

Cryo TEM-based 3D reconstruction of the recombinant expressed human zinc peptidase Meprin β

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Meprins are astacin-type zinc endopeptidases, which have been observed so far exclusively in vertebrates. Based on the structure of their catalytic domains these enzymes are distantly related to matrix metalloproteinases (MMPs)[1]. Typically, meprins are expressed in brush border membranes of intestine and kidney tubules, intestinal leukocytes, and certain cancer cells. This suggests a role in epithelial differentiation and cell migration. For human meprin two subforms are described: Meprin α and meprin β [2].

Although Meprin α and β have an amino acid sequence identity of 44%, they show marked differences in activation, substrate specificity; most drastical deviations are seen in their quaternary structures. From negatively stained electron micrographs it is known that meprin α forms ring- and chain shaped oligomers, up to mega Dalton size. This characterizes meprin α as the largest known secreted protease[3].

In this study a single particle approach was made to elucidate the 3D structure of Meprins. Therefore 7500 particles from Meprin β dimers were carefully selected from Transmission Electron Microscopy (TEM) micrographs and computationally processed. This resulted in a 3D density map with a nominal resolution of 12 Å according to the 0.5 criterion. The resolution was good enough to dock the different domains appearing in Meprin β , preliminarily modelled according to related structures obtained from x-ray crystallographic approaches. The docking did not only match with the shape and size of the obtained density map, but met the requirements set by mutation experiments and a continuous polypeptide chain, too.

Homology modelling of the Meprin α domains and docking according to the orientation chosen for Meprin β , revealed a hydrophobic interface located at both ends of Meprin α , probably promoting the oligomerisation to large structures. Moreover the comparison of Meprin α and Meprin β revealed a difference in the disulfide bonding pattern, too

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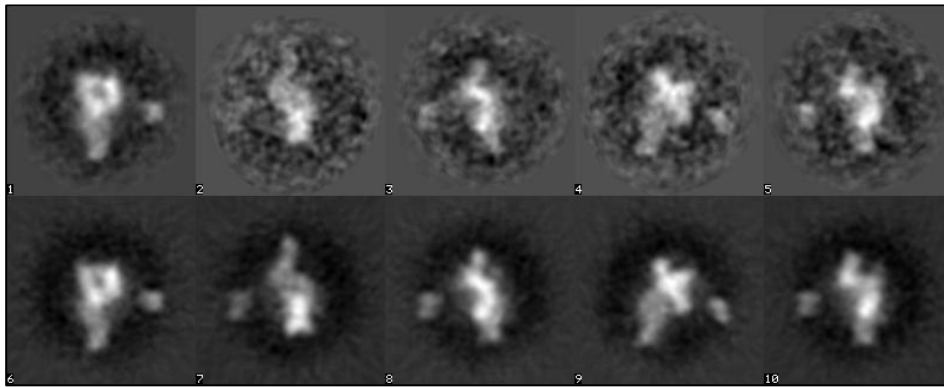


Figure 1: Classsums of Meprin β (upper row) with corresponding reprojections

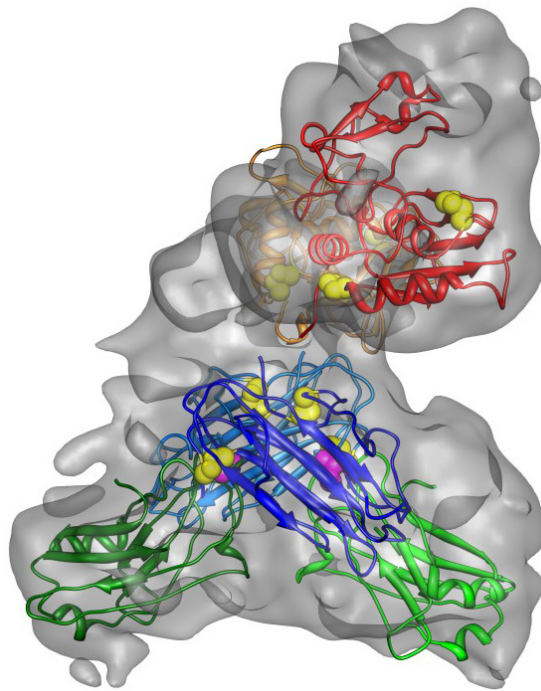


Figure 2: 3D density map of Meprin β with the corresponding domains docked. Intra domain disulfide bonds are in yellow and inter subunit disulfide bonds are in purple.