

# Multivariate Free-Energy Relationships of *O*-GlcNAcase: Insight into the Interaction of Nucleophile and Leaving Groups in Transition State Structures.

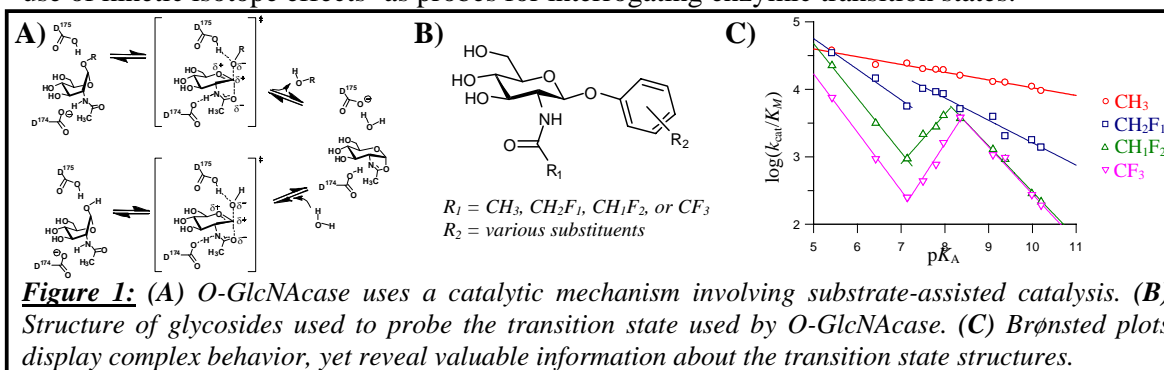
Matthew Macauley<sup>1</sup>, Ian Greig<sup>2</sup>, Rebecca Dennis<sup>3</sup>, Gideon Davies<sup>3</sup>, and David Vocadlo<sup>1</sup>

<sup>1</sup>Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada

<sup>2</sup>Department of Chemistry, University of Bath, Bath, UK

<sup>3</sup>Department of Chemistry, University of York, York, UK

The attachment of a single 2-acetamido-2-deoxy- $\beta$ -D-glucopyranose residue (GlcNAc) to serine and threonine residues of nucleocytoplasmic proteins is an important post-translational modification in higher eukaryotes<sup>1</sup>. An enzyme termed OGT catalyzes the transfer of GlcNAc to target residues while the enzyme termed *O*-GlcNAcase catalyzes the hydrolytic removal of this residue. Inhibitors of *O*-GlcNAcase are proving to be very useful within a cellular context at deducing the roles of this modification. Through a series of kinetic<sup>2</sup> and crystallographic experiments<sup>3</sup>, we have determined that *O*-GlcNAcase uses a catalytic mechanism involving substrate-assisted catalysis. In this unusual catalytic mechanism, the 2-acetamido group on the substrate acts as the nucleophile to displace the leaving group to form an oxazoline intermediate, which is then broken down by attack of water (Fig. 1A). Since this enzyme uses the acetamido group of the substrate as the nucleophile and tolerates a variety of leaving groups, both these groups can both be modulated in a quantifiable manner by altering the substrate structure; the nucleophile by fluorination of the substrate 2-acetamido group and the leaving group by altering substituents of an aryl group (Fig. 1B). Here we describe multivariate free-energy relationships to advance detailed insights into the transition state structure and the interaction of nucleophilicity and leaving group ability. These free-energy relationships (Fig. 1C) will be discussed in the context of charge distribution and transition state poise using the structure of *O*-GlcNAcase. These findings offer guidance for the design of inhibitors of human *O*-GlcNAcase and other glycoside processing enzymes. We expect this method of using multiple free-energy relationships to provide valuable complementation to the use of kinetic isotope effects<sup>4</sup> as probes for interrogating enzymic transition states.



**Figure 1:** (A) *O*-GlcNAcase uses a catalytic mechanism involving substrate-assisted catalysis. (B) Structure of glycosides used to probe the transition state used by *O*-GlcNAcase. (C) Brønsted plots display complex behavior, yet reveal valuable information about the transition state structures.

1 G. W. Hart, M. P. Housley, and C. Slawson, *Nature* **446** (7139), 1017 (2007).

2 M. S. Macauley et al., *The Journal of biological chemistry* **280** (27), 25313 (2005); N. Cetinbas et al., *Biochemistry* **45** (11), 3835 (2006); G. E. Whitworth et al., *Journal of the American Chemical Society* **129** (3), 635 (2007).

3 R. J. Dennis et al., *Nature structural & molecular biology* **13** (4), 365 (2006).

4 V. L. Schramm, *Annual Review of Biochemistry* **67**, 693 (1998).