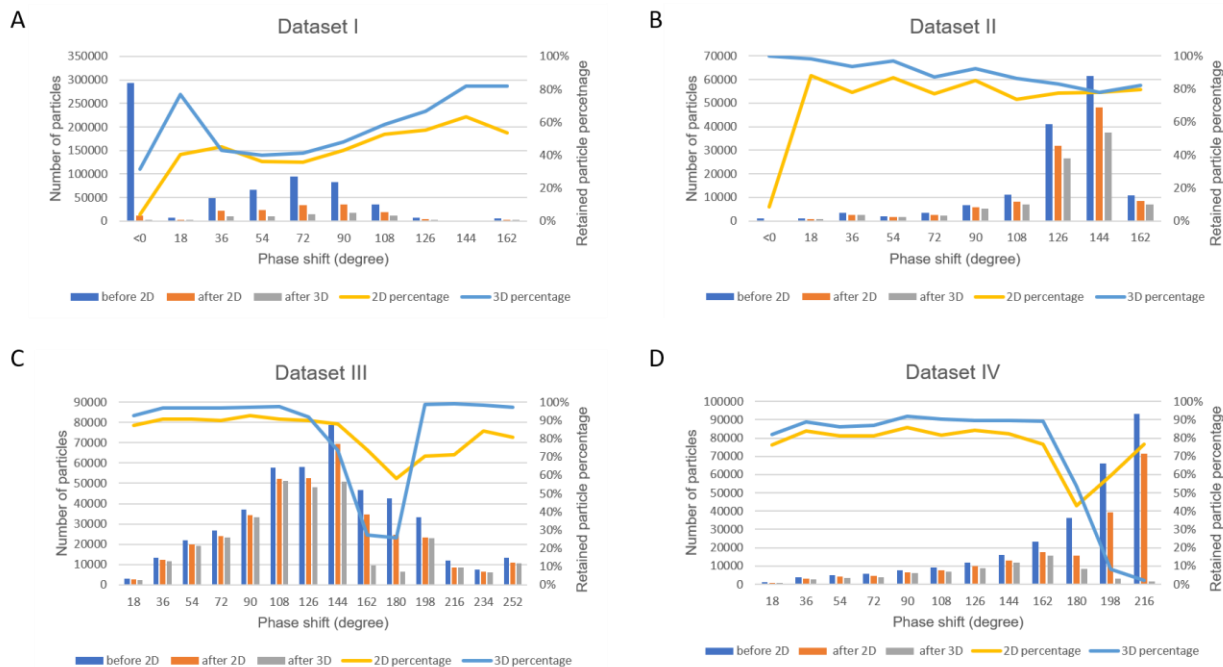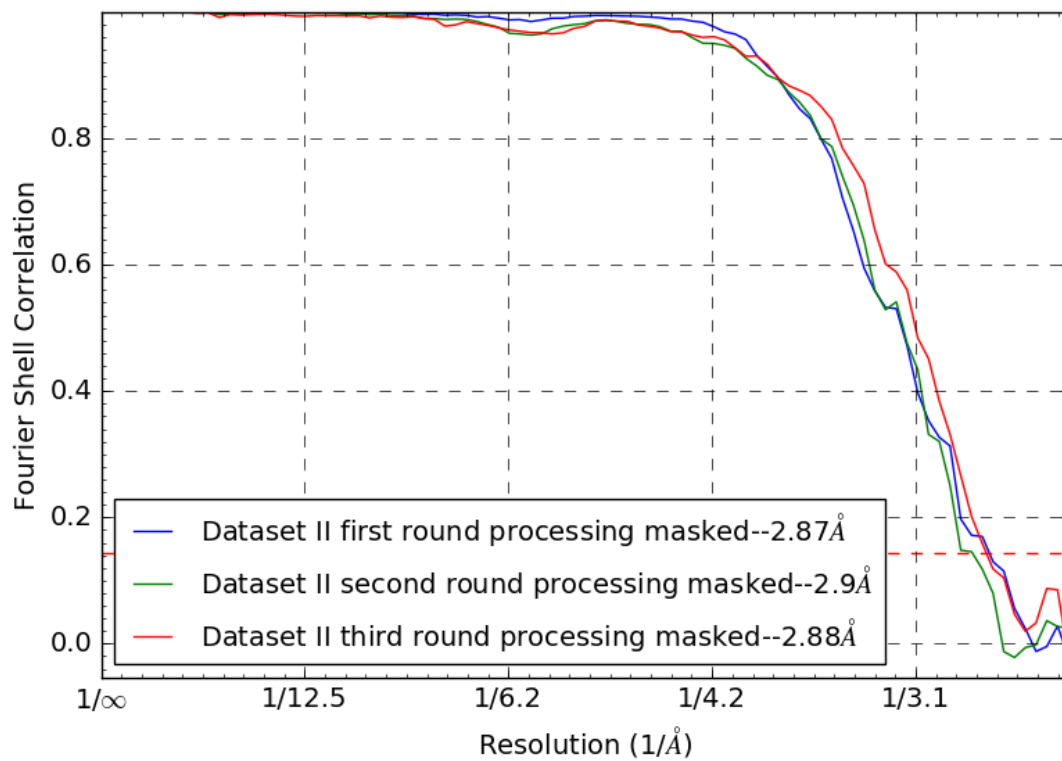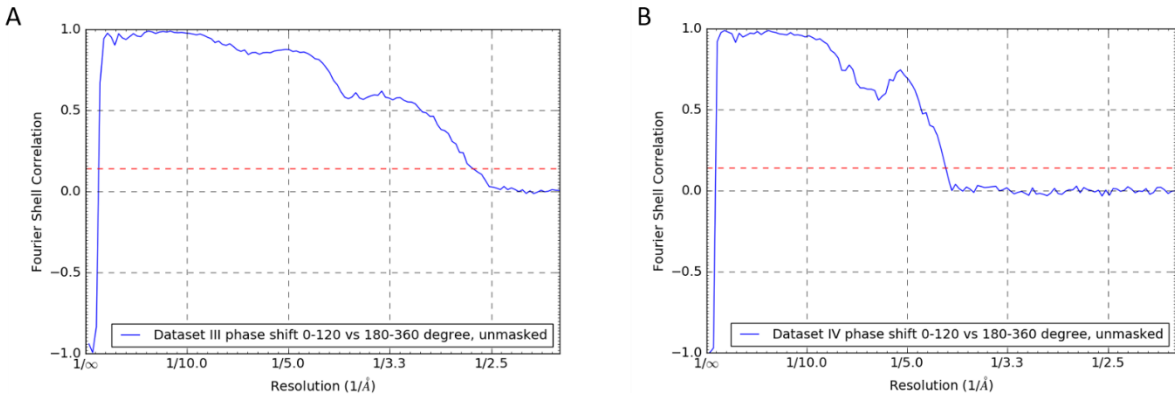**Figure S1.** The *CTFFIND4* estimated phase shift and maximum resolution of each micrograph of the four datasets.

Figure S2. The 2D and 3D classification retained particle percentage of the four datasets. The number of particles in different phase shift bins before 2D classification, after 2D classification, and after 3D classification are represented by the blue, orange and grey bars respectively. The retained particle percentages after 2D classification and 3D classification are shown as the yellow and blue lines respectively. The 2D and 3D classification plot for dataset II used the particle numbers of the third round of processing. The 2D percentage was calculated as the ratio of the number of particles retained after 2D classification and the total number of particles. The 3D percentage was calculated as the ratio of the number of particles retained after 3D classification and the total number of particles.

Figure S3. The Fourier shell correlation (FSC) curves between the JSPR refined half maps of the first, second and third round reconstructions of dataset II.

Figure S4. The FSC between the full unmasked maps reconstructed from 0-120 degree phase shift particles and 180-360 degree phase shift particles in dataset III (**A**) and dataset IV (**B**) showing the negative FSC at low resolutions.