

1 Motivation

The in the original proposal we were trying to overcome a severe preferred orientation of Pannexin in lipid nanodisc. This problem extends to Pannexin in detergent, to a certain degree. Initially we reconstituted Pannexin into MSP lipid nanodiscs with a purified lipid mix. To preserve the natural lipid composition we modified a saposin (SaliPro) direct extraction protocol. This sample is similar to one recently published in a ThermoFisher cryoEM whitepaper.

We intended to improve the angular distribution of the particles by freezing the sample with the Chameleon.

2. Preliminary results

The sample was frozen with two different plunge speeds. For each of the conditions, a small dataset was collected on a Glacios. The images were processed here locally with CryoSparc 3.2.2.

First, we processed the data of the grids that were frozen with the slower speed. After curating exposures and particle picking, ~130000 were classified in 2D (Fig.1). Top and oblique views are present but no sideviews could be found.

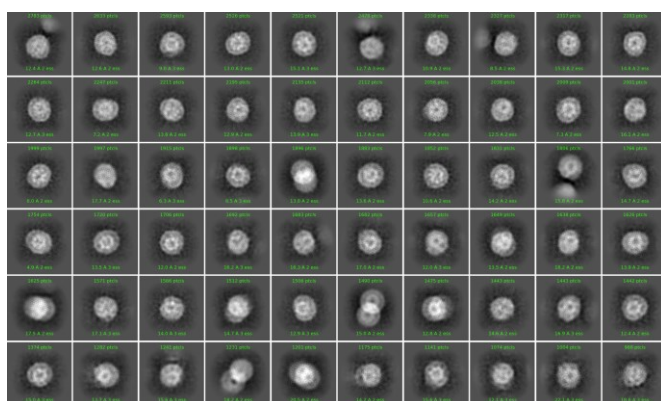


Fig 1

Therefore, I combined the different dataset from the different freezing conditions and obtained ~330000 particles. Top and oblique views show higher resolution features, but sideviews are still missing (Fig.2). Yet, high resolution structures are attainable, based on selected 2D classes (Fig. 3).

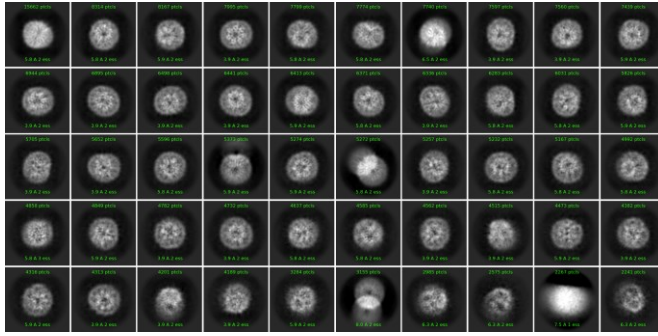


Fig. 2

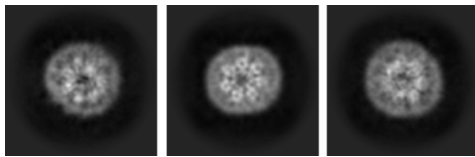


Fig. 3

3. Reasoning for access to high resolution data collection.

Although we failed to obtain sideviews, we would like to explore if the Glacior data is representative for the angular distribution in our sample. Or more importantly, if there is a population of side views that become apparent with a larger dataset.