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
Submission Date: January 1, 2019 12:00 UTC

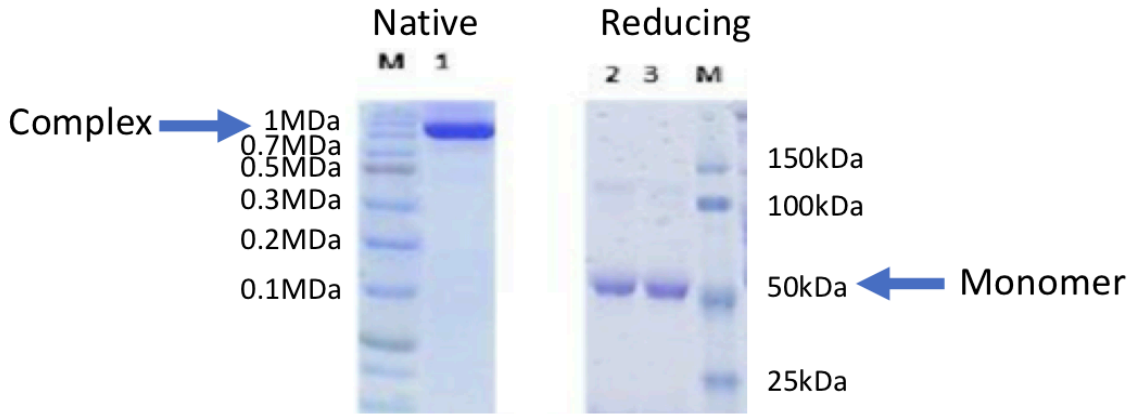
SAMPLE INFORMATION

1) Name/Title of macromolecule of interest: T20S proteasome
2) Molecular weight: 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) Standard storage temperature (in °C): -80C
6) Storage time (max time at storage temperature and/or at RT): 2.5hr at RT before precipitation
7) Highest soluble concentration tested (mg/mL): 13.8 mg/mL without aggregation

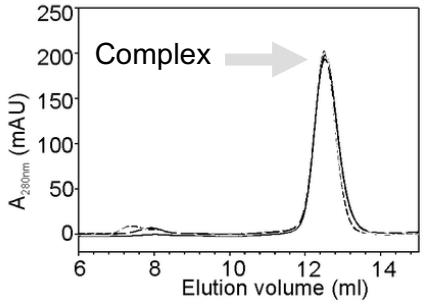
8) Additional information: Stored in aliquots @-80C at 4 mg/mL. After 2.5hr at RT increased air-water interface interaction. Once thawed do not save aliquot.

MOLECULAR BIOLOGY/BIOCHEMISTRY

1) Please provide an annotated SDS-PAGE gel image of the sample being submitted.



2) Please provide an annotated size exclusion chromatogram of the sample being submitted.



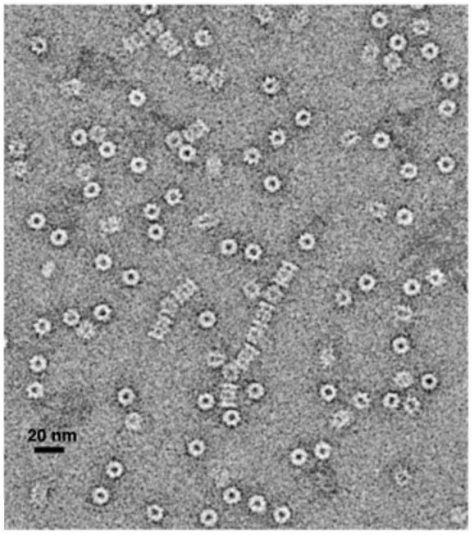
3) Additional information on all chemicals required for stability and/or biochemistry of sample:

DTT crucial for preventing non-specific oligomerization due to engineered cysteines in the construct for previous EPR studies.

ELECTRON MICROSCOPY AND SAMPLE CHARACTERIZATION

1) Negative stain characterization

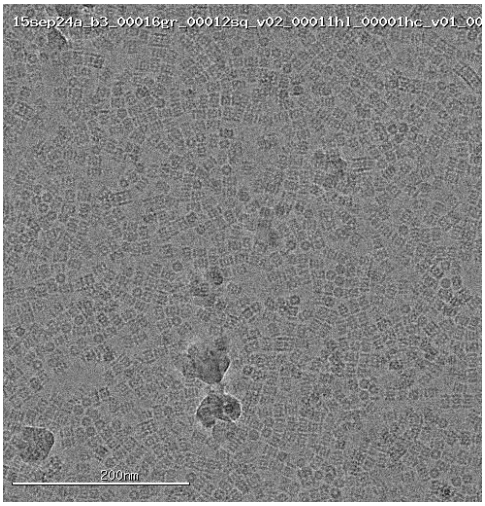
Please provide a representative negative stain micrograph and 2D class averages. Also, include stain used, scale bars and other relevant information.

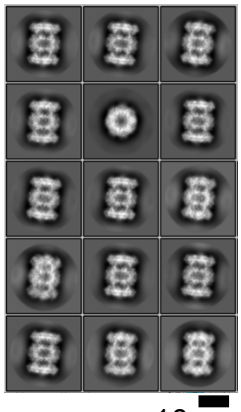


Uranyl acetate at 0.05 mg/mL.
Images collected on a 120 kV T12 with F416 camera at 60kX mag.

2) CryoEM characterization

a) Please provide a representative cryo micrograph, 2D class averages and 3D reconstruction (if available). Also, include scale bars and other relevant information of the experiment.

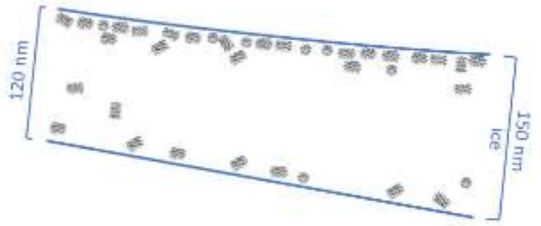




10 nm
Images collected on a 200 kV J2100F with a K2.

b) Please provide plunge freezing protocol used (include plunge freezing device, plunge freezer settings and parameters, grid type used, and sample concentration/buffer 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.

c) Provide your analysis on the issue with your sample preparation and why Chameleon would be helpful.
After taking 3 representative tomograms the sample clearly is interacting with the air-water interface. See representative tomogram below.



SAMPLE GUIDELINES

We make the following recommendations for Chameleon:
1) No less than 50 µl 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?
_x__ 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).