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Combined structural snapshots and metadynamics reveal a substrate-guided SNi-type reaction for polypeptide GalNAc-transferase T2

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Glycosyltransferases (GTs) are an ubiquitous group of enzymes that catalyze the transfer of sugar moieties from activated donors, mostly nucleotide sugars, into a diverse number of acceptor substrates.[1] They are responsible for protein glycosylation, the most recurrent post-translational modification occurring in nature for cell recognition and signaling. GT alterations cause several diseases, such as infection, inflammation, and either normal or abnormal cellular developments. Given their importance in both normal development and pathological conditions, GTs are targets for inhibition and thus their molecular mechanisms of action are the focus of intense study.

In this context, the catalytic mechanism of GTs, specially the retaining ones, have been a controversial subject in recent times. Here using the retaining protein GalNAc-T2, a member of a large family of human glycosyltransferases and responsible for a very abundant post-translational modification, and substrates, we describe different structural snapshots along the catalytic cycle to uncover the reaction coordinates. Furthermore, we combine the experimental atomic information with QM/MM metadynamics to unravel the catalytic mechanism of this retaining enzyme at atomic-electronic detail. Our study reveals key features of substrate recognition, the specificity of acceptor Thr versus Ser residues and a front-face S_Ni-type reaction with substrate-assisted catalysis for the glycosyl transfer.[2]

[1] Lairson, L. L.; Henrissat, B.; Davies, G. J.; Withers, S. G. Glycosyltransferases: structures, functions, and mechanisms. *Annual Review of Biochemistry* **2008**, *77*, 521-555.

[2] Lira-Navarrete, E.; Iglesias-Fernández, J.; Zandberg, W. F.; Compañón, I.; Kong, Y.; Corzana, F.; Clausen, H.; Vocadlo, D.; Peregrina, J. M.; Rovira, C.; Hurtado-Guerrero, R. *Angew Chem. Int. Ed.* **2014**, in press (DOI: 10.1002/anie.201402781 and 10.1002/ange.201402781)