An introduction to national cryoEM center resources

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New York Structural Biology Center

Tuesday, March 17, 2026

NCCAT

NYSBC SEMC

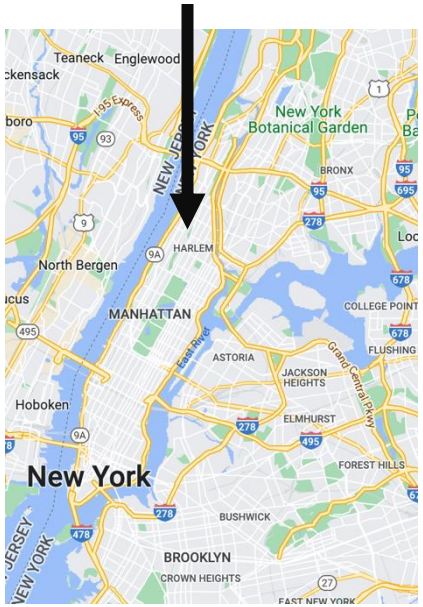
SIMONS ELECTRON MICROSCOPY CENTER

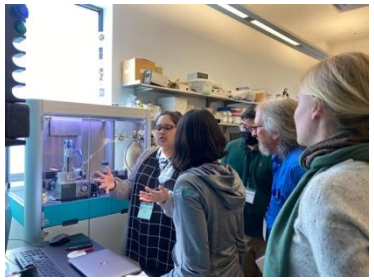
NEW YORK STRUCTURAL BIOLOGY CENTER NYSBC

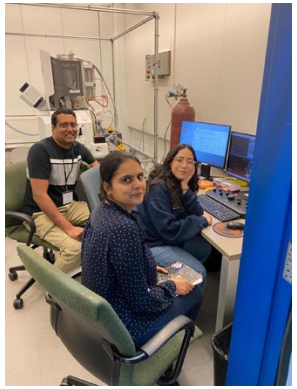
NCCAT & NCITU: Resources for cryoEM and cryoET

NIH Aim: broaden access to high-resolution cryoelectron microscopy (and tomography) for biomedical researchers, and cultivate a skilled workforce through the development and implementation of cryoEM training material

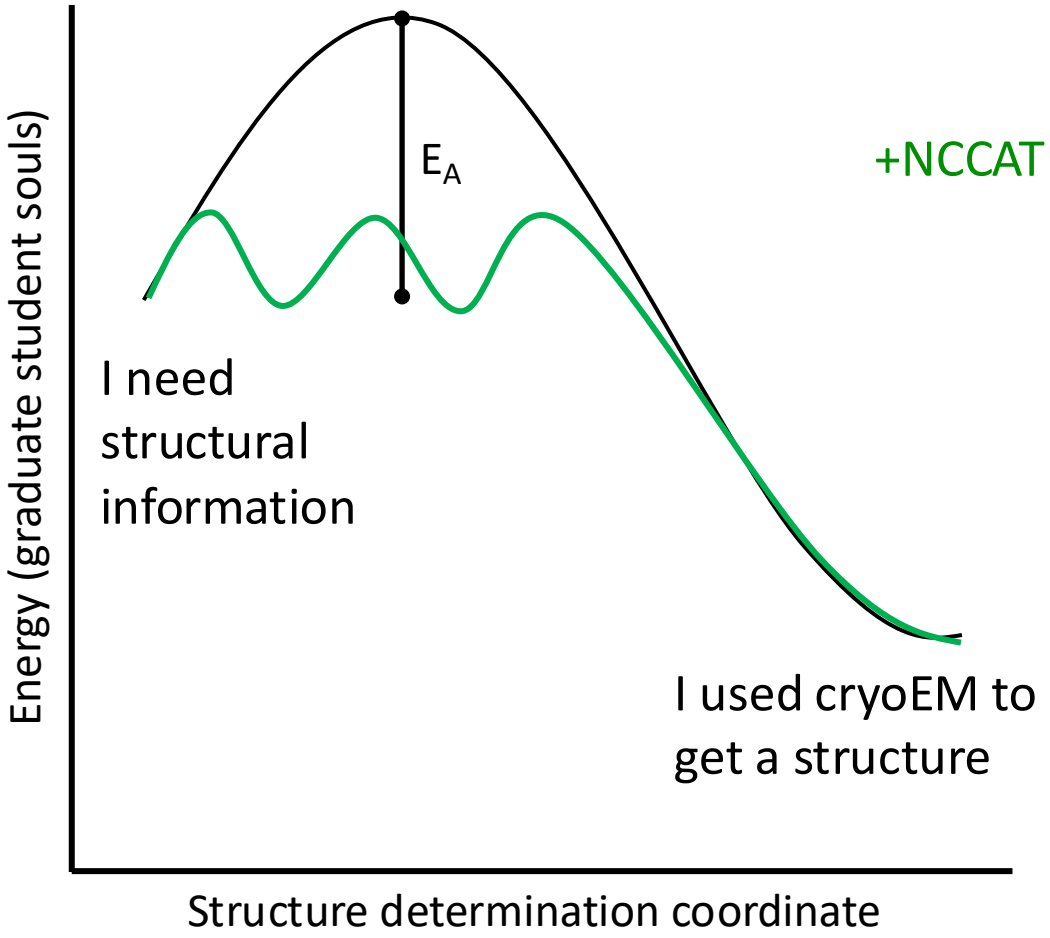
+ very cool people







The national centers mission is to lower barriers for cryoEM use



+NCCAT

E_A = Samples + Instrumentation + Expertise + $$

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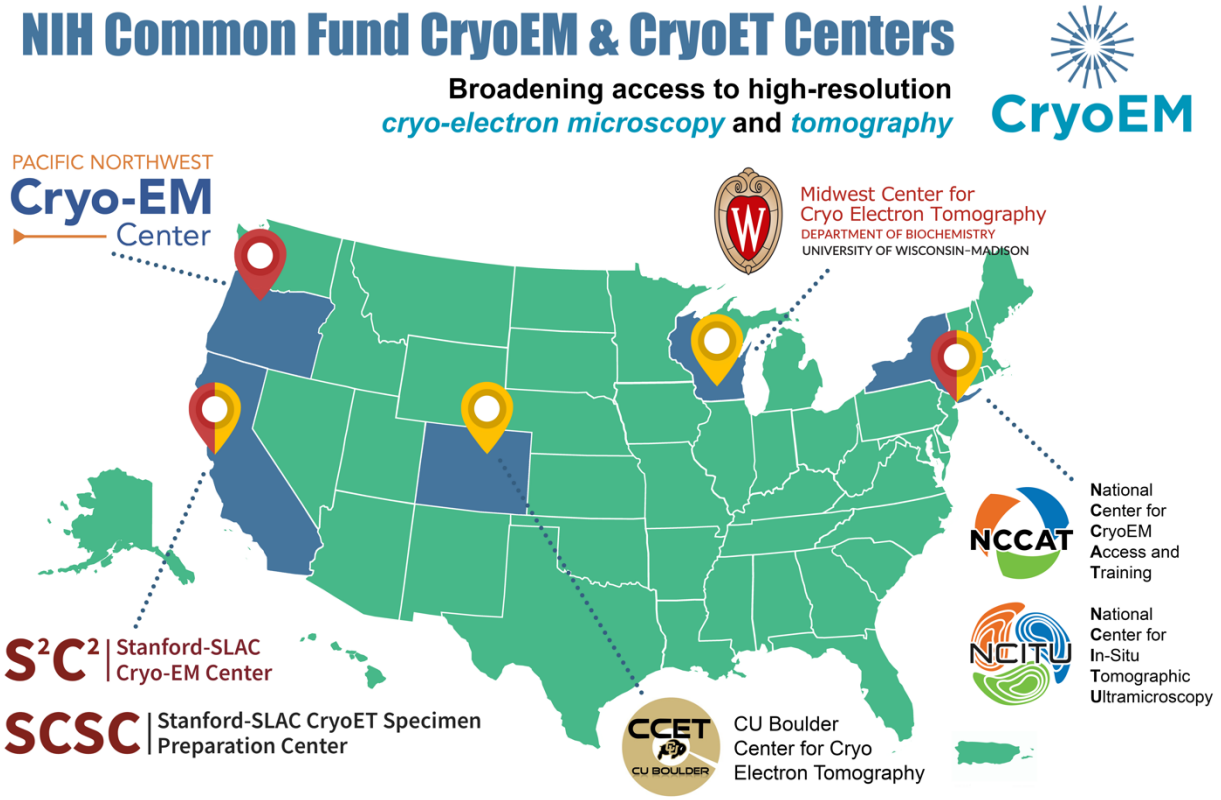
Workshops

Multiple centers share a mission make cryoEM accessible

CryoEM Centers

CryoET Network



Free Curriculum

PURDUE UNIVERSITY CryoVR

THE UNIVERSITY OF UTAH CryoEM 101 CryoET 101

Caltech | Getting Started in Cryo-EM

Yale Cryo-EM Principles

UNIVERSITY OF MICHIGAN cryoEDU

NCI National CryoEM Facility (NCEF)
www.cancer.gov/research/resources/cryoem

The Southeastern Center for Microscopy of MacroMolecular Machines (SECM4)
www.secm4.org

cryoemcenters.org

NCCAT has five avenues of access

General User Proposal 1 (**GUP1/BAG**): Data collection on Krios TEMs for ready samples	General User Proposal 2 (**GUP2**): Blot-free vitrification and screening	General User Proposal 3 (**GUP3**): Grid preparation and screening	Training Proposal 1 (**TP1**): Training researchers to become independent cryoEM users	Training Proposal 2 (**TP2**): Training facility managers
				
	 2-3 days	 1-4 days	 2-10 weeks	 3-5 days

- All access categories require submission of a proposal that is scored by an external review committee to determine priority and duration of scheduling.
- Instrument & training time is at no-cost to the user.

The life of an NCCAT proposal:

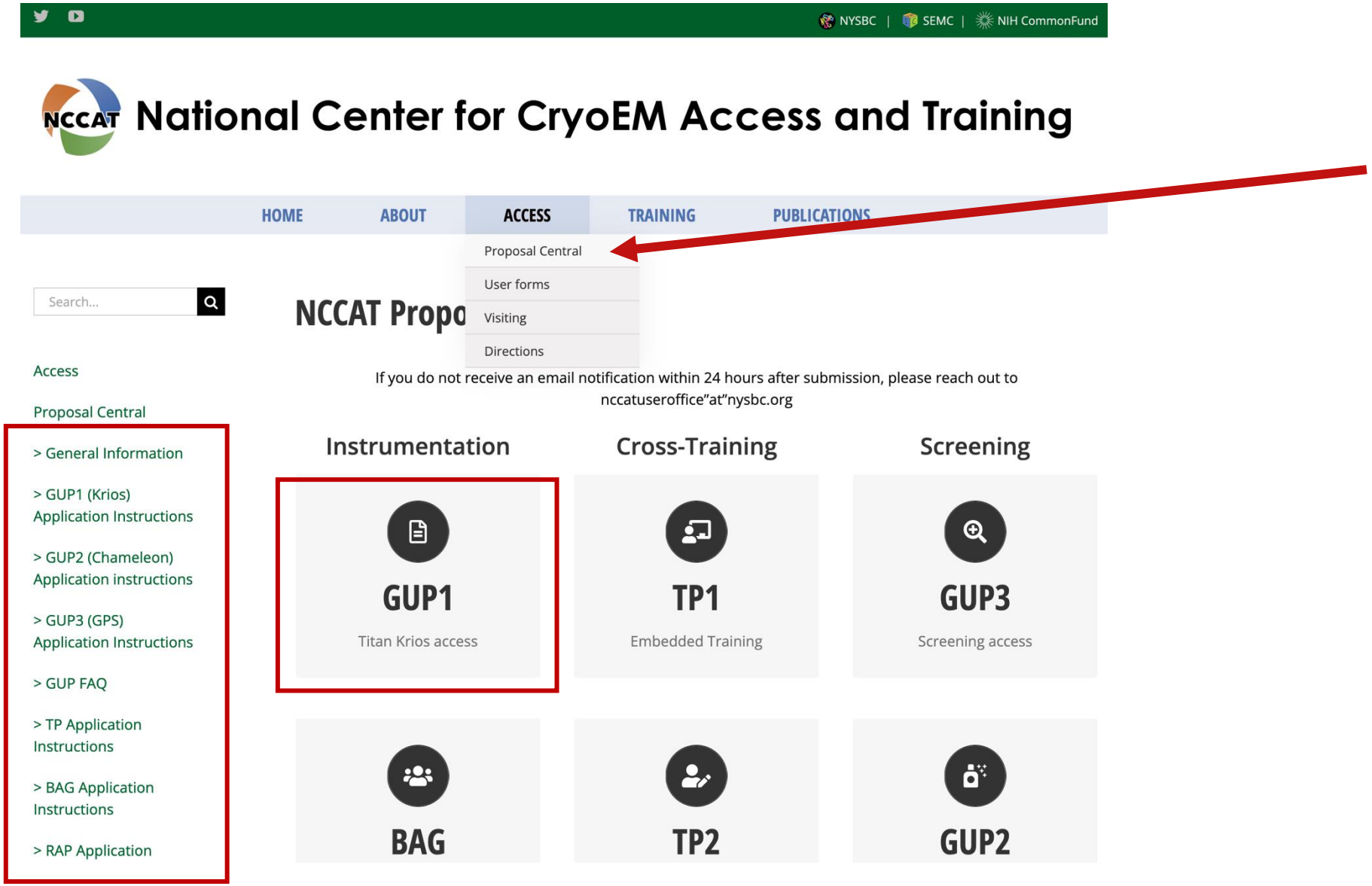
- Quarterly Application deadlines: Jan1, April 1, July 1, October 1
  - 1-2 days after deadline: Applications sent to reviewers.
  - 3-4 weeks after deadline: Reviewer scores returned to user office.
  - 4-6 weeks after deadline: Scores sent back to applicants, user office reaches out to begin scheduling based on scores & instrument availability (pre-session meeting for new users).
  - Once a scored proposal is awarded time, all access is at no cost at point of access (you have to ship your samples, cover travel expenses, lodging, etc).

The life of an NCCAT proposal:

- Once a session is scheduled: Grids must be received 2-2.5 weeks before session time to complete our intake and QC process.

- GUP1/BAG data collection is in-person or remote.
- GUP2/3 and TP are all in person.

- Proposals have up to a 2-year lifetime and will be completed either when the full award is utilized or the lifetime of the proposal ends.


NYSBC | SEMC | NIH CommonFund
National Center for CryoEM Access and Training
HOME
ABOUT
ACCESS
TRAINING
PUBLICATIONS
Proposal Central
User forms
Visiting
Directions
Search...
NCCAT Propo
If you do not receive an email notification within 24 hours after submission, please reach out to nccatuseroffice"at"nysbc.org
Access
Proposal Central
> General Information
> GUP1 (Krios) Application Instructions
> GUP2 (Chameleon) Application instructions
> GUP3 (GPS) Application Instructions
> GUP FAQ
> TP Application Instructions
> BAG Application Instructions
> RAP Application
Instrumentation
Cross-Training
Screening
GUP1
Titan Krios access
TP1
Embedded Training
GUP3
Screening access
BAG
TP2
GUP2

NCCAT application is an online form

NCCAT GUP1 (Krios) Common Application Submission

Fields marked with an * are required

This application applies for Krios access. For more information please see the GUP program or contact nccathelp [at] nysbc.org.

User information fields.

The applicant will be the spokesperson for the proposal and primary correspondence will be directed to this user.

First Name *
Institution/University *
Middle Initial
Mailing Address *
Last Name *
City *
Email *
US States
- Select State -
Phone *
Zip
PI Full Name *
Country *
United States
PI Email *
Lab Name *
eRA commons name *

Enter your NIH eRA commons name here or if you do not have one then you may use an ORCID (https://orcid.org).

Enter your NIH eRA commons name here.

Scientific Document.

Be concise. The combined text in all the proposal fields should be 2 pages (Arial 11pt font) or 1000 word maximum.

Project Title *

Please provide a brief title that describes the project. (80 characters or less)

80 of 80 Character(s) left

Project Abstract *

Abstracts should concisely summarize the project impact. Please note that abstracts and a limited set of project demographics will be sent to the National Institutes of Health (NIH) on a quarterly basis as part of the required project reporting. (150 words or less)

150 of 150 Word(s) left

Aims & Impact *

Please state the specific objectives of your project. Include the scientific and technological importance of your project. (150 words or less)

150 of 150 Word(s) left

Feasibility & Data *

Provide information and preliminary data for the samples associated with this proposal that impact feasibility of cryo-EM studies: molecular weight, stability, homogeneity, sufficient concentrations, etc. Experimental preliminary data may include: SDS-PAGE gels, SEC traces, negative-stain EM, preliminary cryo-EM screening, etc. Reference all uploaded figures and provide a brief description as appropriate. (300 words or less)

300 of 300 Word(s) left

Proposed Experiments *

Describe the work to be conducted at the national center during the awarded project period. For each aim and/or sample under investigation, provide a detailed description of the experiment(s) to be performed and expected outcomes. Strength of justification can affect the overall science and resource scores. (150 words or less)

150 of 150 Word(s) left

Goals & Expectations *

Please describe the goals for this project and resources that would be needed. This section should also include a justification for specific instruments requested, if any, and for the estimated allocation of time for each experiment. (200 words or less)

200 of 200 Word(s) left

Expertise & Resources *

Describe the team's expertise in CryoEM sample preparation, microscope operation, and data interpretation. Please state the available cryoEM and/or computational resources that your team has access to. (200 words or less)

200 of 200 Word(s) left

Figures/Preliminary Results *

Provide scientific figures with captions to reference within the feasibility & data section. (1 page supplemental, pdf)

All attachments should be in pdf format, ideally with a combined size no larger than 10 Mb.

Select Files

NIH Biosketch *

All key personnel named in the application should upload a NIH Biosketch, which should be combined into 1 pdf.

All attachments should be in pdf format, ideally with a combined size no larger than 10 Mb.

Select Files

Additional notes for NCCAT.

To avoid potential conflicts of interest, users have the option to exclude any member of the URC during the application process. If there are individuals you would like to exclude from the review process please include them here. For example, "Please exclude John Smith (Institute A) and Jane Doe (University B) from the review process".

In addition, if there are multiple personnel in the application make sure that each person has uploaded a NIH Biosketch, and in this field state their names and role(s) in the project. For example, "Key personnel: John Smith (primary user), Jane Doe (secondary user), and Careful User (PI)".

Notes for NCCAT.

Voluntary declaration of overlap

Please state if you have submitted this application to other national service centers.

NCCAT and PNCC has established a Memo of Understanding (MOU) in case one center cannot timely serve an application to forward your reviewed application to the other service center. If you would like to learn more contact the NCCAT User Office.

Recaptcha v2

I'm not a robot

Please review and confirm the information all fields before submitting. After submission you should receive a confirmation email. If not, then your application was not processed properly.
If you have any questions please email nccatuseroffice [at] nysbc.org .

The accuracy of this information is a requirement for any NCCAT user. Incomplete or inaccurate information may delay your user registration and project submission. Also, by submitting this application you acknowledge that information may be shared with other national service centers.

Submit

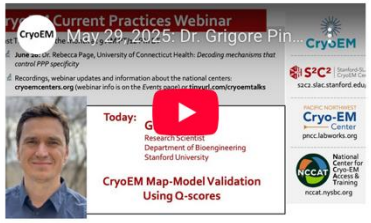
Additional training resources

CryoEM current practices webinar

Some tips & tricks of note:

Sample preparation: Use of liposomes as membrane mimetics. Preparation of small unilamellar vesicles (SUV). Testing carbon/Au/NiTi grid foils with various hole diameters to improve sample distribution. Mixing complex components on grid to improve sample behavior.

Data processing: Topaz picking. Tricks to classify and recenter particles to overcome effects of phospholipid bilayer alignment.



Some tips & tricks of note:

Data analysis: Building atomic models into cryoEM maps. Model validation tools.

Tips & tricks of note:

Data processing: Practicalities of neural network picker software installation and usage. How to choose a particle picker & validation. Neural network picking for single particle analysis, filaments, & tomography. Interplay of particle picking and preferred orientations. Particle centering.

NCCAT | S²C² Stanford-SLAC Cryo-EM Center | PACIFIC NORTHWEST Cryo-EM Center

CryoEM Current Practices Webinar

Using cryo-EM to understand dynamic interactions

Wolfgang Peti, PhD

Professor

University of Connecticut Health Center

12PM EDT / 9AM PDT Thursday, March 28, 2024

As a trained NMR spectroscopist and experienced X-ray crystallographer, I will provide our newly gained experience and insights from using cryo-EM as a routine tool in our laboratory. As an example, I will use a recent complex that we determined by cryo-EM: PP2A:B55-ARPP19. PP2A:B55-ARPP19 is critical as progression through the cell cycle is controlled by regulated and abrupt changes in phosphorylation. Mitotic entry is initiated by increased phosphorylation of mitotic proteins, a process driven by kinases, while mitotic exit is achieved by counteracting dephosphorylation, a process driven by phosphatases, especially PP2A:B55. While the role of kinases in mitotic entry is well-established, recent data have shown that mitosis is only successfully initiated when the counterbalancing phosphatases are also inhibited. For PP2A:B55, inhibition is achieved by the two intrinsically disordered proteins (IDPs), ARPP19 and FAM122A. Despite their critical roles in mitosis, the mechanisms by which they achieve PP2A:B55 inhibition is unknown. Here, we report the single particle cryo-electron microscopy (cryo-EM) structures of PP2A:B55 bound to phosphorylated ARPP19 and FAM122A. Consistent with our complementary NMR spectroscopy studies both IDPs bind PP2A:B55, but do so in highly distinct manners, unexpectedly leveraging multiple distinct binding sites on B55. Our extensive structural, biophysical and biochemical data explain how substrates and inhibitors are recruited to PP2A:B55 and provides a molecular roadmap for the development of therapeutic interventions for PP2A:B55 related diseases.

All are welcome to attend. Registration is at no-cost, but sign-up is required:
https://us02web.zoom.us/webinar/register/WN_EZtYSZ2XTfaVPSQyqDFzfQ

This webinar series is jointly hosted by the NIH Transformative High Resolution CryoEM Program Service Centers: the National Center for CryoEM Access and Training (NCCAT), the Pacific Northwest Center for CryoEM (PNCC), and the Stanford-SLAC CryoEM Center (S2C2) who provide no-cost access to cryoEM instrumentation and training. In this monthly series, we will highlight cryoEM methods and use the Q&A session after the seminar to stimulate discussion of best practices and interesting challenges that will be helpful to researchers new to the field. Representatives from all three service centers will also be on hand to answer questions about the cryoEM resources available to biomedical researchers and how to access them.

Webinar info

cryoemcenters.org/events

What is a cryoEM merit badge?

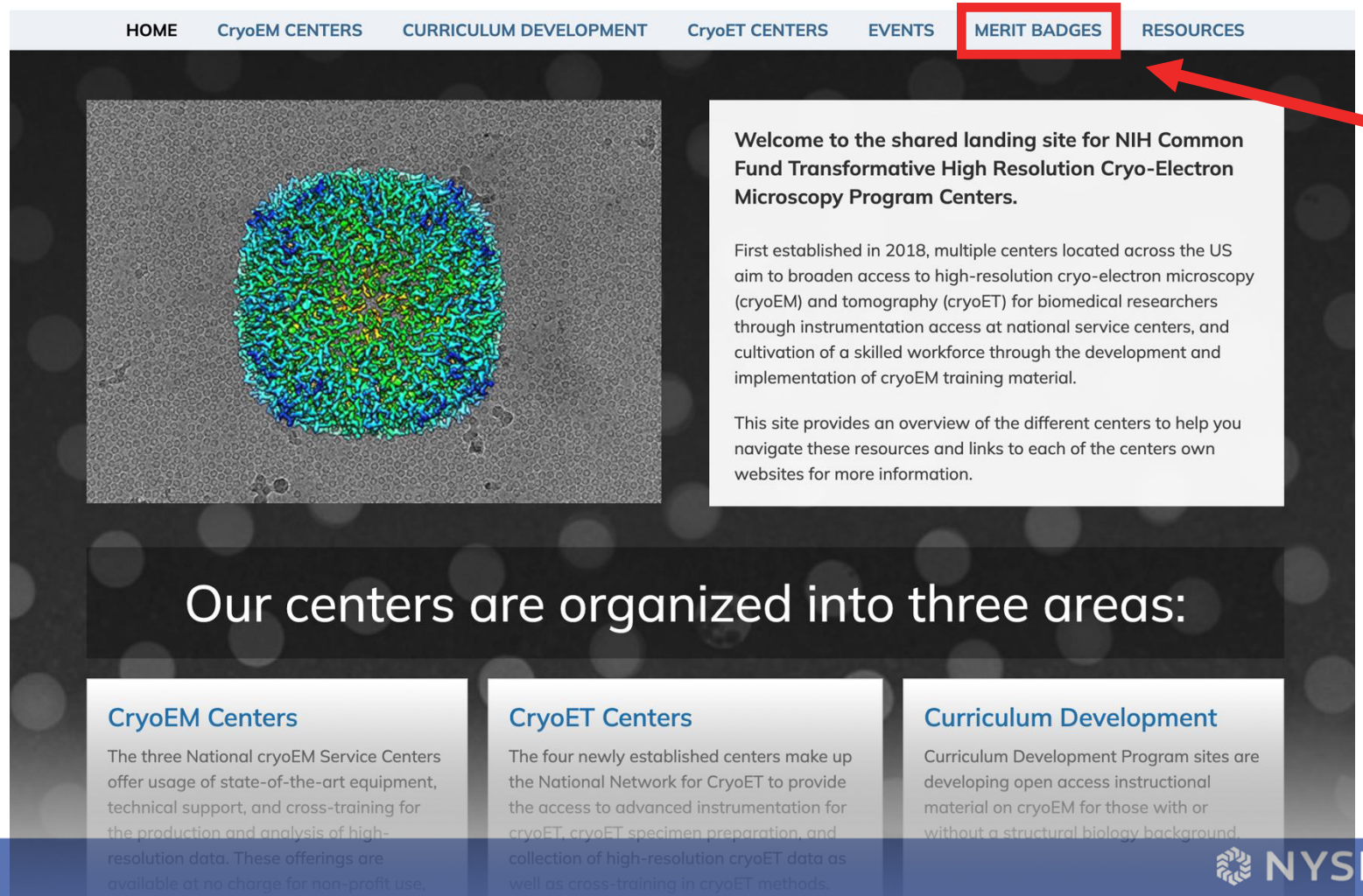
- A proficiency certification that is cross-honored at the national Cryo-EM service centers
  - Standardized level of training
  - Trust but verify independent use of instrumentation for new users

cryoemcenters.org

CryoEM
TRANSFORMATIVE HIGH RESOLUTION CRYO-ELECTRON MICROSCOPY

Broadening access to high-resolution
cryo-electron microscopy and *tomography*

CRYO-EM MERIT BADGE PROJECT



Current badges

Sample Preparation
The start of a cryoEM project

TFS Vitrobot
Plunge freezing and instrument certification for Vitrobot Mark IV

LEARN MORE

Leica EM GP
Plunge freezing and instrument certification for Leica EM GP or GP2

LEARN MORE

Grid Clipping
Grid Clipping Certification

LEARN MORE

Autoloader
Cassette Loading and Autoloader Docking Certification

LEARN MORE

SPT Labtech chameleon
Use of the chameleon for blot free vitrification

LEARN MORE

Cryosol Vitrojet
Use of the Vitrojet for blot free vitrification of clipped autogrids

LEARN MORE

Cryogenic Sample Shipping
Safe transport of vitrified cryoEM grids to a national center for a successful data collection

LEARN MORE

Negative Stain Grid Preparation
Negative staining for macromolecular sample characterization at room temperature

LEARN MORE

Microscope Operations
Microscope use and data collection

SerialEM Screening
SAMPLE SCREENING

Software Operation and Grid Assessment Certification for SerialEM

LEARN MORE

Leginon Screening
SAMPLE SCREENING

Software Operation and Grid Assessment Certification for Leginon

LEARN MORE

EPU Screening
SAMPLE SCREENING

Software Operation and Grid Assessment Certification for EPU

LEARN MORE

SerialEM Data Collection
AUTOMATED COLLECTION

Data Collection Certification for SerialEM

LEARN MORE

Leginon Data Collection
AUTOMATED COLLECTION

Data Collection Certification for Leginon

LEARN MORE

EPU Data Collection
AUTOMATED COLLECTION

Data Collection Certification for EPU

LEARN MORE

TFS Tundra
MICROSCOPE OPERATION AND SOFTWARE CONTROL

Certification for Independent Tundra use

LEARN MORE

Merit Badge Supplement

Advanced TEM session setup checklist
AUTOMATED COLLECTION

A tool for independent autoloader TEM data collection

Open the checklist

MERIT BADGES: THE SOP

TFS Autoloaders

Category: Sample preparation
Sub-category: Cryogenic work

Autoloader grid handling including loading, recover

- + Essential Base Knowledge & SO
- + Knowledge test
- + Demonstration & Center Specific
- + Practice
- + Practical test

Additional information:

Recertification period

- Sample preparation merit badges for cryogeni
- Recertification (to maintain active status) wou front of a center staff member.

Furthering your knowledge

General background on plunge freezing

- Getting Started in CryoEM – Unit 2: Sample Pr Plunge freezing safety modules]

− Essential Base Knowledge & SO

Safety

You must complete all required safety training at training. Coordinate this with the user office or t

Background

Relevant Training Information (to review until yo

- CryoEM101 Chapter 3: Part 2 – Overview o
- Cryo-EM Sample Preparation: Part II – Auto instructions *by Jonathan Ipsaro*
- Workflow Videos:
  - Introduction to Cassette Loading
  - Assembling the Cassette Loading Sta
  - Structure of the Cassette
  - Loading the Cassette
  - Docking
  - Undocking

SOP for Autoloader Loading (for re

- Printable version of the SOP
- Digital version of the SOP

National Center for CryoEM Access and Training | Cryo-EM Stanford-SLAC Cryo-Electron Microscopy | Pacific Northwest National Laboratory | OHSU

CRYO-EM MERIT BADGE PROJECT

Common Autoloader Standard Operating Procedure versio

Authors: Sean Mulligan, Christina Zimanyi, Htet Khant

Created Date: 09/2022

1. **Purpose**
   1.1. To transfer clipped autogrids into a TFS autoloader cassette and use a NanoCab to transfer them into an autoloader system
   1.2. To care for and maintain autoloader tools (autoloader cassettes, transfer stations, and NanoCabs)

2. **Definitions:**
   2.1. An Autoloader system is a robotic system for loading cryo-EM grids into the microscope, in which the user inserts autogrids into a cassette that is loaded into the microscope using a retractable arm.
   2.2. An Autogrid is a Cryo-EM grid secured into a C-clip using clip rings (and prepared by the user through "grid clipping").
   2.3. NanoCab is specially made vacuum insulated capsule that holds a cassette and docks with Autoloader.
   2.4. Liquid Nitrogen (LN_2) is a cryogenic liquid stored under pressure.
   2.5. Definition of terms for tools/equipment can be found in Figure 1.

3. **Supplies & Equipment**
   - ❑ PPE (BSL-1)
     - ❑ Laboratory Coat
     - ❑ Nitrile Gloves
     - ❑ Goggles / Safety Glasses
     - ❑ Cryogenic Gloves
     - ❑ Face Mask
   - ❑ Chemicals/Reagents
     - ❑ Liquid Nitrogen
   - ❑ Table-top Transfer Station assembled with Covers, Metal Block, and Cassette Gripper Handle
   - ❑ NanoCab and Lid
   - ❑ Autogrid Cassette
   - ❑ Autogrid Tweezers
   - ❑ Cassette Tweezers
   - ❑ Standard Fine-tip Tweezers
   - ❑ Autogrid box(es)
     - ❑ Containing vitrified samples on clipped grids
   - ❑ Autogrid Box Opening Tool
   - ❑ Large Forceps
   - ❑ Hair dryer or heat block (if needed to dry tools)

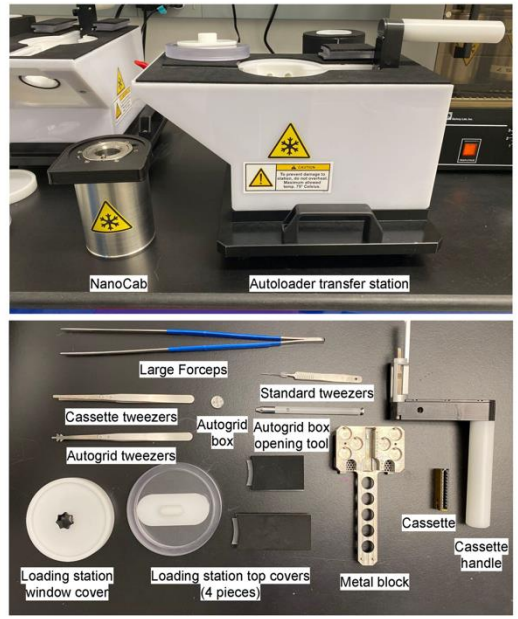


Figure 1. Autoloader transfer station and tools, labeled.

CryoEM Merit Badges were developed with support from NIH Common Fund Merit Badge supplement to 3U24 GM129539-02

Center specific component allows any lab to use our SOP

National Center for CryoEM Access and Training

TFS Autoloaders. NCCAT specific procedures.

Safety

General Precautions:

- Use the Personal Protective Equipment (PPE) provided by NCCAT. Nitrile gloves, goggles (or eyeglasses), and additionally cryo-gloves and a face shield while dispensing LN_2 and a face shield while dispensing ethane.
- The dehumidified room is equipped with an oxygen monitor. If the monitor alarm sounds, you **MUST** stop working, leave the room and leave the door propped open for air replacement. You must wait until air has recirculated and the alarm turns off before reentering the room (this generally takes only a minute or two).
  - If you continue working in the room while the alarm is sounding, you will lose access to the lab.
- Inspect equipment and accessories for weaknesses, cracks or damage before beginning work. If you suspect any damage, or encounter any problems during use, please fix immediately and ask for help from staff if needed.
- Report all accidents, no matter how minor.

Liquid Nitrogen:

- Ask for assistance to fill 4 L LN_2 dewars from the large tanks. Use cryo-gloves for LN_2 tank operation.
- Do not discard of LN_2 on the floor. Empty any used nitrogen into LD10 collection dewars.
- Be careful with any metal or tweezers in vacuum sealed dewars. If a dewar breaks, the release of vacuum can cause glass to shatter around the room.

Mandatory PPE for LN_2 dispensing

CryoEM Merit Badges were developed with support from NIH Common Fund Merit Badge supplement to 3U24 GM129539-02

National Center for CryoEM Access and Training

4.1 and 4.2 Setup

- NanoCabs & empty cassettes are stored in the ovens on the bench in the NCCAT dehumidified room (Fig 1A). Loading stations are stored on the left side of the bench (Fig 1B). NanoCabs are stored upside down.

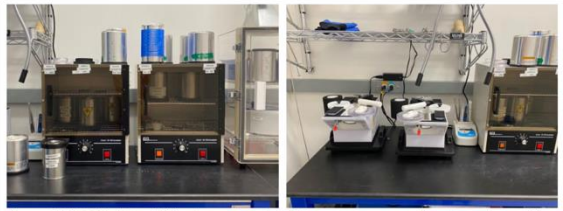


Figure 1. NCCAT dehumidified room bench. A) Two ovens are used to dry and store NanoCabs and empty cassettes. B) Two loading stations are kept on the left end of the bench.

- Each microscope has two designated NanoCabs, clearly labeled (Fig 2A). Each microscope also has designated cassettes, with labels etched onto the bottom (Fig 2B). NanoCabs and cassettes should be stored in the proper oven (labels show in Fig 2C).

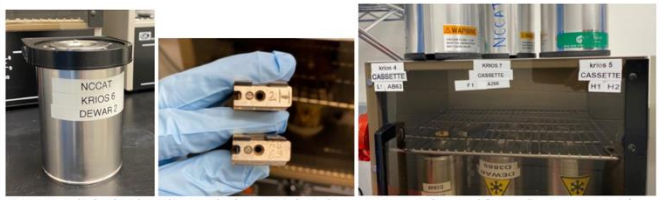


Figure 2. Labels clearly indicate which NanoCab and cassette are to be used for each microscope. A) Each NanoCab is labeled (here the NanoCab for Krios6 is shown). B) The silver end of the cassette has and etched label (here labels "H2: and "AB63" are shown). C) Each oven is labeled with which NanoCabs and cassettes should be placed inside.

- Forceps and grid box opening tools are in the drawers below the lab bench.

CryoEM Merit Badges were developed with support from NIH Common Fund Merit Badge supplement to 3U24 GM129539-02

National Center for CryoEM Access and Training

4.6 Add autogrids to cassette

- **4.6.5 Orientation of autogrids in the cassette:** Our expectation is that grids have been placed into autogrid boxes with the C-clip facing the notch (Fig. 3) Autogrids should be loaded into the cassette with the C-clip facing the first position – if grids are transferred without rotation, they will be in the correct orientation.

C-clip faces the notch in the box. The notch faces towards the left in the loading station, so the C-clip will face towards position 1 when loaded in the cassette.

- **4.6.7. Leave an empty position in the cassette:** A cross-grating grid should have been left on the stage. This grid will go into position 1, so do not load a grid into position 1.

4.9 Cleaning up

- Place all cold tools into a heat block to warm and dry (see image on the right).
- Empty remaining LN_2 in the NanoCab into and LD10. Store NanoCab upside down in the oven in the designated spot.
- If someone needs the loading station after you, leave cold and fully covered.
- If no one else is using the station, remove covers and leave the loading station upright on the bench for the LN_2 to evaporate and dry.
- Empty any unused LN_2 in LD4s into the LD10 collection dewar and invert on the rack to dry.

CryoEM Merit Badges were developed with support from NIH Common Fund Merit Badge supplement to 3U24 GM129539-02

MERIT BADGES: THE QUIZ

CryoEM
TRANSFORMATIVE HIGH RESOLUTION CRYO-ELECTRON MICROSCOPY

Broadening access to high-resolution
cryo-electron microscopy and *tomography*

HOME | CryoEM CENTERS | CURRICULUM DEVELOPMENT | CryoET CENTERS | EVENTS | MERIT BADGES | RESOURCES

< return to all badges

Grid Clipping

Category: Sample preparation
Sub-category: Cryogenic work

Autoloader grid clipping.

- + Essential base knowledge & SOP
- + Knowledge test
- + Demonstration & Center Specific Policies
- + Practice
- + Practical test

Additional information:

Recertification period

- Sample preparation merit badges for cryogenic work are valid for 6 months.
- Recertification (to maintain active status) requires demonstrating proper use and care in fr
  of a center staff member.

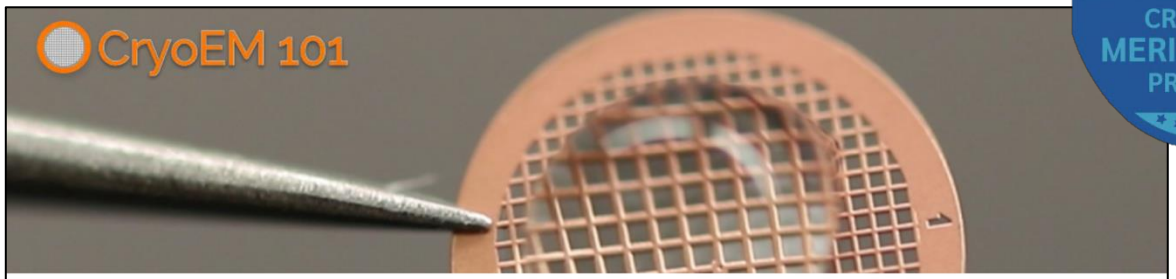
– Knowledge test

The pass/fail knowledge assessment will test understanding of the following concepts:

- Why good clipping is important for your grids and the microscope
- The purpose of PPE for grid clipping
- Keeping your grids vitrified during clipping
- Determining if your grid is clipped properly
- ID tools used for grid clipping

Here is the current quiz version:

Take the AutoGrid Clipping Quiz



Begin Quiz: Merit Badge Knowledge Quiz - TFS Autogrid Clipping

When you're ready, fill in your infomation and click the "Start the Quiz" button

ping station use. You must answer
may take the quiz multiple times.

CRYO-EM MERIT BADGE PROJECT

Merit Badge Knowledge Quiz - TFS Autogrid Clipping

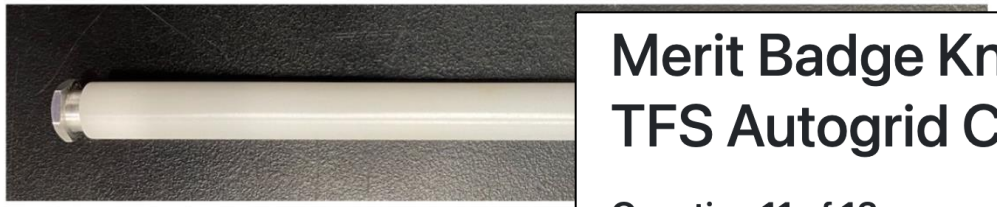
Previous Question | Submit & Next Question

Question 2 of 13:

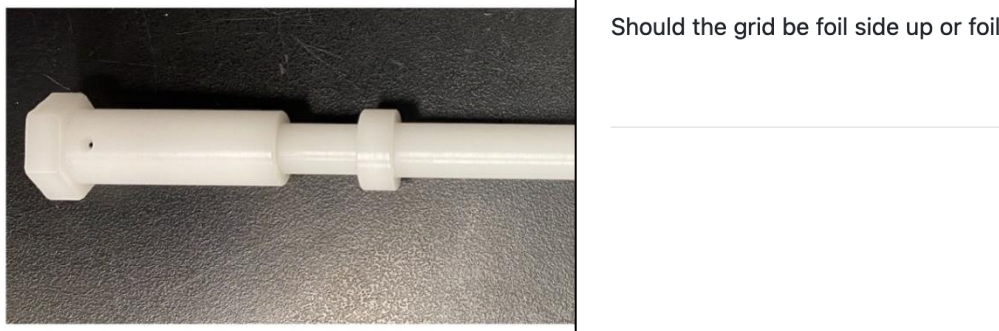
Which of these tools can you use to open the lid of an autogrid box?

Select the correct answer(s) below:

A



B



Merit Badge Knowledge Quiz - TFS Autogrid Clipping

Previous Question | Submit & Next Question

Question 11 of 13:

Should the grid be foil side up or foil side down on the clip ring? Choose all correct answers.

Select the correct answer(s) below:

✔	Answer:
☐	Foil side should always be down.
☐	It doesn't ever matter.
☐	Foil side should always be up.
☐	It depends.

MERIT BADGES: THE INDEPENDENCE CH

SerialEM Screening Practical Evaluation

Supervising Staff: _____ Date: _____
Trainee(s): _____

Section 1: Pre-screening Checks

CRYO-EM MERIT BADGE PROJECT

Section 2: Screening

- ❑ Loads grid
- ❑ Opens Navigator window
- ❑ Saves LMM to correct project path
- ❑ Collects LMM
- ❑ Identifies __ grid-squares of interest
- ❑ Eucentric height calculated for each screened grid-square.
- ❑ Properly centers hole to screen
- ❑ Adjust focus position correctly
- ❑ Defocus target within range
- ❑ Autofocus
- ❑ Acquires Record Image
- ❑ Saves Record Image
- ❑ Repeats screening steps on other grid-squares properly
- ❑ Saves LMM with target notations

Section Score: _____

Supervisor Notes: _____

_____

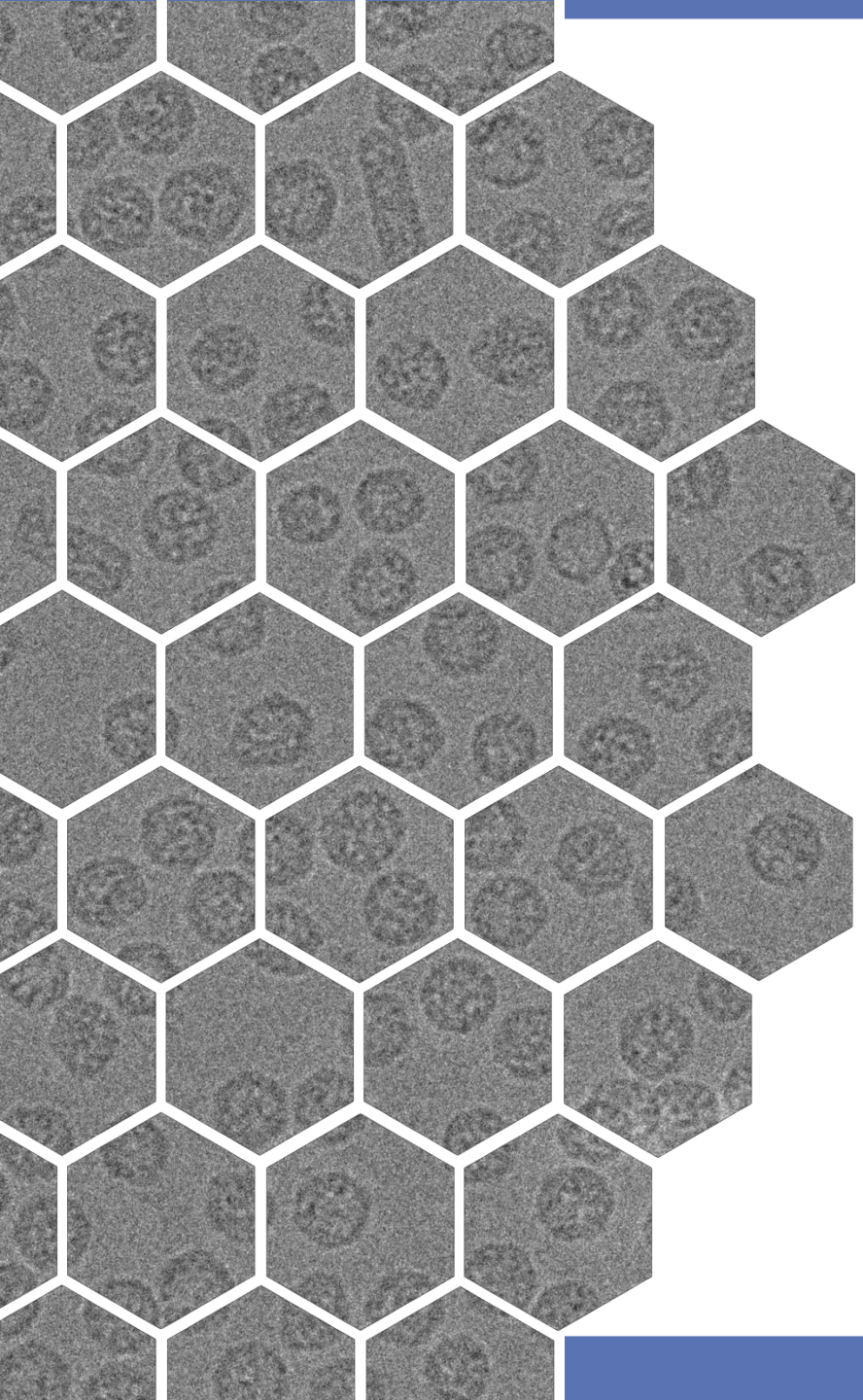
_____

_____

MERIT BADGES

More merit badges are under development

- CryoET:
  - FIB-SEM operations, clipping additions
  - High pressure freezer operations
- Autogrid and Autoscreen workflows in EPU & Leginon
- General LN_2 hygiene
- Tell us what you need/want!



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