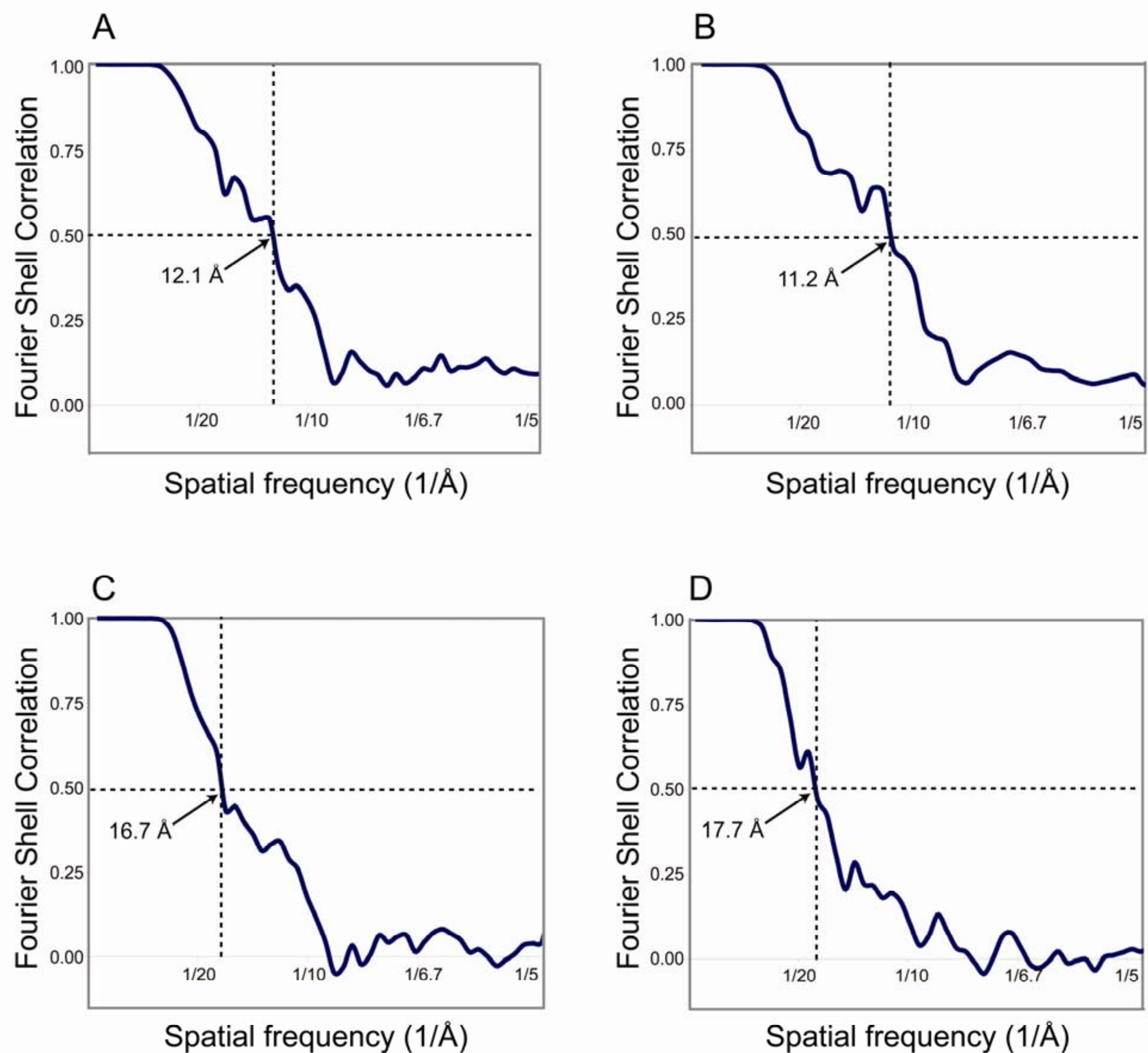
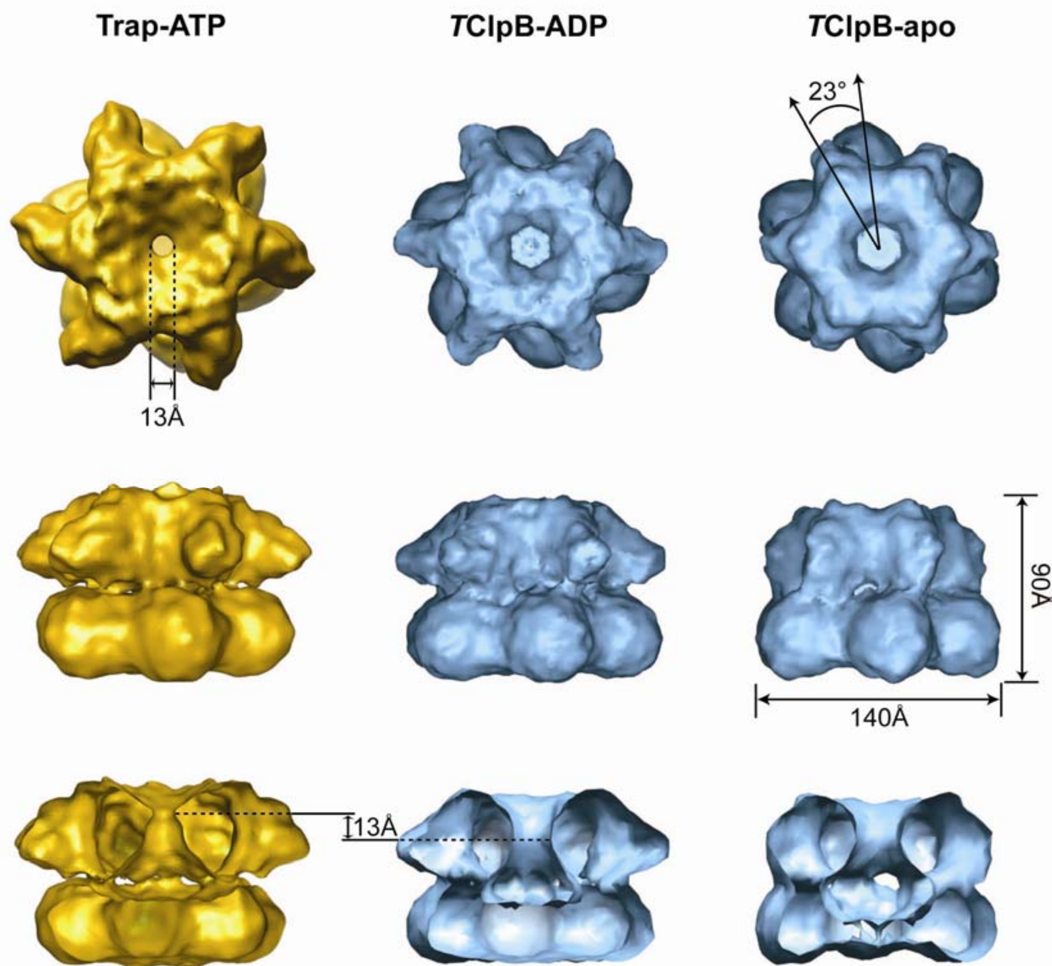


Supplementary Figure 1

Fourier shell correlation between reconstructions from even and odd halves of the data set. A threshold of 0.5 is used to assess the resolution of the final cryo-EM reconstructions: (A) *T*ClpB-AMPPNP, (B) Trap-ATP, (C) *T*ClpB-ADP, (D) and *T*ClpB-apo.



Supplementary Figure 2
Isosurface representation of the *T*ClpB hexamer in different nucleotide states. The figure shows top down, side, and cut-away side views of the Trap-ATP, *T*ClpB-ADP, and *T*ClpB-apo hexamer obtained by cryo-EM and single particle reconstruction techniques. To compare the cryo-EM structure of the Trap-ATP hexamer with those of the ADP and apo states, we low-pass filtered the high-resolution cryo-EM map of the Trap-ATP hexamer (shown in gold) to a resolution comparable to that of the *T*ClpB-ADP hexamer (~17 Å). The cryo-EM maps of the *T*ClpB-ADP and *T*ClpB-apo hexamer are colored cyan and remain unchanged (as depicted in Figure 2). The cryo-EM reconstruction of the Trap-ATP hexamer clearly shows that the pore of the AAA-1 ring is much wider in the ADP and apo states (compare isosurface representations of the different nucleotide states in the top down and cut-away side views). The additional mass at the pore is only seen in the ATP-activated state (represented by the Trap-ATP hexamer) and not in the ADP and apo states. As depicted in Figures 4 and 5A, the additional mass accounts for the D1 loops that are stabilized at the central pore, resulting in a larger, solvent-exposed, upper surface area of the D1 ring, which is critical for high-affinity substrate binding.