Correlates of successful structure solution in cryoEM single particle reconstruction (SPR)

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In cryoEM SPR, particle concentrations and their distributions in supported or unsupported amorphous ice layers correlate with the structure solution success. How particles interact with the support is shaped by buffer conditions and physical properties of particles' surface.

We modulated the interactions between particles and the support by using a His-tag as a modulating feature, by adding small amounts of Ni2+ ions when a His-tag was present to promote its folding, by using graphene oxide grids, and by increasing the concentration of proteins both to support the amorphous ice layer and to saturate the carbon support to increase the probability of the association between particles and the ice layer in the grid holes. We also devised a new method of analyzing the patterns of preferred orientation to preserve information about the polarity of interactions between particles and two water-air interfaces present in the experiment so that we can quantify how our experimental modifications change the nature of interactions for C symmetry groups. We observed asymmetries of distributions even for pure thin ice, indicating that kinetics is an important contributor to observed particle distribution patterns.

We will discuss how these strategies contributed to solving several challenging structures, with molecular mass of particles ranging from 98 kDa to 239 kDa.