

Characterization of core fucosylation via sequential enzymatic treatments of intact glycopeptides and mass spectrometry analysis

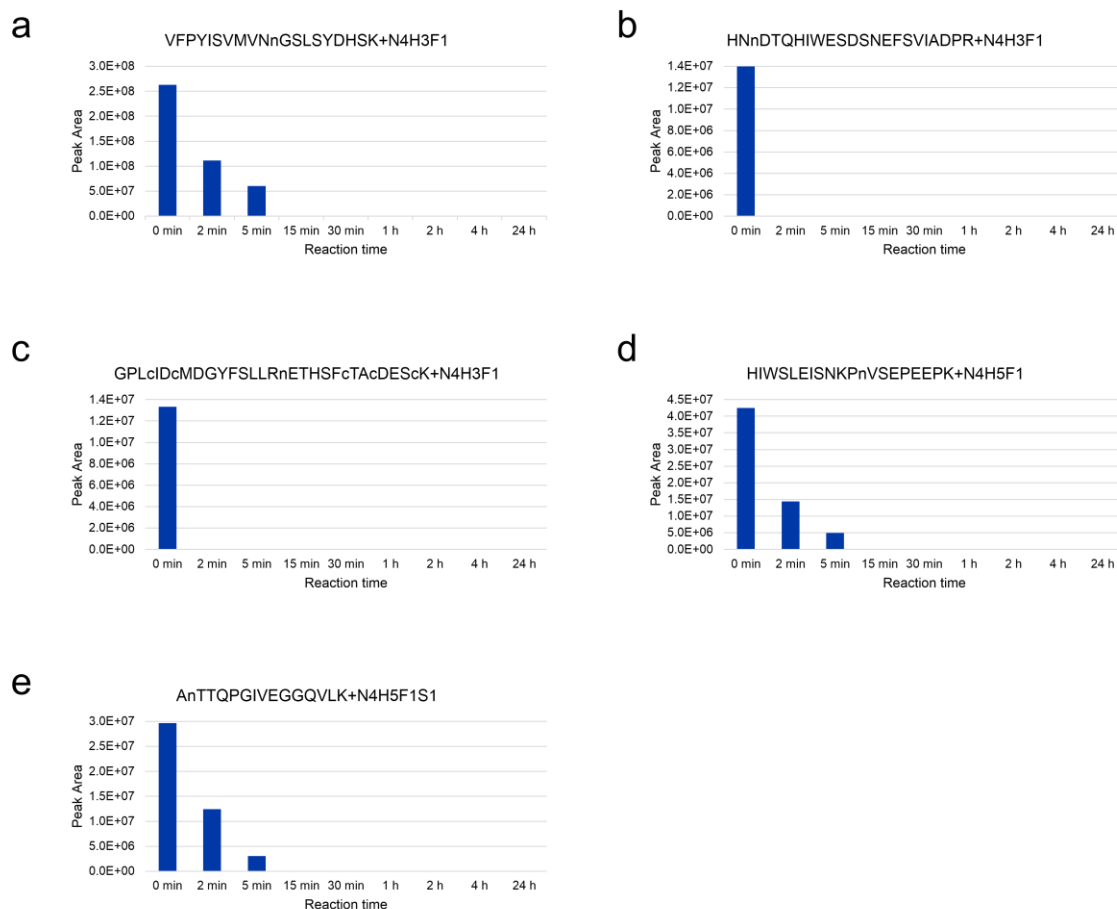
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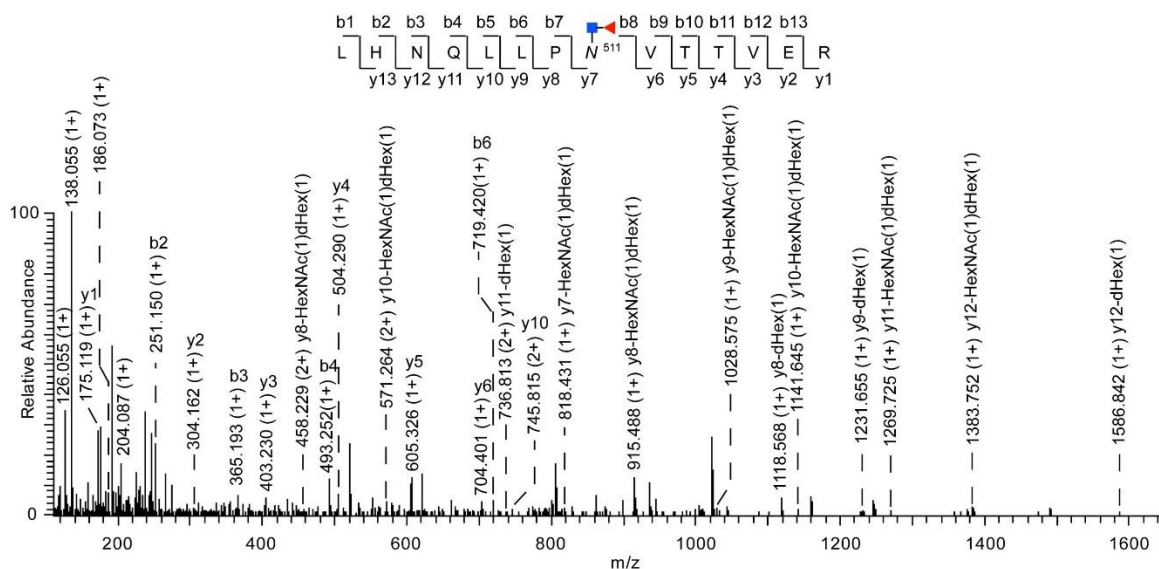
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Supplementary Figure 1. Assessment of the completeness of the enzymatic reaction using Endo F3 on CHO cells. a) The peak area of the CF biantennary glycopeptide (VFPYISVMVNnGSLSYDHSK-N4H3F1, Uniprot ID: G3H177) from LMAN2 at different time points. **b)** The peak area of the CF biantennary glycopeptide (HNNDTQHIWESDSNEFSVIADPR-N4H3F1, Uniprot ID: G3HQM6) from endoplasmin at different time points. **c)** The peak area of the CF biantennary glycopeptide (GPLcIDcMDGYFSLLRnETHSFcTAcDEScK-N4H3F1, Uniprot ID: G3IC16) from protein disulfide-isomerase at different time points. **d)** The peak area of the CF biantennary glycopeptide (HIWSLEISNKPnVSEPEEPK-N4H5F1, Uniprot ID: G3HR95) from carboxypeptidase D at different time points. **e)** The peak area of the CF biantennary glycopeptide (AnTTQPGIVEGGQVLK-N4H5F1S1, Uniprot ID: G3HHR9) from integrin alpha-V at different time points. All detectable intact CF biantennary glycopeptides in the

glycopeptide fraction of the CHO digest were listed in Supplementary Figure 1 and Figure 2. Core fucosylation was evidenced by the ion consisting of the peptide and the glycan moiety (HexNAc(1)dHex(1)). c: carbamidomethylation of cysteine; n: glycosylated site; N: HexNAc; H: Hex; F: fucose; S: sialic acid.



Supplementary Figure 2. MS/MS spectrum of a glycopeptide with CF glycan. MS/MS spectrum of a peptide, LHNQLLPN⁵¹¹VTTVER from glutathione hydrolase 1 proenzyme (GGT1, UniProt ID: P19440). The site (N⁵¹¹) was core fucosylated (attach to HexNAc-Fuc, Endo F3 treated). Keys: b-HexNAc(1)dHex(1) represents a disaccharide, HexNAc(1)dHex(1), is lost from this b ion; y-HexNAc(1)dHex(1) represents a disaccharide, HexNAc(1)dHex(1), is lost from this y ion; b-dHex(1) represents a monosaccharide residue, dHex(1), is lost from this b ion; y-dHex(1) represents a monosaccharide residue, dHex(1), is lost from this y ion; b-HexNAc(1) represents a monosaccharide residue, HexNAc(1), is lost from this b ion; y-HexNAc(1) represents a monosaccharide residue, HexNAc(1), is lost from this y ion.