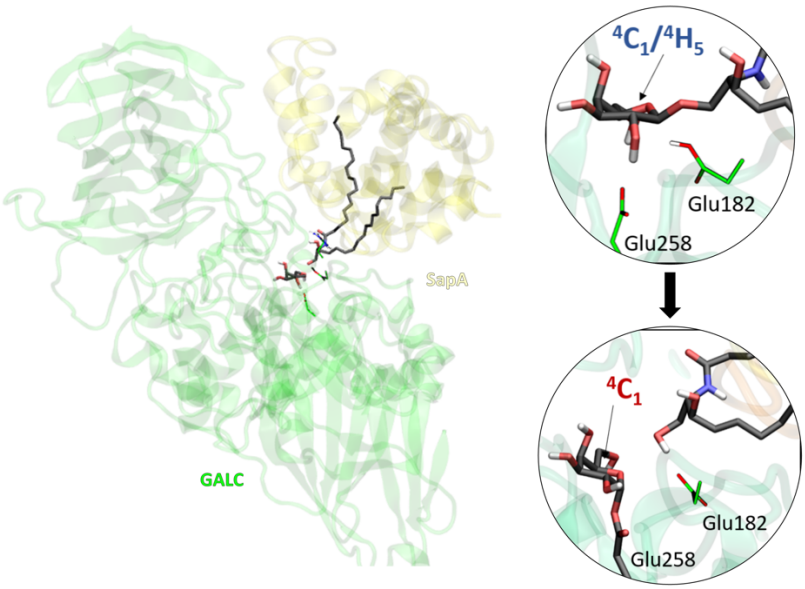
The catalytic reaction mechanism of the β-galactocerebrosidase enzyme deficient in Krabbe disease

Alba NIN-HILL [1], Carme ROVIRA [1,2]

[1] Departament d'Inorgànica i Orgànica and Institut de Química Teòrica i Computacional (IQTC). Universitat de Barcelona. Barcelona. Spain. [2] Institució Catalana de Recerca i Estudis Avançats (ICREA). Barcelona. Spain

albanin@ub.edu

Krabbe disease is a neurodegenerative disorder related to misfunction of Saposin A or β-galactocerebrosidase (GALC), a glycosidase that catalyzes the cleavage of *β*-galactosidic bonds in glycosphingolipids [1]. Here we uncover the catalytic molecular mechanism of GALC in complex with Gal-β-*p*-nitrophenyl, a substrate analogue, using quantum mechanics/molecular mechanics (QM/MM) metadynamics simulations [2]. Our results clarify the unusual chair conformation of the substrate observed in the crystal structure [3] and show that catalysis can take place via two distinct conformational pathways with similar free energy barriers because of leaving group flexibility [4][5]. Moreover, we study the complex of the important agent for GALC activity *in vivo*, the lipid-transfer protein Saposin A (SapA) with GALC. SapA extracts the lipid substrate from the cell membrane, forming a soluble saposin-lipid complex that provides the lipid to GALC. In spite of the relevance of SapA for GALC function, the catalytic reaction mechanism of the GALC-SapA complex with its natural substrate has not been reported. Our simulations show that SapA not only acts as a transport agent but also it helps decreasing the hydrolysis energy barrier by stabilizing the reaction transition state [6]. This mechanistic insight can aid in the design of Krabbe diagnosis probes and GALC conformational chaperones and expand the knowledge of the importance of saposin domains and their interaction with lipid-degrading enzymes.



Bibliographic references:

[1] A.C. Graziano, V. Cardile (2015), Gene (555) 2–13.
[2] A. Ardèvol, C. Rovira (2015), JACS (137) 7528-7547.
[3] C.H. Hill, S.C. Graham, R.J. Read, J.E. Deane (2013), PNAS (110) 20479-20484.
[4] A. Nin-Hill, C. Rovira (2020), ACS Catal. (10) 12091−12097.
[5] M. Morais, A. Nin-Hill, C. Rovira (2023), Curr. Opin. Chem. Biol. Accepted
[6] A. Nin-Hill, C. Rovira (2023) Submitted