1. There are substantial particles that are the half size of the rest of particles. 3D reconstruction of these half size particles at 4.6 angstrom resolution reveal that they are from human presequence protease that has the partially denatured C-terminal half domain (Figure 1 A top, the partially denatured PreP particles are highlighted in red and structure model on the right). However, 2D classification from the particles collected using grid prepared by spotiton eliminated the half particles. This is consistent with the notion that the rapid freezing can outrun the denaturation (Figure 2 bottom).

2. From our recent data collected at NCCAT, we have done substantial 2D and 3D classifications and reconstruction (Figure 1B) to obtained a 6.4 angstrom Coulomb potential density map and open state model and two distinct partial closed state map (4.8 and 7.1 angstrom) that are distinct from the previous solved human PreP closed state structure.

3. We have built the open and partial closed state models (Figure 1C). The goal of this proposal is to collect micrographs from spotiton prepared grids to push the resolution of these three states to beyond 4 angstrom resolution.

Figure 1 Key progress of cryoEM structures of human PreP open and partial closed states. (A) Comparison of 2D classification from micrographs prepared using vitrobot (top) and spotiton. The half PreP from 2D classification is highlighted in red. Right, structure model of PreP-N only structure. (B) Flow chart of 3D classification of PreP. (C) Models of PreP open states and PreP partial closed state prior to further 3D classification to show the fit of map with structure model.