Molecular modeling of glycosaminoglycan-binding regions on the surface of procatepsin K key to its allosteric regulation.

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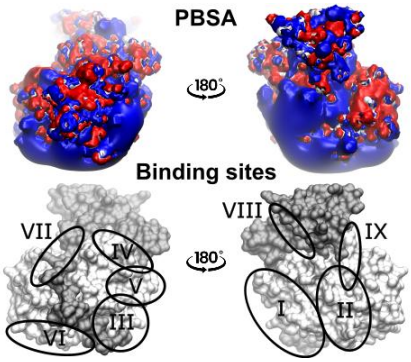
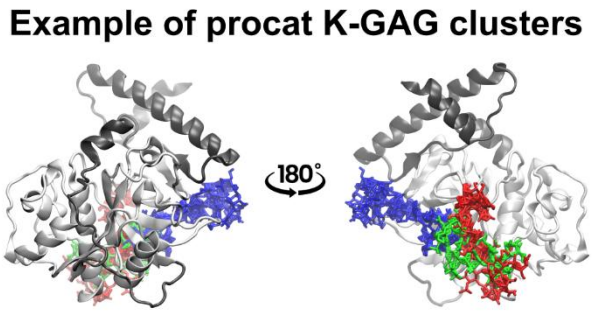
Procathepsin K is the precursor of cathepsin K, a key proteolytic enzyme involved in bone resorption and extracellular matrix remodeling. The allosteric regulation of cathepsin activity by glycosaminoglycans (GAGs) has recently gained scientific interest, yet its exact mechanisms remain unclear. Overexpression or overactivity of cathepsin K is linked to serious health conditions, including osteoporosis, pycnodysostosis, and various tumors, primarily due to its role in bone degradation. The maturation of inactive cathepsin forms is crucial for their activation at the right time and place. GAG binding can induce conformational changes that influence propeptide flexibility, either accelerating maturation or stabilizing structures where the proregion blocks the active site. However, little is known about the allosteric regulation of procathepsin K by GAGs.





This study aimed to screen a set of ligands, identify the most promising candidates, and determine their binding sites on procathepsin K. Molecular modeling techniques, including PBSA (Poisson-Boltzmann Surface Area) electrostatic potential analysis, molecular docking, molecular dynamics (MD) simulations and MM-GBSA (Molecular Mechanics-Generalized Born Surface Area) binding free energy calculations, were employed using AMBER21 package [1]. The set of potential glycosaminoglycan-binding sites was narrowed down to a few of the most promising ones with potential significance for the allosteric regulation of procathepsin K activity. Notably, these sites overlapped with those previously identified for the mature enzyme through both experimental [2, 3] and computational studies [4-7]. The charge and length of the GAG chains were found to influence binding. Chains with lower charge formed complexes with the lowest binding free energy values (the highest stability). In addition, shorter chains were preferred over longer ones at binding sites where the positive electrostatic area was narrower or unfavorably located for longer chains. The obtained results provide a solid foundation for further studies on the allosteric regulation of procathepsin K enzymatic activity by glycosaminoglycans and will serve as a basis for the next stages, where more advanced methods allowing for better sampling of conformational properties will be utilized.

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