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## Open Structure of Intramembrane Protease GlpG

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*The active sites of the intramembrane protease, E. coli rhomboid GlpG, are buried in the lipid bilayer when it is in a closed conformation without a bound substrate. How transmembrane substrates enter the internal and closed active sites remains an open question. Here we describe the crystal structure where the capping L5 has been lifted. The changes following the loop movement surrounding the active sites contribute to a more open conformation. The unexpected experimental results reveal the conformational flexibility and plasticity of GlpG, which may function to accommodate substrate binding and catalysis.*

The first crystallographic analysis of the E. coli rhomboid protease GlpG confirmed that the active sites of intramembrane proteases are positioned in the lipid bilayer. The structure of GlpG contains six transmembrane helices (S1-S6) and is apparently in a closed conformation, where the internal active site is covered tightly by the capping loop L5. A new open-cap conformation crystal structure, owing to the loop L5 movement, has been solved at 2.5Å based on the data collected at the NSLS.

The crystal structure (**Figure 1A**) confirmed that L5 (residues 245-249) was lifted away from the original internal and hydrophilic active sites, and the two side chains (Met-247 and Met-249) were sequentially pulled away from the immediate vicinity of Ser-201. The void left behind was occupied by new water molecules. A neighboring His-150 moved in and overlapped with the original path of L5 main chain. An open trough appears on the top of the membrane-embedded protease (**Figure**

**1B**), which could be where substrate binds.

The oxyanion-binding site in the closed state of GlpG was not obvious because of the two methionine side chains impeding over the active sites. While in the open state of GlpG, water molecules immobilized in the active site slightly adjusted their positions. One water, previously bound between Ser-201, His-150, and Gly-198, had moved to a new location, where it broke off the hydrogen bond with the backbone carbonyl of Gly-198 and formed a new bond with the side chain amide of Asn-154 (**Figure 1C**). This new location corresponds roughly

to where the oxyanion hole for a classic serine protease is.

The loop L5 movement creates a lateral opening (**Figure 2**). In the closed structure, the side chain of Phe-245 is inserted into a gap between transmembrane helices S2 and S5, roughly at the same level of the internal active site. Phe-245 physically separates the membrane-embedded active site from the lipid. In the open-cap structure, Phe-245 became disordered, the lightly inward adjustment from the side chain of Met-249 was not sufficient to bridge the gap. An opening was left in the wall of protein structures that surrounded the active site, exposing internal hydrophilic residues unfavorably to lipid.

This unexpected structure, combined with the previous closed structure, illustrates our thoughts on the role of L5 in the proteolysis process. In the closed structure, the active sites are completely buried inside the membrane protein. The protein structure must become open to



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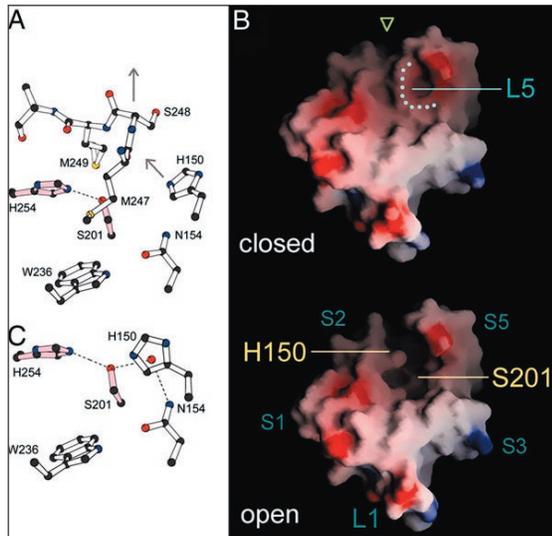


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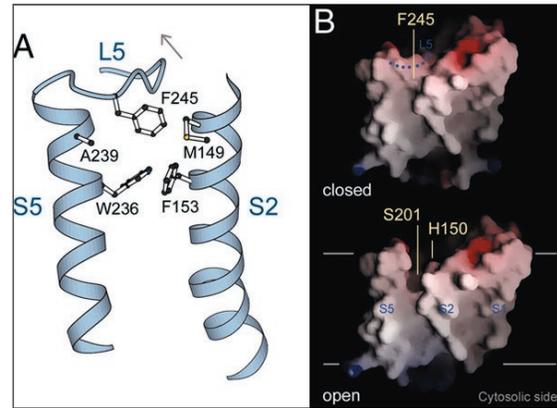
accommodate a substrate. In the open-cap structure, the loop L5 is displaced from its original position. This movement of the L5 cap exposes the putative oxyanion hole

and creates a side portal. The comparison of the two structures implies the following: the docking of substrate transmembrane domain near the S2/S5 gap could

cause the L5 cap to open so that the top portion of the substrate could access the active site.



**Figure 1.** The active site is exposed in the open-cap conformation. (A) Detailed picture of the closed cap and the catalytic dyad of GlpG (back view). (B) Comparison of the surface features of the closed- and open-cap GlpG structures (top view). Blue areas are positively charged, and red areas are negatively charged. The dotted line marks the path of L5 in the closed structure. (C) With the cap lifted, a water molecule (red dot) moves into the putative oxyanion hole.



**Figure 2.** Cap movement creates a lateral opening. (A) Phe-245 is normally inserted between S2 and S5, blocking an entrance to the active site (back view). (B) Molecular surface of GlpG (back view). Blue areas are positively charged, and red are negatively charged. The two horizontal lines mark the hydrophobic region of lipid bilayer. The dotted line depicts the path of L5 in the closed structure.