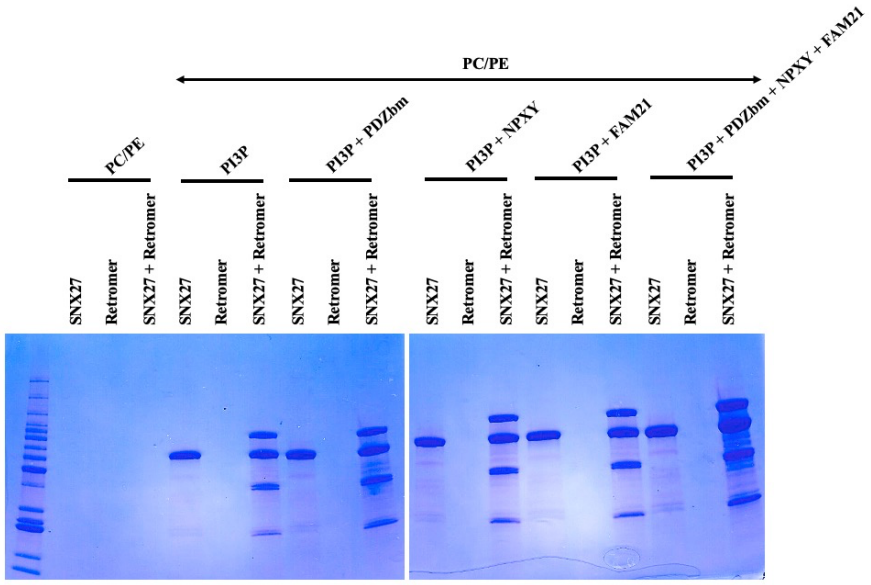

Figure 1. SNX27/retromer reconstitution on membranes. Liposome pelleting assays reveal conditions for recruiting SNX27/retromer efficiently to PI3P-enriched membranes. SNX27 uses its PX domain for membrane recruitment, and retromer requires SNX27 to bind membranes in the presence of PI3P. Addition of cargo and regulatory partners substantially enhances SNX27/retromer recruitment (far right). These data suggest optimal conditions for recruiting SNX27/retromer to PI3P-enriched liposomes for cryoET studies.

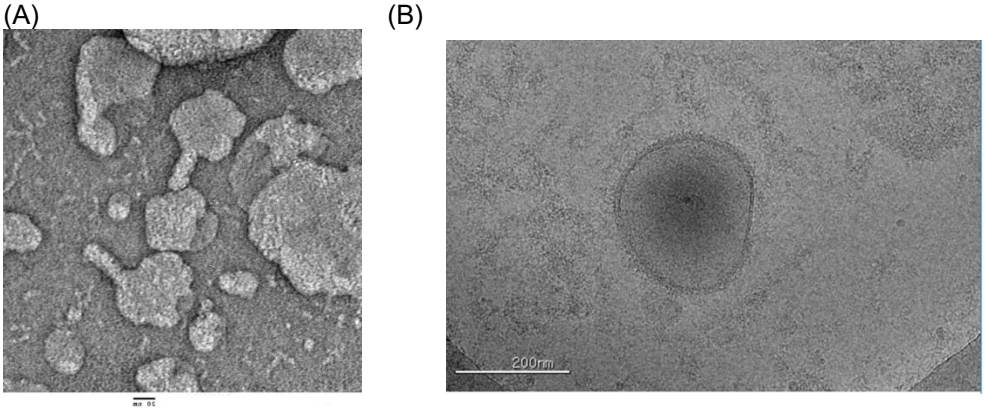

Figure 2. SNX27/retromer liposomes in negative stain and vitreous ice. (A) Negatively stained SNX27/retromer complex forms small tubules emanating from PI3P-enriched liposomes in the presence of cargo. No tubules are formed on control liposomes (data not shown) Scale bar: 20 nm. (B) Example coated liposome (shown here) and coated tubules (not shown in this image) are observed in vitreous ice.

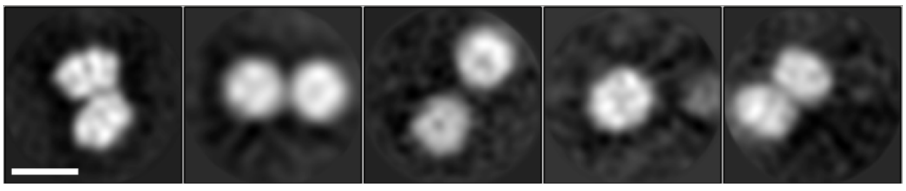

Figure 3. Initial 2D classification of β'-COP (1-604)/Glo3 (230-300) complex. Representative five classes from 5,000 manually picked particles (1.096 Å/pixel bin) following NCCAT data collection (December 2020). Scale bar: 50 Å. We currently have 3.4 million particles after auto-picking; we observe these classes and several others. These data suggest we can successfully pursue single particle structures of the b'-COP subunit with a variety of binding partners.