Structure, Volume *20*

Supplemental Information

An Internal Water-Retention Site in the Rhomboid Intramembrane Protease GlpG Ensures Catalytic Efficiency

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Inventory of Supplemental Information

A. SUPPLEMENTAL DATA: FIGURE LEGENDS

Movie 1: Molecular dynamics simulation of GlpG in a POPE bilayer. The catalytic serine and histidine are shown in space-fill.

Movie 2: Water dynamics during a MD simulation, starting from waters observed in the crystal structure. Note that the majority of crystallographic waters rapidly escaped, with no bulk waters entering to take their place. In contrast, several water molecules were retained near the catalytic center in a localized site (the 'water retention' site). The catalytic serine and histidine are shown in stick format. GlpG is simulated in a POPE bilayer, but phospholipids were removed from the movie for the sake of clarity.

Movie 3: Water dynamics in the active site of GlpG during a MD simulation. Note that the waters are dynamic, but constantly interacting with GlpG (side chains are shown in stick format). Only one water molecule (in blue and white) entered from bulk and only in this one of the four simulations. This water molecule remained at the active site due to its interactions with GlpG residues of the 'water retention' site.

Figure S1: C_α RMSD value versus time for the loops in closed state of simulations GlpG1 (A), GlpG2 (B), open state of simulations GlpG3 (C) and GlpG4 (D).

Figure S2: C_α RMSD value versus time for the helices in closed state of simulations GlpG1 fitted to TM1-TM4+TM6 of open state (A), GlpG2 (B), open state of simulations GlpG3 fitted to closed state (C) and GlpG4 (D).

Figure S3: C_α RMSD value versus time for the loops in closed state of simulations GlpG1 fitted to TM1-TM4+TM6 of open state (A), GlpG2 (B), open state of simulations GlpG3 fitted to closed

state (C) and GlpG4 (D).

Figure S4: MD trajectory of the GlpG center of mass along the Z axis relative to the center of mass of the lipid bilayer. Closed state of simulations GlpG1 (A), GlpG2 (B), open state of simulations GlpG3 (C) and GlpG4 (D).

Figure S5: A mean water density of the triple water cluster and interaction of surrounding residues. The water positions in the x-ray structural models are in black spheres. Closed state of simulation GlpG2 (left) and open state of simulation GlpG4 (right).

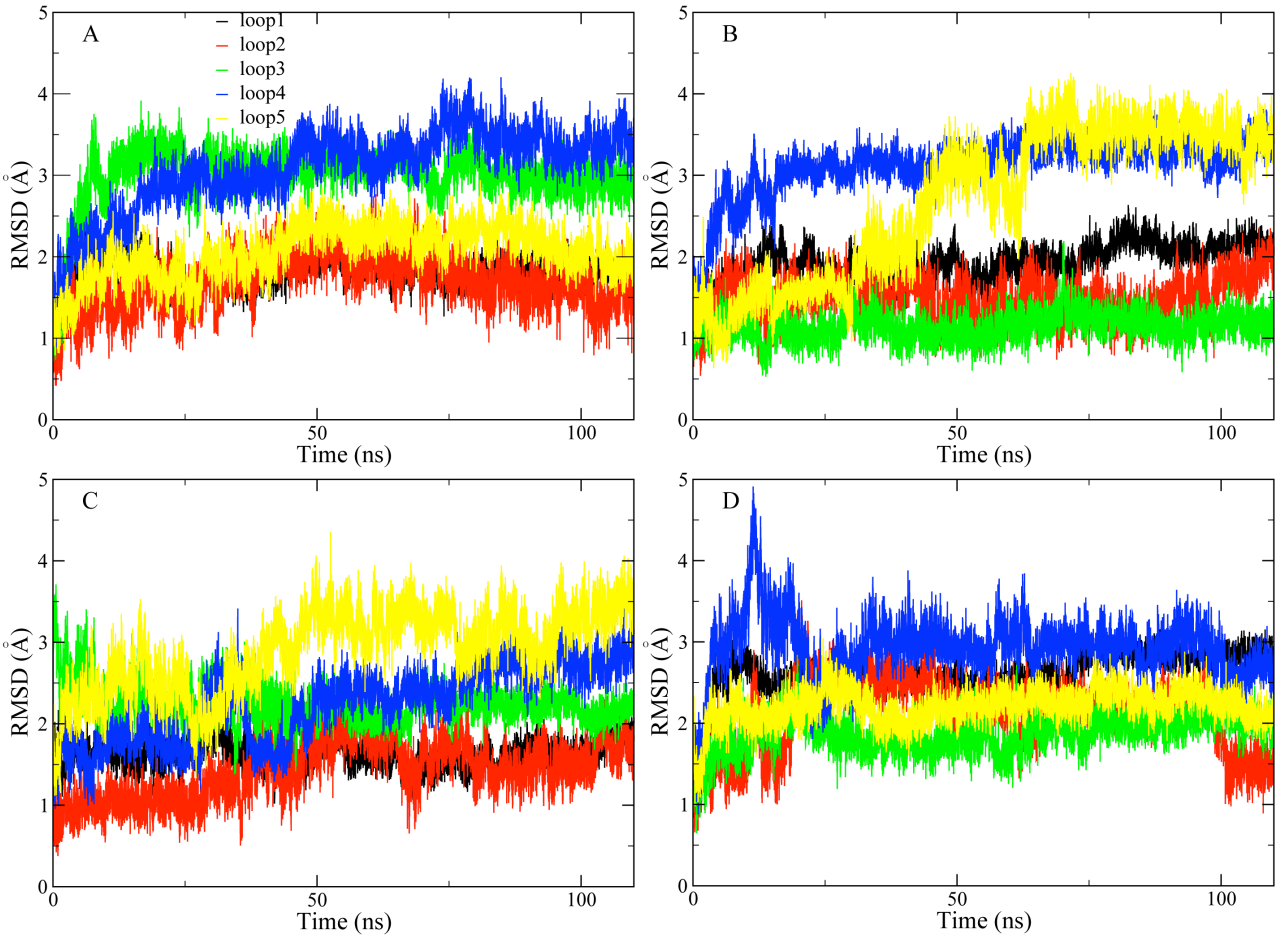
A
B
C
D
loop1
loop2
loop3
loop4
loop5
RMSD (Å)
Time (ns)
0
50
100
1
2
3
4
5

Figure S1.

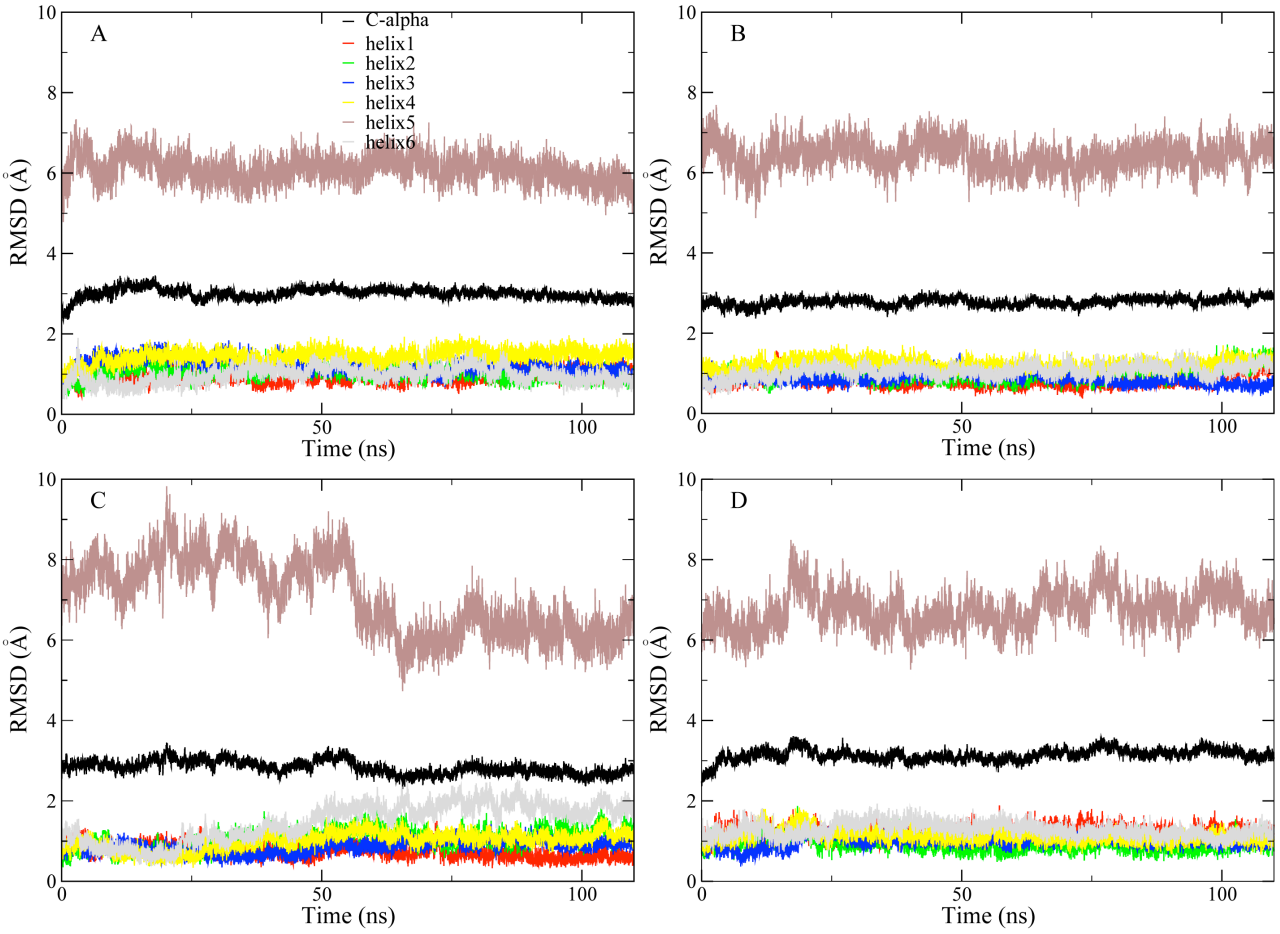
A
B
C
D
C-alpha
helix1
helix2
helix3
helix4
helix5
helix6
RMSD (Å)
Time (ns)
0
2
4
6
8
10
0
50
100

Figure S2.

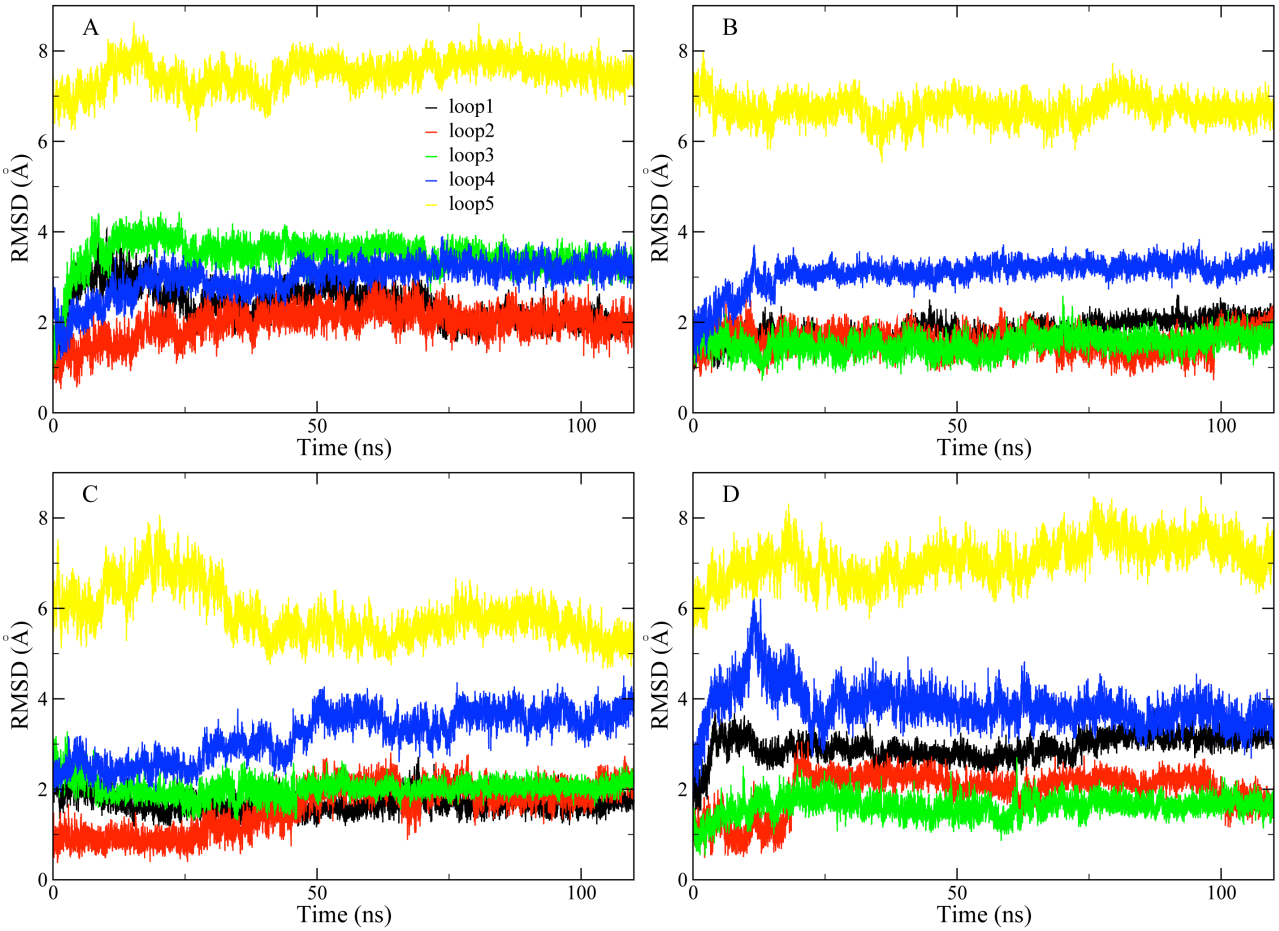
A
B
C
D
loop1
loop2
loop3
loop4
loop5
RMSD (Å)
Time (ns)
0
2
4
6
8
0
50
100

Figure S3.

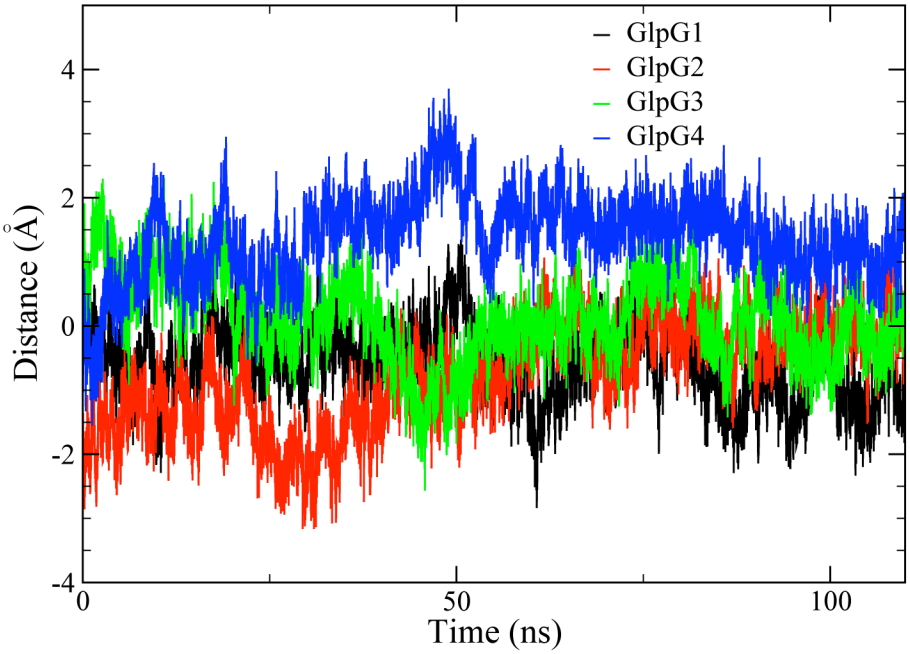

Figure S4.

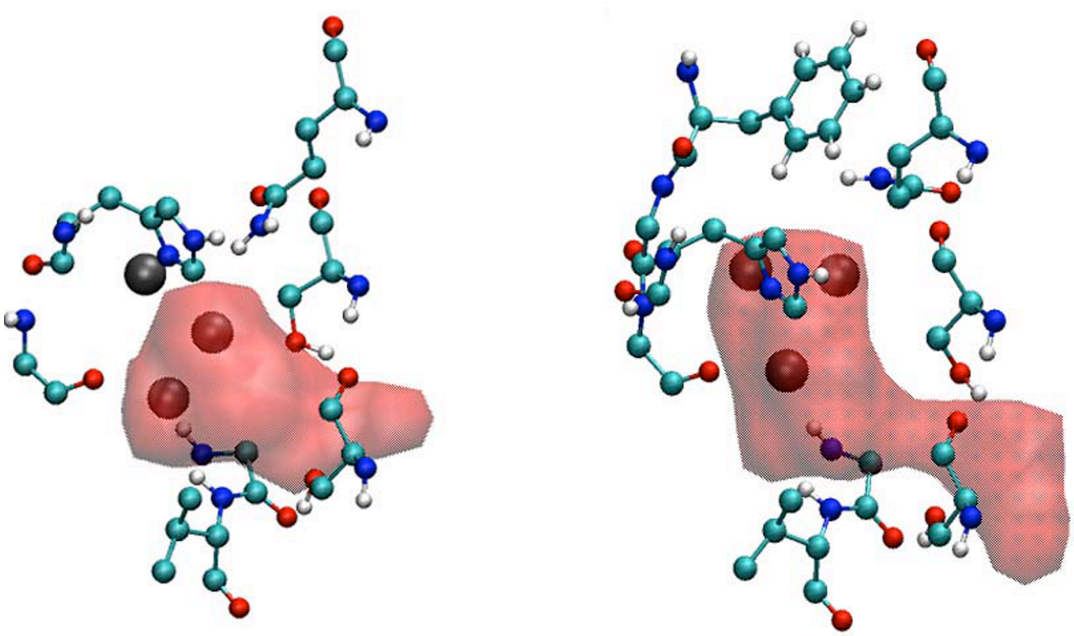

Figure S5.

Table S1: H-bond interactions at the active site (The values indicate percentage of total simulation time for which a given H-bond was present. An interaction is recorded only if it was present for >20% in at least one simulation).

Interaction	GlpG1	GlpG2	GlpG3	GlpG4
H150-Wat:O	34.3	66.7	61.0	74.4
N154-Wat:O	93.5	80.2	55.7	72.4
S201-Wat:O	5.1	7.9	29.8	16.0
S201-H254	99.3	97.4	93.7	96.7
S201-Wat:H	88.4	87.5	83.0	87.8
M247-Wat:H	69.7	41.0	-	-
G198-Wat:H	9.2	24.8	0.7	41.9
N154-Wat:H	-	0.1	52.9	9.3
N154-H145	86.4	87.2	46.4	62.3