Structural and mechanistic characterization of heparan sulfate *N*-deacetylase-*N*-sulfotransferase 1

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Mammalian heparan sulfate (HS) biosynthesis is a complex non-templated process, mediated by multiple enzyme activities in the Golgi complex (polymerases, sulfotransferases, epimerase etc). The interplay between enzymes and substrates involved in HS construction creates diverse polysaccharide sequences, which are essential for key biological processes, including cell adhesion, cytokine signalling and host-pathogen binding.

We have recently investigated the structure and function of heparan sulfate N-deacetylase-N-sulfotransferase (NDST)1, the first enzyme that acts on nascent HS after its polymerization. NDST1 possesses bifunctional deacetylase and sulfotransferase activity and converts N-acetyl-glucosamines in HS to N-sulfo-glucosamines. Whilst a crystal structure of the NDST1 sulfotransferase domain was reported in 1999, the molecular details of the NDST1 deacetylase domain, and how it works alongside the sulfotransferase domain, remain unknown.

Here, we report cryo-EM structures of full length bifunctional NDST1. Our structures show an unusual back-to-back arrangement of the enzyme domains, which imposes strong steric constraints on functional cooperativity. Aided by novel activity modulating nanobodies, we also carried out biochemical and biophysical analysis of NDST1 function. Our results suggest non-catalytic binding must operate alongside catalytic turnover to mediate cooperativity in the bifunctional enzyme. These data shed light on the molecular basis of function