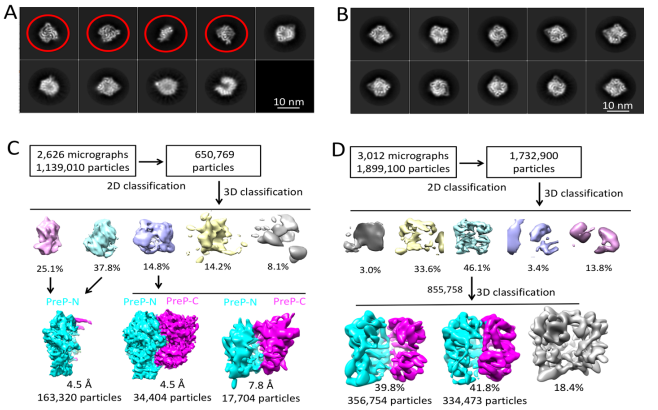
When grids are prepared via Vitrobot, only 1/3 of the PreP particles are intact and the rest is only half of the size of PreP (Figure 1A). 3D classification revealed that the half particles are derived from particles with an intact PreP N domain and a denatured PreP C domain (Figure 1C). However, 2D classification from the particles collected using grid prepared by chameleon completely eliminated the half particles (Figure 1B,D). Currently, the precise reasoning for why the differences in grid preparation by vitrobot vs chameleon affect the denaturation of PreP-C domain remains elusive and we will work with Bridget Carragher and Clint Potter to use cryoET to investigate the localization of denatured PreP particles to further study the mechanism of denaturation.

Figure 1 Comparison of 2D and 3D classification of PreP particles from grids prepared by vitrobot (A,C) or chameleon (B,D).



The 3D classification from this data reveals maps for PreP open and partial open states that have the 4.6 and 4.2 angstrom resolution, respectively (Figure 1D, 2A). These two states are distinct from each other and different from the previously reported closed structure of PreP solved by X-ray crystallography (Figure 2B). We have performed 3D classification of both states. While no new meaningful states can be obtained from PreP open state, the 3D classification of PreP partial open state reveal three distinct states that vary mostly at the catalytic and linker region between PreP-N and PreP-C. Such motions within the catalytic domain of PreP can be interpreted as the key conformational switch necessary for PreP to unfold and select unstable peptides in mitochondria, such as presequences. In this proposal, we will address substrate bound state PreP structure to address the structural basis for the interaction of PreP with its substrates.

Figure 2 The analysis of PreP open and partial open state structures.