Supplementary materials and methods

Yeast strains - We obtained α(Cop1)-TAP, β'(Sec26)-TAP, β(Sec27)-TAP, γ(Sec21)-TAP, δ(Ret2)-TAP, ε(Sec28)-TAP, and ζ(Ret3)-TAP haploid yeast strains (S288C background) from the yeast TAP-tagged collection (Open biosystems). To construct yeast strains with a GFP tag on β', γ, δ, and ε, a C-terminal GFP cassette was amplified by PCR from the plasmid pFA6a-GFP(S65T)-kanMX6[1], and the resulting PCR product was used to transform the α-TAP strain. To construct yeast strains with a GFP tag on α, the C-terminal GFP cassette was amplified by PCR from the plasmid pFA6a-GFP(S65T)-kanMX6, and the resulting PCR product was used to transform the δ-TAP strain. Transformants were selected on G418 plates, and correct integration of the cassette was verified by PCR.

Purification of yeast coatomer - Yeast cells were grown to an OD_{600} of ~4, harvested, and frozen. For a typical purification, 25 g of frozen cells were grinded briefly by hand, resuspended in lysis buffer (10 mM sodium phosphate, pH 7.4, 150 mM NaCl, 0.1% NP40, 2 mM EDTA, 1 mM EGTA, 50 mM NaF, 0.1 mM Na_3VO_4, 10 mM β-mercaptoethanol, 1.5 mM benzamidine, 1 mM PMSF, complete protease inhibitor tablet (Roche)), and lysed by bead beating (10 x 10 sec). The lysate was clarified by centrifugation at 30,000 x g for 30 minutes at 4°C. The supernatant was incubated with IgG sepharose (GE Healthcare) for 1 hour at 4°C in TEV-C buffer, washed, and proteins were eluted by TEV cleavage for 1.5 hours at 16°C. The eluted proteins were concentrated using an Amicon Ultra 50K centrifugation unit (Millipore) and loaded onto a Superose 6 gel filtration column, equilibrated with buffer (20 mM Tris, pH 8.0, 150 mM NaCl, 10% glycerol, 0.005% NP40, 0.5 mM DTT). Purified complexes were immediately examined by EM or TCA-precipitated for SDS-PAGE analysis.

Electron microscopy - Samples were prepared by conventional negative staining with 0.75% (w/v) uranyl formate as described[2]. Raw images were recorded at a nominal magnification of 52,000x and a defocus value of –1.5 μm on a 1K x 1K charge-coupled device (CCD) camera (Gatan) with a Philips CM10 electron microscope operated at 100 kV. Images for digital processing were collected with a Tecnai T12 electron microscope

(FEI, Hillsboro, OR) equipped with an LaB_6 filament and operated at an acceleration voltage of 120 kV. Images were recorded on imaging plates at a nominal magnification of 67,000x and a defocus value of –1.5 μm using low dose procedures. Imaging plates were read out with a scanner (DITABIS) using a step size of 15 μm, a gain setting of 20,000, and a laser power setting of 30%; 2 x 2 pixels were averaged to yield a pixel size of 4.5Å at the specimen level[3]. Cryo-negative staining was performed as described[2]. Samples were embedded in a layer of uranyl formate between two carbon films on Quantifoil R2/1 400 mesh grids (Quantifoil Micro Tools) and frozen in liquid nitrogen. Specimens were examined using a CT-3500 cryo-transfer holder (Gatan) and a Tecnai F20 electron microscope equipped with a field emission electron source (FEI) and operated at an acceleration voltage of 200 kV. The same specimen areas were recorded at tilt angles of 50° and 0° on Kodak SO-163 film under low-dose conditions at a nominal magnification of 50,000 and a defocus value of –2 μm. The negatives were developed for 12 min at 20°C using full-strength Kodak D-19 developer. Micrographs were digitized with a Zeiss SCAI scanner using a step size of 7 μm; 3 x 3 pixels were averaged to yield a pixel size of 4.2Å at the specimen level.

Image processing - For 2D analysis of negatively stained coatomer (α-TAP), 12,515 particles were interactively selected from 168 images using BOXER, the display program associated with the EMAN software package[4]. Using the SPIDER software package[5], the particles were windowed into 80 x 80-pixel images, rotationally and translationally aligned, and subjected to 10 cycles of multi-reference alignment. Each round of multi-reference alignment was followed by K-means classification specifying 100 output classes. The references used for the first multi-reference alignment were randomly chosen from the particle images. For GFP-labeled samples, a total of 3,987 particles were selected for α-GFP/δ-TAP, 8,361 particles for β'-GFP/α-TAP, 9,368 particles for ε-GFP/α-TAP, 3,467 particles for γ-GFP/α-TAP, and 3,267 particles for δ-GFP/α-TAP. The particle images were windowed into 96 x 96-pixel images, and subjected to multi-reference alignment and K-means classification, specifying 200 output classes. For 3D reconstructions of cryo-negatively stained coatomer, a total of 20,561 particle pairs were interactively selected from both the untilted and 50° tilted images (56 pairs) using WEB,

the display program associated with the SPIDER software package. The selected particles were windowed into 90 x 90-pixel images, and the particles from the untilted specimens were classified into 100 classes as described above. 3D reconstructions were independently calculated for 20 classes with the particle images from the tilted specimens using the backprojection, backprojection refinement and angular refinement procedures in SPIDER. For display and further analysis, the density was filtered to 36 Å, the resolution estimated using the Fourier shell correlation (FSC) = 0.5 criterion[6]. A representative map has been deposited in the EMDatabank (accession code EMD-XXXX).

Supplementary Table 1: Mass spectrometry of TAP-purified coatomer (α-TAP).

Subunit	Number of peptides	Coverage by amino acid count (%)
α or Cop1 (136 kDa)	79	59.2
β or Sec26 (109 kDa)	50	42.4
β' or Sec27 (99 kDa)	48	64.2
γ or Sec21(105 kDa)	47	56.1
δ or Ret2 (61 kDa)	25	46.1
ε or Sec28 (34 kDa)	16	68.6
ζ or Ret3 (22 kDa)	14	42.5

Supplementary Figure 1. (**a**) The 100 class averages of negatively stained coatomer (α-TAP**)** obtained from the classification of 12,515 particles. The side length of the panels is 36 nm. (**b**) A pair of corresponding images of cryo-negatively stained coatomer at 0º (*left*) and 50º tilt (*right*). The lines indicate the tilt axis. The scale bar represents 50 nm. (**c**) The 100 class averages of cryo-negatively stained coatomer obtained from the classification of 20,561 particles selected from the images of the untilted specimen. The side length of the panels is 38 nm. (**d**) Fourier shell correlation (FSC) curve of the 3D reconstruction of class 36. According to the FSC = 0.5 criterion, the resolution is estimated to be 36 Å.

Supplementary Figure 2. (**a-e**) Different views of representative 3D reconstructions with the corresponding 2D average shown on the left; (a) class 17, (**b**) class 55, (**c**) class 86, (**d**) class 91, and (**e**) class 17. Side length of the 2D averages is 38 nm. The scale bars represent 5 nm. (**f**) A silver stained gel of purified coatomer with GFP fused to the α subunit (α–GFP) (*left*). Three representative averages of negatively stained α–GFP (*right*). Density for GFP could not be identified. Side length of each panel is 43 nm. (**g**) A silver stained gel of purified coatomer with GFP fused to the β' subunit (β'–GFP) (*left*). Three representative averages of negatively stained β'–GFP (*right*). Density for GFP could not be identified. Side length of each panel is 43 nm. (**h**) A silver stained gel of purified coatomer with GFP fused to the ε subunit (ε–GFP) (*left*). Three representative averages of negatively stained ε–GFP (*right*). Density for GFP could not be identified. Side length of each panel is 43 nm.

References

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