

Duke Krios Cryo-TEM Project Request Submission Form

Please email completed form to: krios@duke.edu

User Name(s): _____ Institution: _____

PI Name: _____ Date: _____

Project Title: _____

Estimated total number of samples to image for this project: _____

Estimated number of images to acquire per sample: _____

Number of anticipated Krios measurement sessions: _____

Note: Only non-hazardous and non-infectious samples will be imaged on the Krios

A) Please provide the following information about the sample(s) and goals for the Krios imaging

a. Sample Description: _____

b. Molecular Weight: _____

c. Symmetry: _____

d. Number of conformations: _____

e. Target Resolution: _____

Provide any additional information that would be helpful in the text box below:

Insert text here

B) Please provide the following for the project (may be multiple samples, multiple sessions):

- 1) A low magnification cryo EM montage (atlas) of the specimen/s to be imaged or of a specimen obtained using similar preparation conditions shown previously to behave analogously in cryo conditions (e.g. the same protein in complex with different ligands, point mutations of same protein).
- 2) Two low magnification cryo EM images showing the ice distribution on an entire grid square from the specimen in #1, above.
- 3) Two intermediate magnification images (~1 nm pixel size) of the areas deemed adequate for data collection from the specimen indicated in #1, above.
- 4) One or more representative high magnification images of a field of view on the specimen indicated in #1, above, at under-focused values between 1-3um.
- 5) Images identifying the particles selected from the corresponding micrographs provided in from the specimen indicated in #4, above.
- 6) 2D class averages or a preliminary 3D reconstruction. Please identify or describe the sample that was used to produce the 2D classes or 3D reconstruction.

Notes:

- 1) The biochemical characterization of the sample to be imaged is highly desirable. This should include at least a gel filtration profile and a stained denaturing gel electrophoresis analysis of the sample. Addition of a non-denaturing electrophoretic run (native gel) is recommended. Applicants are encouraged to submit negative stain analysis of the same specimen, if available.
- 2) For new projects where a 3D reconstruction from cryo-EM data is not yet available, only a single measurement session will be initially approved. If different samples are being imaged, users should provide data for #4 and #5 for each of the samples.

If any of the above conditions are difficult to obtain, please email krios@duke.edu to discuss.