PI: Lauren P. Jackson, Vanderbilt University

Project name. Architecture of mammalian retromer by single particle cryoEM

Abstract. In metazoans, retromer (VPS26/VPS35/VPS29) associates with sorting nexin (SNX) proteins to form coats on endosomal tubules and sort cargo proteins to the trans-Golgi network (TGN) or plasma membrane. This core complex is highly conserved from yeast to humans, but molecular mechanisms of metazoan retromer assembly remain undefined. Here we combine single particle cryo-electron microscopy with biophysical methods to reveal multiple oligomer structures formed by mammalian retromer. Two-dimensional class averages in ice reveal the retromer heterotrimer; a "dimers of trimers"; "tetramer of trimers"; and flat chains. These species are further supported by biophysical studies in solution. We provide cryo-EM reconstructions of all species, including pseudo-atomic resolution detail for key structures. We identify a stable and highly conserved electrostatic interface in dimers formed by interactions between VPS35 C-termini. We have generated a structure-based mutant to disrupt this key interface in vitro and introduce equivalent mutations into *S. cerevisiae* to demonstrate the mutant exhibits a cargo sorting defect. Our structural data reveal a key retromer assembly interface, and complementary functional data in budding yeast imply a conserved assembly mechanism across eukaryotes. These data suggest retromer can act as an adaptable and plastic scaffold to interact with key binding partners and sort multiple cargoes from a common origin to multiple destinations.

Scientific impact. Our lab studies uses biochemical, biophysical, and structural methods to uncover the assembly and regulation of non-clathrin coat complexes important in membrane trafficking. A major outstanding question in the field is **how the mammalian retromer coat assembles** with a variety of sorting nexins to sort cargoes to different destinations. We specifically need near-atomic resolution structural data to understand the molecular details of retromer interactions with a variety of binding partners. Our current data suggest retromer is a highly adaptable and plastic coat that can adopt multiple architectures.

Scientific feasibility. We have already obtained highly pure protein samples and determined structures of multiple retromer oligomers (please see attached data). **Our current structures are limited in resolution, because specific oligomers are found in preferred orientations.**

Technical feasibility. We have determined structures of retromer oligomers in ice, but we lack views for specific oligomers. We **need to collect tilt data** in order to obtain additional views **to improve our 3D reconstructions**.

Resources requested. We likely need 48-72 hours, based on previous data collections.

Geographic/demographics. Vanderbilt has a Polara F30 with direct electron detector, but our previous two data sets come from Krios instruments. We would like to ensure consistency when combining multiple data sets.

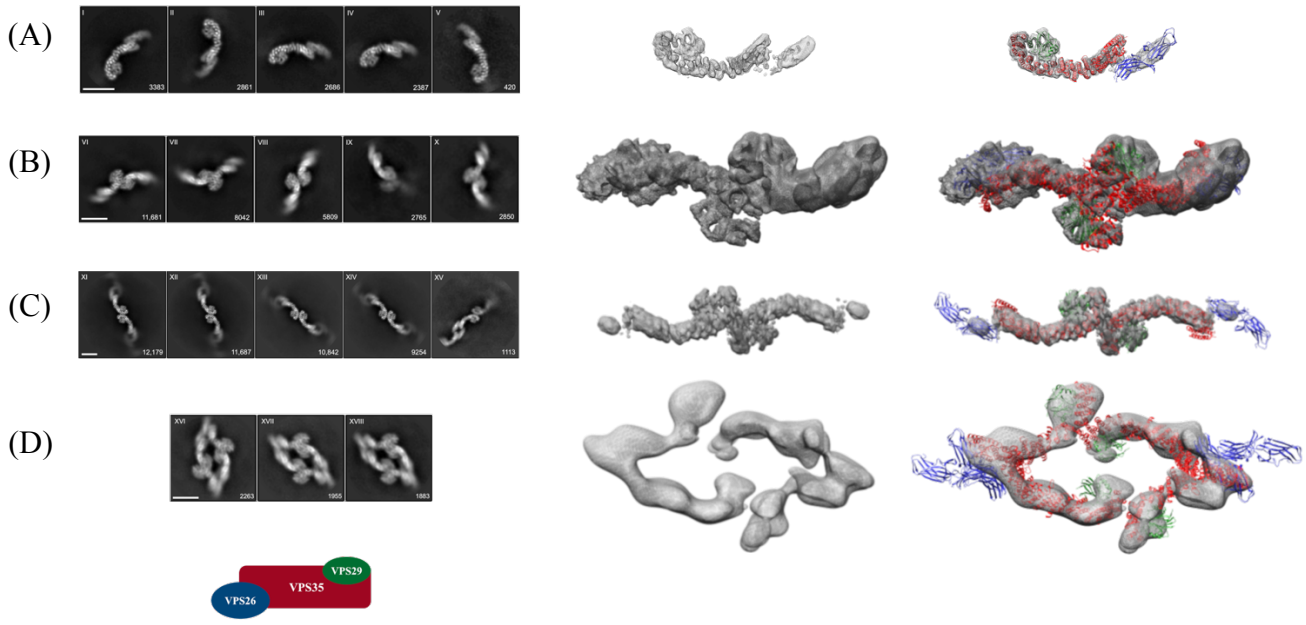

Figure 1. Single particle cryo-EM reconstructions of mammalian retromer. Four retromer species were resolved between 27 and 5 Å: the retromer heterotrimer (A); a dimer of trimers (B); retromer chains, shown as a unit (C); and a tetramer of trimers (D). For each row (A-D), the left-hand column shows representative 2D class averages for the species. The middle column shows the 3D reconstruction, and the right-hand column shows the same reconstruction overlaid with a fitted model generated from partial crystal structures (PDB ID: 2R17, 5F0J). (Scale bars represent 10 nm.)

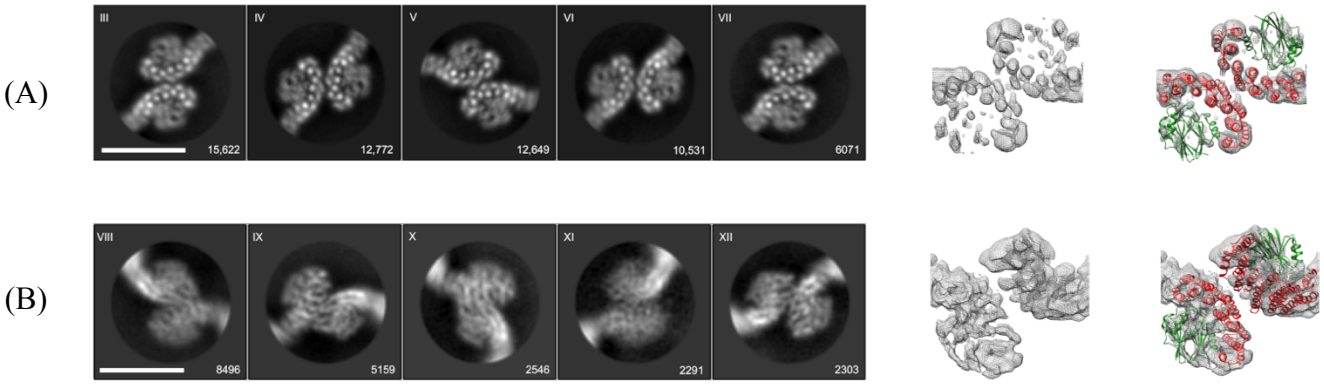

Figure 2. Single particle cryo-EM reconstructions of retromer sub-structures. Retromer forms two different types of dimers mediated by VPS35 subunits. (A) VPS35/VPS35 sub-structure generated from chain species (cf. Figure 1C). (B) VPS35/VPS35 sub-structure generated from dimer species (cf. Figure 1B). For each sub-structure, the left-hand column shows representative 2D class averages. The middle column reveals 3D reconstructions, and the right-hand column shows the same reconstruction overlaid with a fitted model. PDB models 2R17, and 5F0J were used for fitting. (Scale bars represent 10 nm.)