wrinkles of the multiple sheets unanbiguously signal the beginning of the GO substrate.

Supplementary Materials

Methods and Materials

Graphene oxide (GO) grids were made on Quantifoil 200 mesh R 1.2/1.3 holey carbon grids by the method described in the Supplementary Protocol. The GO suspension was purchased from Sigma Aldrich (777676 ALDRICH). We have also successfully produced GO grids using home-made suspensions of GO with larger lateral sheet sizes. For this we used the improved method of synthesizing GO from James Tour's lab, which is described in detail in Marcana et al. (2010). GO grids were used for cryo-EM once they had completely dried, several hours after they were made.

Thermoplasma acidophilum 20S proteasomes were expressed and purified as previously described (Li et al., 2013) and stored in aliquots at -80^{o}C. An aliquot of 20S proteasome was diluted to 0.05 mg/mL in buffer containing 25mM Tris pH=7.5 and 150mM NaCl. 2.5uL of 20S proteasome was applied to a GO grid and allowed to incubate for 30S in the chamber of a Mark III Vitrobot at 100% humidity. The specimen was blotted for 6s with Whatman #1 filter paper using an offset of 0mm and plunged into liquid ethane.

Cryo-EM grids were loaded into a TF30 Polara microscope equipped with a Gatan K2 camera operated in super-resolution mode. Automated data acquisition was performed with SerialEM and 740 micrographs were corrected. Beam-induced motion was corrected in MotionCor2 (Zheng et al., 2017). Per-frame electron dose weighting was performed with MotionCor2 using a nominal electron dose of 1.2 e^{-}/A^{2}*frame and the radiation damage model derived in (Grant and Grigorieff, 2015). Micrographs were visually inspected and segregated into optimal GO coated (73%), overcoated GO (10%), poor ice (10%), or uncoated (7%) images. 540 micrographs with optimal GO coating were selected for continued processing. CTF parameters were estimated with Gctf (Zhang, 2016) and particles were picked with Gautomatch (https://www.mrc-lmb.cam.ac.uk/kzhang/Gautomatch/) using a template of a 20S proteasome side view. Mispicked particles were removed after 2D classification in cryoSPARC (Punjani et al., 2017)and subjected to homogeneous 3D refinement with D7 symmetry. Masking and FSC estimation were performed automatically in cryoSPARC. The resulting map was compared visually to previous 20S proteasomes maps from our lab with UCSF Chimera.