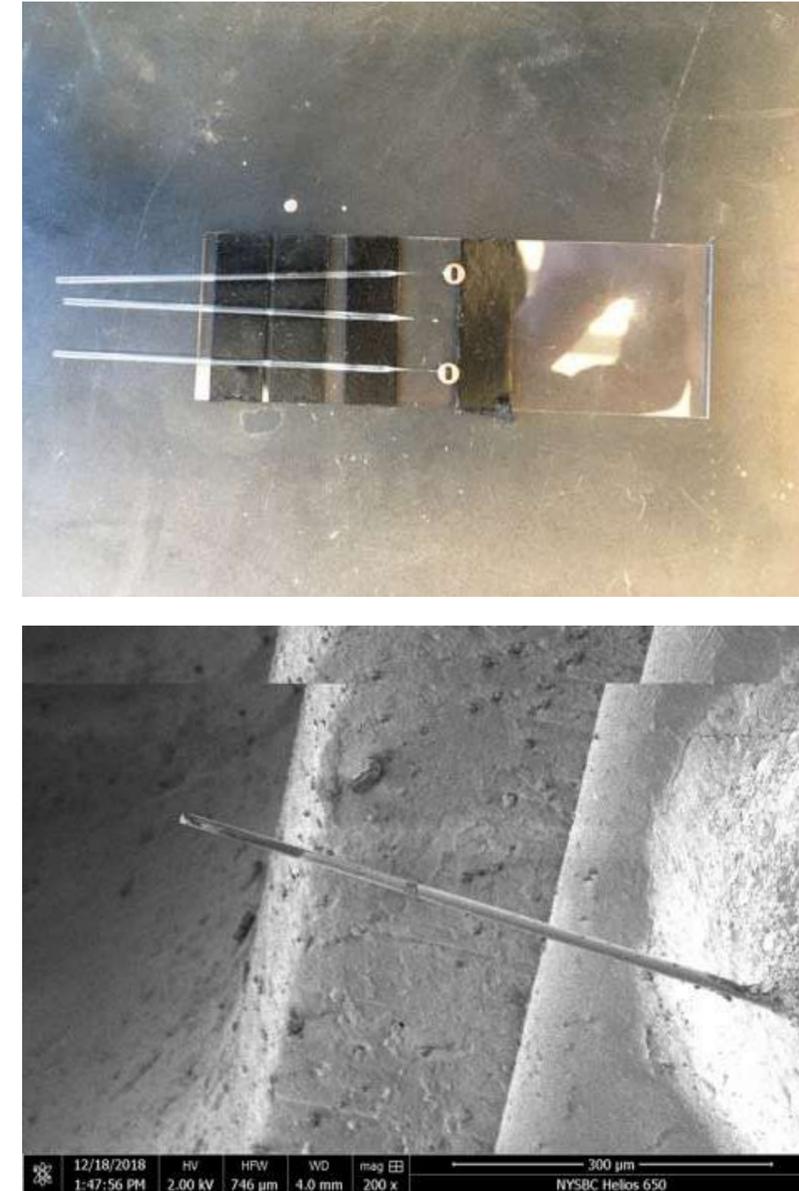
*with
lift-out*



Rods

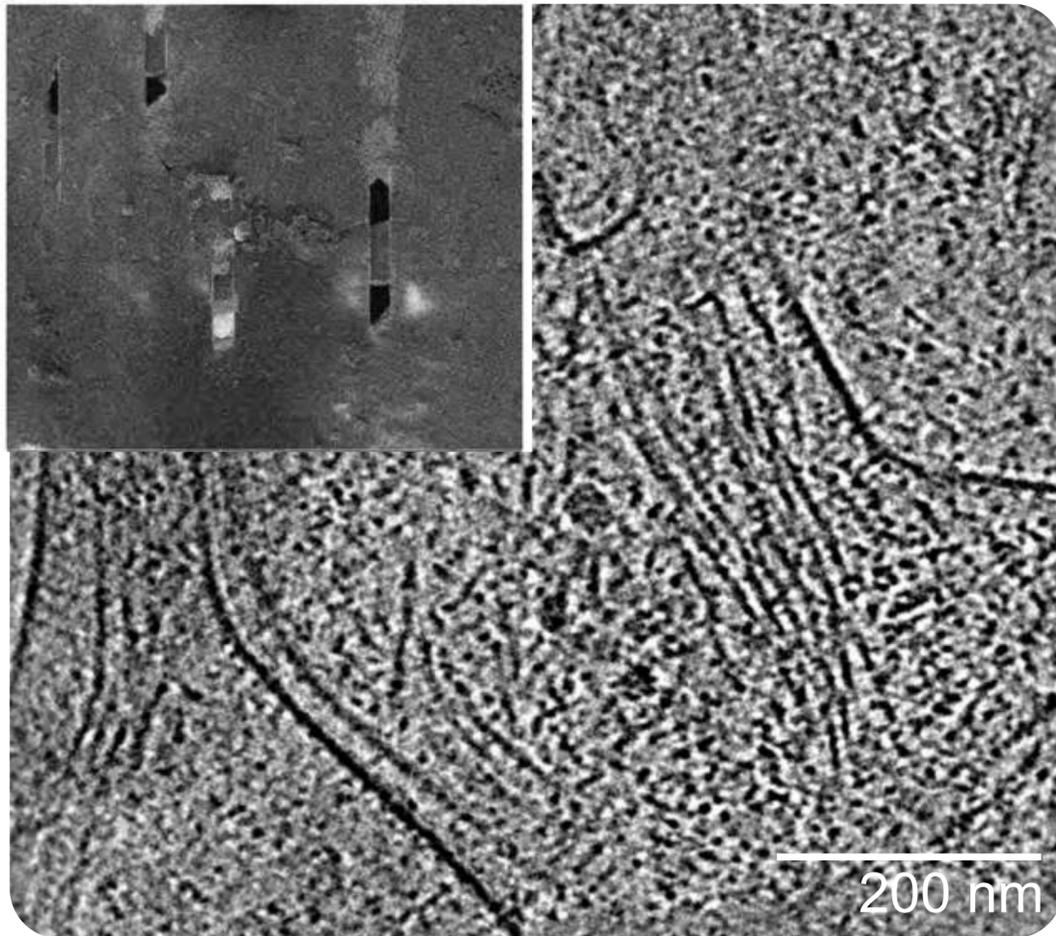


Capillaries



How are samples prepared for cryoEM?

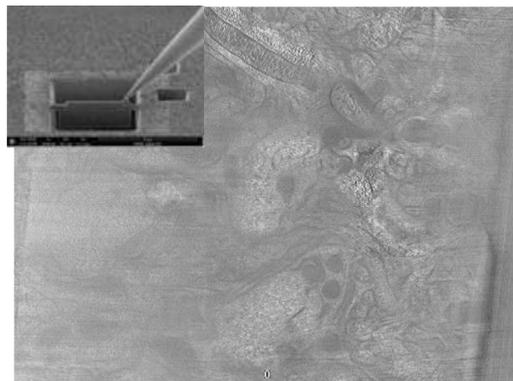
Lamella



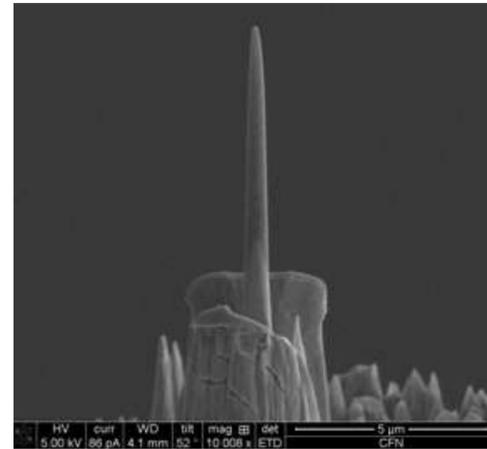
Zach Freyberg,
(Univ. Pittsburg)

*with
lift-out*

Kotaro
Kelley
(NRAMM)

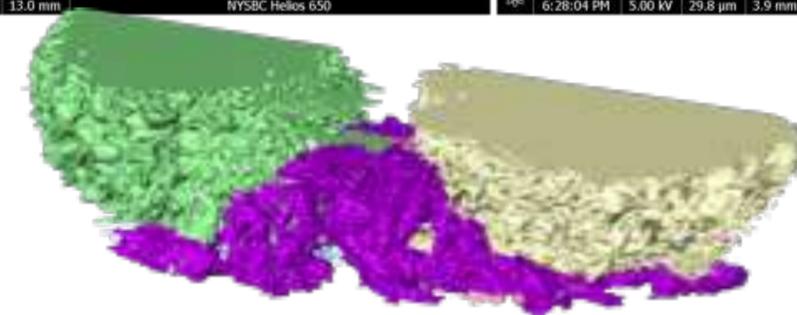
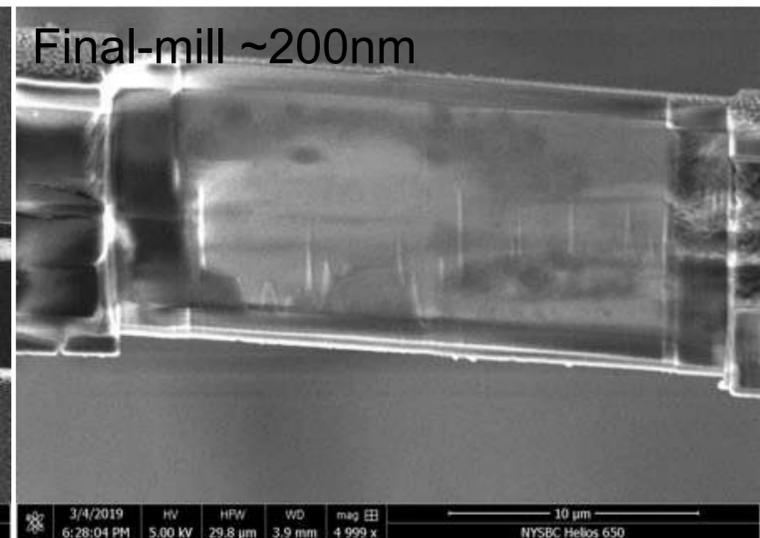
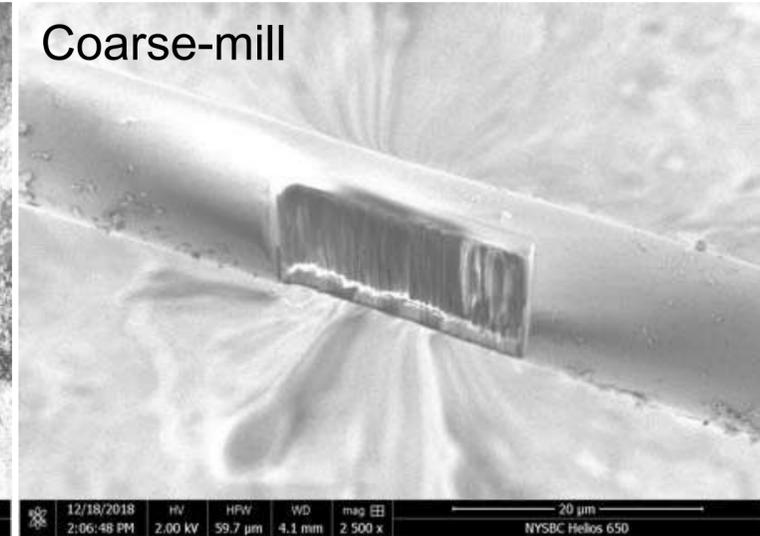
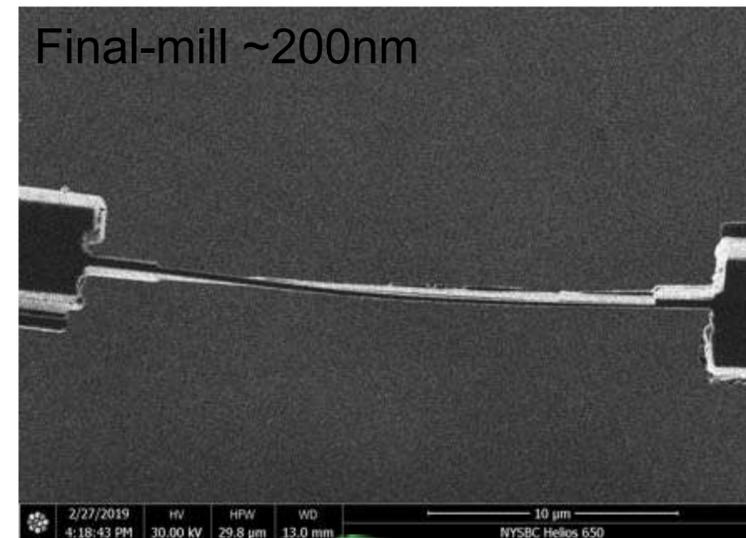
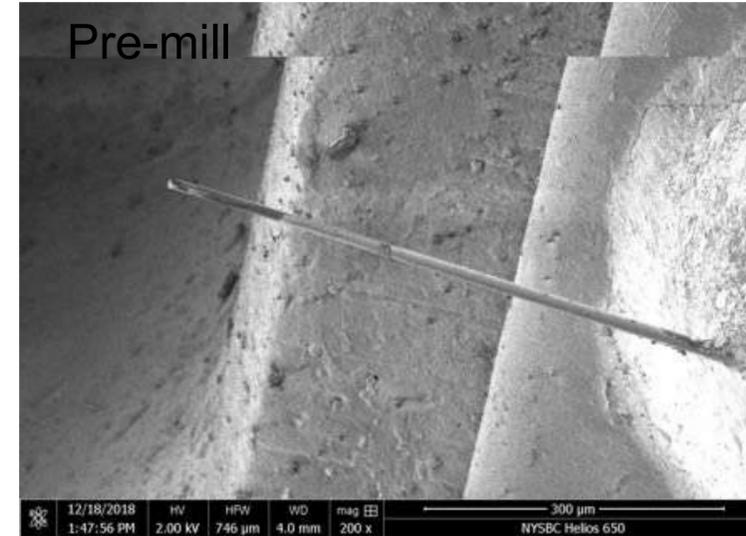


Rods



with Xin
Group
(BNL)

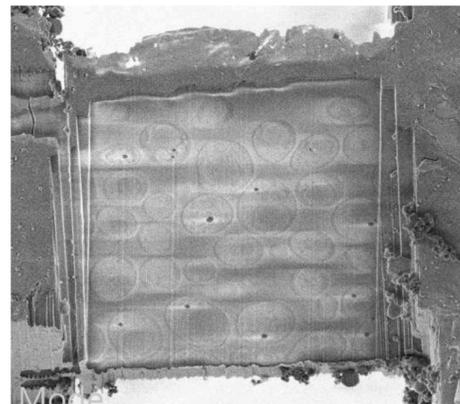
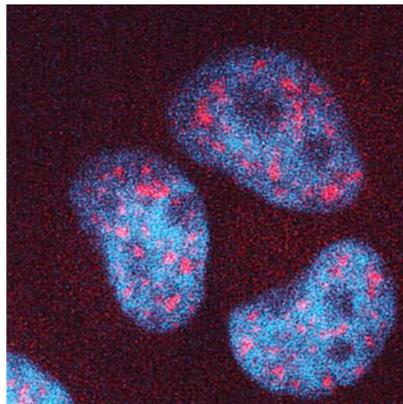
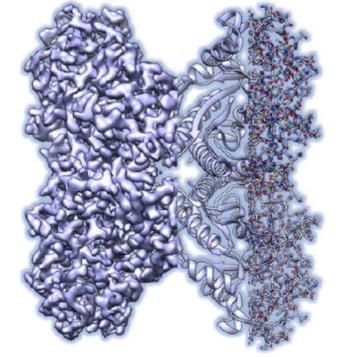
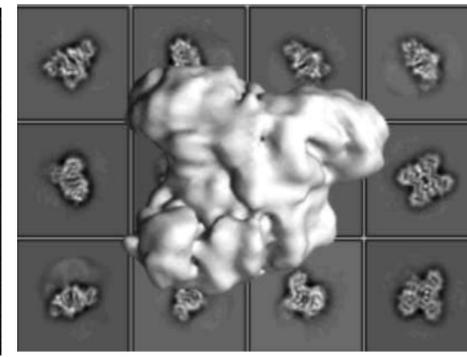
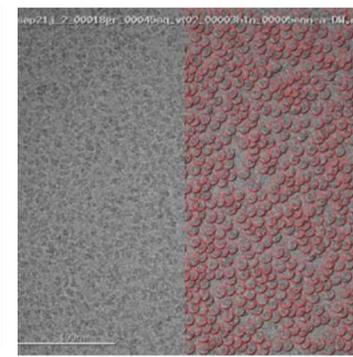
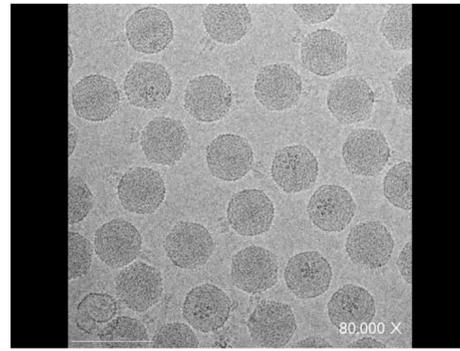
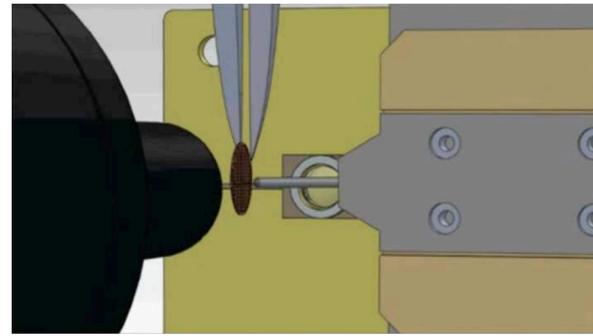
Capillaries



Kotaro
Kelley
(NRAMM)

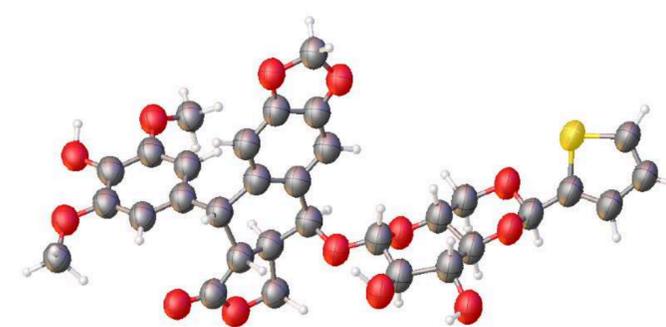
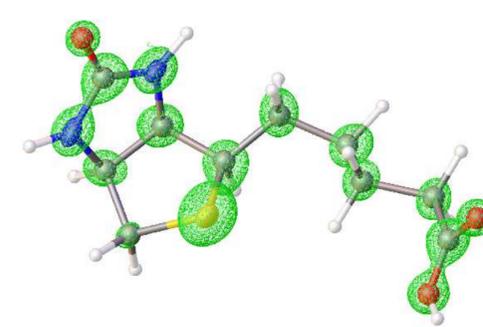
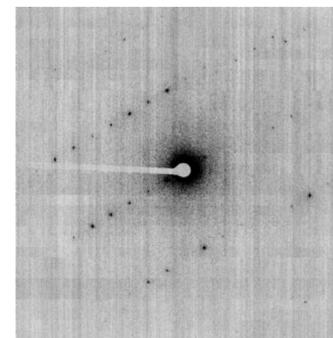
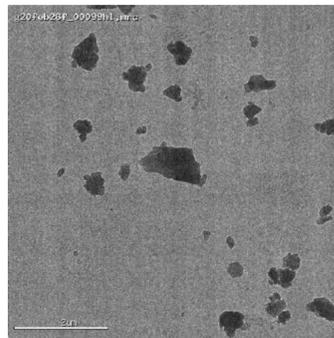
cryoEM: technology on the rise

Single particle cryoEM

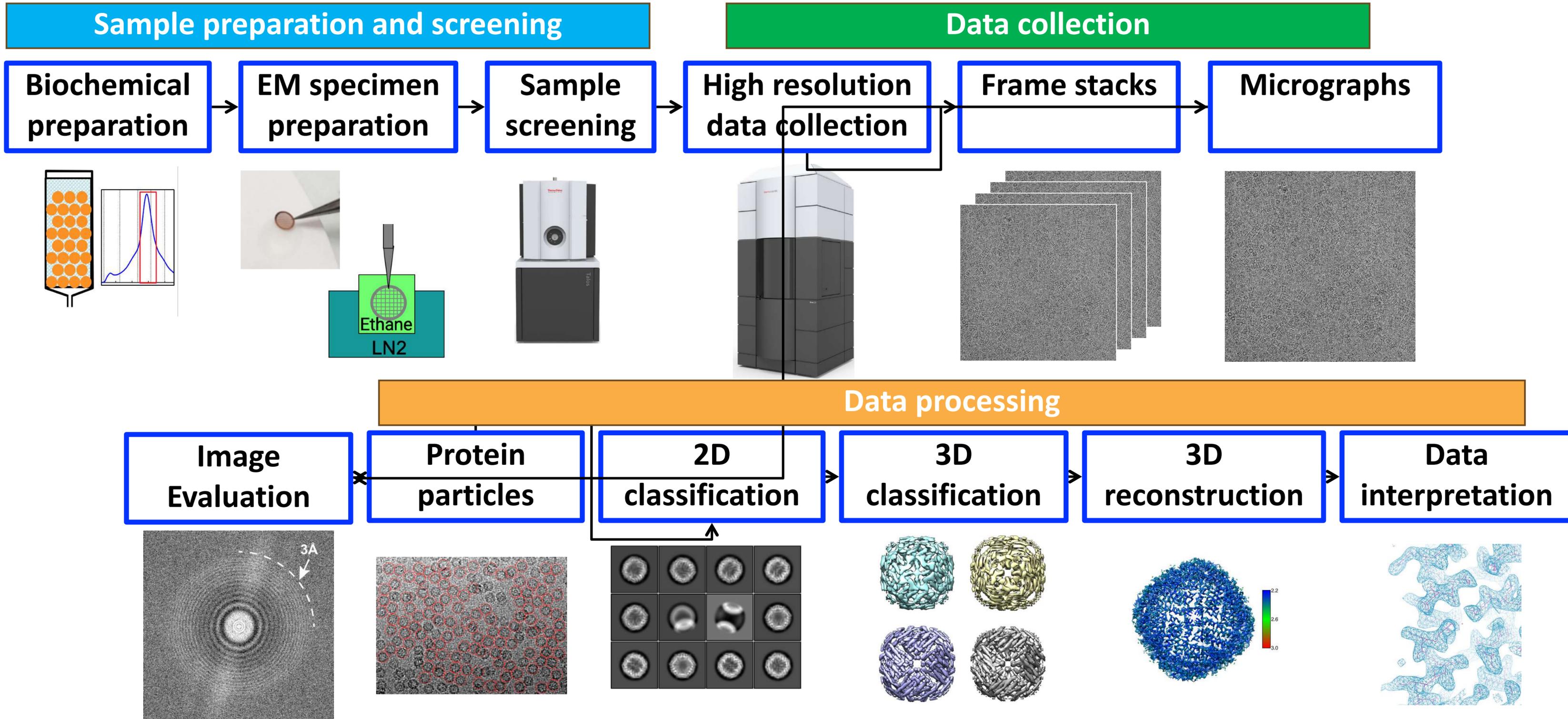


Cryo Electron Tomography (cryoET)

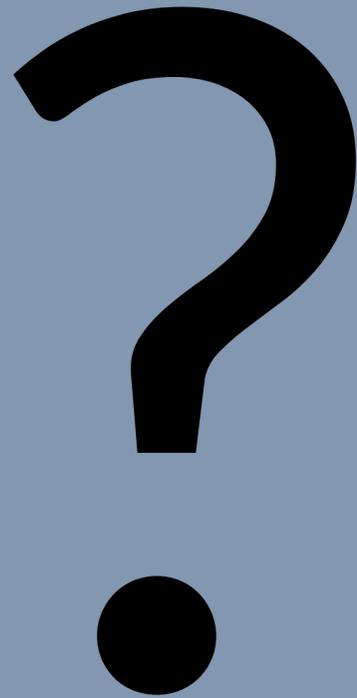
Micro crystal electron diffraction (microED)



The typical SPA workflow has many steps



What issues arise?



Small protein

- VPP
- Thinner ice

Protein denaturation/Dissociation of protein complex

- Continuous carbon film
- Graphene oxide
- Cross-linking (GraFix)

Preferred orientation

- Tilt stage
- Cross-linking
- Detergent
- Glow-discharging conditions
- Support film (Graphene oxide)
- Image analysis (3D classification)

Flexibility

- Focused classification (subtraction)
- Multibody refinement

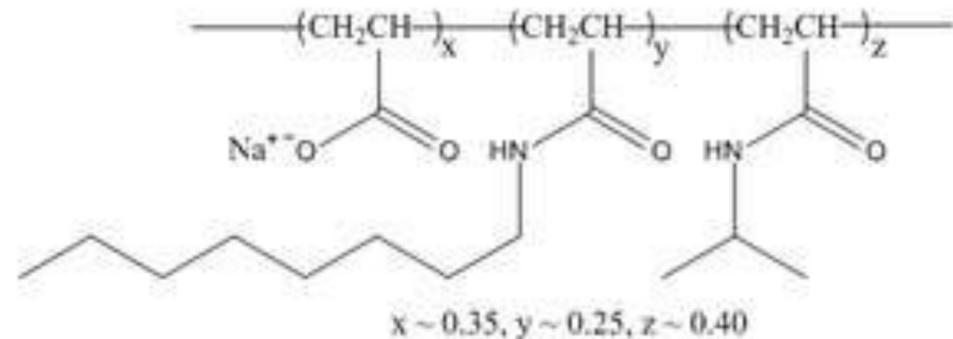
Filamentous protein

- Segmented analysis

Low concentration

- Multiple blots
- Affinity grids

Reagents for improving vitrification of Cryo-EM grids used in single particle analysis.



Molecular Formula:

$(C_{6.2}H_{10.3}O_{1.35}N_{0.65}Na_{0.35})_{35}$

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 μ l	0.41% (w/v)	0.07% (w/v)	non-ionic detergent
Hexadecyl-trimethyl-ammonium Bromide (CTAB)	100 μ l	0.34% (w/v)	0.03% (w/v)	cationic detergent
n-Decyl- β -D-Maltoside (DM)	100 μ l	0.87% (w/v)	0.09% (w/v)	non-ionic detergent
n-Decyl- α -D-Maltoside (DaM)	100 μ l	0.46% (w/v)	0.08% (w/v)	non-ionic detergent
n-Dodecyl- β -D-Maltoside (DDM)	100 μ l	0.09% (w/v)	0.01% (w/v)	non-ionic detergent
Sodium Deoxycholate	100 μ l	1.66% (w/v)	0.17% (w/v)	anionic detergent
Triton X-100	100 μ l	0.15% (w/v)	0.01% (w/v)	non-ionic detergent
Tween 20	100 μ l	1% (w/v)	0.01% (w/v)	non-ionic detergent
CHAPSO	100 μ l	2.5% (w/v)	0.5% (w/v)	zwitterionic detergent
Amphipol A8-35	100 μ l	5% (w/v)		anionic surfactant
Glycerol	1 ml	30% (w/v)		cryoprotectant

- [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.

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

Reagents for improving vitrification of Cryo-EM grids used in single particle analysis.

PDB Release Date	PDB	Protein	Additive
2020-01-08	6PWN	MscS mechanosensitive channel	0.01% f-OM
2019-09-04	6KG7	Piezo2 mechanosensitive channel	0.65 mM f-FC8
2019-08-28	6QTI	Nicotinamide nucleotide proton channel	0.05% CHAPS
2019-08-07	6R7L	SecYEG translocon	0.2% f-OM
2019-02-06	6E0H	TMEM16 scramblase	3 mM f-FC8
2018-12-19	6N3Q	Sec protein-translocation channel complex	3 mM f-FC8
2018-11-07	6H3I	Type 9 secretion system translocon	1.5 mM f-FC8 or 0.7 mM f-OM
2018-10-24	6DMR	TRPV5 ion channel	3 mM f-FC8
2018-10-17	6D3R	CFTR	3 mM f-FC8
2018-09-26	6HJR	Influenza Hemagglutinin	2% Octyl Glucoside
2018-08-08	6FOO	Ryanodine receptor 1	0.2% f-OM
2018-08-01	6CJQ	SthK CNG Potassium channel	3 mM f-FC8
2018-05-23	5YX9	TRPC6 ion channel	0.5 mM f-OM
2018-01-31	6C0V	P-Glycoprotein transporter ABCB1	3 mM f-FC8
2017-12-27	6B5V	TRPV5 ion channel	3 mM f-FC8
2017-12-13	6BPQ	TRPM8 channel	2% DMSO

Glaeser, RM, et al.
(2017) Biophys Rep 3(1), 1-7.

Noble, AJ, et al. (2018) Nat
Methods 15(10), 793-795.

Drulyte, I et al. (2018) Acta
Crystallogr D Struct Biol 74(Pt 6),
560-571.

Chen, J, et al. (2019) J Struct
Biol X Volume 1. DOI: 10.1016/
j.yjsbx.2019.100005

<https://www.anatrace.com/Landing/2020/Mar20-Newsletter>



TO BE CONTINUED

Questions?