



NCCAT pre-award questionnaire

GUP2: Chameleon access

GUP2 ACCESS INFORMATION:

In this early access phase NCCAT has the capacity for one (1) Chameleon grid preparation session per application. The workflow for this access category is as follows: 1) users will ship their sample to NCCAT, 2) our operators will iteratively optimize the experiment to maximize performance of Chameleon, and 3) ship you back one (1) grid box with up to 4 grids with a report. To ensure the highest opportunity for success we require additional information about your sample.

Note, incomplete information may prevent NCCAT from being able to match the correct resources to your project. If you do not have data to complete a section, then please state "No data available". *Do NOT ship any samples until NCCAT has contacted to schedule a session. Details on sample requirement and shipping information will be provided at that time.*

NCCAT PROJECT APPLICATION

Project ID: NCCAT-GUP2-CU190101

Project Name: T20S structural dynamics

Primary User Name: Careful User

eRA Commons User Name: cuser

Institution: Rocksteady University

SAMPLE INFORMATION

1) Name/Title of macromolecule of interest: T20S proteasome

2) Molecular weight and symmetry: 750 kDa, D7 symmetry (monomer ~53.5kDa)

3) Storage buffer: 10 mM Tris, pH 7.4, 138 mM NaCl, 0.01 mM DTT

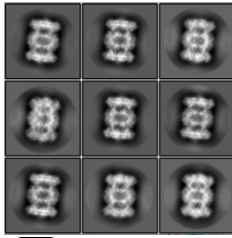
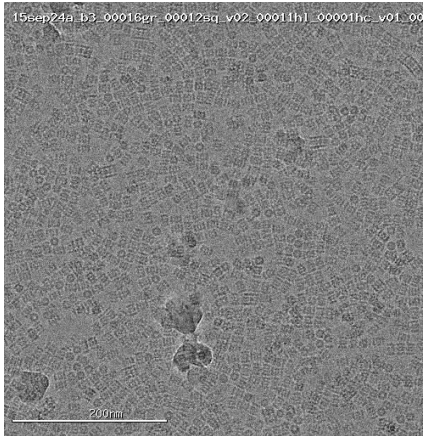
4) Ligands/Binding partners in sample (if applicable): None

5) Concentration of sample provided: 4 mg/mL

6) Please provide cryoEM feedback and plunge freezing protocol used (if available).

Plunge freezing protocol used: -80C stock is thawed in ice and diluted 1 to 3 into TBS, pH 7.4 with a final concentration of 1.3 mg/mL. Within 30 minutes of mixing grids are prepared. After 10 sec glow discharging with H₂ and O₂ with a Solarus at 5W the sample is applied for 60 sec before plunge freezing. A Vitrobot Mark IV is used with 2 sec blot and blot force 2 at RH 100% and 20C.

See example micrograph and class averages below.



10 nm

Images collected on a 200 kV J2100F with a K2.

7) Additional information:

Sample stored in aliquots at -80C at 4 mg/mL. Note, after 2.5hr at RT increased chance of aggregation. Once thawed do not save aliquot.

8) What is the BSL level of the sample?

CHAMELEON SAMPLE GUIDELINES

We make the following recommendations for Chameleon:

- 1) No less than 50 μ l of protein sample.
- 2) High purity, non-aggregated, stable samples.
- 3) Minimal buffer additives (ideally no organic solvents or viscous reagents).
- 4) Include all chemicals/ligands required for protein stability.
- 5) No less than 2.5x the concentration used for plunge freezing. Typically on the order of several mg/mL for standard samples.
- 6) Include 5 mL of dilution buffer that matches the sample storage buffer.
- 7) Do not lyophilize your protein.

Would your sample be able to meet these requirements?

Yes | No (If No, then please clarify.)

CONFIDENTIALITY

Information will not be publicly disclosed regarding any sample applying for (or received for) GUP2: Chameleon access without the expressed written consent of the investigator(s).