

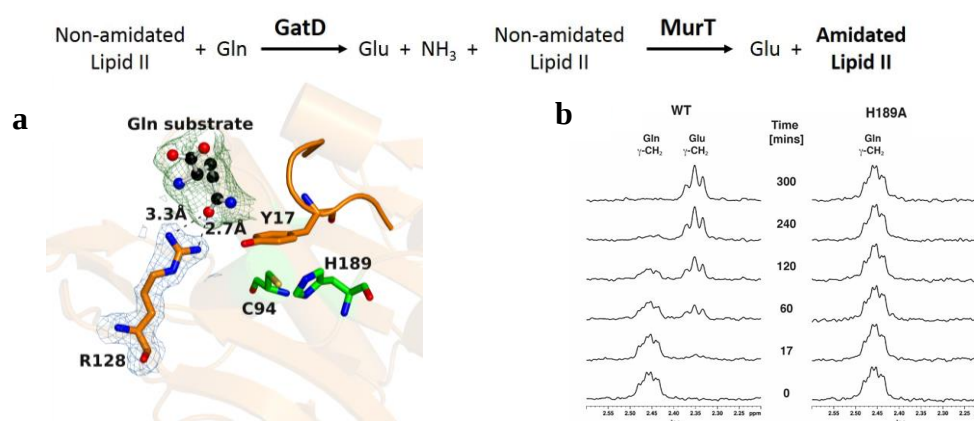
# Crystallographic and functional studies on MurT-GatD shed light in *Staphylococcus aureus* peptidoglycan amidation

F. Leisico<sup>1</sup>, D. Vieira<sup>2</sup>, T. A. Figueiredo<sup>3,4</sup>, M. Silva<sup>1</sup>, E. J. Cabrita<sup>1</sup>, R. G. Sobral<sup>3</sup>, A. M. Ludovice<sup>3</sup>, J. Trincão<sup>2</sup>, M. J. Romão<sup>1</sup>, H. de Lencastre<sup>4,5</sup> and T. Santos-Silva<sup>1</sup>

<sup>1</sup>UCIBIO, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal; <sup>2</sup>Oxford Protein Production Facility, Research Complex at Harwell, Didcot, England and Diamond Light Source, Didcot, England; <sup>3</sup>UCIBIO, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal; <sup>4</sup>Laboratory of Molecular Genetics, Microbiology of Human Pathogens Unit, Instituto de Tecnologia Química e Biológica António Xavier da Universidade Nova de Lisboa, Oeiras, Portugal; <sup>5</sup>Laboratory of Microbiology and Infectious Diseases, The Rockefeller University, New York, USA

Email: franciscoleisico@fct.unl.pt

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 <sup>1</sup>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 were totally inactive. These results revealed the essential role of such residues in glutamine sequestration and deamination 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. Instruct-ERIC infrastructure access was key to establish the expression and purification conditions of MurT-GatD complex.



**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*.

## References

- [1] – J. Gustafson, *et al*, J Bacteriol. **176**, 1460, (1994).
- [2] – T. A. Figueiredo, *et al*, Plos Pathog **8**, e1002508, (2012).
- [3] – D. Munch, *et al*, Plos Pathog **8**, e1002509, (2012).
- [4] – D. Vieira, *et al*, Acta Crystallogr F Struct Biol Commun. **70**, 632, (2014).
- [5] – F. Leisico *et al*, Sci Rep. **8**, 5313, (2018).