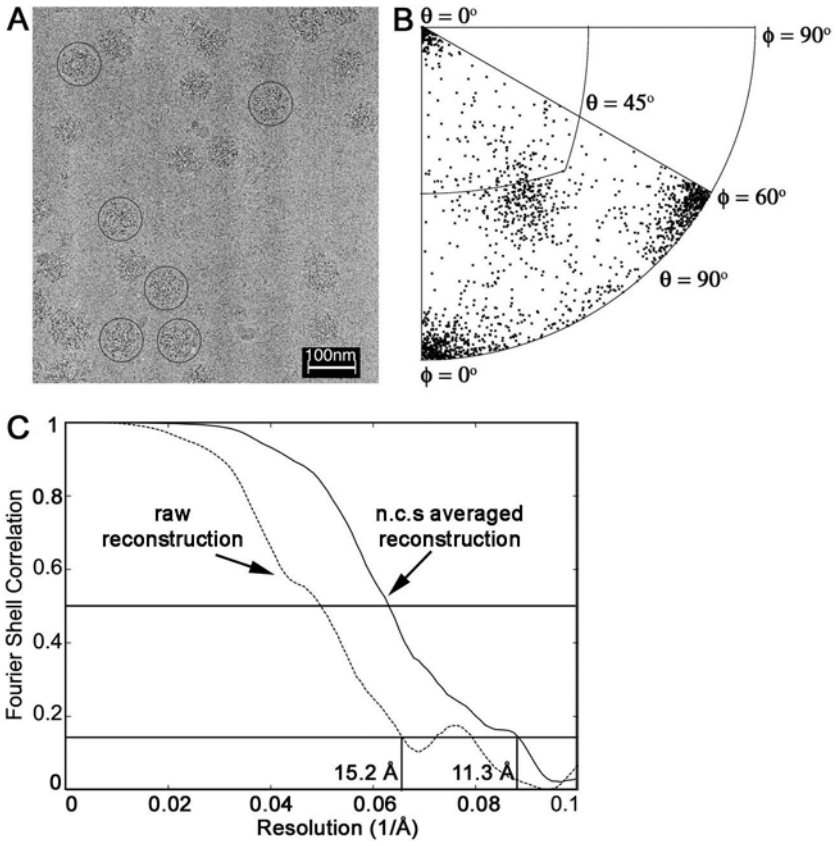
Xing et al Structure of clathrin coat with bound Hsc70 and auxilin: mechanism of Hsc70-facilitated disassembly

Fig. S1. Electron micrograph and statistics of the reconstruction. (A) Field of clathrin coats with bound auxilin(547-910) and Hsc70(1-554). D6 barrels are circled. (B) Distribution of Euler angles for the ~1500 images included in the final reconstruction. (C) Fourier shell correlation, as a function of resolution. We show lines for 0.5 and 0.143, both frequently used criteria for effective resolution. Previous work on clathrin coats using the FREALIGN algorithm (Fotin et al, 2004) suggests that the latter (Rosenthal and Henderson, 2003) is more appropriate here.

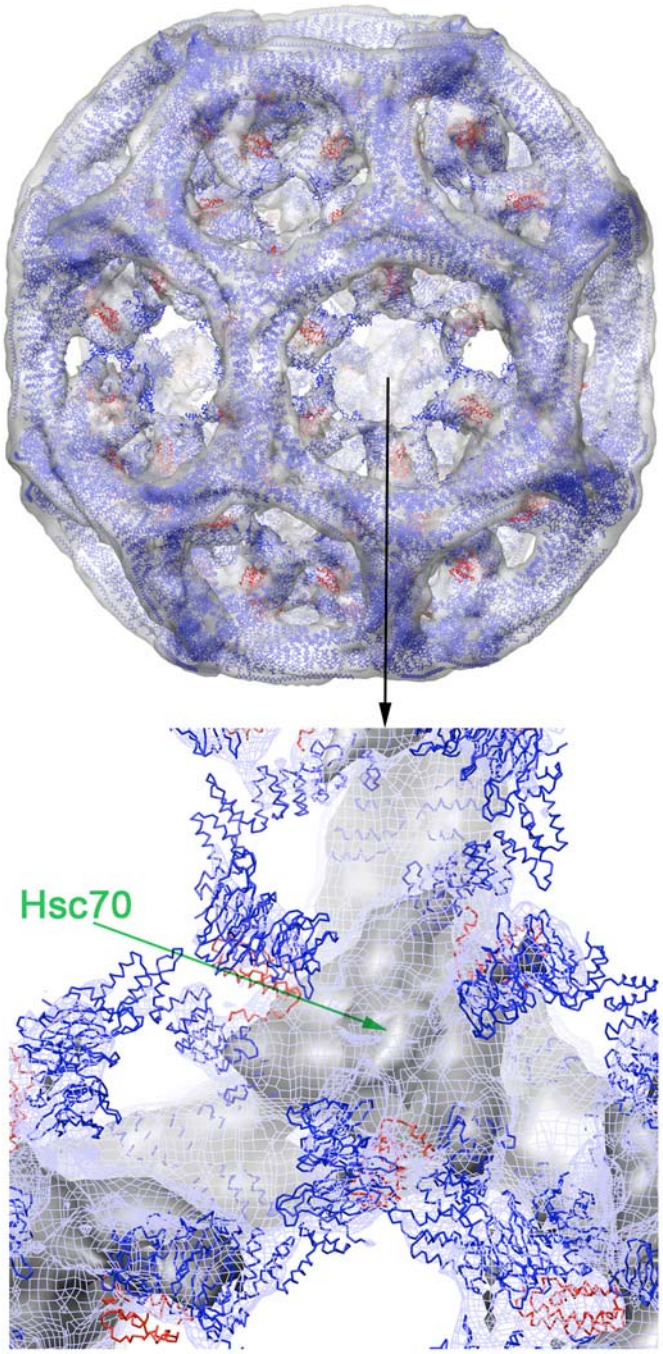


Fotin, A., Cheng, Y., Sliz, P., Grigorieff, N., Harrison, S.C., Kirchhausen, T., and Walz, T. (2004a). Molecular model for a complete clathrin lattice from electron cryomicroscopy. Nature *432*, 573-579.

Rosenthal, P.B. and Henderson, R. (2003). Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. J Mol. Biol. *333*, 721-745.

Xing et al Structure of clathrin coat with bound Hsc70 and auxilin: mechanism of Hsc70-facilitated disassembly

Fig. S2. Map of Hsc70:clathrin D6 barrel at 21 Å resolution.



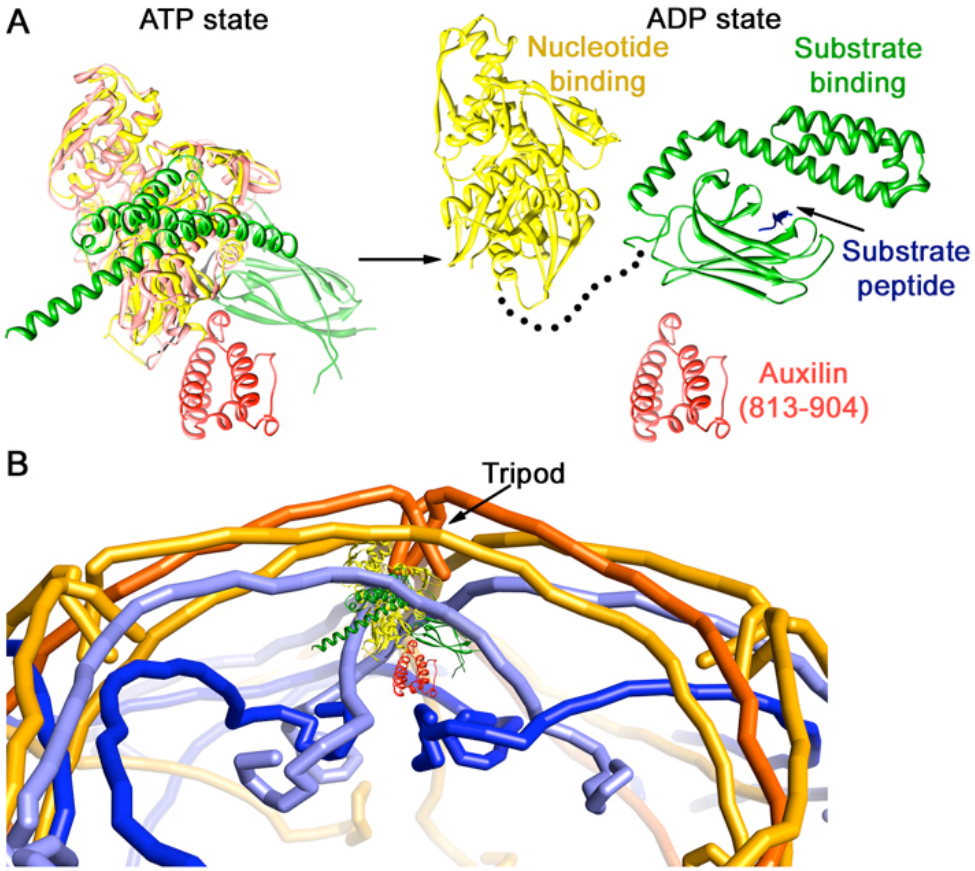
About 7000 particles (from a total of about 14,000 selected at the start of processing) contributed to the map (contoured in translucent gray), which was an intermediate step during refinement of the reconstruction described in this paper. No n.c.s. averaging was performed. The final model, derived from the higher-resolution map, is shown in blue (clathrin) and red (auxilin J-domain). The fit shows that all aspects of the final structure, determined by further selection of low phase-residual images and n.c.s. averaging, are represented in this map. The "zoom" image, contoured at two levels (translucent gray surface and light-blue mesh -- the latter to outline the terminal domains) shows that the density attributed to Hsc70 is clearly present at the higher contour level.

Xing et al Structure of clathrin coat with bound Hsc70 and auxilin: mechanism of Hsc70-facilitated disassembly

Fig. S3. Difference map between auxilin-bound coats with and without Hsc70, calculated as described in Methods and contoured in red (positive) and blue (negative). The map and corresponding model segments are shown for the region around a single vertex. The clathrin model is in brown; the auxilin J-domain model is in gray. The contour level for the map is 4σ; the difference density in the major peak, representing Hsc70 bound tightly to the C-terminal extension of the clathrin heavy chain, is greater than 6σ at its center.

Xing et al Structure of clathrin coat with bound Hsc70 and auxilin: mechanism of Hsc70-facilitated disassembly

Fig. S4. (A) Homology model for the position and orientation of Hsc70:ATP interacting with the J-domain of auxilin at the hub of a clathrin coat (left), just before ATP hydrolysis and clamping onto the C-terminal unstructured tail of a clathrin heavy chain (right). ATP hydrolysis probably also releases Hsc70 from its association with auxilin. (B) The model for Hsc70:ATP:auxilin-J-domain, inserted into the stucture of a clathrin coat by superposing the J-domain on its counterpart in our auxilin(547-910):D6 barrel structure. In constructing the homology model, we have taken the structure of a crosslinked complex between the auxilin J-domain and the Hsc70 NBD as representative of the relative position and orientation of the two components (Jiang et al., 2007) and aligned the NBD in that structure with the NBD of full-length Sse1:ATP (Liu and Hendrickson, 2007).



Jiang, J., Maes, E.G., Taylor, A.B., Wang, L., Hinck, A.P., Lafer, E.M., and Sousa, R. (2007). Structural basis of J cochaperone binding and regulation of Hsp70. Mol Cell *28*, 422-433.

Liu, Q., and Hendrickson, W.A. (2007). Insights into Hsp70 chaperone activity from a crystal structure of the yeast Hsp110 Sse1. Cell *131*, 106-120.