Essentials for a Cryo-EM lab

What do you need?

What can you borrow?

How do you assess a new specimen?

General Advice

Be reasonable, BUT this is the appropriate time to ask for and buy exactly what you need

Decide what is essential and what you can compromise on

- It can be helpful to draw up a list of things that are:
  - Absolutely Required
    - Purchase this for yourself
      - » buy equipment for quality versus “cheaper” – this equipment needs to last you ~5-7 years (or more)
  - Necessary:
    - Share or purchase depending on situation
      - » Make sure this machine/resource is easily accessible to your lab
      - » confirm you really CAN share-i.e. get in writing if equipment is not in a core
      - » If in core, make sure core charges aren’t prohibitive
  - Nice to have, but you don’t really need
    - beg and borrow

HARDWARE

Microscopes and Related Equipment
(discussed in last session)

- Minimum requirements:
  - F20 (or equivalent) equipped with a DED
  - T12 (or equivalent): for screening/negative stain
  - Vitrobot (or equivalent)
  - Glow discharger
  - Carbon evaporator
  - cryo-sample storage tank

  If you are starting out with an F20 (or equivalent) consider asking for resources in your start-up so that you can eventually send samples to a Titan Krios

Wet Lab Equipment

- This is completely person dependent
  - just make sure you get the resources to buy what you need to get the work done
- Should you buy from Ebay?
  - You can get some great deals – but be prepared that you(or someone you hire) may need to “tinker” with the equipment to get it to work
  - Sometimes the older equipment is actually better made than what you can buy new

Computation (minimum requirements – more concrete ideas next session)

- Individual workstations
  - search resources such as the 3DEM listserv to get opinions on current best configurations
  - Buy as needed – since computers are continually improving and getting less expensive
- Ample storage (a never ending issue)
  - Consider buying your own storage and make sure it is expandable
  - Some institutions will have core resources – this may or may not be an affordable long term solution
- Easy access to an affordable computational cluster

“SOFT” RESOURCES

How do you assess a new specimen?

Alternative translation:
How do you tell people -- “No – I can’t work on this project”?

Collaborations

- Collaborations can be fruitful:
  - Lead to new, exciting areas of study
  - Lead to new funding opportunities
    - both grants and money for microscope costs
  - You can become a very popular and essential faculty member at your institution
- Collaborations can be a disaster:
  - **can suck valuable time from your own research and resources (both “brain” time and microscope time)**
  - you may find yourself being treated like a “core” and thought of as a resource rather than colleague
  - You can unexpectedly get enmeshed in nasty political situations with other faculty
    - *i.e.* now you have become an unpopular faculty member

Collaborations

- When to say yes:
  - **It fits into your scientific interests**
  - Project represents a new, exciting question that EM can help answer!
    - you are not directly competing with another lab
    - You are actually answering a question – not just doing busy work
  - The sample already looks beautiful
  - Your collaborator is willing to spend the time to improve the sample (if needed) and has respect for your contribution to the project

Collaborations

- How to say yes?

(make sure to communicate extensively as the project progresses):

- Be **very** upfront with your collaborator that they are not “buying” a resource but are entering into a partnership
  - this includes authorship for yourself and whoever is helping in your lab
  - acknowledgments during presentations
- Be **very** upfront with your collaborator that they will need to help pay for microscope costs
- Be upfront about the limitations of the approach (don’t over promise results)
- Clarify who is responsible for what parts of the project and what the final goals are:
  - Who will collect and process data? Will you train someone from your collaborator’s lab to this? Will they use your computer resources?
    - Be careful of burning your personnel out – they need time to concentrate and make progress on their own projects.
- **Do not minimize your collaborator’s contribution to the project (don’t treat them as a resource!)**
  - Be cognizant that the sample that you are working on probably took years of work to produce

Collaborations

- When to say no:
  - **Project does not fit into your scientific interests**
  - They want a resource – not a collaboration
    - They won't tell you what protein/complex they want you to image or what question they are trying to answer.
    - They express resistance to idea of sharing authorship
  - The sample is not appropriate for single particle EM
    - sample quality is not good enough
    - your potential collaborator does not understand the limitations of the approach

Collaborations

- How to say no (gracefully):
  - Project does not fit into your scientific interests
    - Suggest other EM labs that may be interested in (or are already working on) similar samples/questions
  - They want a resource – not a collaboration
    - Suggest they take their sample to your institutional EM core
  - The sample is not appropriate for single particle EM
    - Ask them to improve the sample and then you can look at it again (they often won't return)
      - However, be specific about what needs to be improved and why --otherwise this approach can still waste your valuable time
    - Send them to your NMR or X-ray crystallography colleagues!

Collaborations

- When in doubt:
  - Discuss with a trusted senior mentor – they may be able to provide some career and scientific perspective, as well as, a history of the project you may not know about

- When a collaboration starts to turn ugly:
  - **Immediately** discuss with a trusted senior mentor – they may be able give advice on how to either repair the situation or to extract yourself from the collaboration with minimal bloodshed