Supporting Information

The Role of Glycosaminoglycans in Procathepsin B Maturation – Molecular Mechanism Elucidated by a Computational Study

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Table S1. Free energies and corresponding probabilities of procathepsin B clusters at 275.0 K obtained from UNRES MREMD simulations.

cluster	ΔG [kcal/mol]	probability, %
1	-9.5	26.96
2	-9.5	25.69
3	-9.3	19.64
4	-9.2	15.95
5	-9.1	11.76

Table S2. MM-GBSA free energy of binding (mean values and standard deviations) in the UNRES model of procatB-HP dp8 and dp12 complexes. The square colours correspond to complex structures and RMSD values shown in Figure 8.

Simulation	ΔG [kcal/mol]
procatB-HP dp8	
■ run 1	9.0 ± 10.0
■ run 2	19.3 ± 14.0
■ run 3	21.7 ± 8.7
■ run 4	-27.2 ± 6.6
■ run 5	-8.6 ± 7.3
■ run 6	-7.0 ± 7.3
■ run 7	7.3 ± 6.8
■ run 8	-2.7 ± 10.4
$average_{HP\ dp8}$	1.5
procatB-HP dp12	
■ run 1	4.1 ± 13.4
■ run 2	-11.6 ± 9.7
■ run 3	-16.9 ± 11.1
■ run 4	-6.5 ± 8.8
■ run 5	27.6 ± 8.7
■ run 6	19.5 ± 7.9
■ run 7	-17.4 ± 10.0

■ run 8	11.7 ± 8.9
$average_{HP\ dp12}$	1.3

Table S3. MM-GBSA free energy of binding (mean values and standard deviations) in the UNRES model of procatB-HP dp8 and dp12 complexes for repeated simulations (number in bracket) in comparison to the original simulation. The square colours correspond to RMSD values shown in Suppoerting Information, Figure S4.

Simulation	ΔG [kcal/mol]
procatB-HP dp8	
■ run 4(1)	22.3 ± 11.5
■ run 4(2)	-17.6 ± 11.5
■ run 4(3)	10.5 ± 12.4
■ run 4(4)	18.5 ± 10.2
■ run 4	-27.2 ± 6.6
procatB-HP dp12	
■ run 7(1)	-0.3 ± 19.4
■ run 7(2)	-20.5 ± 10.5
■ run 7(3)	19.7 ± 13.3
■ run 7(4)	21.5 ± 15.8
■ run 7	-17.4 ± 10.0

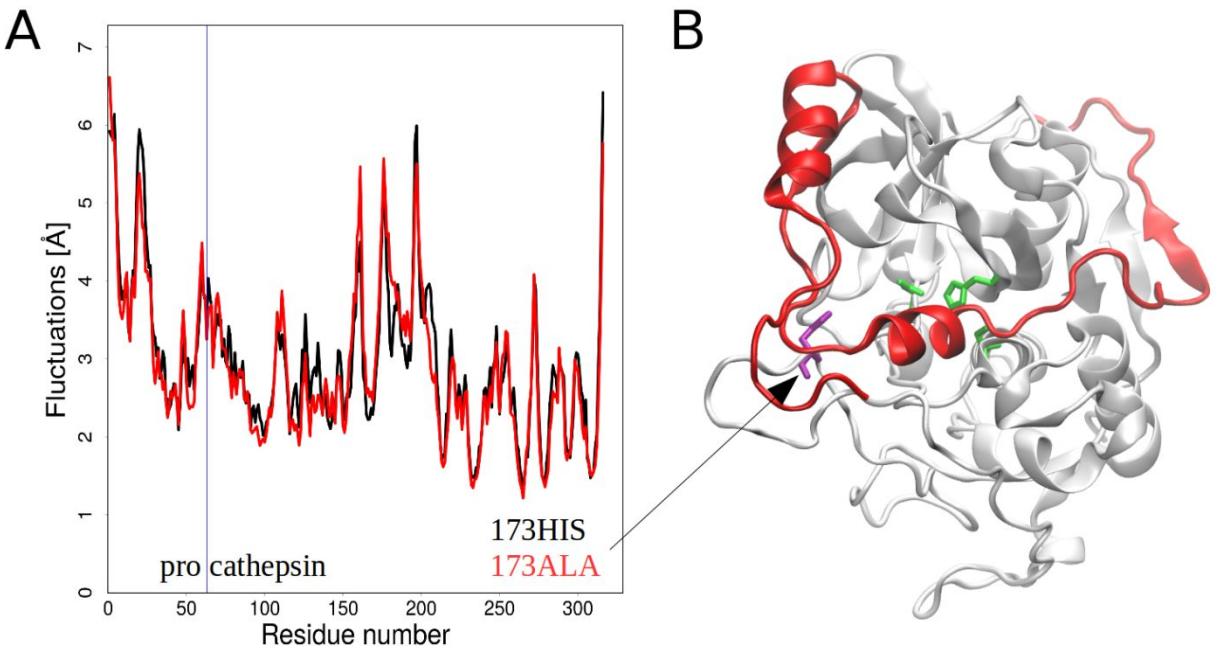

Figure S1. Residue fluctuations of procatB and its HIS173ALA mutant (A) and location of this residue in procatB structure, shown in purple sticks (B). The propeptide and cathepsin are in red and white cartoon, respectively. The active site residues CYS92, HIS262, ASN282 are in green sticks.

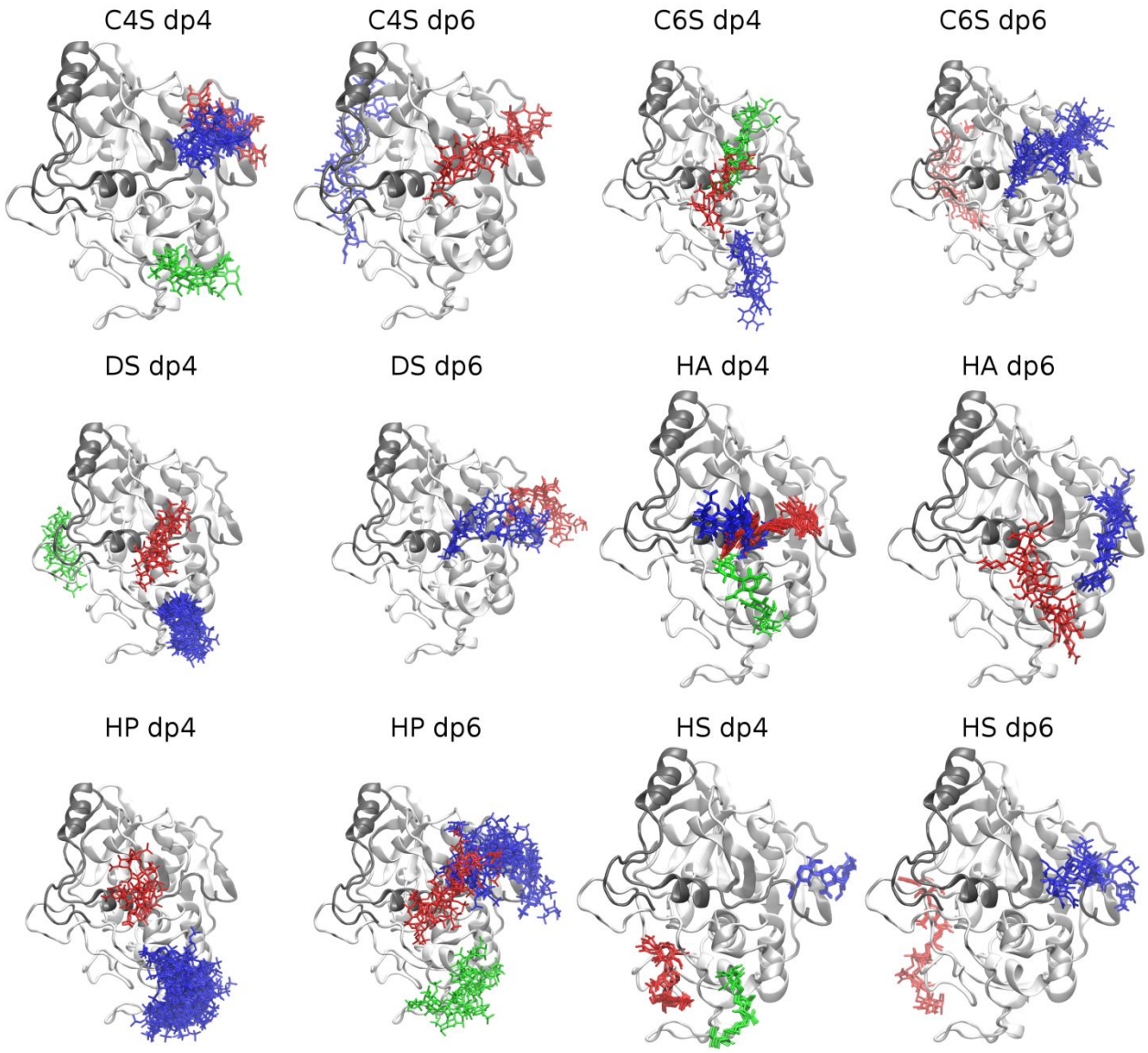

Figure S2. The procatB-GAG dp4 and dp6 structures obtained from molecular docking. The propeptide part and the cathepsin part of the procathepsin are in grey and white cartoon, respectively. The docking solutions are in blue, red and green sticks, respectively. The colours of sticks stand for the size of clusters with blue, red and green being the first, second and third most populated cluster, respectively.

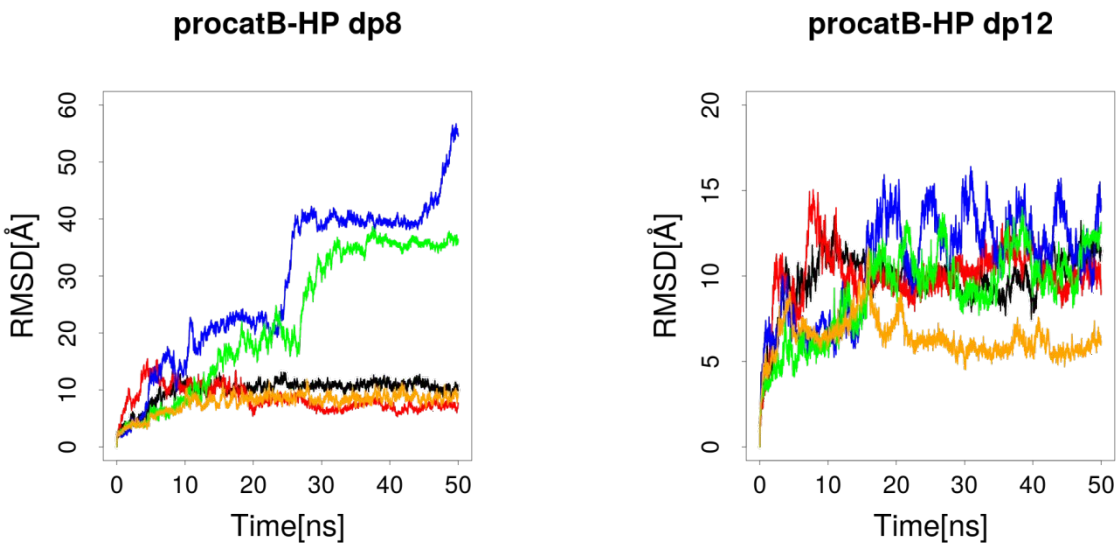

Figure S3. RMSD results of complexes consisting of UNRES model of procatB with the active site accessible and HP dp8/dp12. The colours correspond to MM-GBSA values shown in Supporting Information, Table S3.

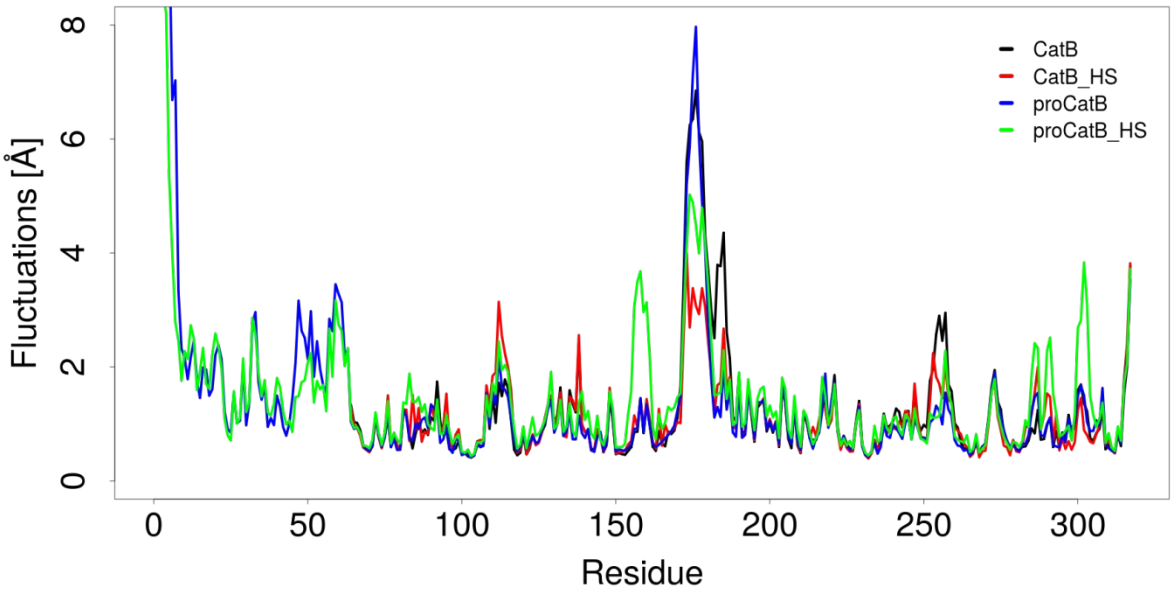

Figure S4. The residue fluctuations of unbound (pro)catB and in HS dp4 complexes from 1μs MD simulation.