|  |  |  |
| --- | --- | --- |
|  |  | **NCCAT pre-award questionnaire** GUP2: Chameleon access |

# GUP2 access information:

In this access phase NCCAT has the capacity for up to two (2) Chameleon grid preparation sessions 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 up to two (2) grid boxes with up to 4 grids per grid box 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:**

**Project Name:**

**Primary User Name:**

**eRA Commons User Name:**

**Institution:**

**Submission Date:**

# Sample information

**1) Name/Title of macromolecule of interest:**

**2) Molecular weight:**

**3) Storage buffer:**

**4) Ligands/Binding partners in sample (if applicable):**

**5) Standard storage temperature (in oC):**

**6) Storage time (max time at storage temperature and/or at RT):**

**7) Highest soluble concentration tested (mg/mL):**

**8) Additional information:**

# Molecular biology/Biochemistry

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

*<insert image here>*

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

*<insert image here>*

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

# 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.**

*<insert image here>*

## *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.**

*<insert image here>*

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

**c) Provide your analysis on the issue with your sample preparation and why Chameleon would be helpful.**

#  Sample Guidelines

**We make the following recommendations for Chameleon:**

**1) No less than 20 μl of protein sample, but larger amounts are preferred.**

**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).