Poster Presentation

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Structural snapshots of GALC: The Krabbe Disease enzyme

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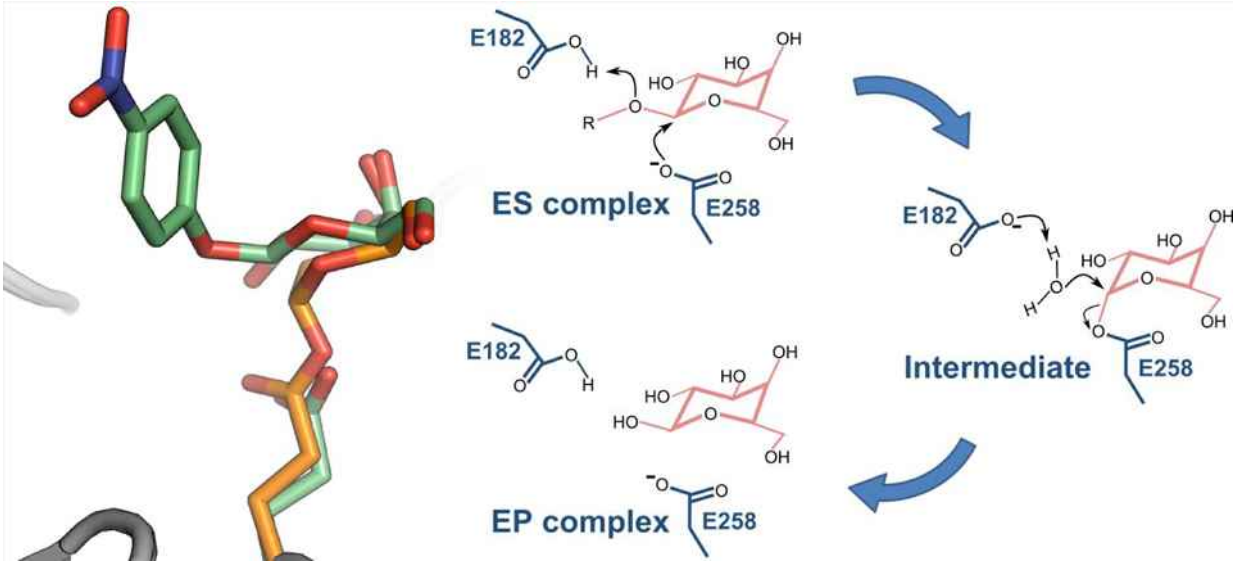
Glycosphingolipids are ubiquitous components of mammalian cell membranes, and defects in their catabolism by lysosomal enzymes cause a diverse array of diseases. Deficiencies in the enzyme β-galactocerebrosidase (GALC) cause Krabbe disease, a devastating genetic disorder characterized by widespread demyelination and rapid, fatal neurodegeneration[1]. Many disease-causing mutations throughout the GALC gene have been identified; rather than rendering the enzyme catalytically incompetent, many of these are predicted to destabilise the protein, compromising lysosomal delivery by causing retention in the endoplasmic reticulum. In these cases, pharmacological chaperone therapy (PCT) may be an appropriate treatment strategy, whereby the specific binding of a small molecule may stabilise folding of the mutant protein sufficiently to correct trafficking defects. Here we present a series of high resolution crystal structures that illustrate key steps in the catalytic cycle of GALC, including enzyme-substrate, enzyme-intermediate and enzyme-product complexes[2]. These structures reveal active site conformational changes accompanying the catalytic steps and provide key mechanistic insights. Building on this, we identify two candidate molecules that stabilise GALC by specific binding to the active site. The nature of several disease-causing missense mutations is also explored using secretion and activity assays, and we show that mutations can be classified into two groups based on defects in catalysis or trafficking. Overall, this study provides an atomic framework for the rational design of GALC pharmacological chaperones, and helps to identify which mutations may be suitable for PCT, bringing the prospect of a drug-based therapy one step closer.

[1] *D. Wenger, M. Rafi, P. Luzi, Human Mutation, 1997, 10, 268-279,* ***[2]*** *C. Hill, S. Graham, R. Read et al, PNAS, 2013, 110(51), 20479-20484*



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