



Figure 1. Retromer heterotrimer reconstructions. Local resolution comparison across heterotrimer reconstructions without (top reconstruction; N=26,369 particles) and with (bottom panel; N=43,843) tilt data. Resolution becomes more uniform across the heterotrimer when more particles and tilt data are included. We think tilt data substantially improves reconstructions because of preferred orientation of these particles.

Published structures (Kendall et al, <i>Structure</i> 2020)	heterotrimer	dimer	curved VPS35/ VPS35 sub-structure	flat VPS35/VPS35 sub-structure
Total particles (autopicked)	439,646	439,646	439,646	439,646
Box size (Å)	252x252	395x395	180x180	180x180
Particles in 2D classification	29,771	31,202	34,156	78,994
Particles in final 3D model	26,369	31,022	32,435	69,195
Symmetry	C1	C1	C1	C2
Map resolution (Masked FSC 0.143, RELION)	5.7 Å	9.3 Å	5.3 Å	4.9 Å
B-factor	-212	-100 (user imposed)	-58	-56

Table 1. Data summary from published structures.

<i>Combined datasets</i>	heterotrimer	dimer	curved VPS35/ VPS35 sub-structure	flat VPS35/VPS35 sub-structure
Total particles (autopicked)	646,672	646,672	646,672	646,672
Box size (Å)	241x241	307x307	180x180	180x180
Particles in 2D classification	72,796	77,003	77,003	121,912
Particles in final 3D model	43,843	43,007	43,007	69,381
Symmetry	C1	C1	C1	C1
Map resolution (Masked FSC 0.143, RELION)	4.8 Å	6.6 Å	4.5 Å	4.4 Å
B-factor	-114	-162	-66	-226

Table 2. Data summary from reconstructions including tilt data (15 degrees). We see resolution improvements, and most importantly, our maps look better when tilt data are included.