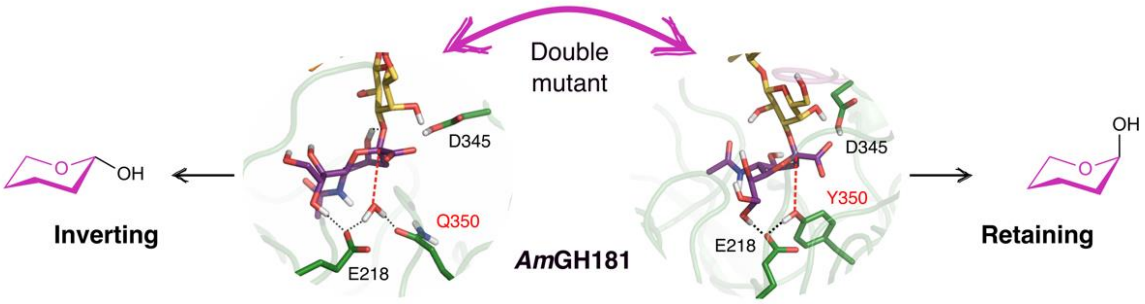
ENGINEERING THE INVERTING MECHANISM OF A HIGHLY SPECIFIC SIALIDASE TO RETAINING

Marina Corbella[a,b], Michael Jakob Pichler[b], Sebastian Meier[c], Tine Sofie Neilsen[b], Sanchari Banerjee[b], Jens Preben Morth[b], Maher Abou Hachem[b], Carme Rovira[a,d]

[a]Departament de Química Inorgànica i Orgànica (Secció de Química Orgànica) and Institut de Química Teòrica i Computacional (IQTCUB), Universitat de Barcelona, 08028 Barcelona, Spain
marina.corbemo@gmail.com
[b]Department of Biotechnology and Biomedicine, Technical University of Denmark, DK-2800, Kgs. Lyngby, Denmark
[c]Department of Chemistry, Technical University of Denmark, DK-2800, Kgs. Lyngby, Denmark
[d]Institució Catalana de Recerca i Estudis Avançats (ICREA), 08020 Barcelona, Spain

Akkermansia muciniphila is a dedicated mucin degrading gut symbiont, which is associated to beneficial impacts on host metabolism and body weight. This bacterium possess a battery of enzymes that collectively degrade mucin, the main constituents of the mucosal layer. This process starts with the action of exoglycosidases that remove terminal caps from muicn *O*-glcyans. Uniqely, the sialidase *Am*GH181 from *A. Muciniphila*, the founding memebr of a recently discovered family, exhbits high selectivty to the sialyl T-antigen [1]. Combining mutagenesis, X-ray crystallography and QM/MM metadynamics simulations [2,3], we show that specificity is established via a flexible tryptophan-histidine pair, forming a “sugar tang” that positions the sialyl T-antigen for catalysis. Hydrolysis of the sialyl-T antigen proceeds via a single-step S_N2 reaction via a $^{3,6}B/^{3}S_O \rightarrow [^{3,6}B]^{\ddagger} \rightarrow {}^{3,6}B/^{3}S_O \rightarrow {}^{2}C_5$ conformational itinerary of the sialyl unit at subsite *-1*, resulting in inversion of anomeric configuration. For the first time, we altered the reaction stereochemistry by a double mutation, introducing a tyrosine residue to perform nucleophilic attack on the C2 of the sialyl unit at subsite *-1* [4]. The retaining mutant acquired trans-sialidase activity and performed synthesis of sialyl-oligosaccharides at remarkably high yields, pioneering an innovative strategy for harnessing inverting glycosidases for oligosaccharide synthesis.



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