

**Supplementary information, Figure S3** Work flow for the processing of the high-resolution proteasome-ADP-AIFx cryo-EM data. Initially, 1,121,573 particles autopicked by RELION 1.3 were classified by 2D and 3D classifications to sort out clean particles, generating a map with an overall resolution of 4.6 Å. Further 3D classification and per-particle motion corrections were performed to improve the resolution to 4.2 Å. More details are available in **Materials and Methods**.