Supporting Information

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SI Materials and Methods

Cell Culture. The *Chlamydomonas reinhardtii mat3-4* strain CC-3994 (69) was acquired from the Chlamydomonas Resource Center (University of Minnesota) and grown with constant light and normal atmosphere aeration in Tris-acetate-phosphate (TAP) medium. The small size of *mat3-4* cells facilitated complete vitrification by plunge-freezing.

Vitrification and cryo-FIB Milling. Plunge-freezing and cryo-focused ion beam milling were performed as previously described (3, 70). Using a Vitrobot Mark 4 (FEI), cells were blotted onto carbon-coated 200-mesh copper grids (Quantifoil Micro Tools), which were immediately plunged into a liquid ethane/propane mixture. Vitrified grids were mounted into modified Autogrids (FEI), which were loaded either into a Scios FIB/SEM dual-beam microscope (FEI) or onto a custom-made 360° rotatable cryo-stage (6, 71) in a Quanta 3D FEG FIB/SEM dual-beam microscope (FEI). After coating samples with organometallic platinum (72) using the in situ gas injection system (GIS, FEI), thin lamellas were milled using the Ga^+ ion beam at 30 kV and a shallow 8°–12° angle.

Cryo-ET. Cryo-electron tomography was performed on a 300-kV Titan Krios microscope (FEI) with a postcolumn energy filter (968 Quantum, Gatan) and a direct detection camera (Gatan, K2 Summit). Low-dose tilt-series acquisition (<100 e/Å^2 cumulative dose) was controlled by SerialEM software (73) with 2° tilt increments, −5 μm defocus, and 14,600× magnification (pixel size of 3.4 Å). The tomogram in Fig. 4 *F–I* was acquired with a Volta phase plate (68), providing enhanced contrast.

Tomogram Reconstruction and Segmentation. IMOD software (74) was used for patch tracking alignment and weighted back-projection reconstruction of the tomograms. Fourier analysis of the intracisternal protein array was performed with Fiji (ImageJ, National Institutes of Health). Tomogram segmentation was performed with Amira software (FEI Visualization Sciences Group).

Template Matching and Subtomogram Averaging. Template matching was performed with PyTom software (75). Tomographic volumes were searched with a low-pass–filtered average of the protein array structure, cross-correlation peaks were extracted, and corresponding subtomograms were generated. The initial template was a single subvolume containing two cisterna membranes connected by a protein array, whereas later iterations used a subtomogram average of the protein array as a template. The extracted subtomograms were visually checked in Matlab (MathWorks) with TOM software (76) and then aligned and averaged in real-space with PyTom.

The subtomogram average shown in Fig. 3 was calculated using 244 subvolumes from a single tomogram. Peaks in the autocorrelation of the subtomogram average revealed the periodicity of the subvolume. This information was then used to generate a subtomogram average with translational symmetry in both the *x* and *y* dimensions, which is displayed in Fig. 3 *A–E*. The average was rendered in 3D with UCSF Chimera (77) and segmented with Amira. Subvolumes from seven tomograms were used to produce the subtomogram average in Fig. S1*A*, which shows the average lateral repeat of all other arrays in our dataset.

The heat map in Fig. 3*F* was determined by template matching. At every position with a positive hit, a sphere with a diameter smaller than the subtomogram size was inserted into a volume and masked with a 3D segmentation of the Golgi cisternae. The result was then projected along the *z* axis and subsequently normalized. To ease visualization, this projection was overlaid on an outline of the central slice of the Golgi.

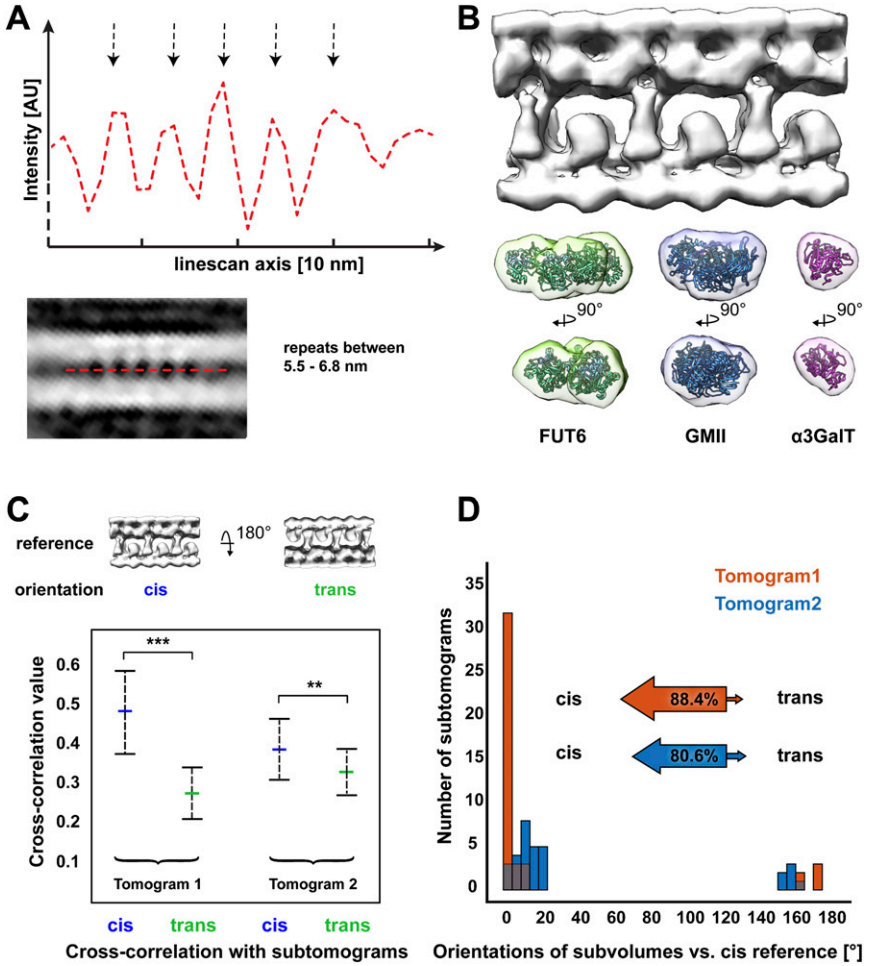
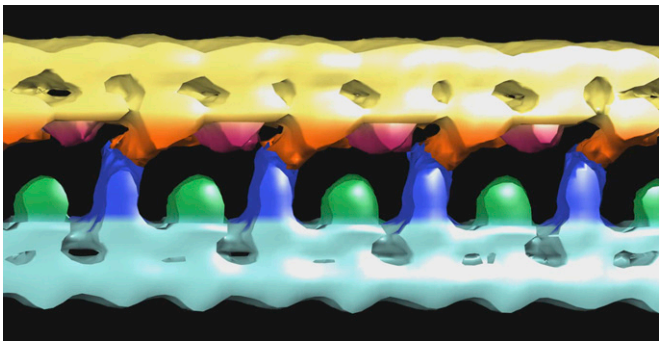
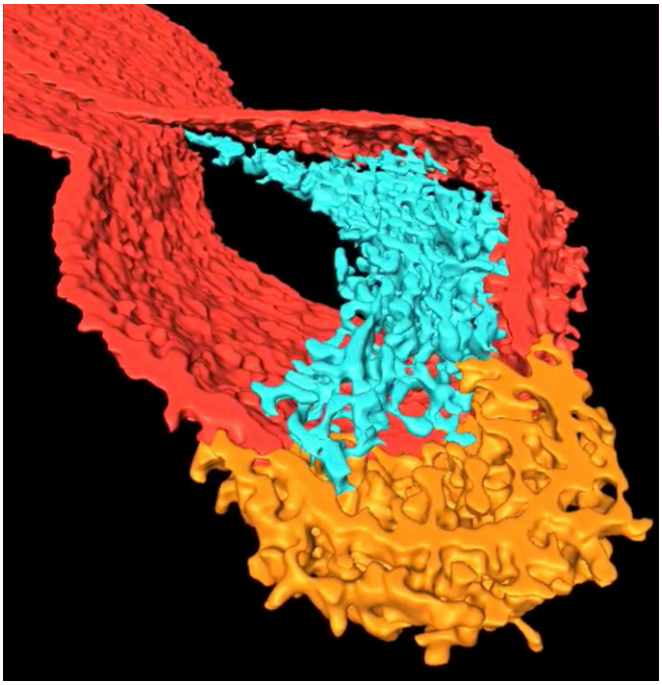


Fig. S1. (*A*) Subtomogram average of extracted array structures from tomograms 2–8 (194 subvolumes), which were not used to generate the structure in Fig. 3. Mass is shown in white. A linescan through the central *xy* slice reveals an average lateral repeat in the range of 5.5–6.8 nm. This is consistent with the 5.9-nm repeat of the highly ordered array in tomogram 1 (Figs. 1 and 3). Arrows: intensity peak positions from the linescan corresponding to luminal densities in the average. (*B*) The subtomogram average of the array in tomogram 1 (Fig. 3) is displayed to scale with the crystal structures of three glycosyltransferase luminal catalytic domains, fucosyltransferase FUT6 (PDB: 2NZW) (78), mannosidase GMII (PDB: 1HTY) (79), and galactosyltransferase α3GalT (PDB: 1G8O) (80). These structures are comparable in size with the luminal densities identified in the array average. (*C*) Cross-correlation values were calculated by comparing the reference structure (from Fig. 3) in the cis and trans orientations to the individual subtomograms from tomogram 1 (containing the highly ordered array, Figs. 1 and 3) and tomogram 2 (another high quality tomogram, Fig. 2). The arrays from both tomograms show a high degree of asymmetry, favoring the cis orientation- tomogram 1 at a significance level of 0.1% (***) and tomogram 2 at 1% (**). Error bars, SD. (*D*) Histograms showing the final orientations of each subvolume relative to the cis reference used for alignment; 88.4% of the arrays from tomogram 1 (red) and 80.6% of the arrays from tomogram 2 (blue) were in the cis orientation (larger projections from the cis-sides of the cisternae), the same orientation as the highly ordered array shown in Figs. 1 and 3.



Movie S1. Subtomogram average of a trans-Golgi intracisternal protein array. Sequential sections through the subtomogram average (mass is white), followed by tour of the 3D structure (cis-side bilayer and embedded proteins, cyan; trans-side bilayer and embedded proteins, yellow; long and short luminal projections from the cis-side bilayer, blue and green; luminal projections from the trans-side bilayer, magenta and orange). Corresponds to Fig. 3 *A–E*.

Movie S1



Movie S2. Intracisternal filament bundle in the trans-Golgi near a COPI-coated bud. Sequential sections back and forth through the tomographic volume in orthographic view, followed by a tour of the 3D segmentation in perspective view. Golgi membrane, dark orange; COPI-coated bud, yellow; filament bundle, blue. The tomogram was 2× binned. Unbinned pixel size, 3.4 Å. Corresponds to Fig. 4 *A–D*.

Movie S2