

Figure S1 Comparisons of the heterochromatin, inner plate, outer plate, corona, and cytoplasm in an unbound PtK1 kinetochore. **(A)** Projection image of the centromere region of a chromosome in a nocodazole-treated PtK1 cell. The areas corresponding to the segmentation in B-F are indicated by contours with colors matching the surface rendering in B - F. Scale bar = 100 nm. **(B) - (F)** Edge views of the heterochromatin (purple), cytoplasm (green), inner plate (blue), outer plate (yellow), and corona (pink). Scale bar for (B), (C), (B'), (C') = 25 nm. Scale bar for (D)- (F), (D')-(F') = 50 nm. **(B')-** **(F')** Face views of the heterochromatin, cytoplasm, inner plate, outer plate,

and corona. The heterochromatin appears as a disordered volume composed of fibers and small knobs, approximately 11-12 nm in diameter (white arrowheads in B' and D'). The corona region is composed of sparsely arrayed fibers (Fig. S2 F, F'). The cytoplasmic region is composed of spheres and rod-like structures that are roughly the size of individual or short strings of ribosomes (Figs. S2C, and C'). Note that only the outer plate has fibers that are coplanar with the outer plate viewed edge-on. Only the outer plate has coplanar fibers that form a distinct disk.

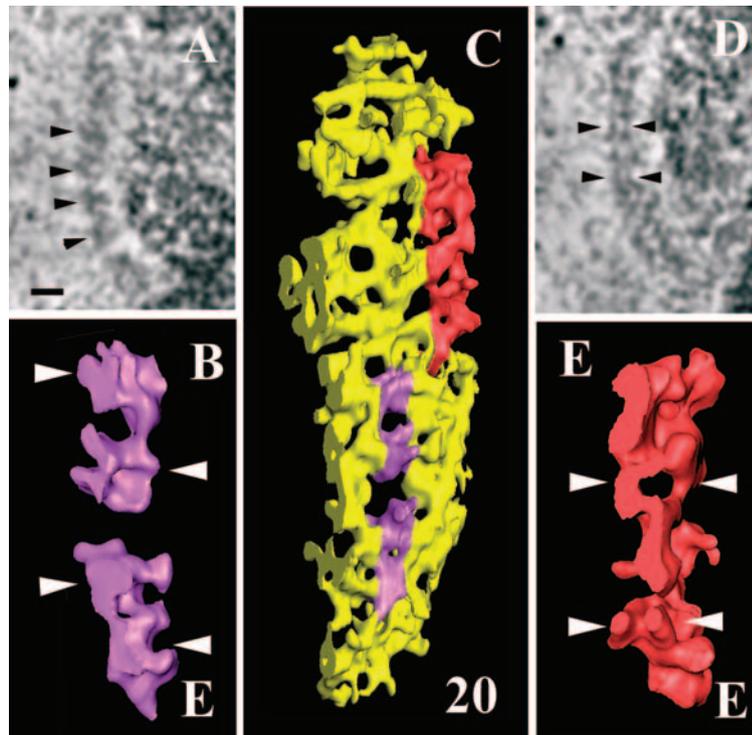


Figure S2 The outer plate does not contain distinct unit motifs, or a double-layer organization. **(A)** and **(D)**: Edge-view slices from the 3D reconstruction of an unbound kinetochore, with arrowheads indicating regions that give the appearance of a subunit **(A)** or double-layer **(D)** organization to the outer plate. Scale bar = 50 nm (see Movie 3). **(B)** Surface rendering of the area

indicated in **(A)**. Arrowheads point out regions that appear as subunits in 2D views. **(C)** Surface-rendered view of the whole unbound kinetochore, with the repetitive and double layer parts shown in purple and red, respectively (See Movie 3). **(E)** Surface rendering of areas indicated in **(D)**. Arrowheads point out the region that appears double-layered in 2D views.

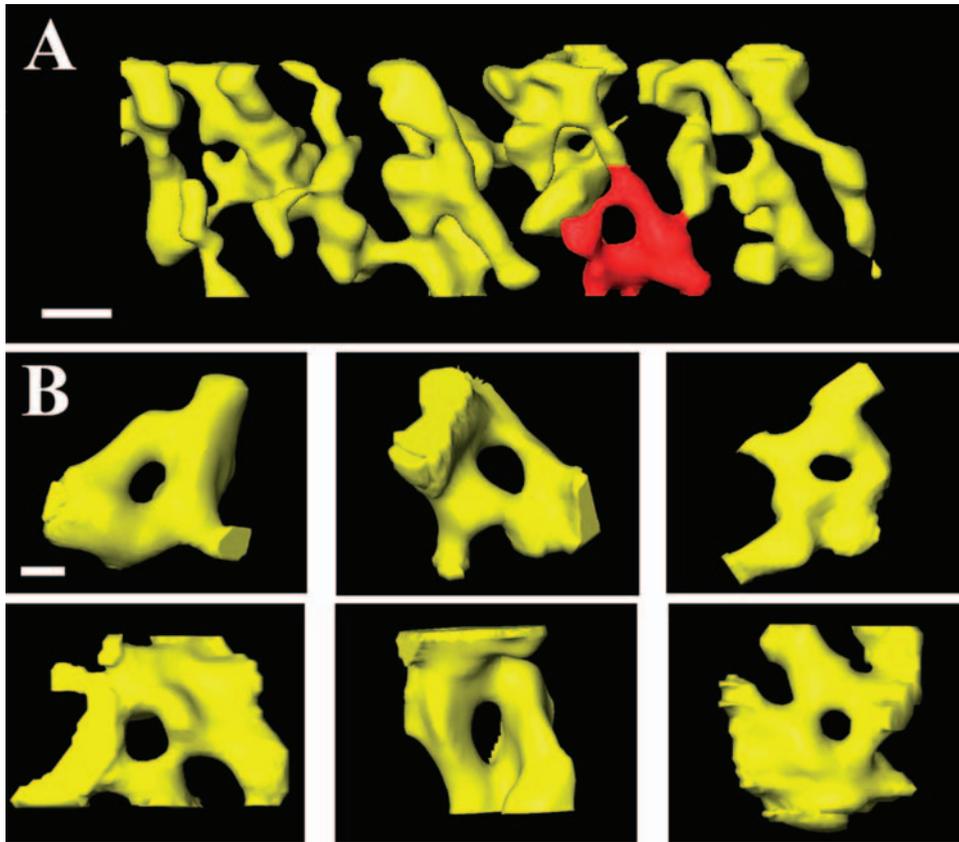


Figure S3 Ring-like structures found on the kinetochore outer plate. **(A)** A ring-like structure in an unbound kinetochore outer plate is shown in red.

Scale bar = 30 nm. **(B)** Six ring-like structures isolated from four different kinetochores. Scale bar = 10 nm.

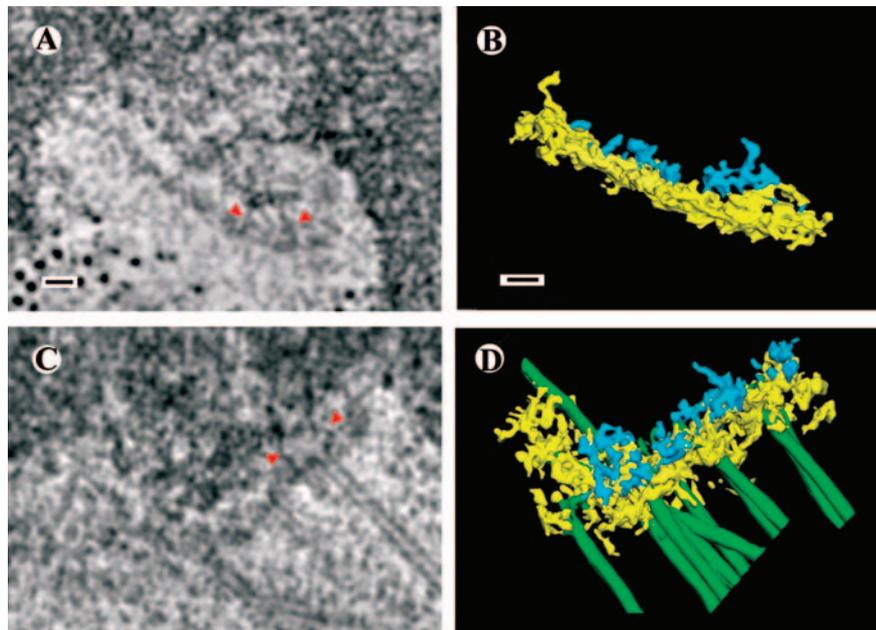


Figure S4 Fibrous connections between the outer plate and the inner centromere. **(A), (C)**: 1.6 nm thick edge-view slices from tomographic reconstructions of unbound (A), and bound (C) kinetochores. Red arrowheads point to fibers connecting the outer plate to the inner

centromere. **(B), (D)** Surface renderings of the reconstructions of the same kinetochores. Connecting fibers are shown in blue, outer plate in yellow, and MTs in green. The unbound kinetochore used in (A) and (B) is from a cell treated with nocodazole to remove all MTs.

SUPPLEMENTARY MOVIES

Movie 1.

Serial face-view slices through the 3D reconstruction of the kinetochore shown in Figs. 1B-D. Left panel: same edge view-slice as shown in Fig. 1C. Red arrows point to the kinetochore outer plate. Right panel: series of 1.6 nm-thick face-view slices through the kinetochore. The vertical line in the left panel indicates the position of the slice being shown in the right panel. Note the distinct arrangement of long fibers in the area of the outer plate.

Movie 2.

Rotation of the surface-rendered model of the outer plate shown in Fig. 1B-D.

Movie 3.

Edge-view slices through the 3D reconstruction of kinetochore shown in Fig. S2, and rotation of the surface-rendered model. Purple arrows in the tomographic slices indicate areas giving the impression of repetitive units, while red arrows in other slices indicate areas giving the impression of a double-layer organization. These areas are shown in purple and red in the surface-rendered model.

Movie 4.

Edge-view slices through the 3D reconstruction of kinetochore with attached kMTs shown in Fig. 2E-H, and rotation of the surface-rendered model. This version of the model shows two laterally associated MTs in orange (bottom of the kinetochore) not shown in Fig. 2E-H.

Movie 5.

Face-view slices through the 3D reconstruction of the kinetochore shown in Fig. 3 and rotation of the surface-rendered model. Blue and red arrows indicated the plus-end tips of two kMTs.

Movie 6.

Arrangement of fibers in the outer plate after kMT attachment. Rotation of the surface-rendered model of the outer plate from a bound kinetochore with the kMTs digitally removed for enhanced viewing of the fibrous network. This is the same kinetochore as illustrated in Movie 4 and Fig 2E-H and 2J,K.