First insights of peptidoglycan amidation in Gram-positive bacteria - crystallographic and functional studies on *Staphylococcus aureus* MurT-GatD protein complex

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Peptidoglycan amidation is a key structural property of the bacterial cell wall and it has been associated to mechanisms of antibiotic resistance in Gram-positive bacteria [1]. The amidation reaction is carried out by the enzymatic complex MurT-GatD in a two-step reaction: the glutaminase GatD produces ammonia that is then transferred to MurT, where the peptidoglycan precursor lipid II is amidated [2, 3]. In this work, combining the first crystal structure of GatD [4], from *Staphylococcus aureus*, and activity studies performed by ^{1}H-NMR spectroscopy, we showed the functional determinants for the glutaminase reaction of MurT-GatD [5]. The protein complex presented glutaminase activity even in the absence of lipid II and the mutants R128A, C94A and H189A where totally inactive. These results revealed the essential role of such residues in glutamine sequestration and deamiation reaction. Given the ubiquitous presence of GatD, these results reveal significant insights into the molecular basis of the so far undisclosed amidation mechanism, contributing to the development of alternative therapeutics to fight bacterial infections.

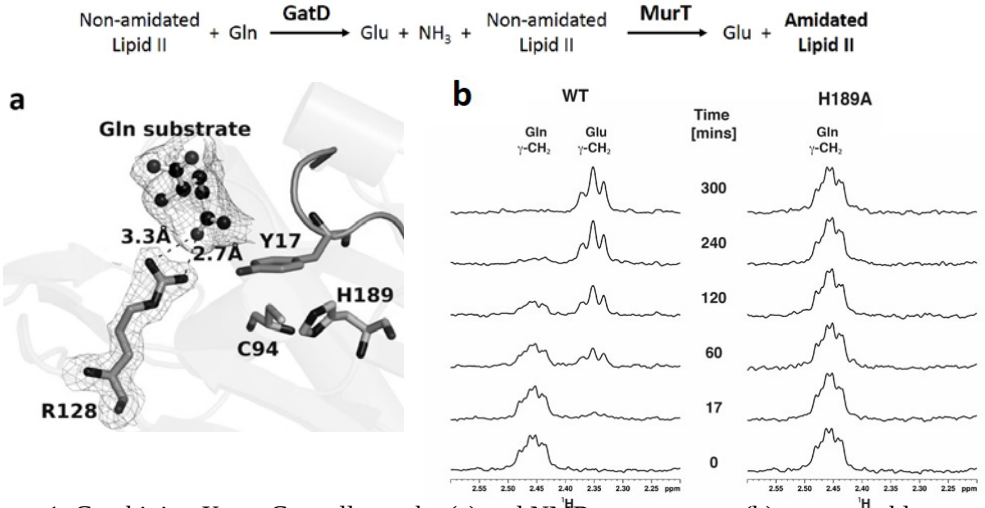

Figure 1: Combining X-ray Crystallography (a) and NMR spectroscopy (b) we were able to unravel the active site and substrate sequestration of GatD, the glutaminase component of MurT-GatD protein complex from *Staphylococcus aureus*. Access to iNEXT platform was determinant for complex expression and stabilization.

References

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