Chameleon Workshop – SciLifeLab 2025

Sample considerations and requirements

Potential benefits of short dispense-to-plunge time

chameleon has the unique capability to freeze grids with a very short sample dispense-to-plunge time. The fastest the instrument can achieve is 54ms and we routinely freeze grids in 100-300ms range. It has been demonstrated in selected cases, “fast grids” made by chameleon can help with:

1. **Preferential Orientation**
   Grids frozen quickly may provide a higher proportion of rare views of the specimen. This can improve the resolution of the final reconstruction by providing better angular sampling within a smaller dataset.
2. **Denaturation at the Air-Water Interface**
   Grids frozen quickly may alleviate partial or full denaturation effects seen due to interactions at the air-water interface denaturation.

Sample concentration

Spraying generally requires your protein sample to be more concentrated. Our experience with chameleon is that optimal sample concentration correlates with 2 factors:

1. **Target Protein Size**
   For smaller proteins (<~450kDa), as a rule of thumb **start with double the concentration that would be used on a blot-freezing device**. For larger proteins, sample concentration likely needs to be higher than this.

2. **Dispense-to-Plunge Time**
   The shorter the dispense-to-plunge time is (i.e. the faster a grid is prepared), the more concentrated your sample will need to be in order to give similar particle density.

We recommend that you bring a reasonably concentrated sample together with sample buffer so we have a bit of flexibility to decide how much dilution is necessary when freezing.

Sample volume

The minimum volume recommended for aspiration is 3µl, and there is a 2µl dead volume. Therefore, the minimum volume required for each sample is 5µl. However, since this will be the first attempt to freeze your sample, we recommend that for each sample you supply us with **2 aliquots of 10µl each and 500ul of sample buffer** in case the sample requires optimization.

Special applications

We know that some of you are interested in application variants from the canonical Cham usage. As examples, we can try to run photoactivation by flashing a LED while plunging the grid or to freeze oxygen sensitive specimens (https://doi.org/10.1101/2024.07.19.604374). **Please let us know if you want to try something special.**

Please fill out the sample sheet below to provide us with details about your samples. This is to help us understand how best to work with your samples. We will select 6-8 samples to run during the workshop, but we will also discuss all the other cases for you to try in the future if there is no time during the current workshop.

Sample Information	
Sample Name	
Protein Class	☐ Soluble ☐ Membrane ☐ Filamentous
Molecular Weight	kDa
Sample concentration	mg/ml
Buffer Composition	
Buffer (e.g. 20mM Tris)	
pH	
Salt (e.g. 100mM NaCl)	
Detergent (e.g. 0.01% (w/v) LMNG)	
Glycerol (e.g. 0.05% (v/v))	
Other components (e.g. 1mM GTP / 2mM $MgCl_2$)	

Sample History	
Sample concentration used previously for cryo grids preparation	mg/ml
Freezing instrument(s) used previously (e.g. Vitrobot, Leica GP)	
Grid Type Used Previously	
Base Grid Material	☐ Copper ☐ Gold ☐ Others:____
Mesh Size	
Foil Material	☐ Carbon ☐ Gold ☐ Others:____
Foil Type (Size) (e.g. holey carbon (1.2/1.3))	
Support layer	

(e.g. continuous carbon/graphene oxide)	
Challenges Experienced in Previous Grid Preparation	
Preferential Orientation	☐ Yes ☐ No
Air-Water Interface Denaturation	☐ Yes ☐ No
Others (please specify)	
Please describe previous attempts to optimize grid preparation (e.g. negative staining? crosslinking? etc)	

Reference Micrographs

*If possible, please include a scalebar