UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Master 2 internship project
Year 2021-2022

Laboratory/Institute: Institut de Biologie Structurale **Director:** Winfried Weissenhorn
Team: Biomolecular NMR group/Pneumococcus group **Head of the team:** Brutscher B/Morlot C

Name and status of the scientists in charge of the project:
Bougault Catherine / Zapun André **HDR: yes ☑ no ☐**
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Program of the Master's degree in Biology:

☐ Immunology, Microbiology, Infectious Diseases ☑ Structural Biology of Pathogens
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology

Title of the project: Mechanisms of peptidoglycan transpeptidation

Objectives (up to 3 lines):

The aim is to better understand the transpeptidation reactions responsible for the cross-linking of the peptidoglycan, a key component of the bacterial cell envelope.

Abstract (up to 10 lines):

Peptidoglycan (PG) is the essential and main constituent of the bacterial envelope, conferring strength and shape to the cells. PG is a giant molecular mesh constituted of chains of repeated disaccharides cross-linked by peptide bridges, which are formed by two types of transpeptidation reaction catalyzed by two enzyme families. Linkage between the third residue of one peptide and the fourth residue of the other peptide (3,4) are formed by PBPs (penicillin-binding proteins), whereas linkage between the third residues of two peptides (3,3) are formed by the L,D-transpeptidases. PBPs can be inhibited by beta-lactam antibiotics. L,D-transpeptidases can play a role in the resistance to beta-lactams and are particularly important in some pathogens such as *Mycobacterium tuberculosis*. These important reactions have been difficult to study due to the lack of defined in vitro substrate. We have now access to synthetic PG fragments of defined length and peptide composition prepared by chemist collaborators. The project is to use such compounds and recombinant PBPs and L,D-transpeptidases to characterize the requirements of the transpeptidase reaction of the different classes of enzymes and to understand structurally the interaction between these enzymes and their substrates.

Methods (up to 3 lines):

Recombinant enzymes will be produced and purified to carry out in vitro enzymatic assays. Substrate/enzyme interactions will be investigated by NMR, requiring the preparation of isotopically labeled proteins.

Up to 3 relevant publications of the team:

Substitutions in PBP2b from β-Lactam-resistant *Streptococcus pneumoniae* have different effects on enzymatic activity and drug reactivity. Calvez, … , Zapun. 2017 J Biol Chem. 292:2854 doi: 10.1074/jbc.M116.764696.
Recognition of peptidoglycan fragments by the transpeptidase PBP4 From *Staphylococcus aureus*. Maya-Martinez, …, Bougault, …, Simorre. 2019 Front Microbiol. 9:3223 doi: 10.3389/fmicb.2018.03223.
Acyl acceptor recognition by *Enterococcus faecium* L,D-transpeptidase Ldt_{fm}. Triboulet, Bougault, …, Simorre. 2015 Mol Microbiol 98:90. doi: 10.1111/mmi.13104.

Requested domains of expertise (up to 5 keywords):

Recombinant protein expression and purification; NMR; enzymology.