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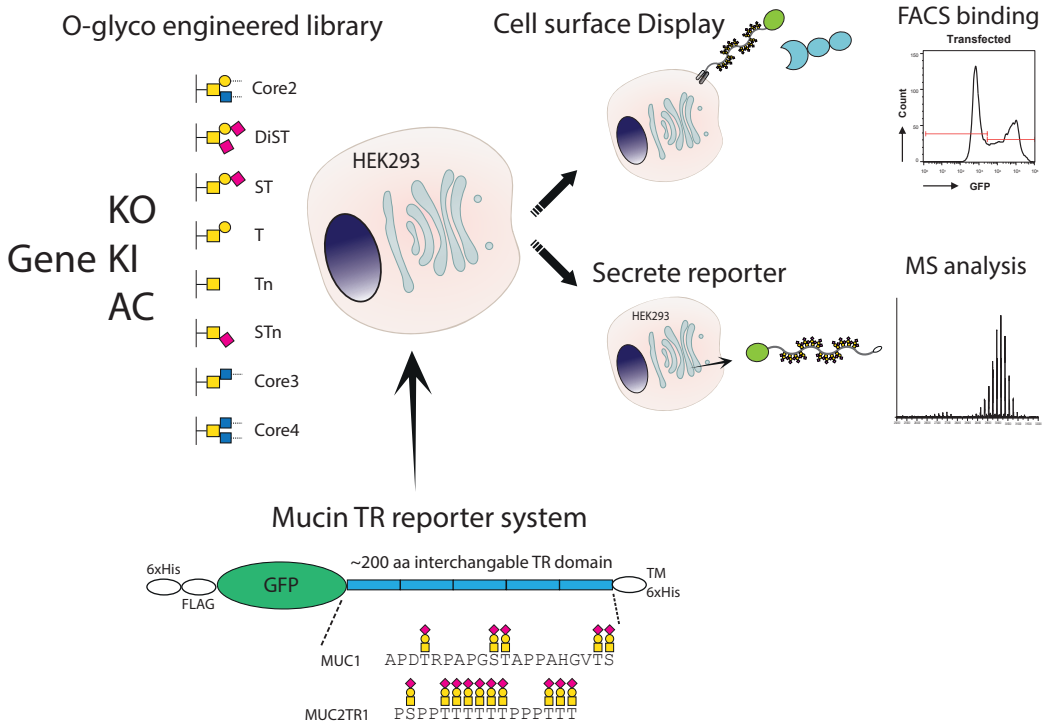
Grant Title: Display of the Human Mucinome with Defined O-Glycans by Gene Engineered Cells

(a) Abstract

Mucins are a large family of heavily O-glycosylated proteins that cover all mucosal surfaces and are the major macromolecules in body fluids. Mucins serve in containment, barrier, clearance and replenishing of the microbiota. Mucins are defined by their tandem repeat (TR) regions densely decorated with O-glycans. The mucin TRs contain molecular cues that guide interactions with microbiota and serve to orient microbiomes, and that these molecular cues consist of higher order assembly of multiple O-glycans with distinct structures and patterns created by characteristic TR sequences. Here, we sought to capture the molecular cues contained in human mucin TRs and enable molecular dissection of these cues.



We developed a cell-based platform for the display and production of representative mucin TRs with defined O-glycans using precise gene engineering. We found that mucin TR reporters could readily be produced as highly homogeneous molecules with essentially complete O-glycan occupancies and with distinct O-glycan structures in amounts that enabled us to characterize the simplest reporters by intact mass spectrometry (MS), and hence circumvent the longstanding obstacles with protease digestion and bottom-up analysis of mucins. We demonstrated utility of the cell-based mucin display through probing and dissecting binding specificities of microbial adhesins, influenza virus, and Siglecs, as well as dissecting the substrate specificities of microbial O-glycopeptidases (mucinases). The cell-based mucin display platform widely opens the mucin and microbiome fields for studies with libraries of homogeneous mucin TR glycodomains, and for entirely new approaches to test and dissect the biophysical properties and the informational content of human mucins.