Figures/preliminary results

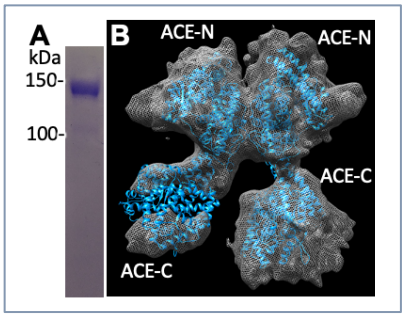

Figure 1 Initial cryoEM analysis of ACE. A. SDS page of purified ACE. B. 3D classification of ACE. Extra-densities are mostly from N-linked glycosylations. It is worth noting that ACE-C domains have substantially poor density in comparison to ACE-N, suggesting the denaturation or high structural heterogeneity.

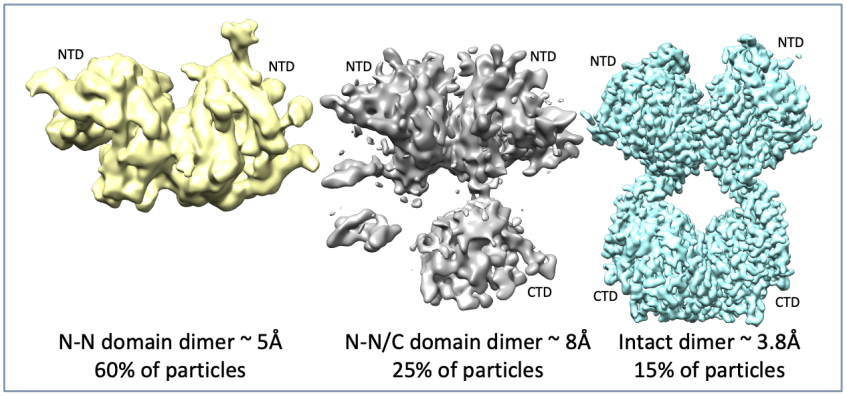

Figure 2 3D classification from the optimized buffer condition that better maintains ACE stability. Of three classes, the most common structure (60% particle) is consistent with that contains only two ACE-N domains. The second (25%) is that contains both ACE-N domains and only one ACE-C. The least one is the dimeric ACE that contains all four ACE catalytic domains. Our data suggests the preferential denaturation of ACE-C domains. We propose to combine the combination of the optimization of buffer conditions and grid making methodologies to reduce the denaturation of ACE-C.

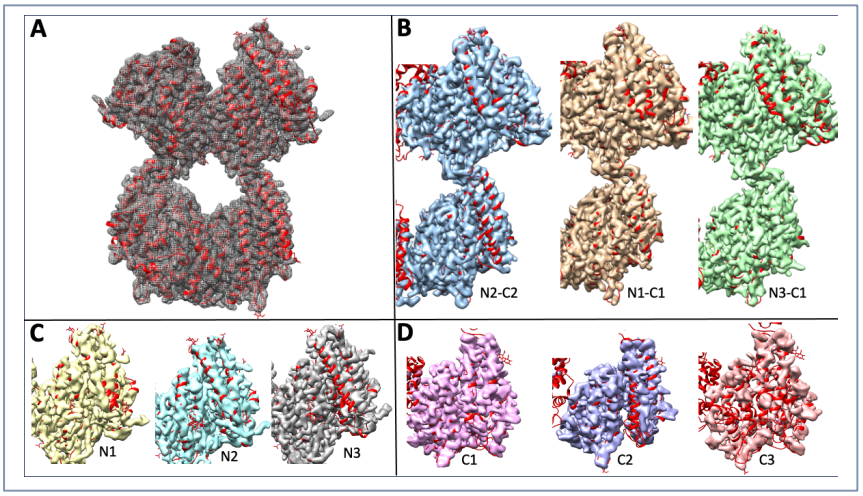

Figure 3 Structural heterogeneity analysis of ACE. A. Overall structure of ACE dimer with Coulomb potential map and structural model in ribbon representation. **B.** Three classes from the focused refinement that represent ACE monomer. **C.** Three classes from the focused refinement that represent ACE -N. **B.** Three classes from the focused refinement that represent ACE-C. Together, this shows the inter-domain variability between ACE-N and ACE-C within each ACE monomer and the structural heterogeneity of individual ACE catalytic domains.