Structural and mechanistic insights into the cleavage of clustered *O*-glycan by mucinases

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Mucinases of human gut bacteria cleave peptide bonds in mucins strictly depending on the presence of a neighboring *O*-glycan. The *Akkermansia muciniphila* AM0627 cleaves specifically in between contiguous (bis) *O*-glycans of defined structure, suggesting that this enzyme may recognize clustered *O*-glycan patches. Here, we report the structure and molecular mechanism of AM0627 in complex with a glycopeptide containing a bis-T (Gal-GalNAc) *O*-glycan, revealing that AM0627 recognizes both the sugar moieties and the peptide sequence. Interestingly, AM0627 prefers bis-T over bis-Tn (GalNAc) *O*-glycopeptide substrates, with the first GalNAc residue being critical for cleavage, and follows a mechanism relying on a nucleophilic water molecule and a catalytic base Glu residue. Structural comparison among mucinases identifies a conserved Tyr, engaged in sugar-p interactions in both AM0627 and the *Bacteroides thetaiotaomicron* BT4244 mucinase, as responsible for the common activity of these two mucinases with bis-T/Tn substrates. Our work illustrates how mucinases, through tremendous flexibility, adapt to the diversity in *O*-glycan distribution in mucins.

Bibliographic references:
Taleb, V., Liao, Q., Narimatsu, Y. et al. (2022), Nat Commun 13, 4324.