from cell membrane microdomains that had distinct glycan signatures. The following study further revealed that microvesicles released from human cancer cell lines had a glycomic pattern that featured in high mannose, poly-LacNAc and complex, and the feature was very different from that of parent membrane. It suggested a potential biogenesis pathway of microvesicles that mediated by glycosylation. In this study, we characterized a MV cargo glycoprotein EWI-2 that had N-link glycan dependent recruiting pathway. Removal of carbohydrate attached on the protein severely disrupted its incorporation within MV. Besides, the overall alteration of glycome changed the recruitment of a few MV-enriched glycoprotein and its associated proteins. Taken together, our data provided evidence that N-link glycan directs glycoprotein sorting in MV and serves as a determinant of MV cargo selection signal.

(77) **Aminated Human Milk Fucooligosaccharide Library and Corresponding High Density Neoglycoproteins**

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Using ammonium carbamate, 12 main individual fucooligosaccharides (FOS) from human milk (2'-FL, 3-FL, DFL, LNFP I, II, and III, LND I, MFLNH III, DFLNH a, DFLNnH, TFLNH, and MF (1-3)iLNO were converted into the corresponding glycosylamines. Acylation with N-hydroxysuccinimide ester of N-t-BOC-glycine gave, after deprotection, FOS-Gly derivatives with a free amino group on a multimilligram scale with 70% yields. The same modification of the total pool of delactosylated neutral human milk OS gave rise to a mixture of the corresponding N-t-BOC-Gly-OS. After HPLC and deprotection, a library of 25 individual FOS-Gly derivatives was obtained. These included, together with 12 main FOS: LND II, LNnD I, MFLNH I and II, MFLNnH I and II, MFpLNH IV, DFLNH b and c, DFpLNH II, DF(1-2,1-3)iLNO, TF(1-2,1-2,1-3) iLNO, and PFiLNO. Five Gly-OS core-based derivatives, including lactose, LNT, LNnT, LNH, and LNnH, were also prepared. By carbodiimide chemistry, BSA was derivitized with ethylenediamine, and succinylated with the corresponding anhydride to give suc-BSA. Using carbodiimide coupling, 10 suc-BSA-FOS_n neoglycoproteins with a high oligosaccharide substitution ($n > 40$) were prepared. Interaction of suc-BSA-FOS_n with fucolectins: *Laburnum anagyroides* bark lectin (LABA), perch (*Perca fluviatilis*) oocyte lectin (PFL), sander (*Lucioperca lucioperca*) oocyte lectin (LLL), and *Lotus tetragonolobus* agglutinin (LTA) was studied. Another application of FOS-Gly includes glycoarrays and glycochips preparation.

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(78) **Enhanced Mannosyl-phosphorylation in Glyco-engineered Yeast by Heterologous Expression of YlMPO1**

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Some portions of *N*-glycans are phosphorylated in yeast by the dual action of two proteins encoded by *MNN4* and *MNN6*. Mnn4 protein (Mnn4p) has been known to be a positive regulator of Mnn6p, a real mannosyltransferase adding mannosyl-phosphate residues to glycan. In the present study, *YlMPO1*, *Yarrowia lypolytica* homologue of *MNN4*, was used to increase the mannosyl-phosphorylation of *N*-Glycans in *Saccharomyces cerevisiae* without the help of Mnn4p or Mnn6p. *N*-glycan analysis of yeast cell wall mannoproteins clearly showed that the *YlMPO1* expression greatly increases the amount of mannosyl-phosphorylated glycans in *ScMNN4* and *ScMNN6* double deletion mutant as well as wild-type. Furthermore, increased amounts of mannosyl-phosphorylated glycans were much higher upon the heterologous expression of *YlMPO1* compared to recombinant overexpression of *ScMNN4*. This strategy employing recombinant expression of *YlMPO1* was applied to the glyco-engineered *S. cerevisieae* strain in which yeast-specific glycosylation pathway was abolished. The resulting glycans containing highly increased amount of mannosyl-phosphates were converted to mammalian-type *N*-glycans with mannose-6-phosphate by uncapping the outer mannose residue. This glyco-engineered yeast will have the promise for the production of therapeutic enzymes containing a high content of mannose-6-phosphates for lysosomal storage diseases.

(79) **Investigation of the Factors That Affect Glucose Unit Generation in HILIC-based Analyses**

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Analysis of such complex structures is typically achieved using a HILIC-based approach, which allows for separation of both neutral and charged glycan structures within a single chromatographic window. In an effort to standardize glycan separation data for curation in databases, glycan peaks are often converted to glucose unit values, which aid in normalizing variability typically observed between instruments and labs. However, adjustment to chromatographic conditions within the separation method can affect the glucose unit values, therein leading to the possibility of erroneous glycan assignments. Here, we present a study of the impact of modifying a number of chromatographic parameters for N-linked glycan separation. Factors such as temperature, gradient slope, mobile