Score and comments

Project Name: CryoEM analysis of dimeric human insulin degrading enzyme dimer in complex with an insulin
Project ID: NCCAT-GUP1-WT190701
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Institution: The University of Chicago
Submission Date: 07/01/2019
Proposal Review
GUP Score: 1.7000000000000002
URC Comments:

Reviewer 1: ['The applicant has generated several cryo-EM structures of IDE. The applicant has experience with refining the biochemical conditions needed for the preparation of grids. The applicant has also determined that Spotiton (chameleon) is an essential tool in the pipeline for good cryo-EM sample preparation. In this proposal, the applicant requests time for data collection (2-days on the Krios). This is well reasoned. The applicant also expressed that the knowledge gained during the process will be returned to the home institute in order to support the on-going development of the cryo-EM community and resource.']

Reviewer 2: ['It is unclear how much conformational variability to expect and if all these states will be computationally resolvable.']

Figure below shows the progress of CryoEM analysis of chameleon-prepared grids that contain 2:1 molar ratio IDE to insulin. Different from previous cryoEM analysis when insulin was in 5-fold molar excess of IDE where all IDE molecule within IDE dimer is in the closed state, our preliminary 2D classification reveals that IDE dimer under this condition exhibits the substantial conformational heterogeneity where IDE dimer with one in open state and the other in the closed state is the dominant state. Typical micrographs. Left is from grid that was plunged at 133 millisecond and right was at 74 millisecond. Data was collected using F20 and Teitz 4K camera. B. preliminary 2D classification.