Glycosynthase-based synthesis of peptidoglycan oligosaccharides to decipher bacterial cell division

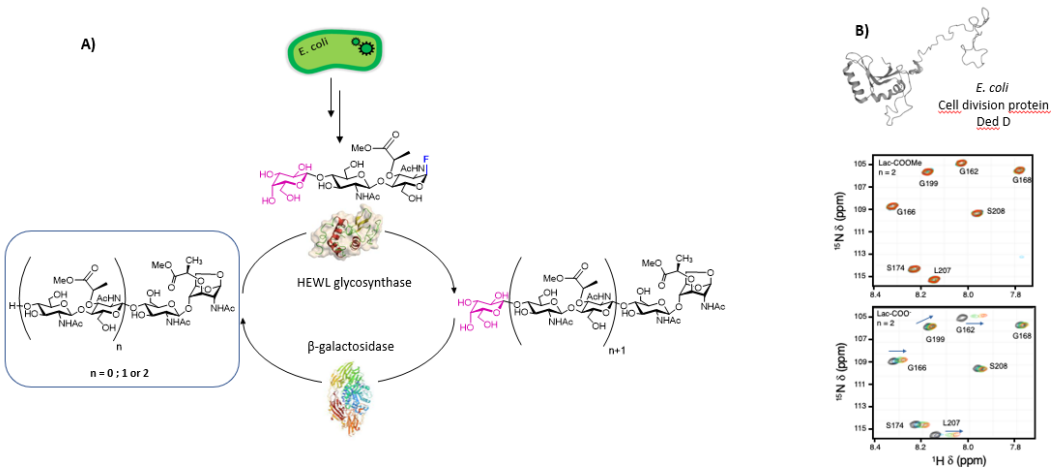
Dindet Steve-Evanes KOFFI TEKI [1], Antoine ROUSSEAU [1], Louis BRIGANDAT [2], Emeline RICHARD [1], Isabel AYALA [2], Jean-Pierre SIMORRE [2], Sylvie ARMAND [1], Sylvain COTTAZ [1], Catherine BOUGAULT [2], Sébastien FORT [1]

[1] Univ. Grenoble Alpes, CNRS, CERMAV, Grenoble, France, [2] Univ. Grenoble Alpes, CNRS, CEA, IBS, Grenoble, France

evanes.teki@cermav.cnrs.fr

Most antibiotics used in human medicine inhibit the biosynthesis of peptidoglycan, an essential component of the bacterial wall. However, antibiotic resistance is rising to dangerously high levels, becoming one of the biggest threats to global health.1 Combating this burden therefore requires the detailed study of peptidoglycan metabolism and implies the need to produce well-defined molecular probes. 2,3

In this context we devised a glycosynthase-based chemo-enzymatic synthesis of peptidoglycan oligosaccharides using the D52S mutant of hen egg-white lysozyme (D52S HEWL).4 Size-control of the oligosaccharides during glycosylation was achieved using a non-polymerizable donor. A galactosyl group, whose introduction and deprotection can be done enzymatically was chosen as temporary protecting group. Trisaccharide Gal-GlcNAc-1,6-anhMurNAc was produced by metabolically engineering E. coli cells and chemically fluorinated at the reducing end to provide the target donor. Successive rounds of glycosylation using D52S HEWL followed by degalactosylation using a commercial β-galactosidase afforded the expected tetra-, hexa- and octasaccharides in 60-70% yields. These compounds were used to decipher the impact of the charge on the lactoyl group of MurNAc, of the anhydro at the reducing end of the oligosaccharide, and of the oligosaccharide chain length on the interaction with E. coli DedD, a SPOR-domain-containing protein involved in bacterial cell-division.5 The information retrieved using NMR spectroscopy in this study sheds a new light on the role of this protein.



A) Glycosynthase-based synthesis of peptidoglycan oligosaccharides; B) Characterization of E. coli Ded D-oligosaccharides interaction by NMR

This work was supported by the French National Research Agency (ANR) through Glyco_SWIM (ANR-20-CE07-0012-01)

Bibliographic references:

(1) O'Neill (2016), J. Nat. Rev. Drug Discov (15), 526–526.
(2) M. Lee, D. Hesek, E. Lastochkin; D. A. Dik, B. Boggess, S. Mobashery (2017), ChemBioChem (18), 1696–1702.
(3) N. Wang, H. Hasegawa, C. Y. Huang, K. Fukase, Y. Fujimoto (2017), Chem. Asian J (12), 27–30.
(4) A. Rousseau, S. Armand, S. Cottaz, S. Fort (2021), Chem. Eur. J (27), 17637–17646.
(5) M. Pazos, K. Peters, A. Boes, Y. Safaei, C. Kenward, N. A. Caveney, C. Laguri, E. Breukink, et al. (2021), mBio, e02796-20