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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.