Supplementary Figure 3. Supervised classification of the ribosome dataset

Based on the assumption that the heterogeneity in the data entailed a ratchet motion, we used two reference structures from a previous study (Valle et al. 2003, Cell 114, 123-34) with ribosomes before (A) and after (B) ratcheting. To avoid any bias introduced by the presence of the ligands, we removed all tRNA and EF-G density from these maps. A superposition of both maps illustrates the ratcheting movement (C). The two reference maps were projected according to an even angular distribution with a sampling rate of 15 degrees, and a standard projection matching protocol was used to correlate each of the experimental images with the projection libraries of both references. A histogram of the resulting cross-correlation differences (CC2-CC1: cross-correlation with a reference after ratcheting minus cross-correlation with a reference before ratcheting) appeared unimodal, without an indication for clustering. We then divided the data into five classes arbitrarily (1-5), but ensuring roughly equal numbers of particles in each class (D).