

	<i>3KE mutant and substructure</i>	<i>Dimer and VPS35/VPS35 substructure</i>	<i>Heterotrimer and VPS35/VPS35 substructure</i>		
Microscope	ThermoFisher Titan Krios G3i Vanderbilt V-CEM	Titan Krios Krios2 NRAMM Data collection 1	Titan Krios Krios2 NRAMM Data collection 1	Titan Krios Krios3 NRAMM Data collection 2	Titan Krios Krios3 NRAMM Data collection 3
Cs	2.7 mm	2.7 mm	2.7 mm	2.7 mm	2.7 mm
Voltage	300 keV	300 keV	300 keV	300 keV	300 keV
Detector	ThermoFisher Falcon 3 direct electron detector	Gatan K2 Summit direct electron detector	Gatan K2 Summit direct electron detector		
Magnification	120,000x	105,000x	105,000x	105,000x	105,000x
Pixel size	0.6811Å/pix	1.096Å/pix	1.096Å/pix	1.06Å/pix (rescaled to 1.096Å/pix, motioncorr2)	1.06Å/pix (rescaled to 1.096Å/pix, motioncorr2)
Dose rate	1.4e ⁻ /Å ² /sec	~8e ⁻ /Å ² /sec	~8e ⁻ /Å ² /sec	~8e ⁻ /Å ² /sec	~8e ⁻ /Å ² /sec
Total dose	50e ⁻ /Å ²	69.34e ⁻ /Å ²	69.34e ⁻ /Å ²	73.92e ⁻ /Å ²	73.92e ⁻ /Å ²
Tilt	+/-30°	N/A	N/A	N/A	+/-15°
Defocus range	-0.8 to -2.6µm	-0.7 to 2.6µm	-0.7 to 2.6µm	-0.8 to -4.4µm	-0.8 to -4.7µm
Number of micrographs	4,791	1,480	1,480	1,299	891
Total particles (autopicked)	275,633	533,231	(250,500 particles selected from combined datasets; Kendall <i>et al.</i> , 2020)		207,026
Box size (Å)	436x436 Å (3KE) 215x215 Å (substructure)	351x351Å (dimer) 220x220 Å (substructure)	241x241Å (heterotrimer) 180x180 Å (substructure)		
Particles in 2D classification	154,533 (3KE) 19,386 (substructure)	509,447 (dimer) 288,247 (substructure)	72,295 (heterotrimer) 69,381 (substructure)		
Particles in final 3D model	40,957 (3KE) 19,386 (substructure)	288,247 (dimer) 268,764 (substructure)	43,808 (heterotrimer) 69,381 (substructure)		
Symmetry	C1	C1	C1		
Map resolution (masked FSC 0.143, RELION)	7.1 Å (3KE) 6.7 Å (substructure)	7.0 Å (dimer) 6.5 Å (substructure)	4.9 Å (heterotrimer) 4.5 Å (substructure)		
B-factor (RELION)	Not determined	Not determined	-114 (heterotrimer) -226 (substructure)		
EMDB code	EMD-26341 (3KE) EMD-26340 (substructure)	EMD-26342 (dimer) EMD-26343 (substructure)	EMD-24964 (heterotrimer) EMD-26345 (substructure)		
PDB code	N/A	N/A	XXXX		

Table 1. Data collection and processing parameters. Summary of data collection and processing parameters for all structures. The heterotrimer is presented here for completeness (Chen *et al.*, *Sci Advances* 7, 2021).