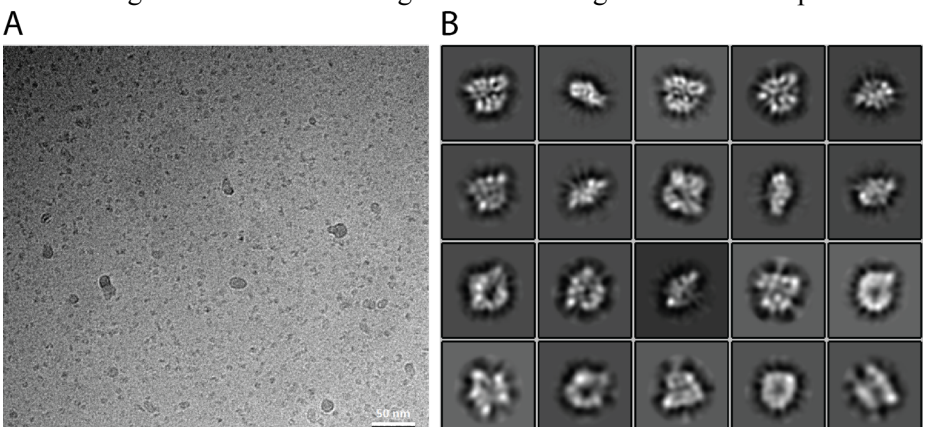
Progress in single particle analysis of human PreP

1. CryoEM analysis of human PreP alone

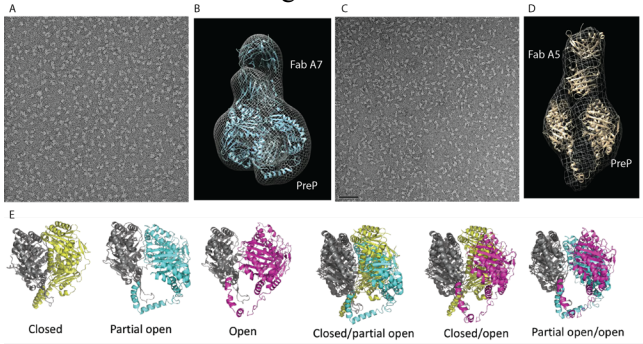
Figure 1 CryoEM analysis of PreP alone structure. (A) Screenshot of micrograph collected using FEI Talos 200 kV at 120,000 magnification. (B) 2D classification of PreP alone. 57K particles are used and resolution limit is set at 7 Angstrom under the setting that CTF was ignored until first peak.



Recombinant human PreP was expressed in *E. coli* and purified using Ni-NTA, Source Q anion exchange, and S200 columns according to King et al Structure 22:996-10017, 2014. The purified human PreP alone behaves as a monomer based on size exclusion column, consistent with the SAXS analysis. Human PreP has 16 cysteine residues, which contributes to its high sensitivity to oxidative inactivation. PreP is a Zinc metalloprotease and the presence of EDTA can strip zinc ion, rendering it catalytic inactive. We thus vary the reducing agents (beta-mercaptoethanol, dithiothreitol DTT), or Tris(2-carboxyethyl)phosphine) (TCEP) and metal chelator (EDTA) in addition to the conventional conditions, i.e., NaCl (50-300 mM), buffers (Tris, Hepes), the type of grid (Au, Cu), and blotting time controlled by vitribot for the grid preparation. We found that the quality of PreP particles in the cryoEM micrograph is highly sensitive to the variations in NaCl concentration, the specific reducing agent, and EDTA. By optimizing these variables, we have found conditions that are optimal to image the monomeric PreP particles in the PreP alone condition (Figure 1A). We have collected ~800 micrographs under one of such condition and performed 2D classification using Relion (Figure 1B). Using Relion 3.0 and initial model generated by stochastic gradient descent, at least two distinct classes of maps were found. The initial modeling reveals that such maps can be interpreted as two distinct open state PreP. However, the resolution is not sufficient for reliable model building and refinement.

2. Negative stain analysis of Fab-bound PreP.

Figure 2 Negative stain analysis of Fab-bound PreP. (A) Presentative micrograph of Fab-A7 bound PreP. (B) Coulomb potential map of Fab-A7 bound PreP with a partial open-state model. (C) Presentative micrograph of Fab-A5-bound PreP. (D) Coulomb potential map of Fab-A5 bound PreP with an open-state model. (E) Comparison of PreP closed state solved by crystallography with the putative partial open and open models derived from negative stain EM studies.



Working with Bridget Carragher and Clint Potter at New York Structural Biology Center, we have shown that Fab-assisted cryoEM analysis is an effective means to elucidate the structure of human IDE, a metalloprotease that is distally related to PreP (Zhang et al eLife 7:e33572, 2018). Fab can increase the size of PreP by approximately 50%, break the pseudo-symmetry existed in PreP, and serve as the fiducial marker. Working with Tony Kossiakoff, we have screened and identified 8 distinct synthetic Fab that can be expressed and purified in *E. coli*. We have collected negative stain micrographs of two Fab-bound PreP complex using FEI Tecnai F30 200 kV and constructed the initial PreP partial open and open state models based on the Coulomb potential maps (Figure 2). The effort to identify and optimize the grip conditions for cryoEM analysis is underway.