Supplementary Material

Figure S1

A. EM image of 50 μg/ml SecA in the buffer: 20mM Tris-Ac, pH 7.5, 1mM DTT. The protein was negative stained by 1% Uranyl Acetate and was observed in Philips CM120 TEM. Some individual particles were marked by squares. The bar is 50nm. Particles were apparently observed, and didn't show any preferred orientation.

B. Some typical particles extracted from A. The size of the box is 20 nm.

C. The surface representation of the 3D reconstruction from negative stained sample. A stable 3D model was obtained after 8 iterations of alignment/classification/3D reconstruction cycles including 2535 particles. The resolution test performed on two independent data sets gave a 3.2 nm for DPR criterion. The surface was rendered and displayed by VMD software. The views were shown in the same orientation as assigned to the raw particles in panel B. View 2 is obtained after the 90° rotation of view 1 around the horizontal axis, and view 3 is obtained after 45° rotation of view 2 around the perpendicular axis. The three domains in two subunits were designated 1, 2, 3 and 1',2',3' ,same as figure 1E. The bar is 5 nm.

Figure S2

A. A plot representing the Euler angle distribution of classified particles within the asymmetric triangle. The brightness of each point indicates the number of particles used in the class-average in that orientation on a log scale. The relatively uniformed distribution indicates that there is no missing cone in the Fourier space.

B. The comparison of 96 projections and corresponding class averages. The label "-1" means this image is a projection map. The numbers at the left corner of images represent the particle numbers in this class.

Figure S3

A The SecA 3D model refined without any symmetry imposed. Each view is obtained after the 90° rotation operation around the horizontal axis as shown between these views.

B. The SecA 3D model refined with 2-fold symmetry imposed. These two models look similar, indicating the SecA has genuine 2-fold symmetry.

Figure S4

The structure accuracy was assessed by comparison of class averages with projections from the EM map at representative orientations. The top panel (A) is the initial classification averages; the middle panel (B) is the class averages in the projection-matching refinement; the bottom panel (C) is the projection map of the reconstructed model. The size of the box is 18.4 nm.

Figure S1

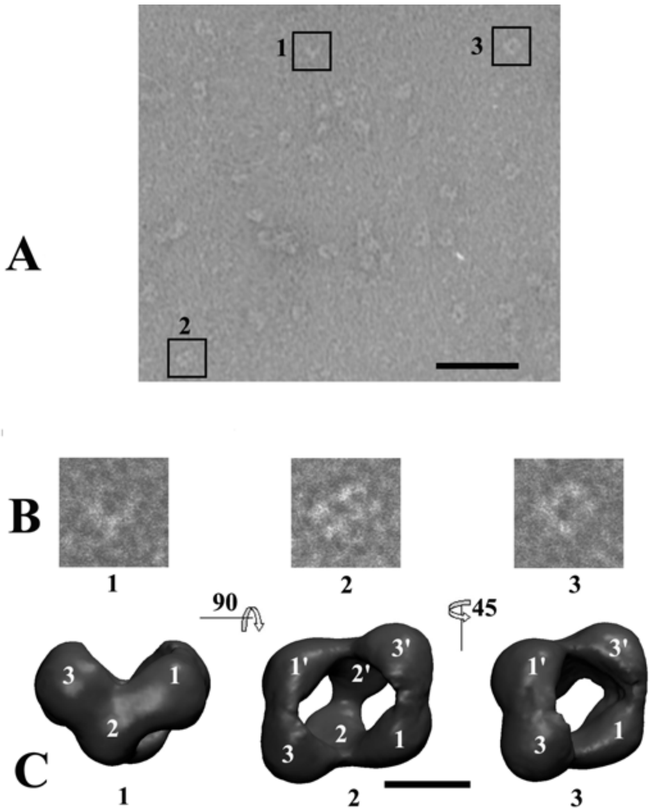
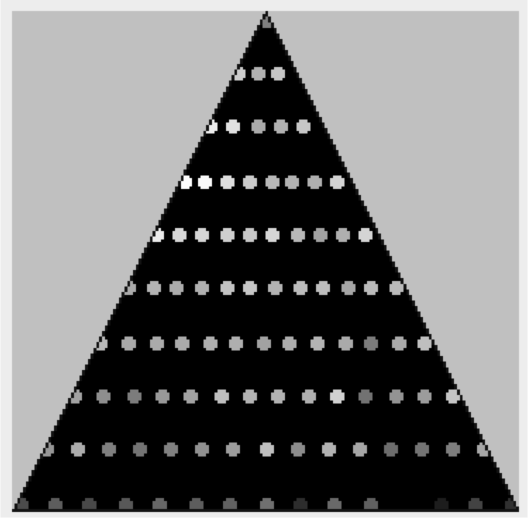
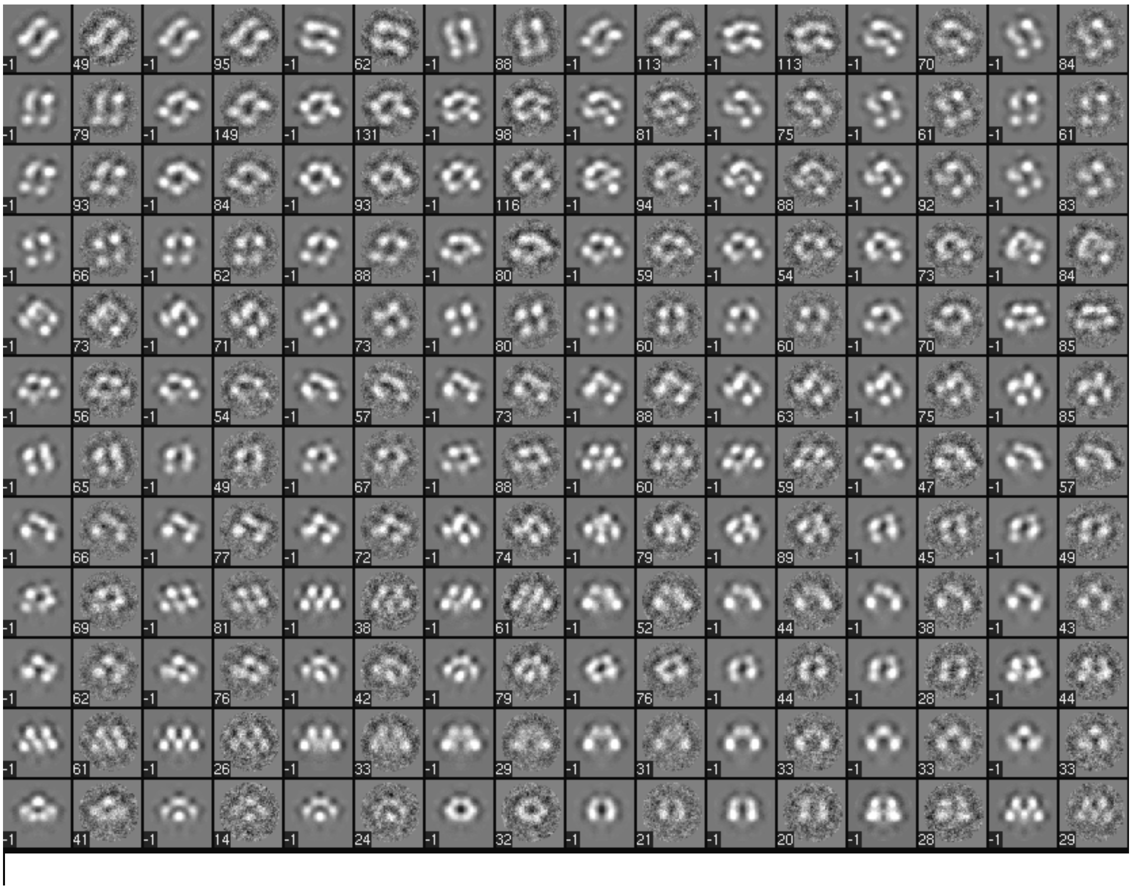


Figure S2

A



B.


-1 49 -1 95 -1 62 -1 88 -1 113 -1 113 -1 70 -1 84
-1 79 -1 149 -1 131 -1 98 -1 81 -1 75 -1 61 -1 61
-1 93 -1 84 -1 93 -1 116 -1 94 -1 88 -1 92 -1 83
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-1 62 -1 76 -1 42 -1 79 -1 76 -1 44 -1 28 -1 44
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-1 41 -1 14 -1 24 -1 32 -1 21 -1 20 -1 28 -1 29

Figure S3

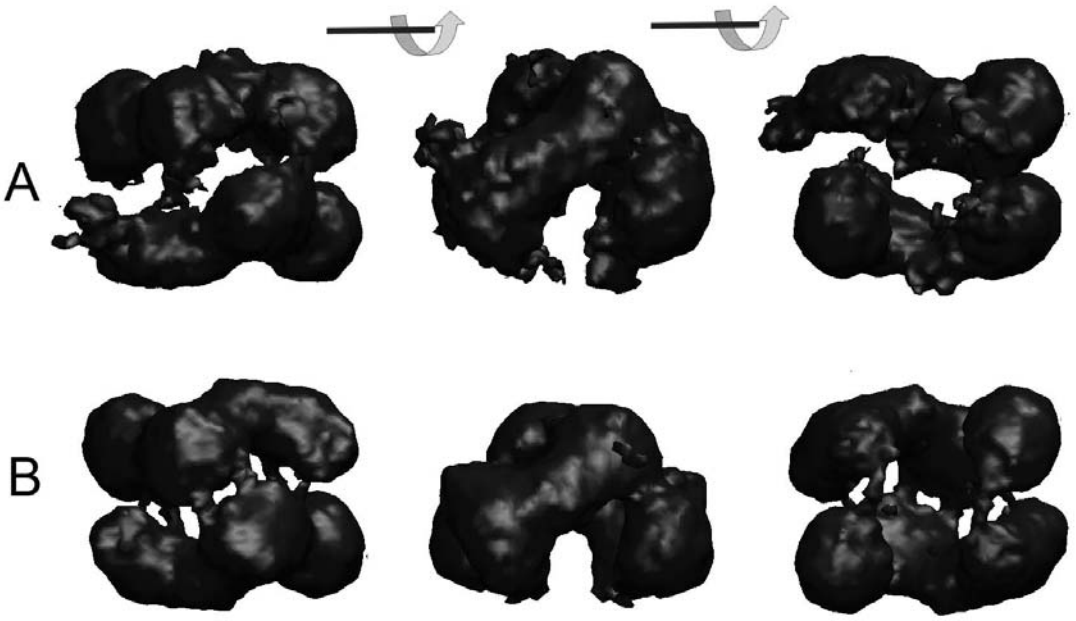

Figure S4