Supplementary Material

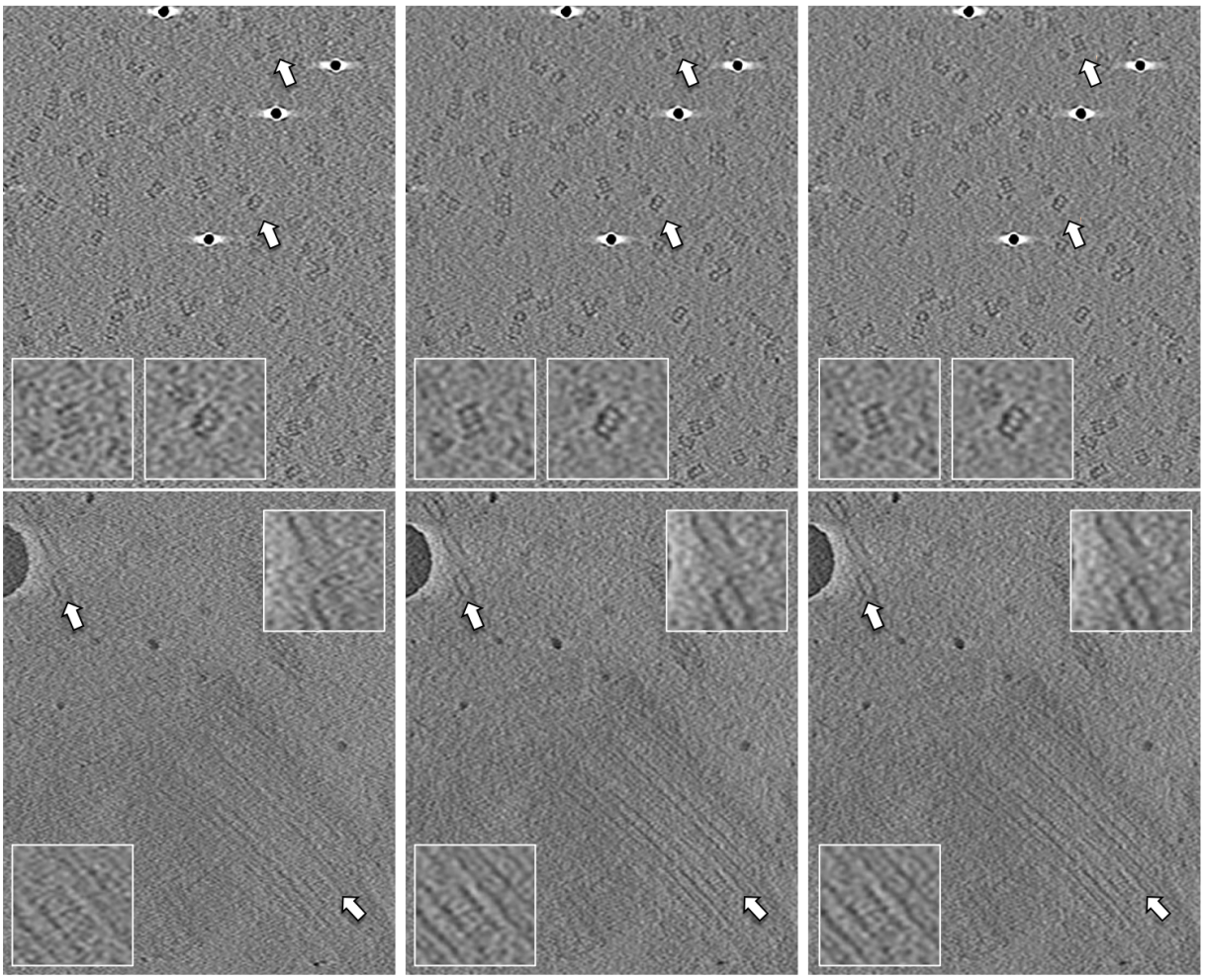

Figure S1: **Effect of motion-compensation in tomograms**. Tomograms obtained from representative proteasome (top) and BB (bottom) tilt-series with the standard alignment (left) and the motion-compensation methods: polynomial approach (centre) and the new one based on residual interpolation (right). Arrows point to some areas (also magnified in the insets) where the improvements of the motion-compensation methods with respect to the standard alignment are noticeable. The proteasomes (top) and microtubules of the BBs (bottom) look sharper, cleaner and better defined than the standard alignment (left panels) after consideration of the motion (centre and right panels). The difference between the two motion-compensation methods (centre and right panels) is negligible, demonstrating that the new method based on the residual interpolation behaves similarly to the polynomial approach.

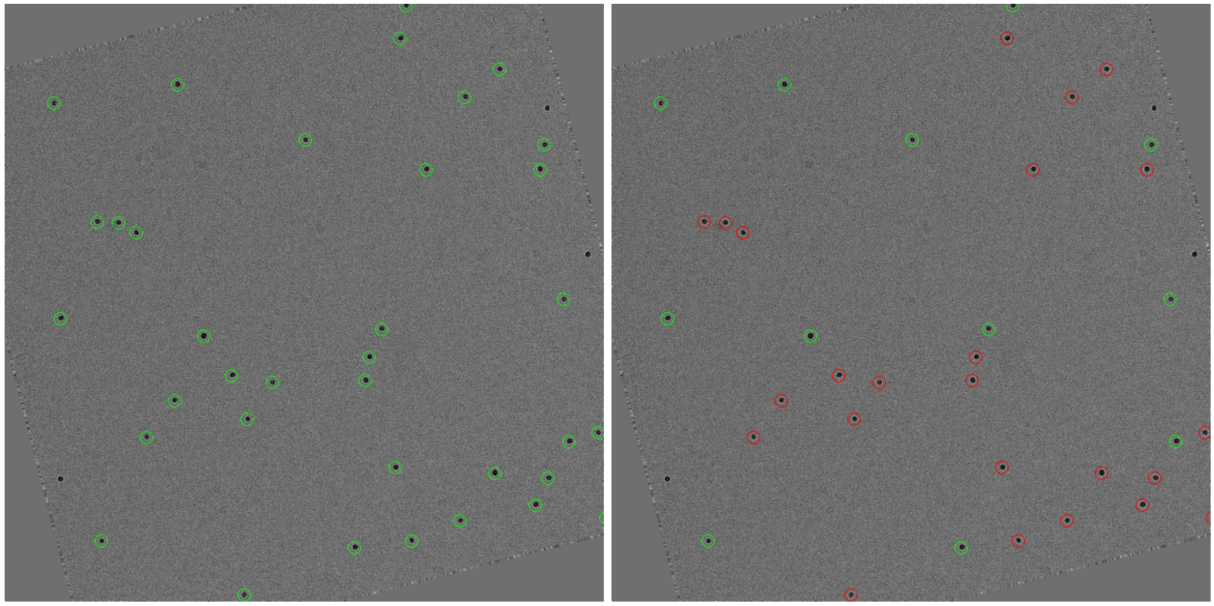

Figure S2: **Selection of fiducials to analyze the performance of the motion-compensation methods**. Left: Full set of fiducials used in the motion-compensation alignment of a tilt-series (green circles). Right: Subsets of 12, 9 and 6 fiducials distributed across the field of view were selected (green circles; only a subset of 12 fiducials is presented here), and the remaining fiducials (red circles) were used for cross-validation assessment.

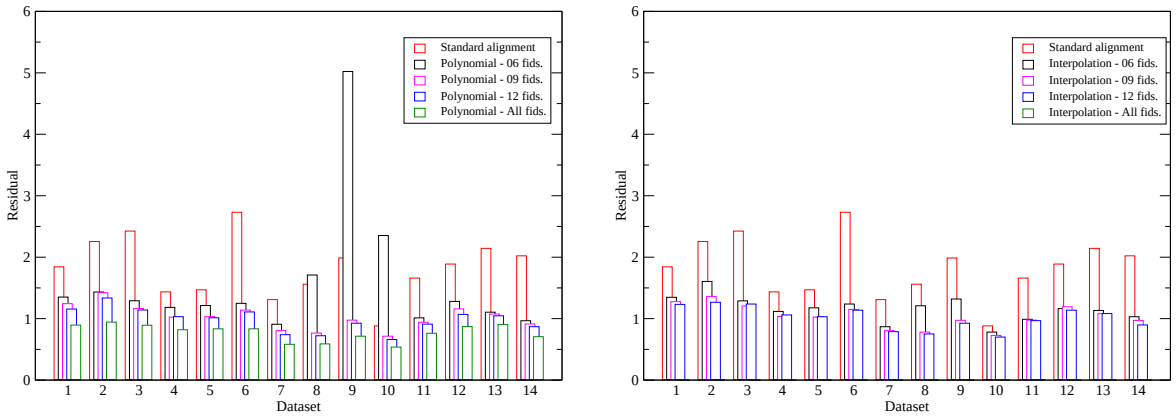

Figure S3: **Cross-validation residual for individual proteasome tilt-series with different fiducial subsets**. Subsets of 6, 9 and 12 fiducials were selected from the whole set to model the sample motion and the remaining fiducials were used for cross-validation assessment based on their mean residual. The results from the polynomial (left) and the TPS interpolation (right) methods are shown. As a reference, the residual obtained from motion modelling (green) and that from the standard alignment (red) using the whole set of fiducials are shown. In general, the more fiducials in the selected subset, the lower the validation residual. With the subset of 6 fiducials, over-fitting problems were found in several tilt-series using the polynomial method, identified as validation residual being higher than that from the standard alignment (tilt-series #8, #9, #10). In the TPS interpolation method, the validation residual always improved with respect to the standard alignment.

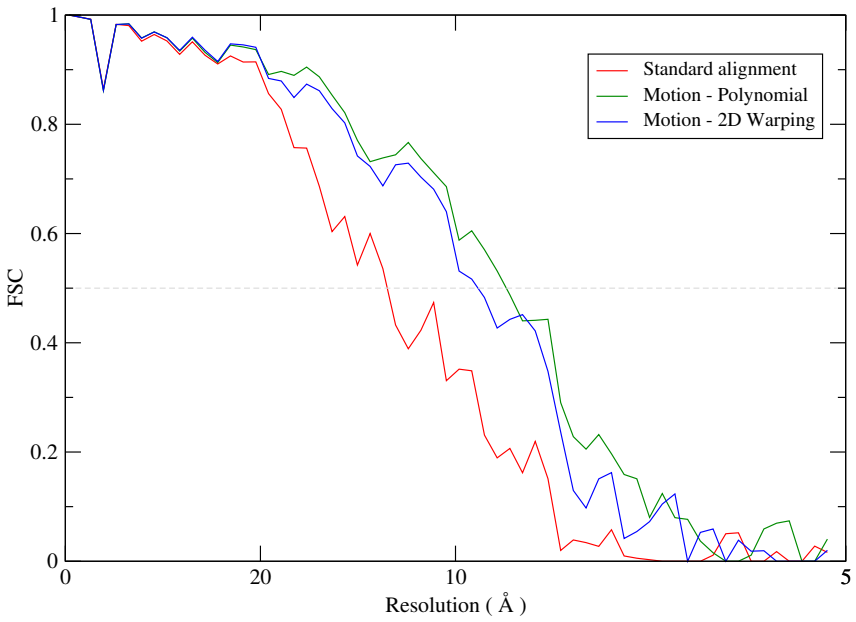

Figure S4: **FSC curves from subtomogram averaging of the proteasome dataset using 2D warping of images of the tilt-series**. Images of the tilt-series were warped to compensate for the sample deformation and produce pseudo-perfectly aligned tilt-series. The residuals at the 2D fiducial positions in the projection images were interpolated (Fig. 2A in the main text) and used to warp the images, similarly to (Nejadasl et al. (2013)). The resulting motion-corrected tilt-series were subjected to the standard tomographic workflow, using tomo3d (Agulleiro and Fernandez (2011)) to compute the tomograms. Subsequent subtomogram averaging followed as described in the main text and in (Fernandez et al. (2018)). The resulting FSC (blue curve) shows that this strategy helps improve the resolution with respect to the standard alignment applied to the original, unwarped tilt-series (red curve). However, the improvement is lower than that obtained with motion-compensation applied at the 3D space of the tomogram (green curve, obtained with the polynomial motion modelling (Fernandez et al. (2018)).

References:

Agulleiro and Fernandez (2011) Bioinformatics 27:582-583.
Fernandez et al. (2018) J. Struct. Biol. 202:200-209.
Nejadasl et al. (2013) J. Synchrotron Radiat. 20:58-66.