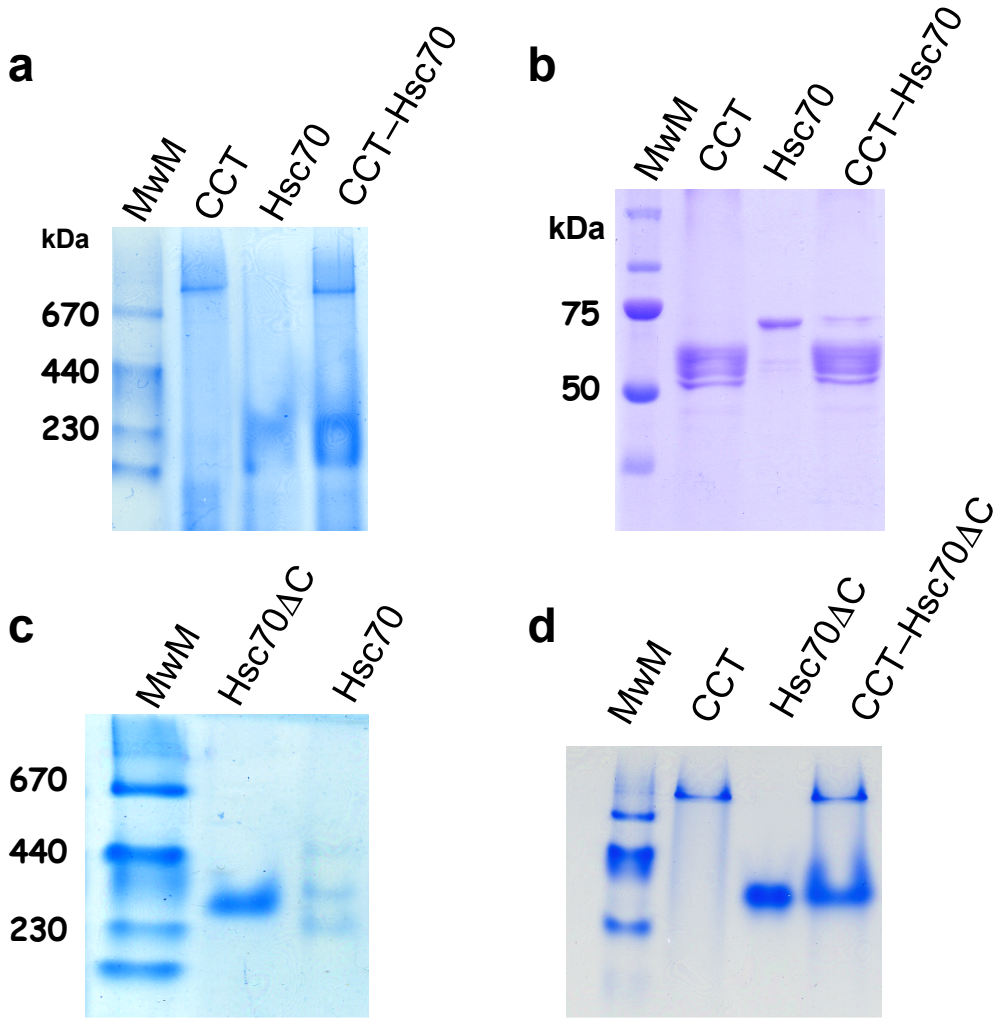
The structure of CCT-Hsc70$_{NBD}$ suggests a mechanism for Hsp70 delivery of substrates to the chaperonin

Jorge Cuéllar, Jaime Martín-Benito, Sjors H.W. Scheres, Rui Sousa,
Fernando Moro, Eduardo López-Viñas, Paulino Gómez-Puertas,
Arturo Muga, José L. Carrascosa and José M. Valpuesta



Supplementary Fig. 1. Hsc70/Hsp70 binding to CCT. (**a**) Aliquots of Hsc70, CCT and a mixture of CCT and Hsc70 were run on a native gel. The CCT band, either uncomplexed or complexed to Hsc70 runs with a different mobility than that of Hsc70. (**b**) The CCT band corresponding to the putative CCT:Hsc70 complex was excised from the gel and loaded onto an SDS gel, which reveals, besides the group of bands corresponding to the 8 different subunits of the CCT oligomer (not all the bands are visible due to the limited resolution of this particular type of gel), the band corresponding to Hsc70. (**c**) The full-length Hsc70 tends to aggregate (revealed in a native gel by the presence of multiple bands) due to the presence of a C-terminal oligomerization domain. The removal of this 10 kDa sequence generates a mutant (Hsc70ΔC) which binds and folds unfolded proteins, has normal ATPase activity[16] but does not aggregate (as revealed by the presence of a single band in the native gel). (**d**) A CCT-binding experiment performed in a native gel as described in a), but with Hsc70ΔC.

a

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  1 MSKGPAVGID LGTTYSCVGV FQHGKVEIIA NDQGNRTTPS YVAFTDTERL
 51 IGDAAKNQVA MNPTNTVFDA KRLIGRRFDD AVVQSDMKHW PFMVVNDAGR
101 PKVQVEYKGE TKSFYPEEVS SMVLTKMKEI AEAYLGKTVT NAVVTVPAYF
151 NDSQRQATKD AGTIAGLNVL RIINEPTAAA IAYGLDKKVG AERNVLIFDL
201 GGGTFDVSIL TIAAGIFEVK STAGDTHLGG EDFDNRMVNH FIAEFKRKHK
251 KDISENKRAV RRLRTACERA KRTLSSSTQA SIEIDSLYEG IDFYTSITRA
301 RFEELNADLF RGTLDPVEKA LRDAKLDKSQ IHDIVLVGGS TRIPKIQKLL
351 QDFFNGKELN KSINPDEAVA YGAAVQAAIL SGDKSENVQD LLLLDVTPLS
401 LGIETAGGVM TVLIKRNTTI PTKQTQTFTT YSDNQPGVLI QVYEGERAMT
451 KDNNLLGKFE LTGIPPAPRG VPQIEVTFDI DANGILNVSA VDKSTGKENK
501 ITITNDKGRL SKEDIERMVQ EAEKYKAEDE KQRDKVSSKN SLESYAFNMK
551 ATVE
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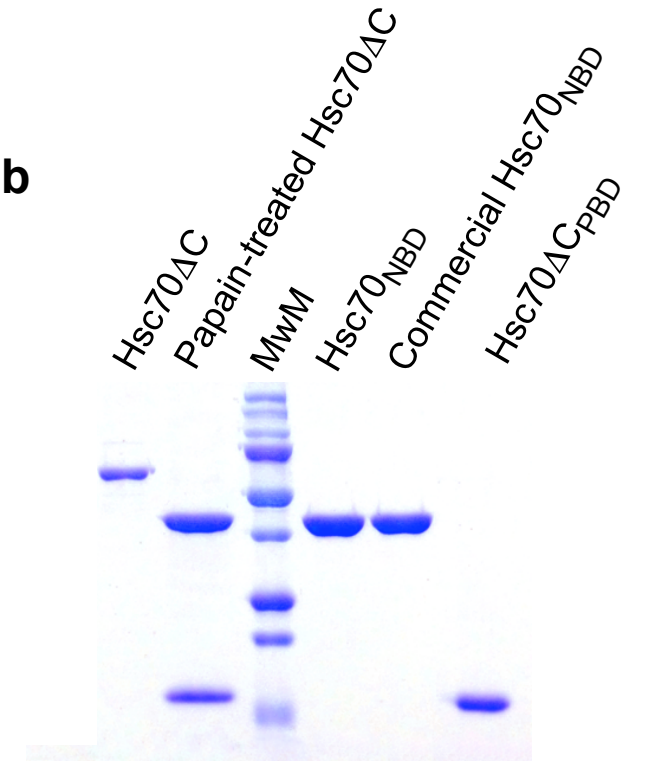
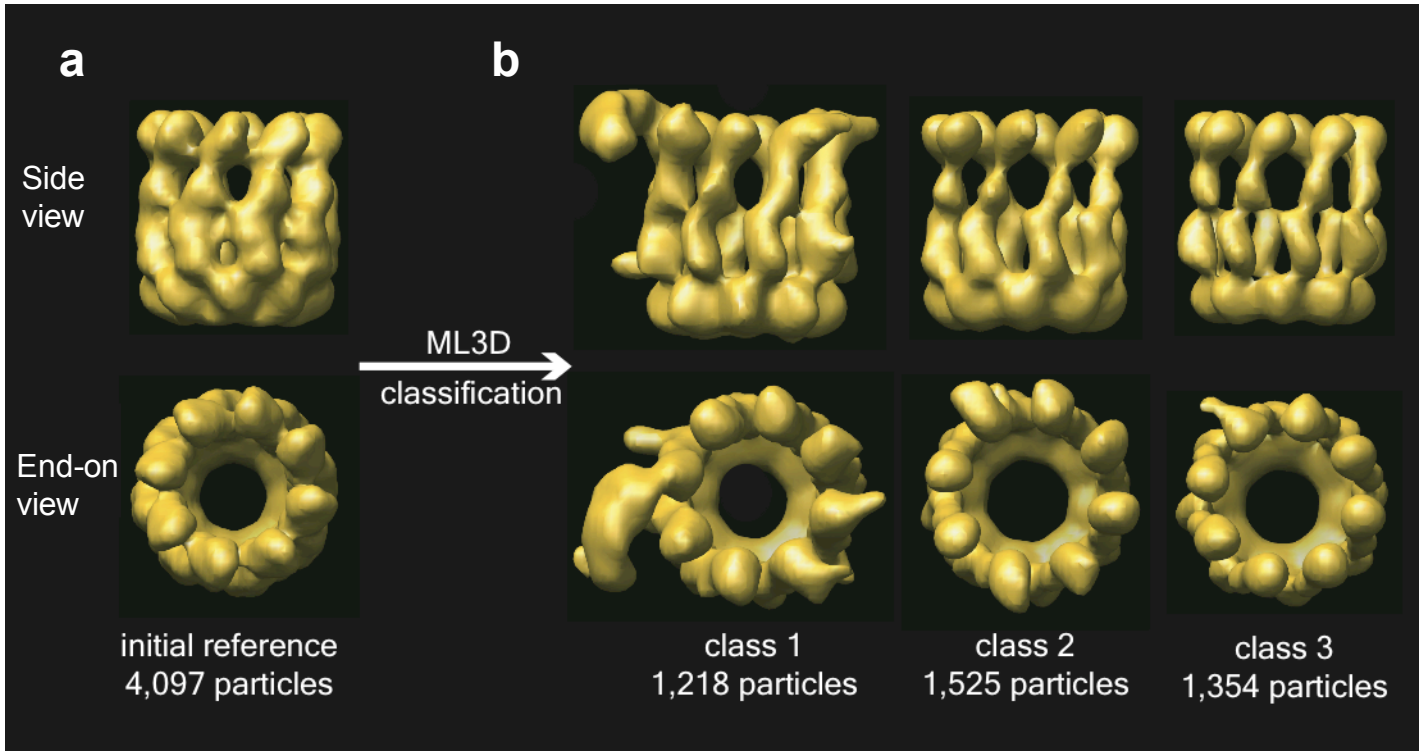
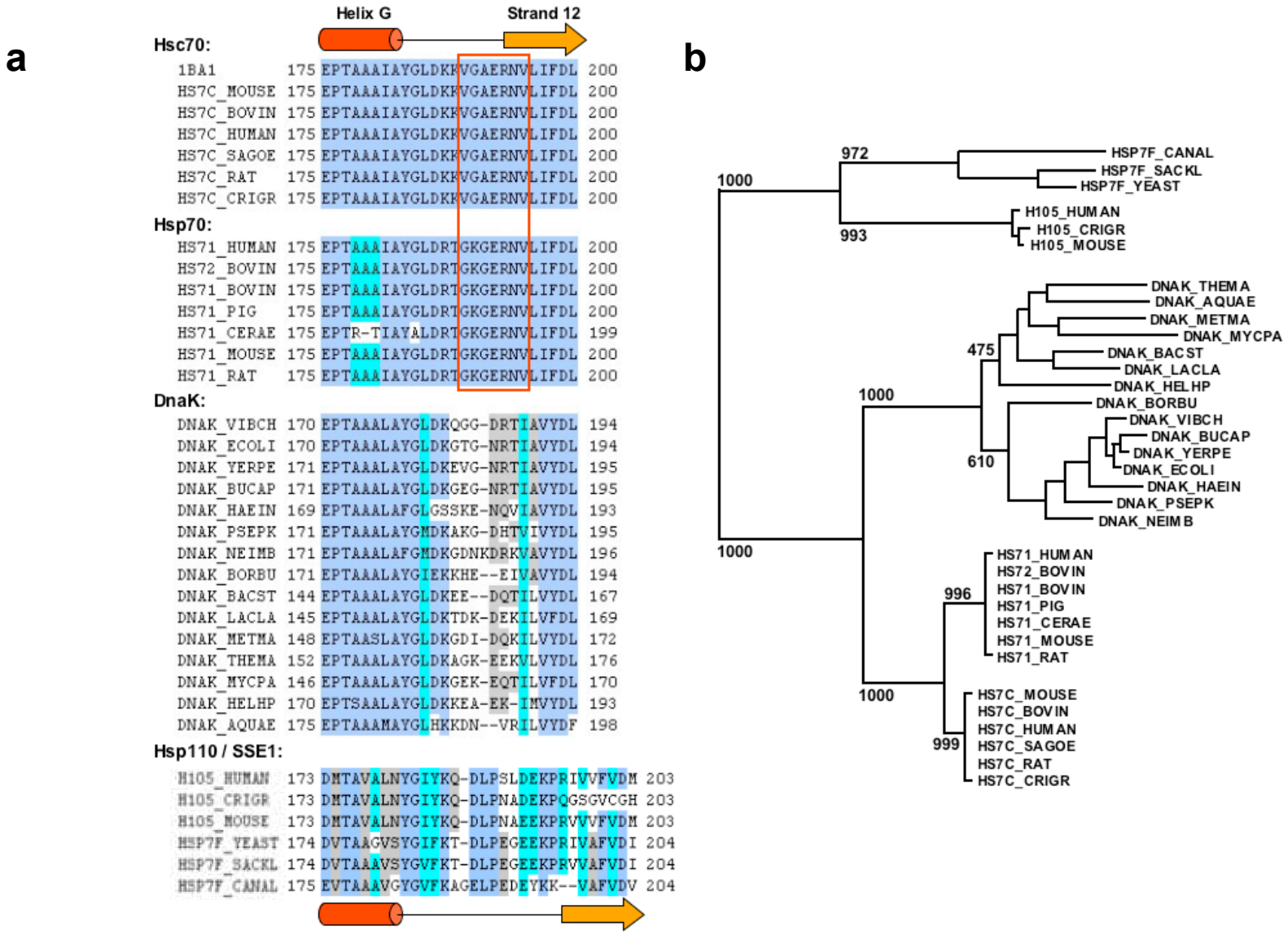
b



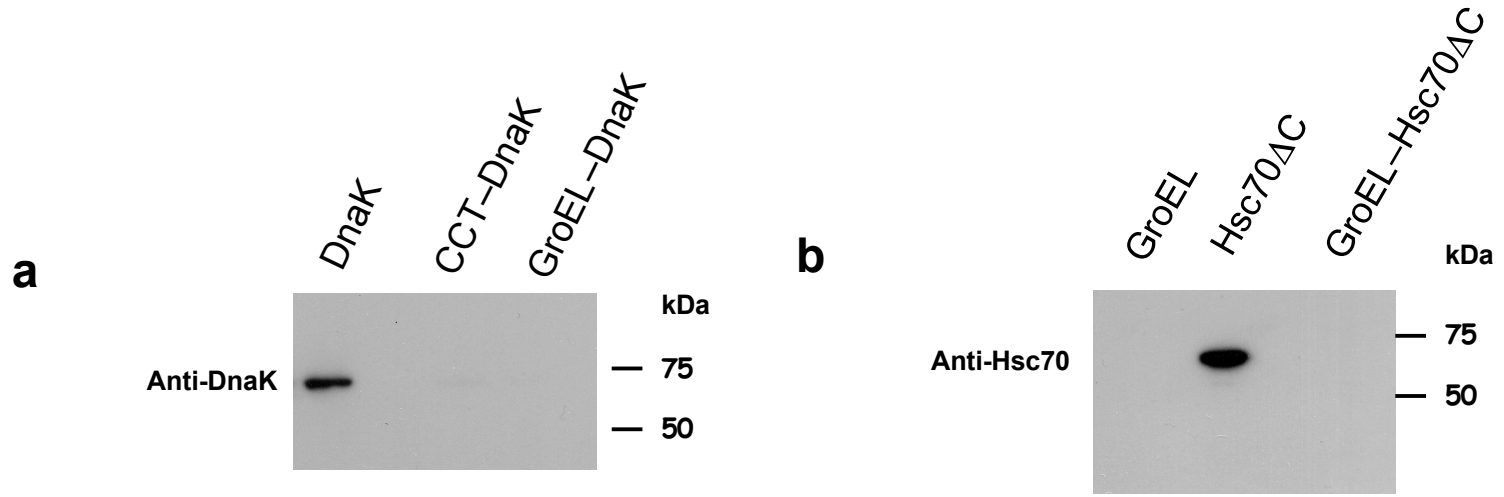
Supplementary Fig. 2. Proteolysis of Hsc70ΔC in two domains. The proteolysis of $Hsc70_{NBD}$ generated two fragments, the N-terminal ATPase domain ($Hsc70_{NBD}$; ~45 kda) and the C-terminal, peptide binding domain ($Hsc70\Delta C_{PBD}$; ~15 kda). (**a**) Protein sequence of human Hsc70ΔC. The yellow box points to the proteolysis site of papain, and the green and red residues are those that match the $Hsc70_{NBD}$ and $Hsc70\Delta C_{PBD}$ sequences, respectively, after the Hsc70 was treated with papain, the two fragments purified and subjected to mass spectrometry of the trypsin-generated fragments. (**b**) SDS gel that shows the two purified Hsc70 fragments, the $Hsc70_{NBD}$ and the $Hsc70\Delta C_{PBD}$ domains. A commercially available $Hsc70_{NBD}$ is also loaded for comparison with our $Hsc70_{NBD}$.



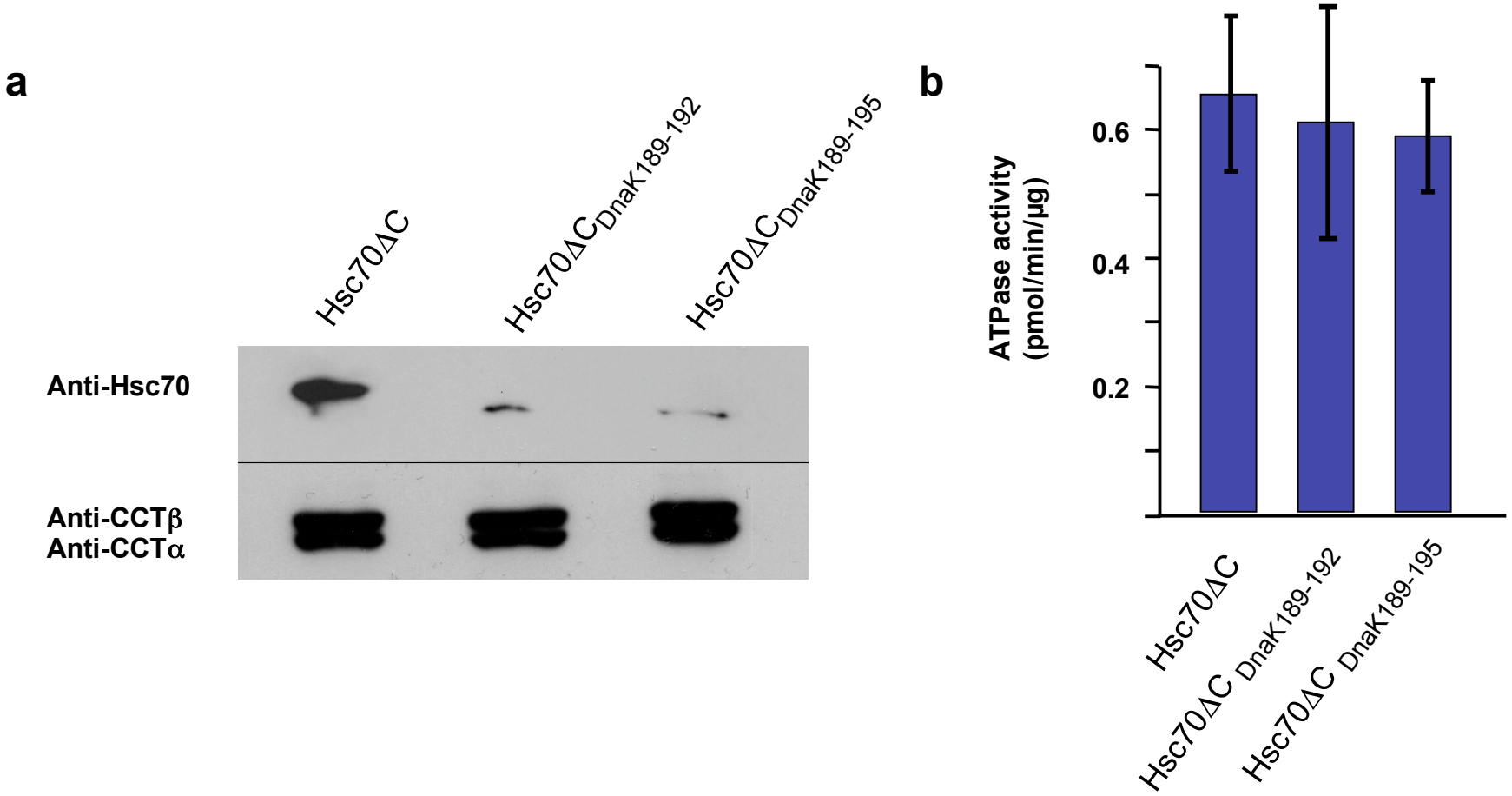
Supplementary Fig.3. Maximum-likelihood classification of the structurally heterogeneous cryo-EM data set. A preliminary reconstruction of the entire data set of 4097 particles (**a**) was used as initial reference to generate the seeds for a ML3D classification as described in the Methods section. The resulting classes (**b**) were interpreted as CCT:Hsc70$_{NBD}$ complexes (class 1), and uncomplexed CCT particles (classes 2 and 3). The number of particles pertaining to each class are indicated.



Supplementary Fig.4. Multiple sequence alignment of Hsc70/Hsp70/DnaK/Hsp110/SSE1 family of proteins. (**a**) Homologous sequences belonging to the Hsc70/Hsp70/DnaK/Hsp110/SSE1 family of proteins. (**b**) Phylogenetic tree represents the possible evolutive relationships among proteins of the Hsc70/Hsp70/DnaK/Hsp110/SSE1 families, showing the contrast among homogeneity of Hsc70 and Hsp70 families compared to heterogeneity in DnaK sequences.



Supplementary Fig.5. Specific binding of Hsc70 to CCT. (**a**) DnaK, the prokaryotic homologue of Hsc70, was incubated with either CCT or GroEL, run on a native gel and the band corresponding to the chaperonin (either CCT or GroEL) was excised and loaded onto a SDS gel. The gel was subsequently treated with a monoclonal antibody that reacts specifically to DnaK (left lane), which was run as a positive control. The experiment shows that DnaK does not interact with neither CCT nor GroEL. (**b**) The same kind of experiment in which Hsc70ΔC was incubated with GroEL. The solution was run on a native gel and the band corresponding to GroEL was excised and loaded onto a SDS gel. The gel was subsequently treated with a monoclonal antibody that reacts specifically to Hsc70/Hsp70 (central lane). The experiment reveals that Hsc70 does not interact with GroEL.



Supplementary Fig.6. CCT binding assay of Hsc70ΔC. (**a**) One of the CCT-binding assays of Hsc70ΔC and the $Hsc70\Delta C_{DnaK189-192}$ and $Hsc70\Delta C_{DnaK189-195}$ mutants, as described in Fig. 5b. CCT was mixed with identical amounts of the three Hsc70 variants. (**b**) The ATPase activity of several of the mutants used in this work was performed as a test of its functional viability. All the mutants revealed similar activity.