Supplementary Information

Transmission electron microscopy enables the reconstruction of the catenane and ring forms of CS_2 hydrolase

Joseph C.-Y. Wang,*[a] Adam Zlotnick,[a] and Jasmin Mecinović*[b]

[a] *Molecular & Cellular Biochemistry Department, Simon Hall 217, 212 S. Hawthorne Drive, Indiana University, Bloomington, IN 47405*

[b] *Institute for Molecules and Materials, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands.*

SI Methods

Transmission electron microscopy and image reconstruction.

Specimens were prepared by applying 4 μl of protein solutions on glow-discharged 300-mesh carbon-coated copper grids (EMS), washed with deionized water, negative stained with 2% (w/v) uranyl acetate (for catenane assembly) or 0.75% (w/v) uranyl formate (for ring assembly), and blotted to dry. The images were acquired at a nominal magnification of 60,000x using a JEOL JEM-3200FS TEM operated at 300 kV under low-dose condition (≤20 $e^-/Å^2$) on a Gatan UltraScan 4000 4k x 4k CCD camera (equivalent to 1.84 Å per pixel at the specimen space). An in-column zero-loss energy filter was applied using slit width of 20 eV to improve image contrast. Particles were semi-manually extracted in a 140 x 140 pixel box for catenane assembly and 120 x 120 pixel box for ring assembly.[1] Contrast transfer function of the images were measured by CTFFIND3[2] and corrected by flipping the phase using EMAN.[3] Reference-free classification was carried out using iterative hierarchical clustering method (CL2D) in Xmipp.[4, 5]

The *de novo* initial models were obtained using startAny program (part of EMAN package). Orientation determination and 3-D reconstruction were carried out by EMAN software packages. The resolution was estimated to be 23 Å for catenane assembly and 15 Å for ring assembly based on a Fourier shell correlation cutoff of 0.5. Visualization of the 3-D reconstruction and molecule docking were done using UCSF Chimera.[6]

SI Figure Legends

Supplementary Figure 1

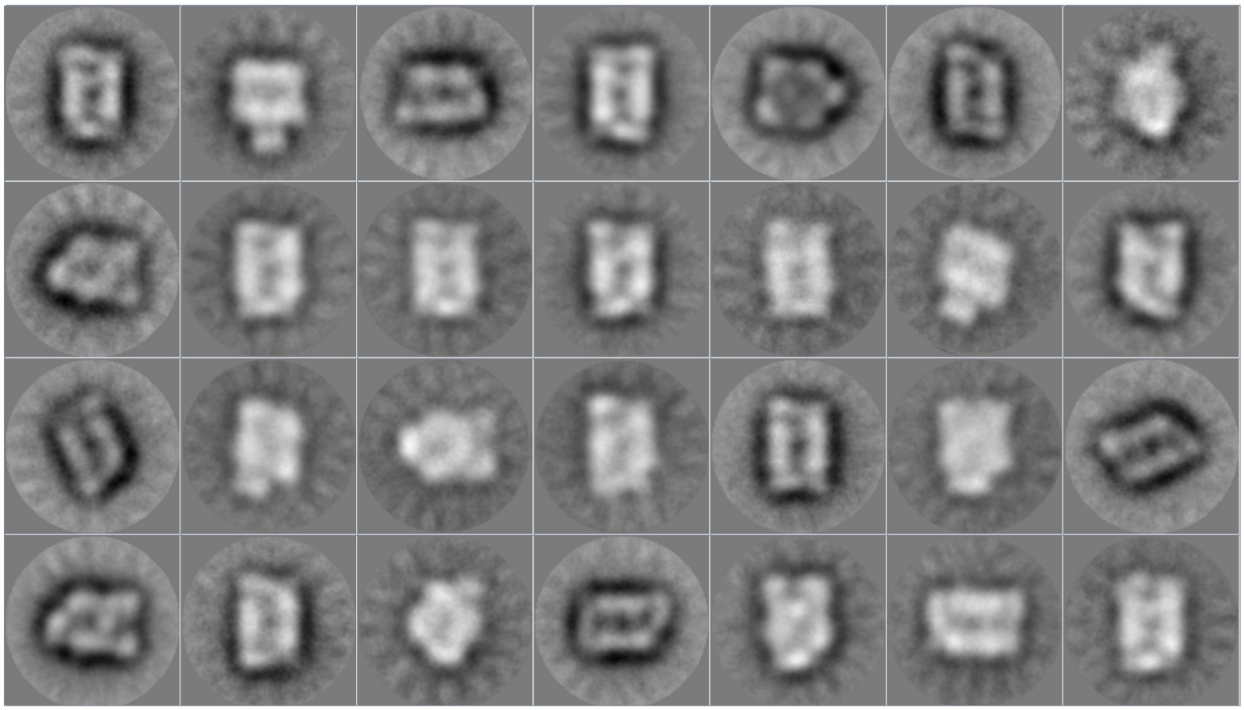

Figure S1. Class averages of the catenane assembly form of CS_2 hydrolase. The resulting class averages of the catenane assembly shows the major orientations within the TEM specimen existed in side views and tilted views.

Supplementary Figure 2

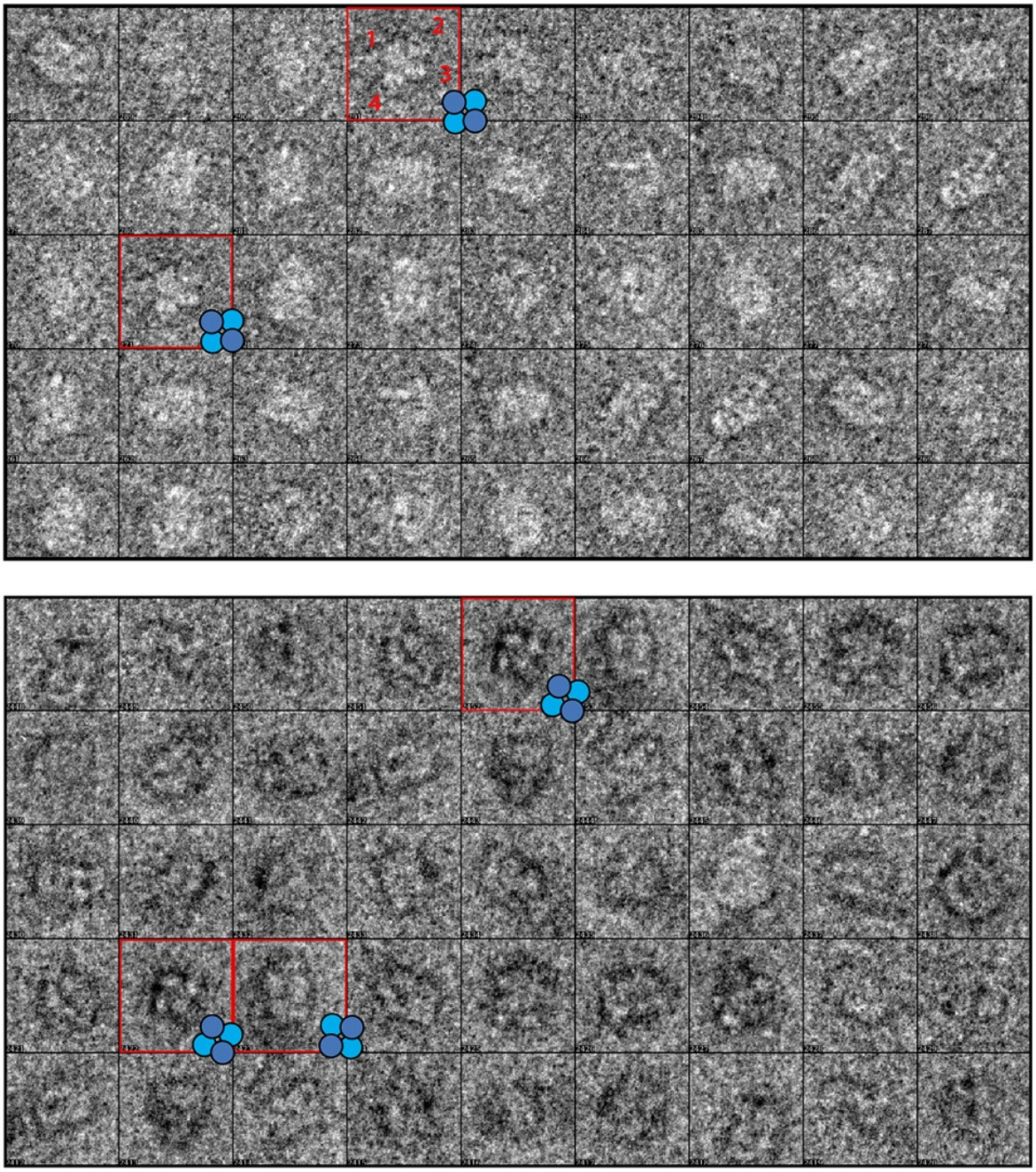

Figure S2. Selected gallery of the extracted catenane particles from EM micrograph. Each square box represents one catenane particle extracted from the EM micrographs. The red box shows a particle that can be identified as the top/bottom view orientation with the cartoon illustration. Each circle represents a density blob of dimeric CS_2 hydrolase at the corner. Dark blue circle denotes the ring at the top and light blue circle denotes the ring at the bottom. Note the particles with top/bottom views are relatively few compared to other orientations.

Supplementary Figure 3

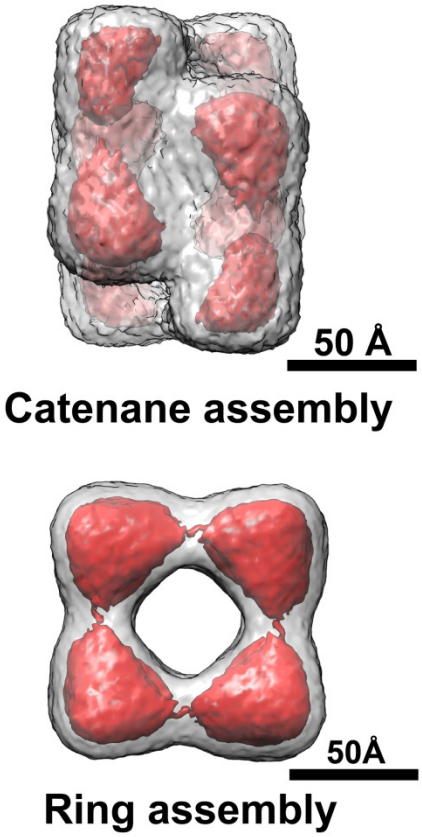

Figure S3. Isosurface representations of the catenane assembly and ring assembly of CS_2 hydrolase rendered at different contour levels. The transparent gray contour represents the estimated full mass of the molecule assembly. The red contour shows the molecule rendered at the higher contour level. Note that the more rigid and/or ordered structural elements remain when rendering at the higher contour level.

SI References

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