UNRAVELLING THE MOLECULAR BASIS OF A4GALT CATALYSIS AND INHIBITION: TOWARDS A NEW THERAPEUTIC APPROACH FOR FABRY DISEASE

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Fabry disease is a rare and severe genetic lysosomal disorder caused by a deficiency of α-galactosidase A. This deficiency leads to the accumulation of the sphingolipid globotriaosylceramide (Gb3) and its deacylated form globotriaosylsphingosine (LysoGb3), resulting in progressive heart and kidney dysfunction as well as chronic pain. Current treatments, including enzyme replacement therapy (Fabrazyme® or Replagal®) [1] and the pharmacological chaperone Galafold® [2], have limited efficacy, as patients often continue to suffer disease progression. An alternative therapeutic approach is the inhibition of lactosylceramide 4-α-galactosyltransferase (A4GALT), the enzyme responsible for synthesizing Gb3. However, no A4GALT inhibitors have been reported to date.

In this multidisciplinary project, we have elucidated the enzyme's substrates recognition motifs and catalytic mechanism, enabling the rational design of novel inhibitors. Using molecular dynamics simulations with an Alphafold-derived A4GALT model, we identified key interactions governing substrate binding, which were experimentally validated through mutagenesis and lipidomics. Additionally, we modelled the enzyme's reaction mechanism using QM/MM metadynamics, revealing that α4GalT follows an S_Ni-type reaction mechanism [3], where the UDP-galactose phosphate group acts as a general base. Based on these insights, we designed, synthesised and experimentally validated several acceptor analogues [4].

This duo speaker presentation will highlight how an integrative approach –combining metadynamics, cell biology, organic chemistry and biochemistry– advances our understanding of galactosyltransferases and facilitates the rational design of mechanism-based inhibitors for therapeutic applications.

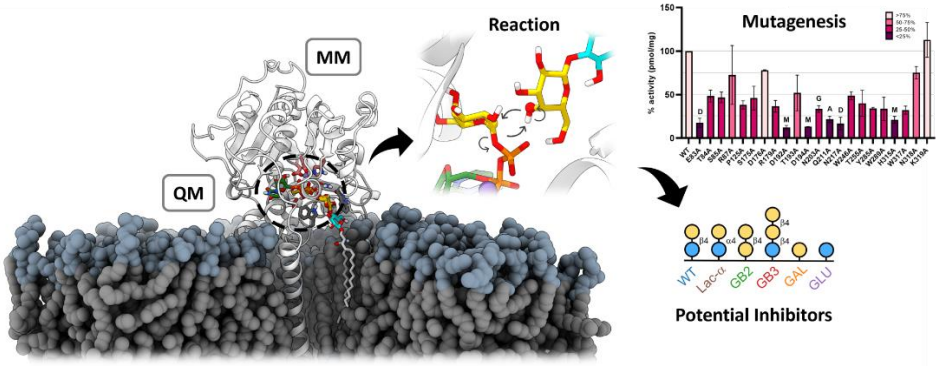

Figure 1. Structure of the Michaelis complex of the A4GALT enzyme and the delimited QM and MM regions, reaction mechanism, A4GALT mutagenesis and potential inhibitors.

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