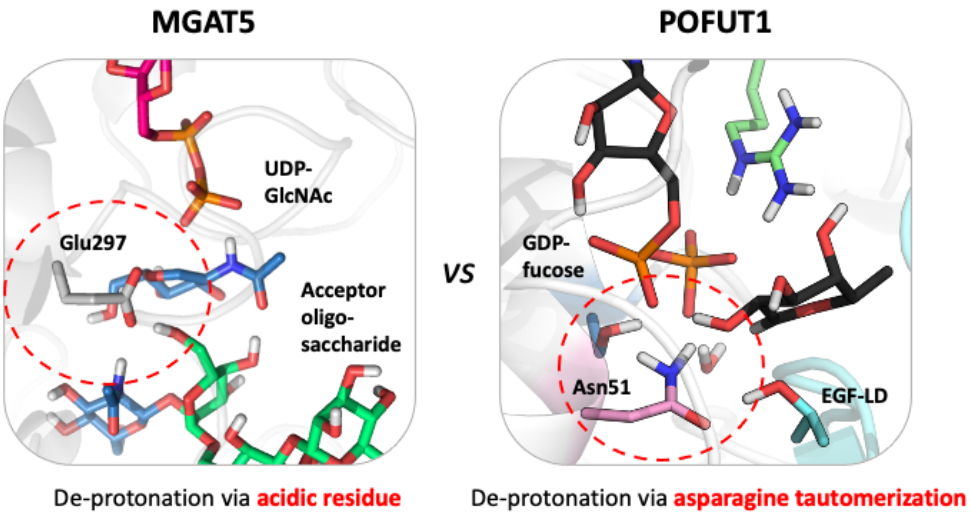
Uncovering catalytic mechanisms of inverting glycosyltransferases involved in protein glycosylation

Beatriz PINIELLO [1], John F. DARBY [2], Amelia K. GILIO [2], Christian ROTH [2], Elena BLAGOVA [2], Roderick E. HUBBARD [2], Erandi LIRA-NAVARRETE [3], Hideyuki TAKEUCHI [4], Megumi TAKmEUCHI [4], Robert S. HALTIWANGER [4], Liang WU [2], Gideon J. DAVIES [2], Ramón HURTADO-GUERRERO [3], Carme ROVIRA [1]

[1] University of Barcelona SPAIN, [2] University of York UK, [3] University of Zaragoza SPAIN, [4] University of Georgia, Athens USA

b.piniello@ub.edu

Post-translational protein modifications, such as N- and O-glycosylation, are essential to life. Glycosylation defects are the cause of several diseases and, therefore, there is a great interest in deciphering the mechanism of action of the enzymes responsible for the synthesis of protein glycoconjugates. Two of these enzymes are α-mannoside β-1,6-N-acetylglucosaminyltransferase V (MGAT5, also known as GnT-V) and protein O-fucosyltransferase 1 (POFUT1). MGAT5 catalyzes the transfer of an N-acetylglucosamine moiety to the core α-1,6-mannose of an N-glycan and its activity is connected to oncogenic behavior in cells. POFUT1 catalyzes the transfer of one moiety of L-fucose to a threonine or serine residue in the surface of epidermic growth factor-like domains (EGF-LD), small peptides involved in the Notch signaling cascade, and it is related to diseases such as colorectal cancer, leukemia and Dowling-Degos disease. We have recently uncovered the catalytic mechanism of these two enzymes by means of quantum mechanics/molecular mechanics (QM/MM) and metadynamics methods. Our simulations show that whereas MGAT5 follows a classical SN2 mechanism in which an acidic residue (Glu297) acts as the catalytic base [1], POFUT1 follows an unusual mechanism involving a proton shuttle through a conserved active site asparagine [2]. Site-directed mutagenesis experiments are in line with our results. These findings widen our understanding of these enzymes and will aid inhibitor development to correct disease associated O-glycosylation.



Bibliographic references:

[1] J.F. Darby, A.K. Gillio, B. Piniello, C. Roth, E. Blagova, R.E. Hubbard, C. Rovira, G.J. Davies, L. Wu. (2020), ACS Catalysis, 10 8590-8596.

[2] B. Piniello, E. Lira-Navarrete, H. Takeuchi, M. Takeuchi, R.S. Haltiwanger, R. Hurtado-Guerrero, C. Rovira (2021), ACS Catalysis, 11 9926-9932