Getting started in Cryo-EM at

# Harvard Cryo-EM Center for Structure Biology

02 May 2024

Megan Mayer

# The Harvard Center for CryoEM (HC$^2$EM) and the Molecular EM Suite (MEMS) are both located in the basement of the Seeley G Mudd Building

Stephen Harrison, PhD
Faculty Director

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Facility Director

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Senior Cryo-EM Scientist

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Cryo-ET Specialist

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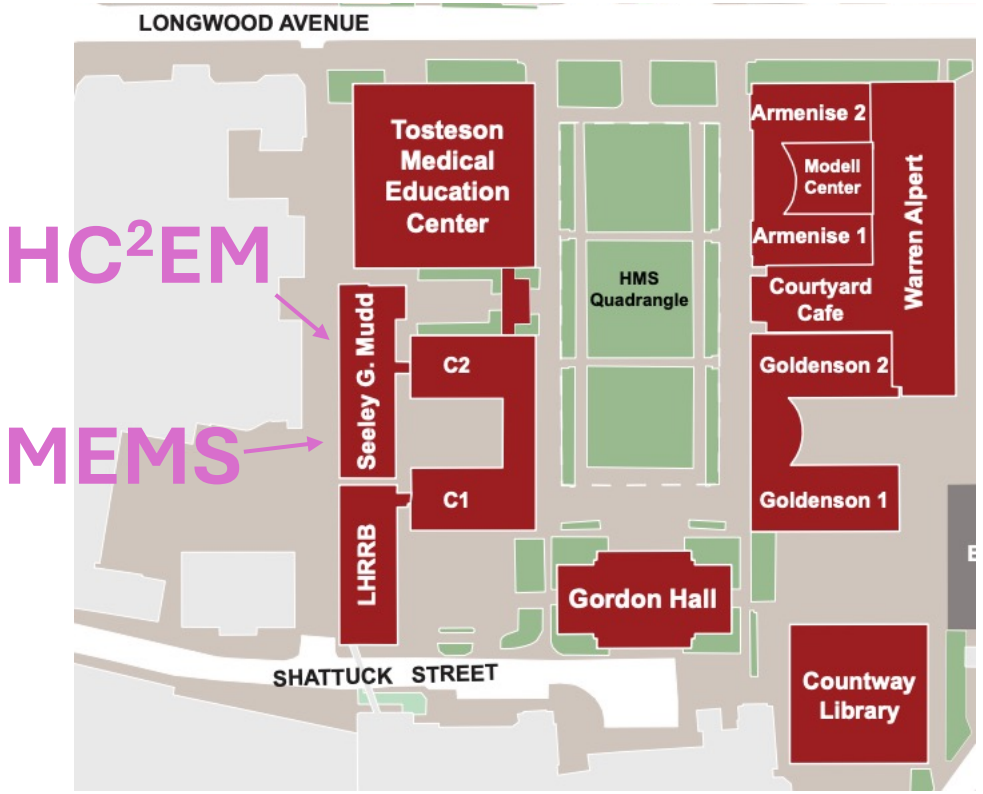
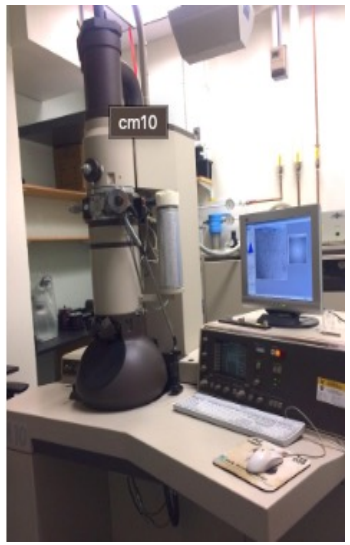
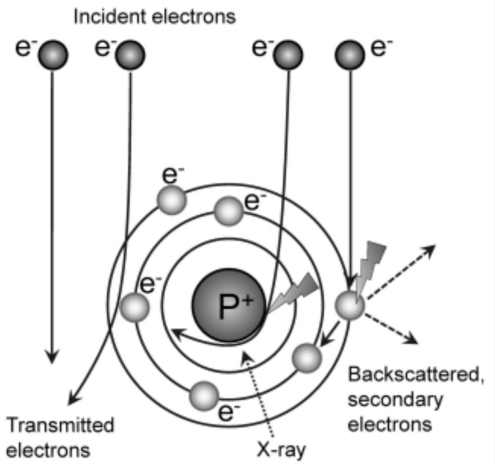
Cryo-EM Computational Specialist

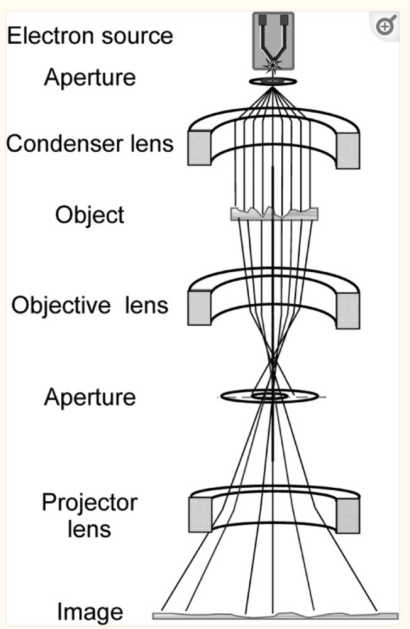


# CM10, T12, Talos Arctica, Titan Krios are all transmission electron microscopes.







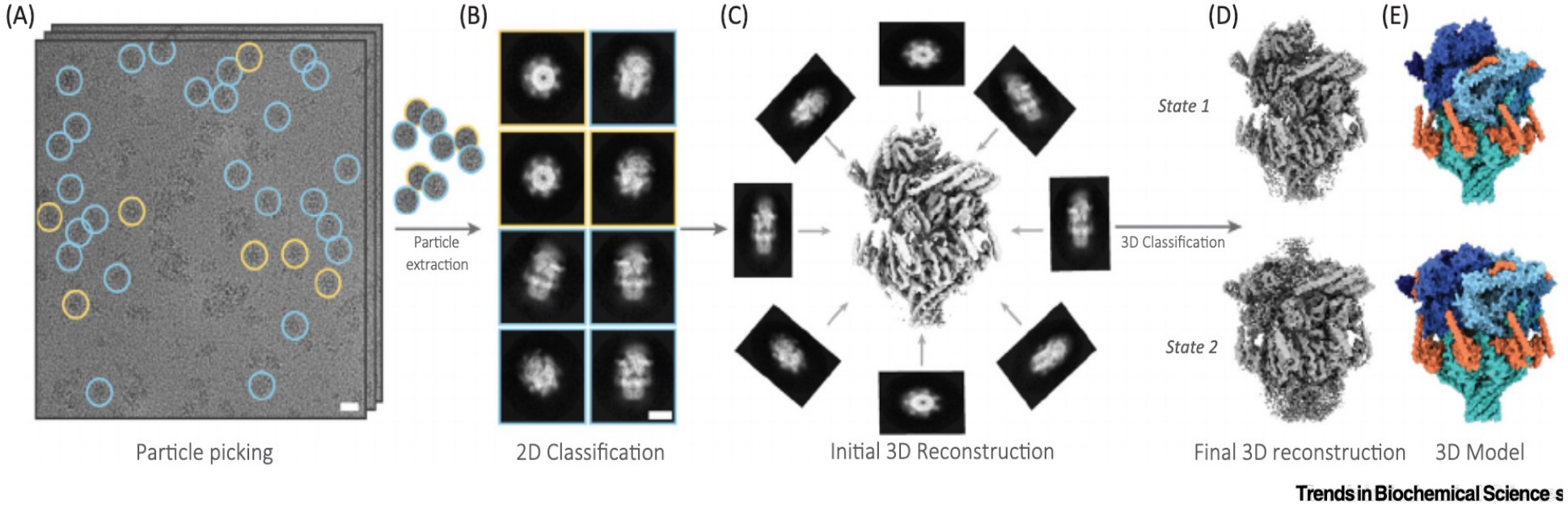

| | |
|---|---|
| **CM10/T12** | Tabletop microscopes<br>Negative Stain EM<br>Cryo-EM grid screening<br>1 grid at a time |
| **Talos Arctica** | 200 keV microscope<br>12 grids at a time<br>High resolution data collection |
| **Titan Krios** | 300 keV microscope<br>Energy filter<br>12 grids at a time<br>*Highest* resolution data collection |

**There are 2 types of cryo-EM experiments: single-particle analysis (SPA) or cryo-electron tomography (Cryo-ET)**

## Single particle analysis (SPA)

- Capable of atomic or near atomic resolution structural information
- Typically requires purified, homogeneous macromolecules
- Macromolecules must form stable complexes that are larger than ~90 kDa

HSP101




DOI: 10.1016/j.tibs.2021.10.006

**There are 2 types of cryo-EM experiments: single-particle analysis (SPA) or cryo-electron tomography (Cryo-ET)**

## Cryo-ET

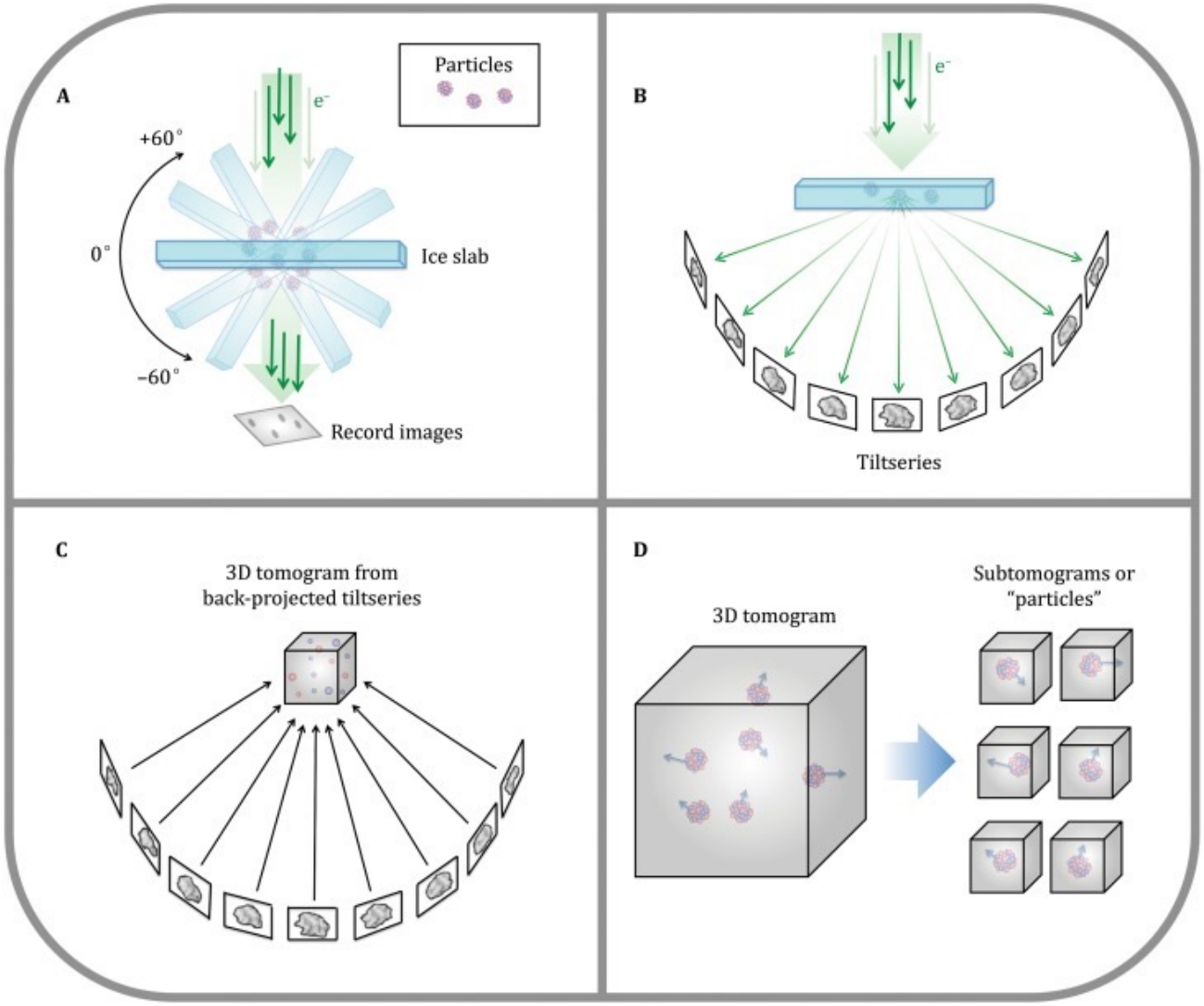
•3D structures of biological molecules can be obtained in large, irregular macromolecular assemblies or *in situ,* without the need for purification.

•biological specimen is tilted to record 2D images from -60 to +60 degrees

•Typically lower resolution and more challenging because:

- thicker samples=more noise
- tilted data is lower resolution
- Missing wedge (-60 to +60 tilts)
- Slower Data collection

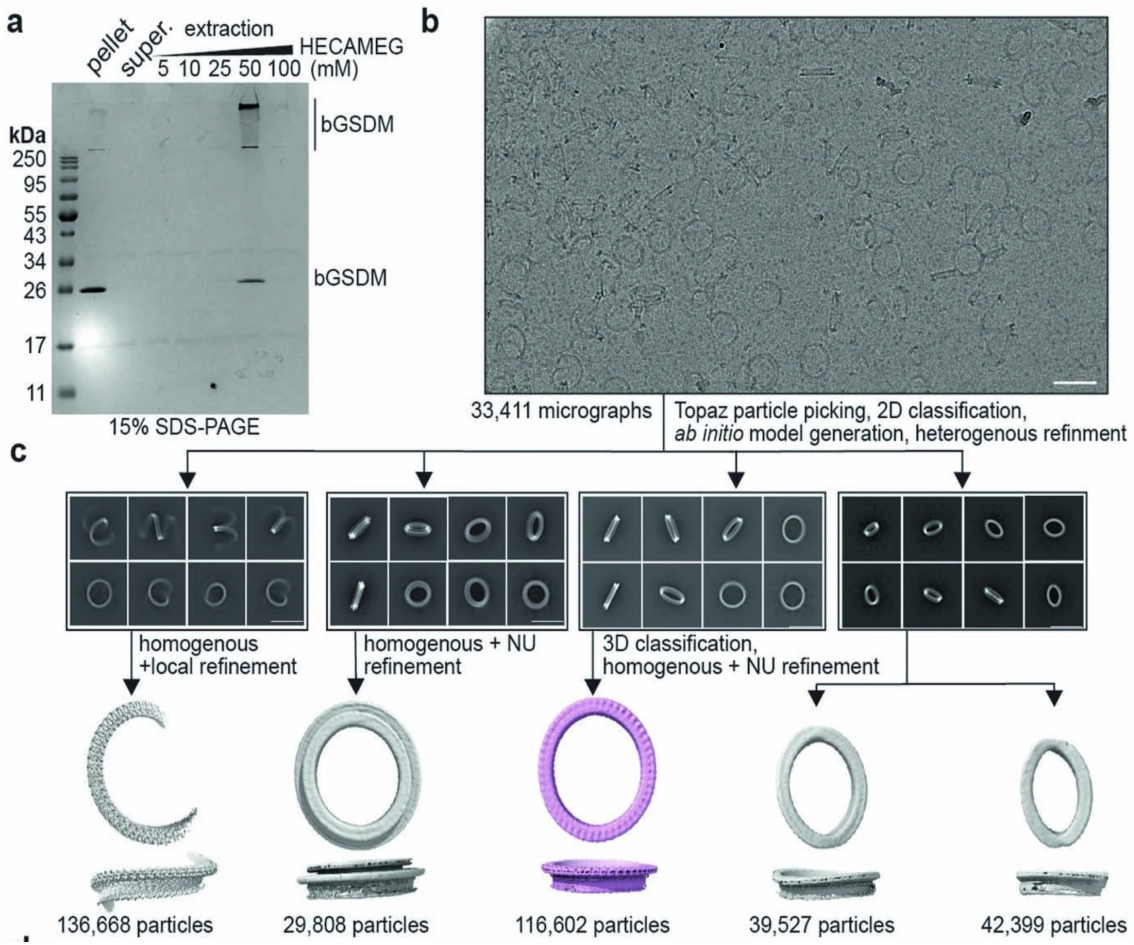
~50-100 tomograms per day vs. 10,000 SPA images per night on Krios




DOI: 10.1016/j.tibs.2021.10.006

Different cryo-EM workfows result in different types of data analysis: Segmentation, STA, and SPA

## Single Particle Analysis

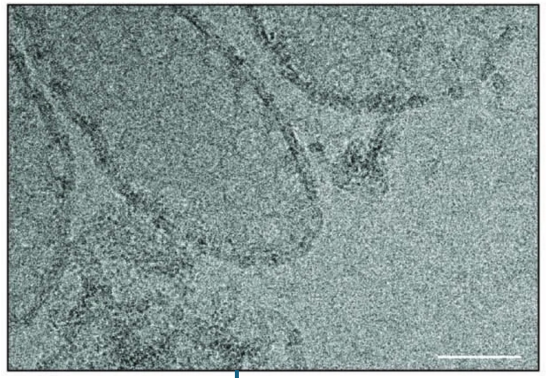


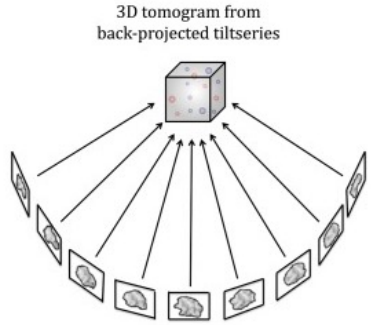

## Single Particle Averaging

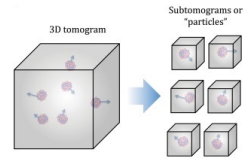
Johnson et al. 2023

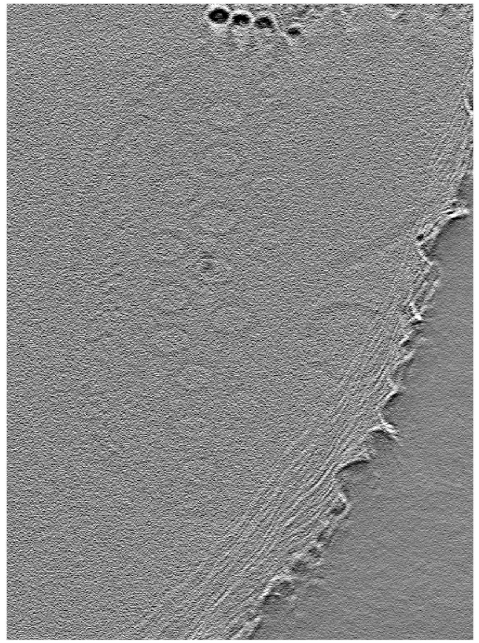
## Cryo-ET

Tilts series reconstruction

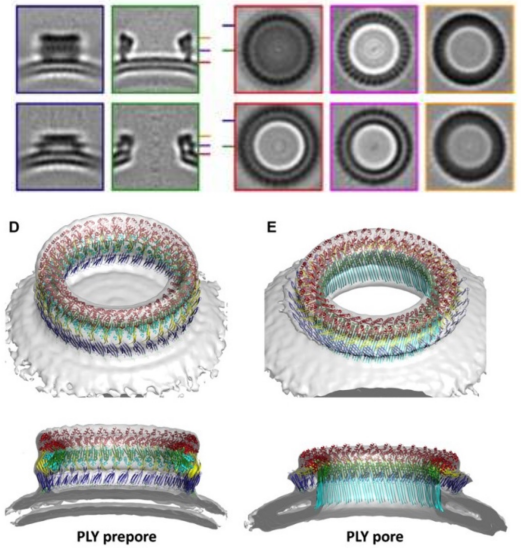




## Segmentation



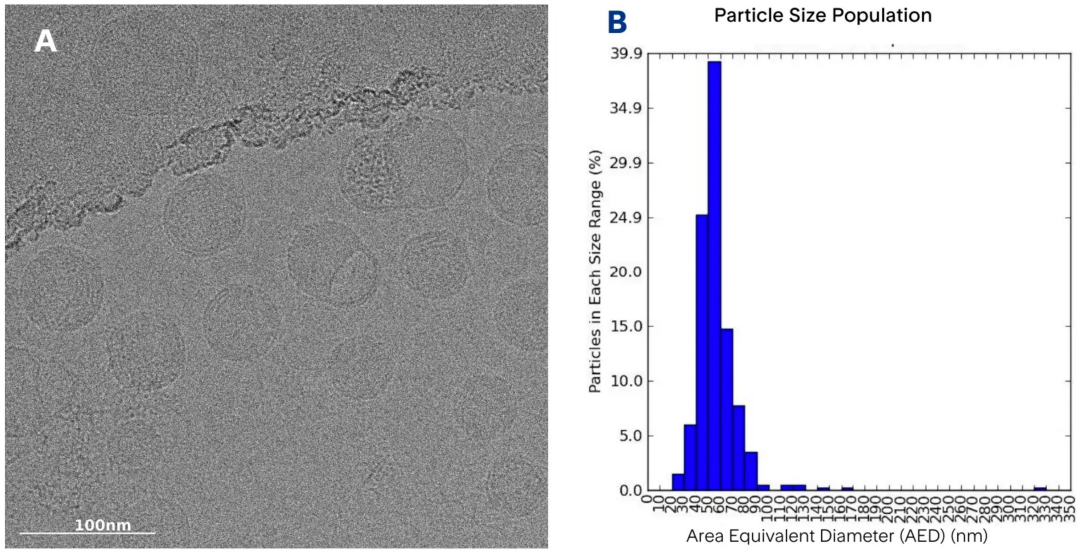

## Sub-Tomogram Averaging

Johnson et al. 2020, Pee et al. 2017

# What technique should you use?

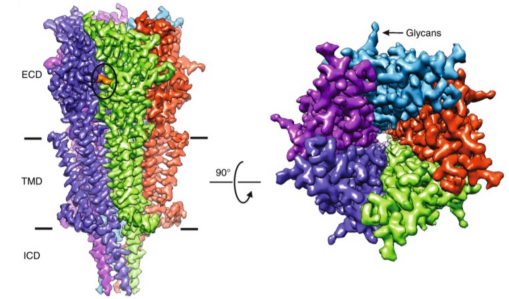
**What is your biological question that you are trying to answer?**

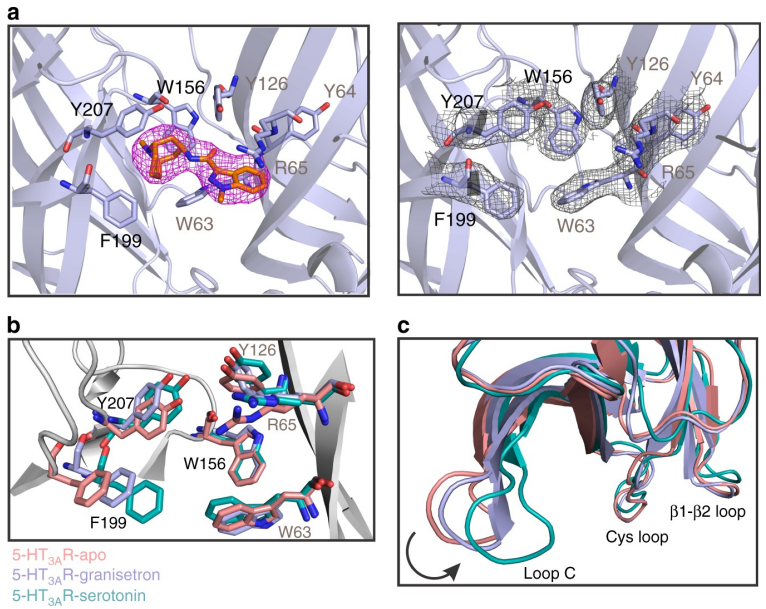
- What resolution do you need to reach?
- Can you purify your protein complex of interest to obtain high resolution structure? Is it stable?
- What is your project timeline?




**Lipid nanoparticle characterization** only requires 2D images- an afternoon screening on the T12 or Talos Arctica !

NanoImagingServices.com




**Small molecule interacting with Seratonin Receptor:**

a few screening sessions on the T12 or Talos Arctica, and then at least one high-resolution data collection on the Titan Krios.

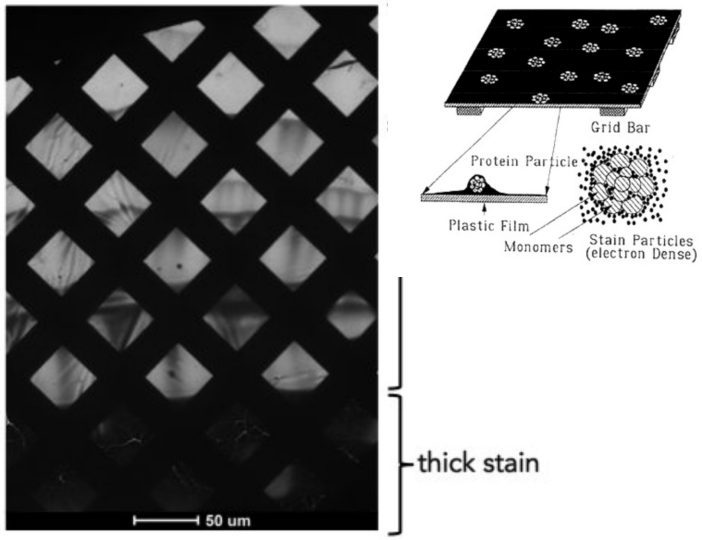
Basak et al. 2019

# Supplies to purchase for negative stain VS. cryoEM (they're different!)

1. Negative stain OR cryoEM grids
2. Your own Grid handling tweezers
3. Buttons to store cryoEM grids

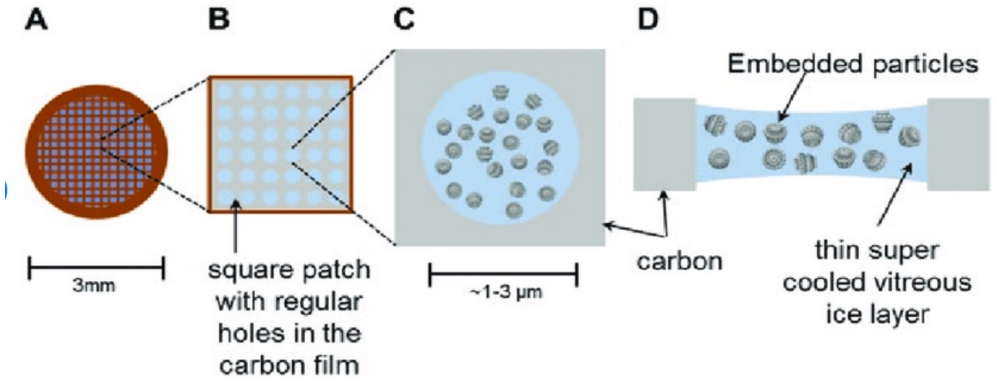
Negative stain grids have continuous carbon –sample is fixed with heavy metal stain.

Carbon is about 20-30 nm thick (useless for cryoEM grid screening)



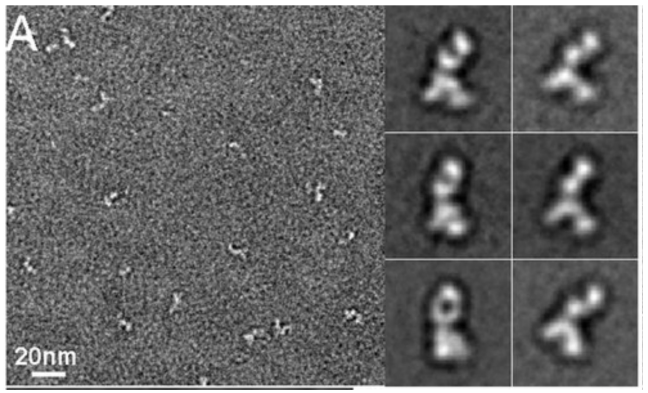

CryoEM grids have micro-patterned holes where your sample is fixed in amorphous ice

Ultrathin carbon layers may be necessary for some samples (3nm thick carbon at most)



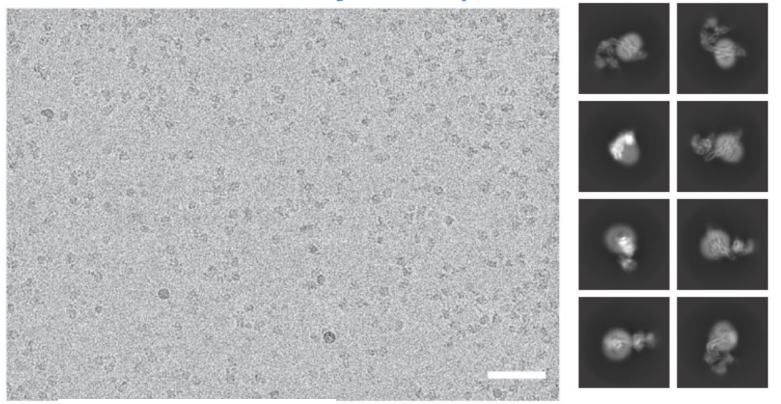

https://doi.org/10.1007/978-1-4939-7033-9_28

Negative stain 2D classes of FAB-Protein complex




DOI: 10.1016/j.str.2012.02.017

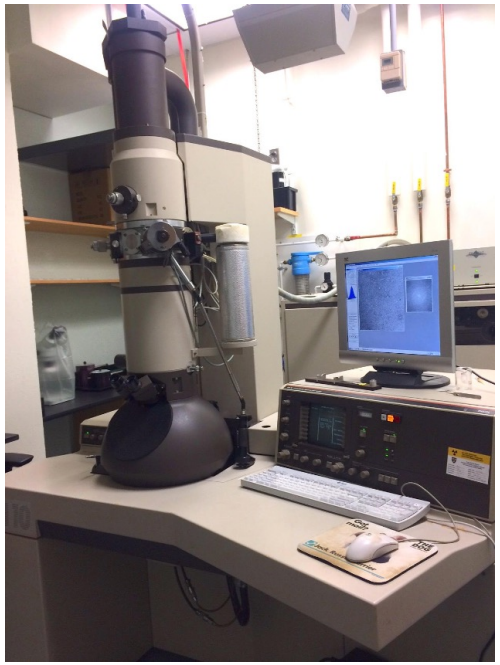
CryoEM 2D classes of AT1R-Nanobody complex

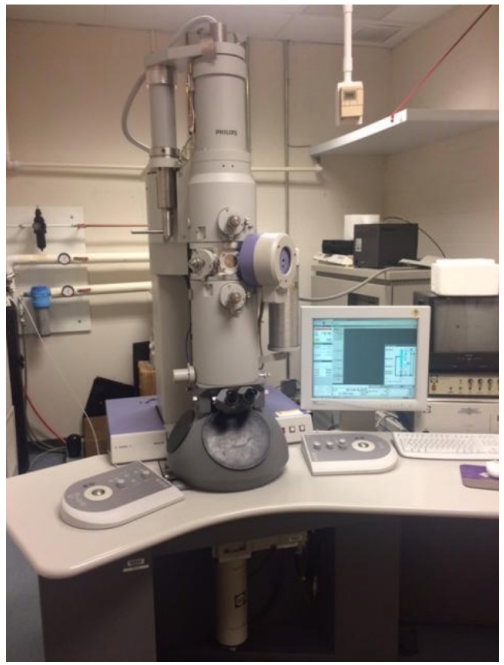


Skiba et al. 2023, bioarXiv

# How do I get started in negative stain EM for SPA?

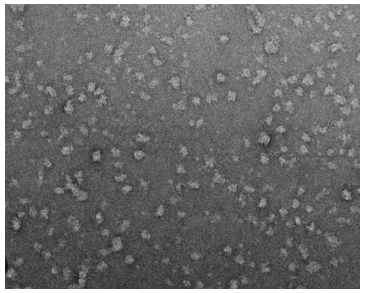
1. NON-HMS labs need Harvard Sponsored Role (HSR)
2. Create a PPMS account for the MEMS
3. Request training consultation **inside PPMS**
4. Start out with the CM10 training  (negative stain only)
5. Move on to the T12 (cryo-capabilities)

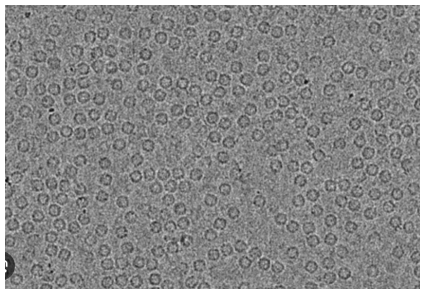




For more info: https://cryoem.hms.harvard.edu/mems-training

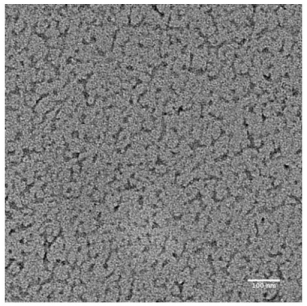
Examples of **good** negative stain images that show suitability for cryoEM ☺

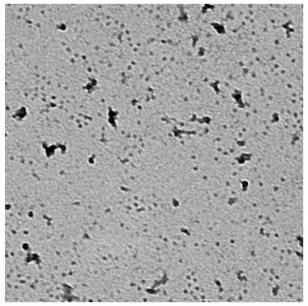




Uniform, homogenous, in-tact.

Examples of **bad** negative stain images that do not show suitability for cryoEM ☹



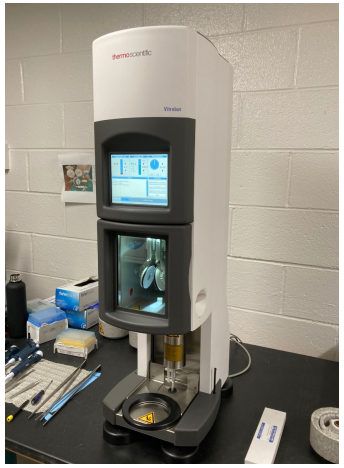


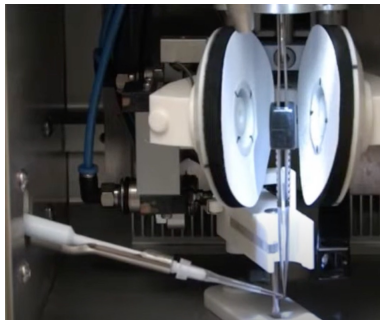
Non-uniform, heterogenous, splotchy

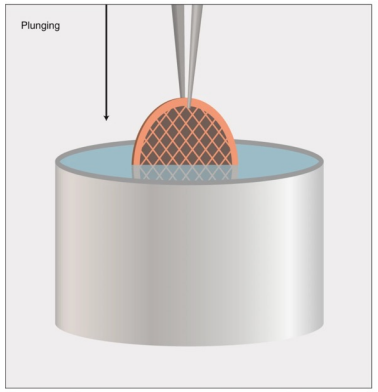
# How do I get started in making and screening grids for cryo-EM?

1. NON-HMS Labs need a Harvard Sponsored Role (HSR)
2. Create a PPMS account for the HC$^2$EM
3. Request training consultation **inside PPMS**
4. Start with vitrobot training (either with Staff or an experienced lab member)
5. Screen your first cryoEM grids on the T12 **OR** Talos Arctica (whatever has shortest wait time)

## Freeze sample on CryoEM grids Using Vitrobot

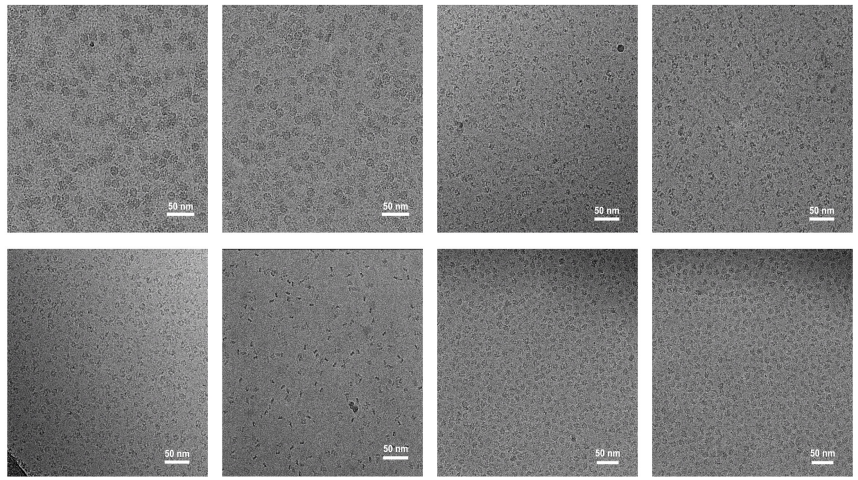






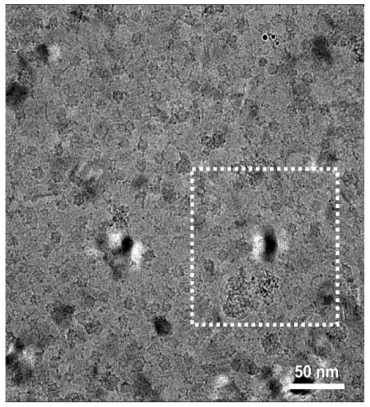

## Screen grids on T12 or Talos

## Examples of **good** cryoEM images that are ready for data collection and 2D classification ☺

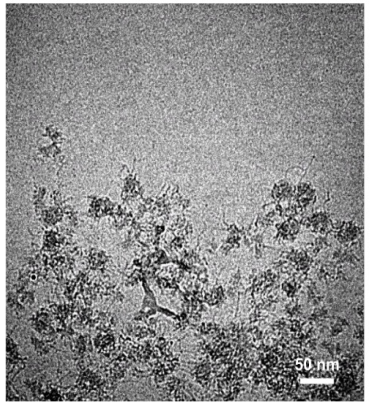


# Common problems during sample optimization for Cryo-EM

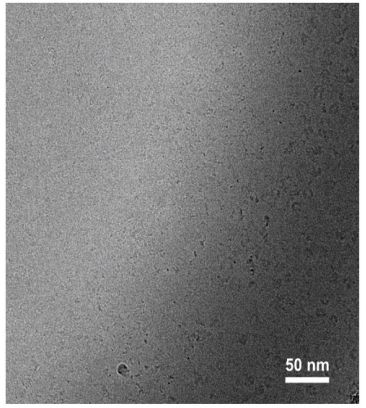
## Examples of **non-ideal** cryoEM images that need further optimization



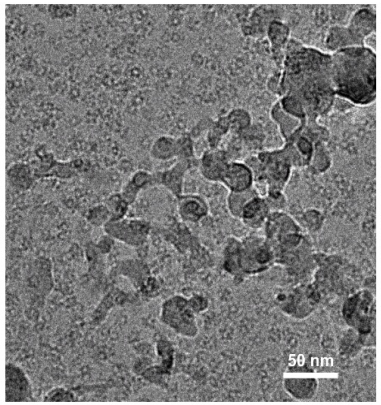

Devitrified ice
Make sure ethane is cold
Make sure your tools are cooled before touching grid



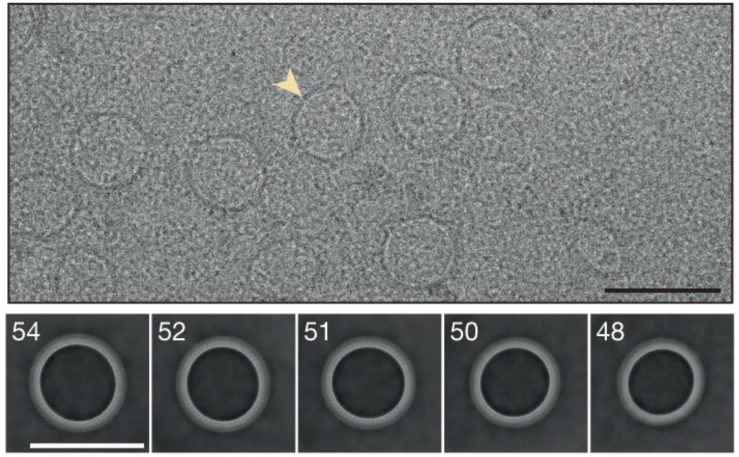

Aggregation
Try detergent panels (TFS makes a kit)
Try a few different grid types (Ultrafoil Gold)




Low concentration
Try concentrating your sample or imaging in thicker ice.
Do negative stain



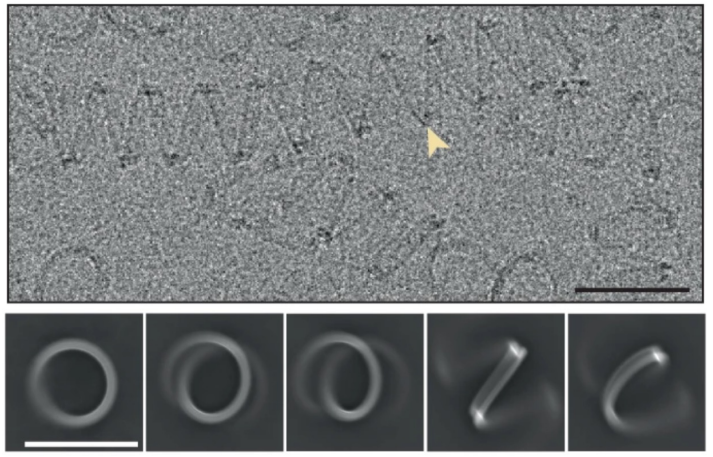

Ice contamination
Don't breathe on your sample

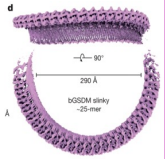



## Preferred orientation →

Things you could try:

Tilted data collection (easiest method)
Detergent panel
subtomogram averaging





Johnson et al. 2023, Nature.

Reserving Talos Microscope time for full day screening + data collection requires a project proposal.

## TALOS PROPOSAL REQUIREMENTS

**Your Name:**
**Your PI's Name:**
**Your collaborator Name(s):**
**Collaborator PI's Name(s):**
**Project Name (3-5 words):**

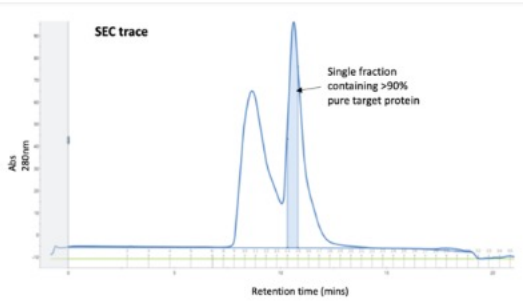
**Describe specimen, biological relevance, and brief background (maximum 500 words):**

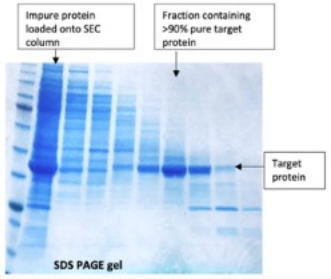
**Specimen molecular weight:**
**Specimen dimensions:**

**Specimen screening on the Talos Arctica requires biochemistry-related files (evidence of biochemical homogeneity such SDS page, SEC traces etc.) and negative stain images or 2D classes:**

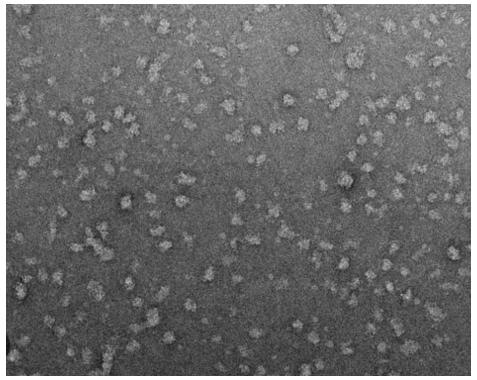
**Phase plate (yes or no):**
**Requested number of sessions (up to 4) on the Talos Arctica:**

SEC Trace (Native)




SDS-PAGE Gel



Screening images From CM10, T12, or Talos?

SEC MALS?

Mass Photometry?

**Submit A Project Proposal Here → cryoem.hms.harvard.edu/proposal**

**Cryo-ET proposal has some slight differences.**

# Reserving Krios Microscope time for full day SPA data collection and/or cryo-ET lamellae screening requires a proposal

## KRIOS PROPOSAL REQUIREMENTS

**Your Name:**
**Your PI's Name:**
**Your collaborator Name(s):**
**Collaborator PI's Name(s):**
**Project Name (3-5 words):**

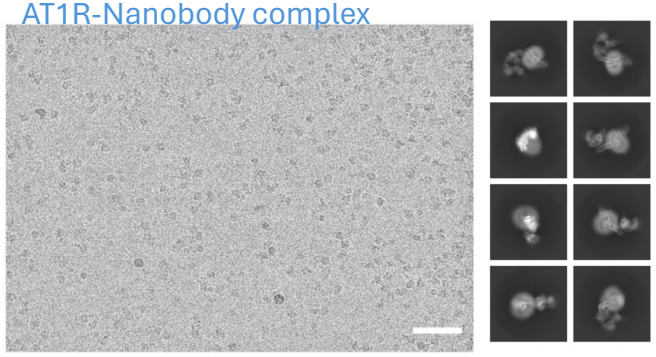
**Describe specimen, biological relevance, and brief background:**

**Specimen molecular weight:**
**Specimen dimensions:**

**Data collection on either the Talos Arctica or Titan Krios requires upload of cryo images (with scale bars) or 2D classes with a description of your previous TEM usage and processing scheme:**

**Requested microscope (Talos Arctica or Titan Krios):**
**Phase plate (yes or no):**
**Requested number of sessions (up to 4):**

CryoEM 2D classes of AT1R-Nanobody complex



Skiba et al. 2023, bioarXiv

## CryoET PROPOSAL REQUIREMENTS

**Your Name:**
**Your PI's Name:**
**Your collaborator Name(s):**
**Collaborator PI's Name(s):**
**Project Name (3-5 words):**

**Describe specimen, biological relevance, brief background, and goal of tomography study:**

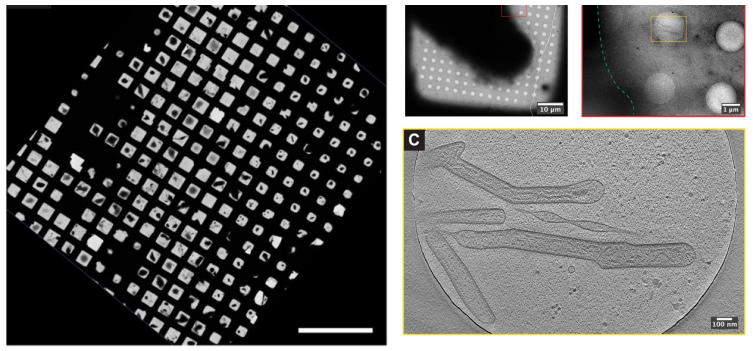
**Biosafety level and disposal:**

**Planned data processing scheme (tomograms, subtomogram averaging, etc.):**

**Atlas images, medium magnification montages, and/or screening images to demonstrate decent ice and distribution of specimen described:**

**Requested microscope (Talos Arctica or Titan Krios):**
**Phase plate (yes or no):**
**Requested number of sessions (up to 4):**

### Eukaryotic Cells in Vitreous, thin ice




RSV-infected Eukaryotic cell. Sibert et el. 2021, BioarXiv

## Summary

**Have your biological question ready, and hypothesis what you will find.**

**Plan out your project with your advisor; seek advice from labs with cryo-EM expertise; or seek consultation with staff at the center.**

**Preliminary biochemical characterization will make or break your project. Cryo-EM is not a tool to help you figure out what your sample is.**

**Negative stain and MEMS CM10/T12 screening is the fastest and most efficient way to make sure your sample is ready for SPA cryo-EM.**

**Talk to Conny about how to get a cell on a grid for CryoET.**

**The proposal process is a tool to make sure your project is on track for success.**

## Resources and links to get started now!

**Our website:** cryoem.hms.harvard.edu
*These slides will be posted on: cryoem.hms.harvard.edu/blog*

**Get a PPMS Account**: cryoem.hms.harvard.edu/requesting-ppms-account

**Request MEMS Training:** cryoem.hms.harvard.edu/requesting-ppms-account

**Request CryoEM Training**: cryoem.hms.harvard.edu/new-user-training

**Submit a Project Proposal for CryoEM:** cryoem.hms.harvard.edu/proposal

**Protocols for CryoEM Data collection Training**: cryoem.hms.harvard.edu/protocols

Grant Jensen's lectures series, as well as CryoEM101.org, are a great reference for getting started with cryoEM concepts.


# Thank you!

Requests for next topics: send email to cryoEM@crystal.Harvard.edu