

[SEMC Krios] Rapid Access Application 2018

Request For Applications (RFA) for Rapid Access.

All active users from our member institutions will be allowed to apply for EM time. The minimum requirement would be for users to have screened similar cryo sample conditions (including preliminary data processing) for the grids that they will be loading into the instrument.

Details of open beta time:

- 1) Each interested user should email emg@nysbc.org and their institutional representative an application. They must state they are applying for rapid access time and submit an application. The application should include: a) Leginon project ID (if you do not have one please create one), b) Leginon IDs of the personnel that will be using the instrumentation, and c) a brief update of the progress of the project including why it is ready for the Krios (please include preliminary cryo data). Awarded times are non-transferrable.
- 2) All Krios users should have a project registered with the center and must fill out a survey at the end of their session. If surveys are not completed within a week of the session, then no future instrument sessions will be granted at the center until the survey is completed.
- 3) User's grids must be dropped off and clipped before entering the rapid access queue.
- 4) Applications will be reviewed on a rolling basis depending on availability of instrumentation and ability to match the instrument requested with the project. If more than 1-person from the institution applies we will consult with your IR. We may not cycle through every user or institution during rapid access because there are limited slots available.
- 5) Rapid access users should leave contact information including a phone number, such that we may notify the user at a moment's notice. Any user changeovers between users must occur between 10am- 2pm during business days. The first priority is the health of the instrument. 24-hr access is a privilege and may be granted to users that display sufficient mastery of the instrumentation. We will make every effort to give the user as much lead time before their session. If the user is unavailable to make use of their opportunity they may lose their spot in the queue and we will skip to the next user. We will be as accommodating as possible, but the second priority is to ensure maximum use of the instrumentation.
- 6) For further details or questions please email emg@nysbc.org and your institutional representative.

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Example application

Email emg@nysbc.org the following:

- 1) Title of the email should be: “[SEMC Krios (1,2 or 3)] S 2018: Rapid access application”
- 2) State the Leginon Project ID(s). If you do not have one please register a project at http://emg.nysbc.org/semc_content/submitProject.html.
- 3) State the Leginon User ID(s) of the personnel that will be using the Krios.
- 4) Provide a brief update (300 word limit) on the progress of the project with why it is ready for the Krios (e.g. representative micrographs, 2D class averages and, if available, a preliminary 3D reconstruction). Links to the Appion session ID(s) may also be used.

Example Application:

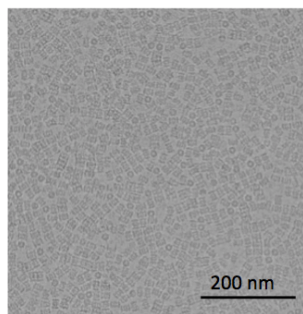
Leginon Project: T20S proteasome dynamic studies

Leginon Project ID: 153

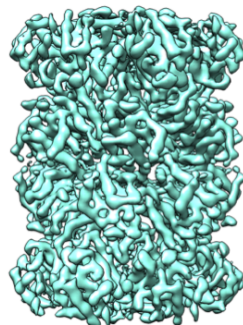
Leginon User ID: Careful User (cuser)

We are interested in the domain interactions between the subunits of the T20S. There are two types of experiments we would like to do:

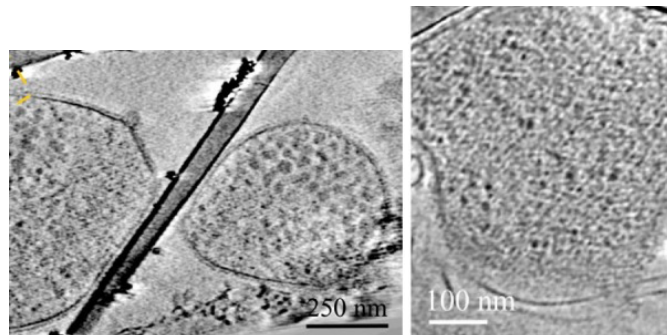
- 1) In our submitted manuscript we have a 2.8Å structure, but the reviewers wanted additional evidence of the β K382- α E15 salt bridge that was an important point in the paper, as well as a ligand-binding interface that impacts activity. Biochemical experiments are underway and we have 4 cryo grids ready to go (we froze 4 grids with the same protocol and screened 1 of them). Included are: a representative micrograph, 2D class averages and initial reconstruction with 25 micrographs from this screen. We are currently around 7-8Å on the TF20/4K CMOS and have saved this grid. This processing may also be found in Appion experiment ID=1094.
- 2) In addition, we have over-expressed this proteasome in *T. acidophilum* cells that we have plunge frozen and inspected on the TF20. To address the reviewers concern that the structure we have solved is not in a biochemically meaningful we would like to do *in situ* reconstructions using cryo-ET and sub-tomogram averaging on Krios#2. We have had some initial success with plunge freezing the cells, but the main area of interest is in the thickest part of the cell and we hope 300 kV and the energy filter will reduce inelastic scattering.



Box size 25x25nm



FSC_{0.143}=7.5Å



Sub-tomogram avg/cryo-ET project grid screen.

TF20/4K CMOS Single particle project grid screen.