Title: **Sortase mediated ligation.**

Student: Víctor Lauroba Manrique

Date: June 2020

Supervisor/s: Dr. Miquel Pons Valles
Departament de Química Inorgànica i Orgànica

Sortases are transpeptidases (enzymes) that covalently anchor proteins to the peptidoglycan of Gram-positive bacteria's cell wall. They recognise and cleave a pentapeptide motif within the cell wall sorting signal (CWSS) of their target substrates. Afterwards, they covalently link it to a N-terminal amino group or lysine side chain forming a peptide or isopeptide bond, respectively; resulting in their attachment to the bacteria cell wall. The most studied and characterized sortase is class A sortase from *Staphylococcus aureus* (Sa-SrtA). This sortase cleave the C-terminal LPXTG (with X being any aminoacid) pentapeptide motif of their target substrate between their Thr-Gly bond. Then, a cross-linked oligoglycine called lipid-II is covalently linked via peptide bond to the protein substrate. Finally the resulting substrate-lipid-II is anchored to the peptidoglycan of the bacteria cell.

Sortases have a specific His-Cys-Arg catalytic triad in their active site. If this triad is properly charged, sortases are able to catalyse a cysteine transpeptidase mechanism that enable them to develop their physiological functions; anchoring surface proteins to the bacteria cell wall, assembling and polymerization of pilin subunits and anchoring iron acquisition proteins to the cell envelope are some examples. Although there are different classes of sortases (A-F), all of them perform their catalysis following the similar mechanism.

Sortases play an important role in infections and pathogenesis as most of the proteins they bind to the cell wall are virulent. It has been emphasized the finding of sortase inhibitors in order to avoid the infection course of these enzymes.

In addition, this transpeptidase reaction called Sortase Mediated Ligation (SML) has led to many applications involving anchoring proteins of interest, cell to cell ligations, immunologic treatments and reengineering of protein structures, as well as industrial applications.