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Abstract

Many nuclear and cytosolic proteins are transiently glycosylated by an enzyme known as O-GlcNAc transferase (OGT), which transfers N-acetylglucosamine from UDP-GlcNAc to selected serine and threonine residues. O-GlcNAcylation affects such diverse cellular processes as transcription, translation, organelle targeting, and protein-protein interactions,¹ and is believed to play a role in a variety of signaling cascades that mediate glucose homeostasis and stress responses.² Specific inhibitors of OGT could be valuable tools to probe the biological functions of O-GlcNAcylation, but the inability to obtain significant quantities of enzyme, combined with the lack of a high-throughput assay, has impeded efforts to identify such compounds.³ We have developed conditions to express large quantities of the catalytic domain of active OGT for the first time, and we report a high-throughput donor displacement assay for the enzyme along with the discovery of a set of small-molecule inhibitors. This work lays the foundation for both structural and functional analysis of the catalytic domain of OGT.