**Reference Number:** 120072
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**Organization:** Bio21 Institute, University of Melbourne
**Project Period:** April, 2012 - March, 2013
**Grant Title:** Structural insight into inhibition of N-glycan processing endomannosidase

**(a) Abstract:**

Abnormal protein glycosylation is a hallmark of diseases such as cancer and viral infection. Significant interest has been directed at inhibition of glycoprotein N-glycan biosynthesis, through inhibitors targeted at the *exo*-acting glycoside hydrolases such as glucosidase I and II, which remove a single sugar residue at a time (Figure). However, the presence of the endomannosidase pathway provides an alternative route to such structures, subverting the effect of these inhibitors. Efforts to study mammalian endomannosidase, which uniquely removes more than one sugar at a time from glucosylated N-glycans, are thwarted by the difficulties of expressing and handling this membrane-associated enzyme. This project sought to study bacterial models of human endomannosidase, which can be recombinantly-expressed in soluble form and have been crystallized. In doing so it was hoped that we could accelerate inhibitor development to be ultimately applied to the mammalian enzyme.

Inspired by the established monosaccharide-based inhibitors of *exo*-mannosidases, isofagomine and kifunensine, we have chemically-synthesized new inhibitors of endomannosidase, a 'blocked' glucosyl-isofagomine and Man-isofagomine. The blocked glucosyl-isofagomine was shown to be an effective inhibitor of endomannosidase, and promises to be -glucosidase resistant and thus might possess favourable properties for use in cellular studies. Man-isofagomine was evaluated as a ligand for the bacterial enzyme by isothermal titration calorimetry, and was found to be a better inhibitor than any previously described endomannosidase inhibitors. Using X-ray crystallography, structures of Man-isofagomine and the blocked glucosyl-isofagomine bound to a bacterial endomannosidase were determined, providing insight into the structural basis of inhibition. Finally, we have shown that Man-isofagomine is an effective inhibitor of the mammalian enzyme, validating the concept of using the bacterial enzyme as a model for the mammalian enzyme. This work will provide a foundation for the exploitation of endomannosidase inhibition in cellular research and medicine through providing new inhibitors that can be used in cell biology and lead compounds for future drug development efforts.

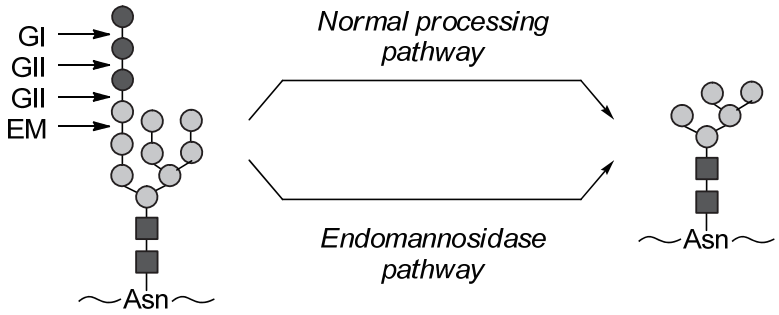


**Figure.** Summary of pathways for N-linked glycan processing. In the normal pathway, glucosidase I and II (GI, GII) sequentially remove individual sugar residues, prior to exomannosidase action. In the Endomannosidase pathway, the enzyme endomannosidase (EM) removes the glucosylated mannose residue, prior to further processing by exomannosidases.