Workflow
@
CryoEM Shared Resource
Howard Hughes Medical Institute
Janelia Research Campus

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janelia.org/support-team/cryo-electron-microscopy

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Two types of work

Routine data collection for users	Collaboration projects with collaborators
We focus mainly on instrument operation and data collection.	We cover the entire cryoEM workflow from grids preparation to map generation.

To serve labs with cryoEM experiences and easy access to grids preparation/screening equipment.

Receive: ready-to-image cryo grids
Deliver: large quantity of raw data

To serve labs with limited experience in cryoEM or no easy access to equipment.

Receive: solution samples
Deliver: 3D density map or feedback to improve sample

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Routine data collection for users

Users send cryoEM images to qualify for data collection time
Users send frozen grids to the facility
Facility staff often provide suggestions for optimization if necessary

Grids clipping and loading into the microscope.
Facility staff take care of aligning and operating microscope, optimizing imaging condition/parameters and setting up automated data collection.
Users pick acquisition targets. (can be done remotely if not present on site)
Facility staff monitor the data collection process and do quality check regularly.
Data management and transfer

Users take full responsibility
Facility staff often provide assistance by request (motion correction, CTF estimation)

Users' full responsibility

Facility Staff
Users

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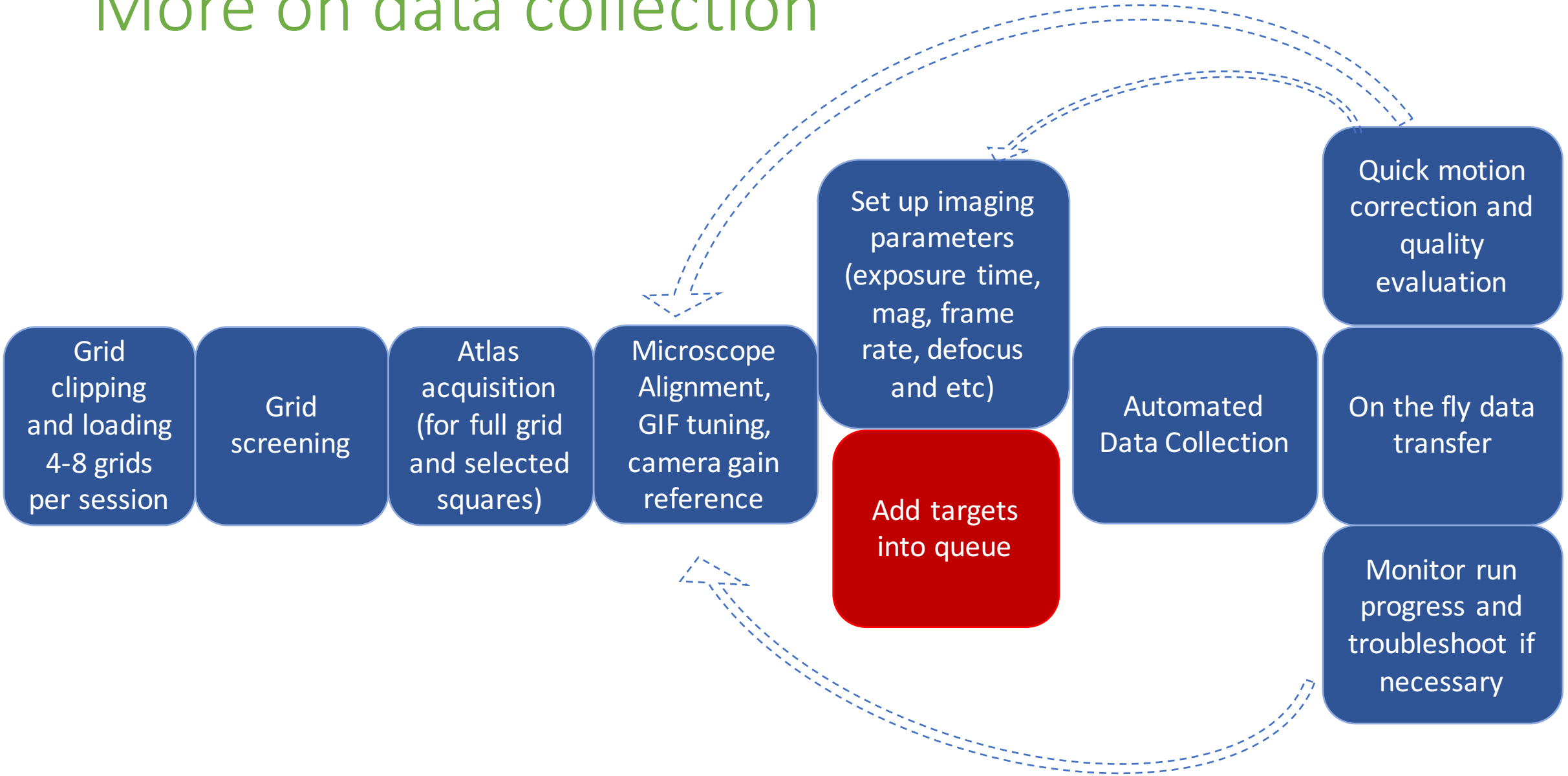
More on data collection



Facility Staff

Users

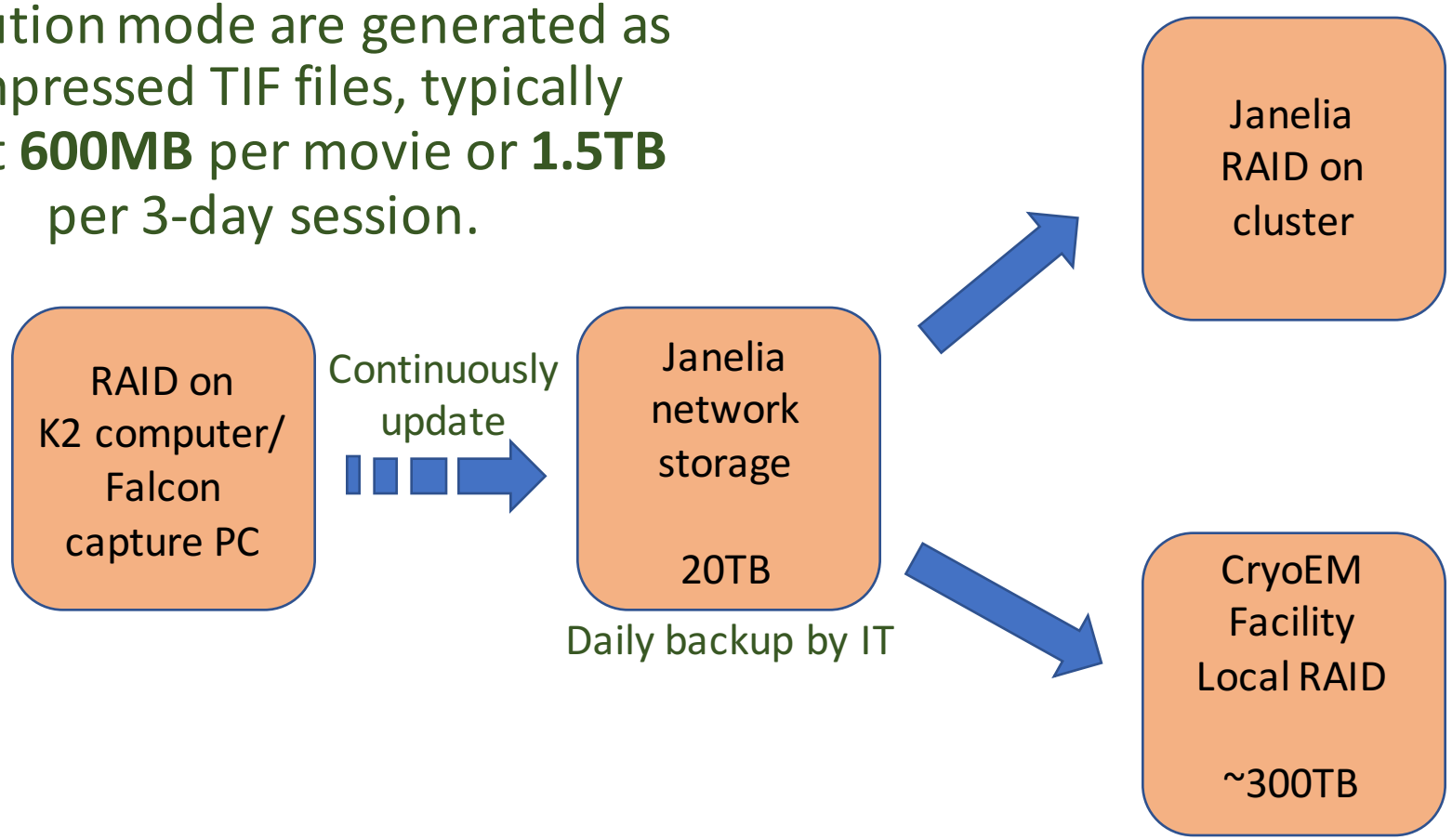
Data Transfer to Users

- rsync via ssh directly to remote server.
  - Yay: allow users to process data on-the-fly.
  - Nay: remote firewall and security policy. A guest account with password is required.
- Portable Hard Drives
  - Yay: easy to set up.
  - Nay: takes time.

We currently don’t grant access to users to download from our server due to Janelia network security policy.

Data Management and Storage

Raw movie stacks with super-resolution mode are generated as compressed TIF files, typically about **600MB** per movie or **1.5TB** per 3-day session.



Rapid read/write disks for data processing, i.e. motion correction.

Data are kept for ~2 months

10G network either on fiber optics or copper

Post data collection follow-up

- Book keeping: parameters such as total dose, exposure time, magnification, grid type, issues encountered and etc.
- Voluntary user feedbacks.
- Discussions and suggestions for next session if needed.
- When data is published, user should send a link to the paper as well as a representative figure to the facility.

Collaboration projects

Collaborators send solution samples to the facility
Facility staff screen samples by negative stain or cryo and provide suggestions to optimize protein preparation.
Multiple iterations might be needed till suitable cryo grids are obtained.

Facility staff carry out the data collection. Collaborators are welcome to join the data collection and on-the-fly early processing (help pick particles).

Facility staff lead in data processing but interact closely with collaborators.

Facility staff send collaborators intermediate results and final maps.
Collaborators analyze maps, build atomic models, and interpret results.

Facility Staff
Collaborators

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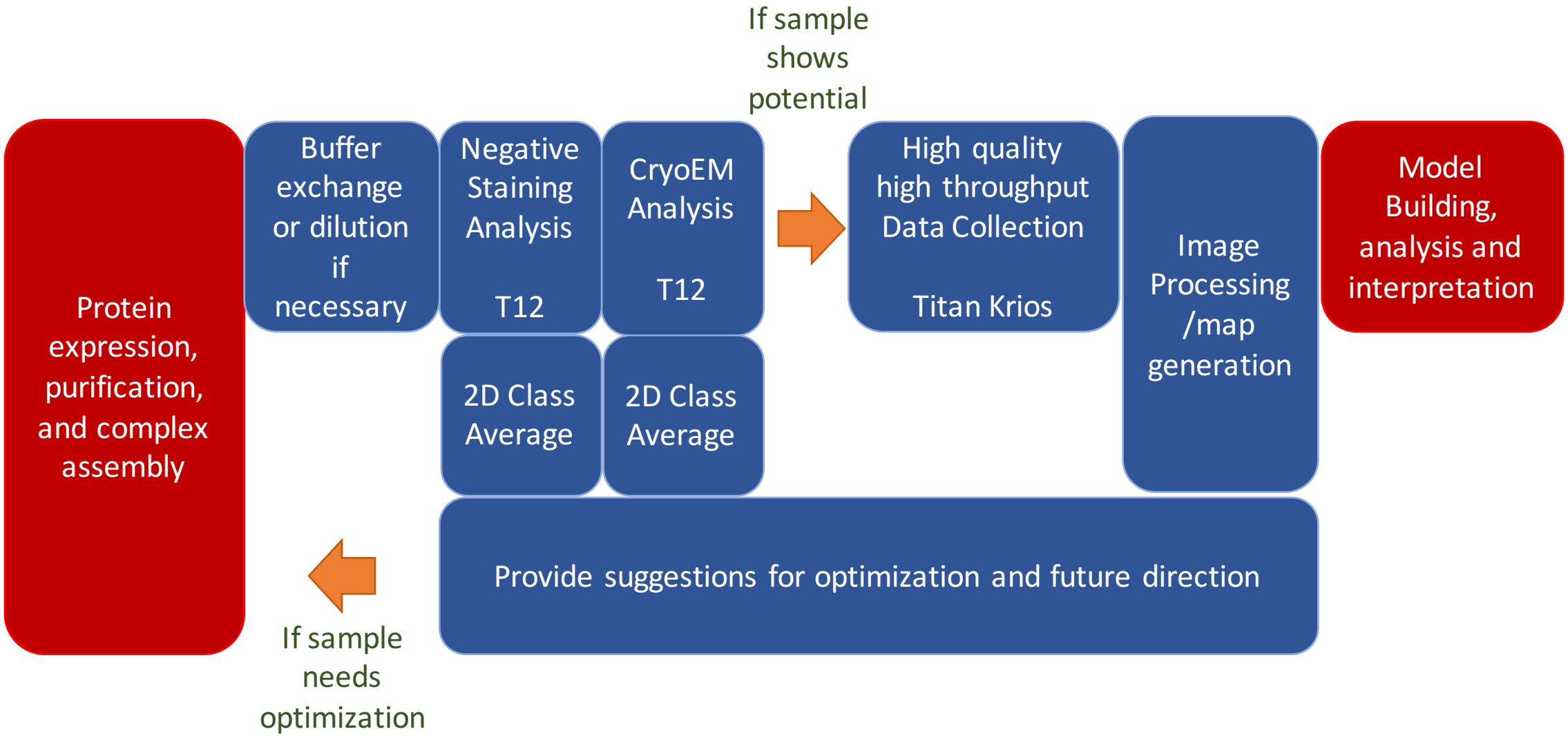
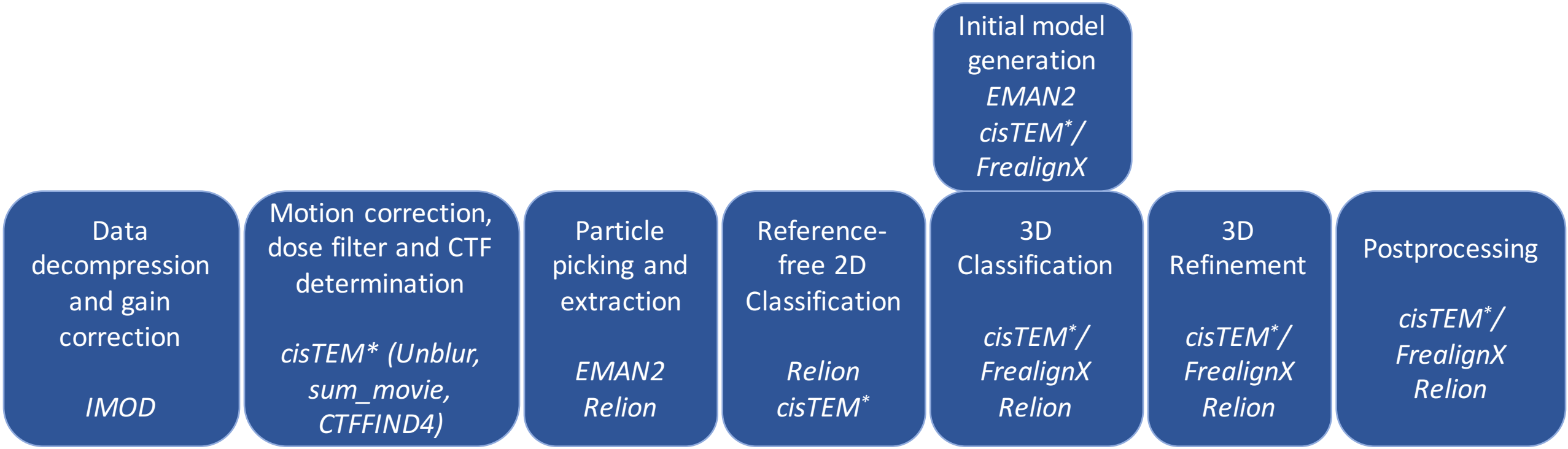
The key determinant: sample preparation



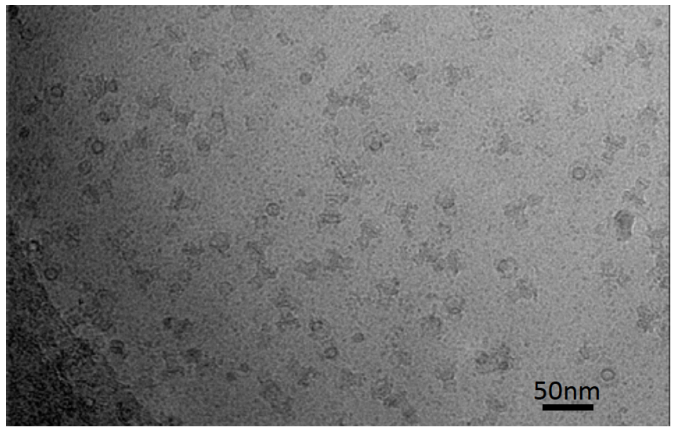
Facility Staff
Collaborators

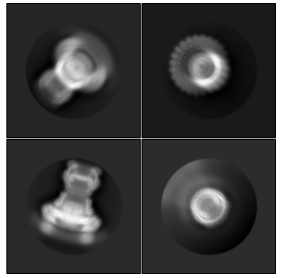
One typical image processing workflow

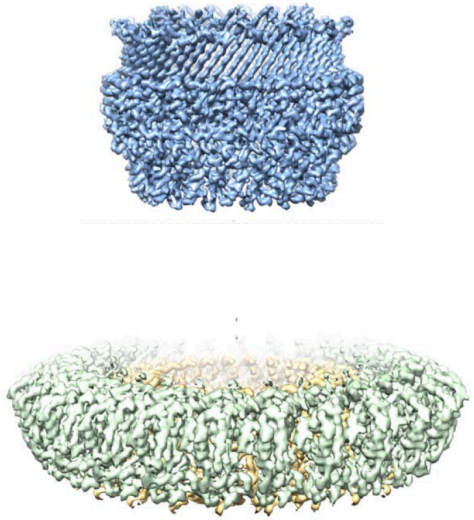


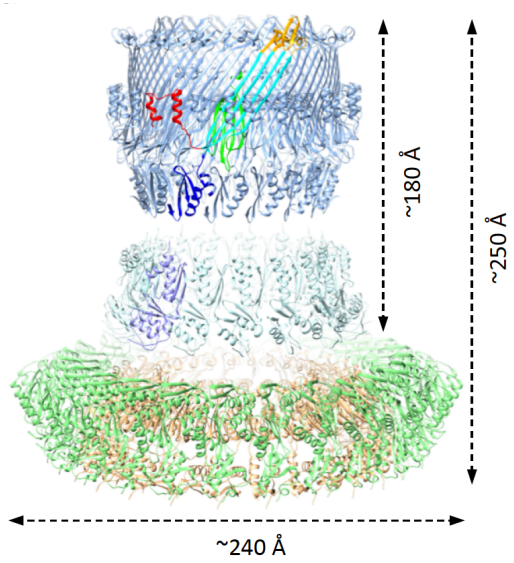
**cisTEM* from Niko Grigorieff's lab is a complete package from raw movie stack to 3D classification and refinement with a GUI interface for single particle cryoEM. *Unblur, sum_movie, ctffind4* and *FrealginX* are all part of *cisTEM* which also includes 2D classification and more.

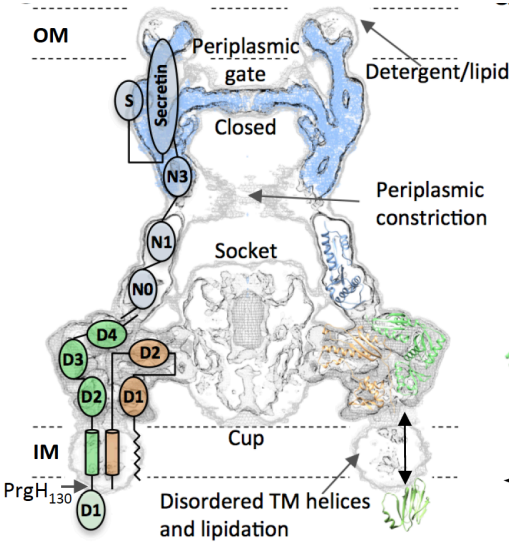
One example: T3SS Basal Body Complex











Worrall LJ, *, Hong C*, Vuckovic M, Deng W, Bergeron JRC, Majewski DD, Huang RK, Spreter T, Finlay BB, Yu Z#, Strynadka NCJ#, ***Nature***, Dec 2016

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- All users

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