

Supplementary data

N-Glycan Profile of Cell Membrane as Probe for Lipopolysaccharide-induced Microglial Neuroinflammation Uncovers the Effects of Common Fatty Acid Supplementation

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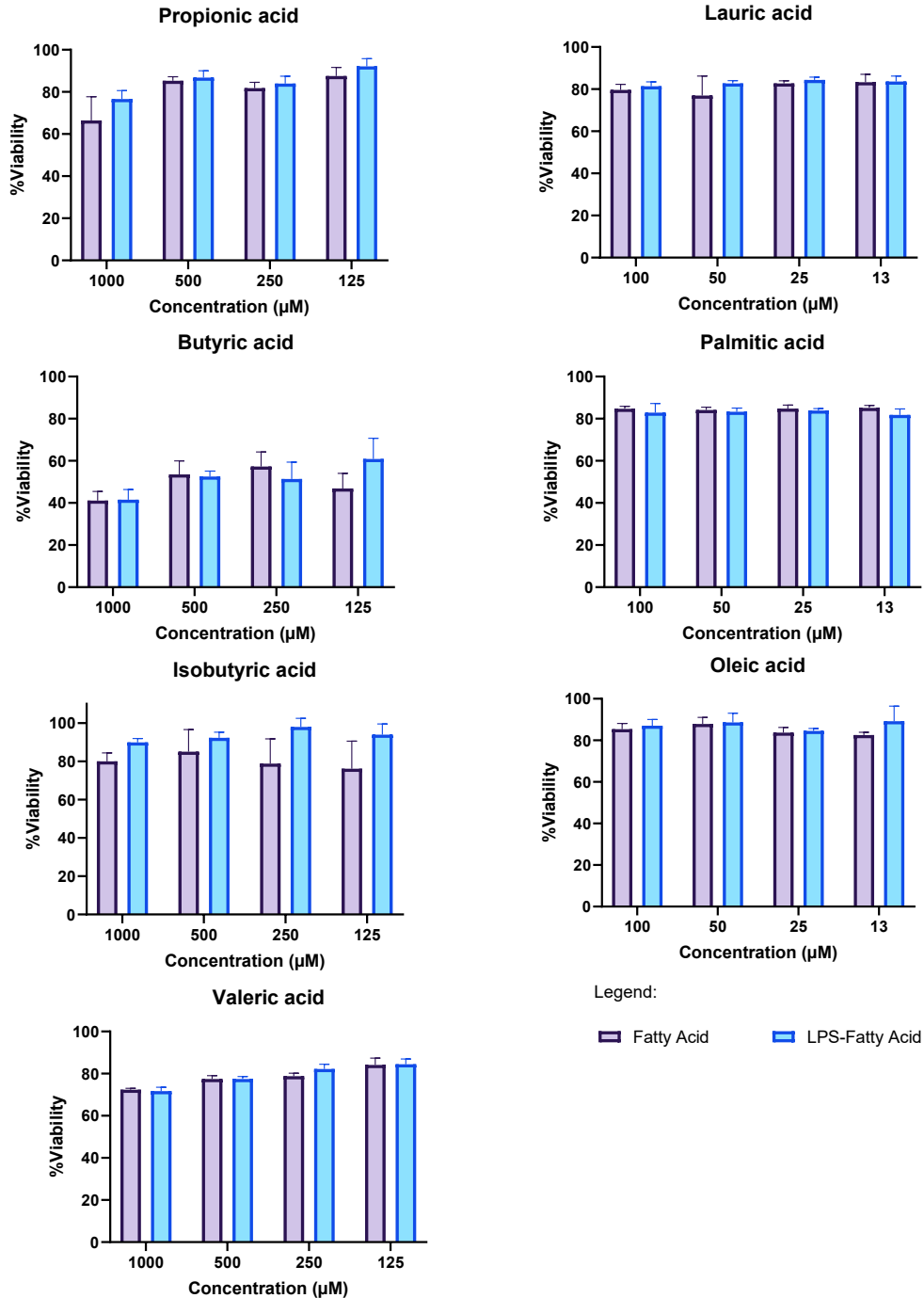
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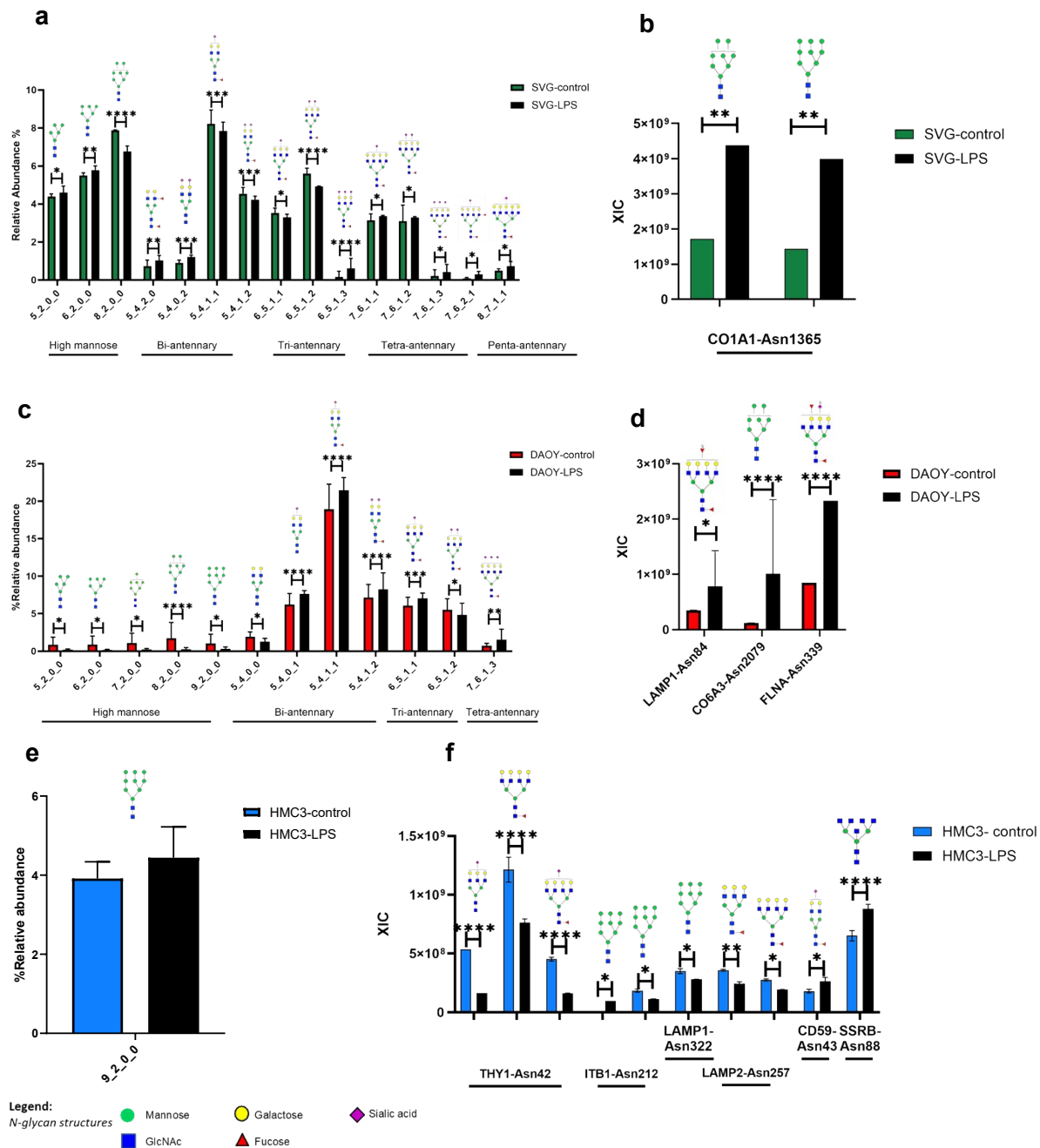
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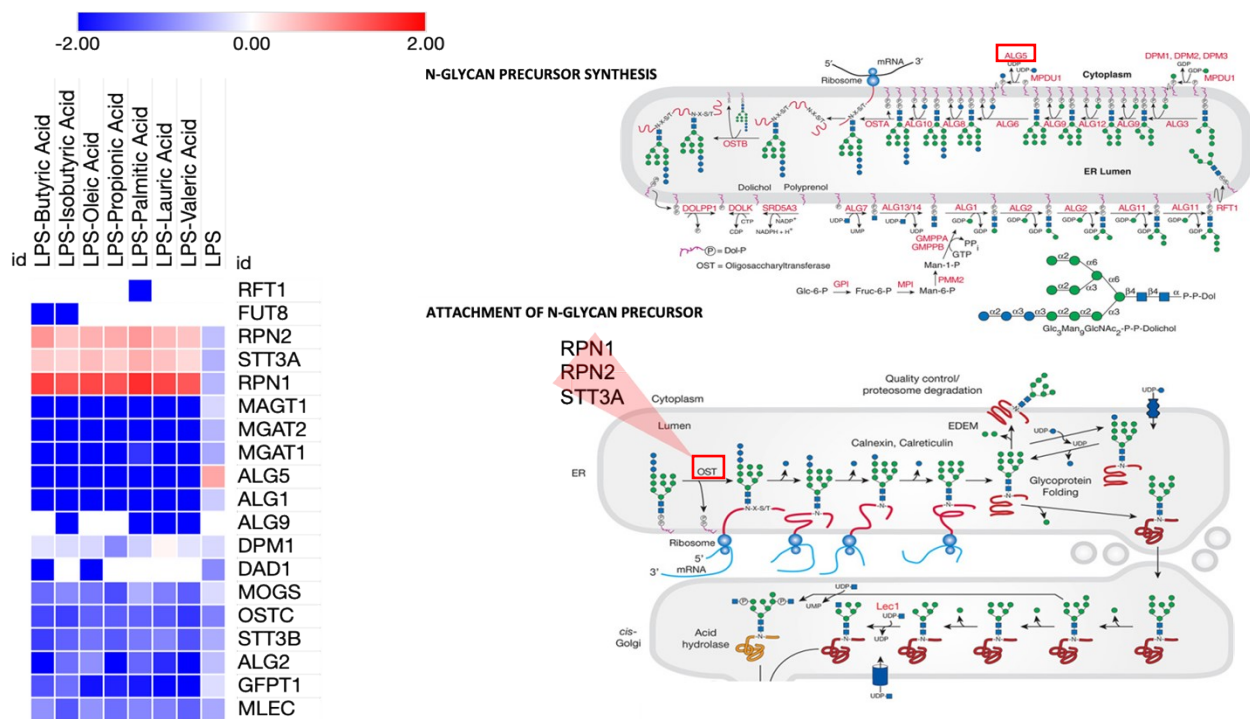
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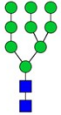
Supplementary Figure 1. Microglial cell viability against fatty acid and LPS-fatty acid treatments. Bar graph illustrating cell viability assay using the MTS assay of the HMC3 microglial cells after 24h of incubation with select fatty acids (violet bar) or with LPS-fatty acid (blue bar).

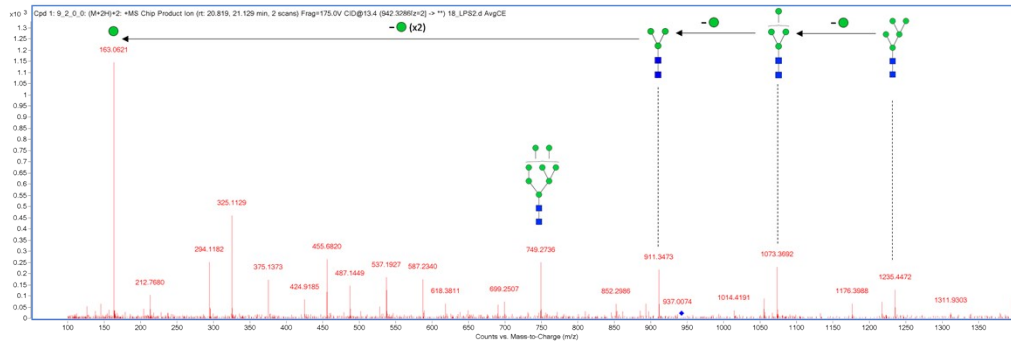


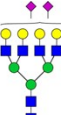
Supplementary Figure 2. Significantly different N-glycans and site-specific N-glycopeptide of *in vitro* neuroinflammation cell models (4a) N-glycans and (4b) N-glycopeptides in astroglia, SVG p12 cells; (4c) N-glycans and (4d) N-glycopeptides in medulloblastoma, DAOY cells; (4e) N-glycans and (4f) N-glycopeptides in microglia, HMC3 cells.

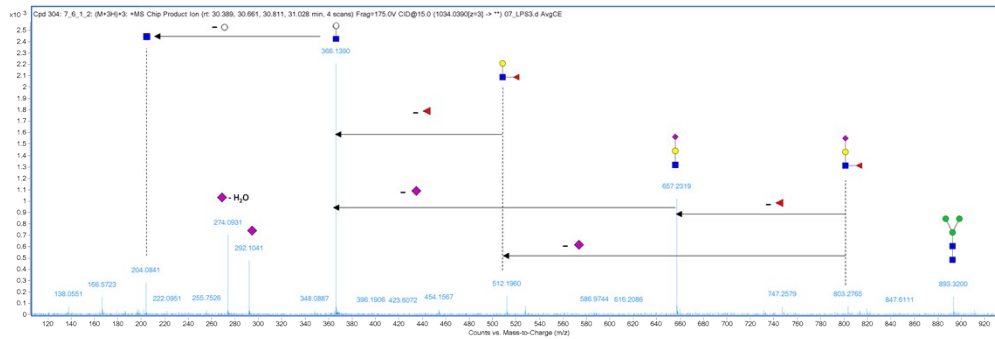


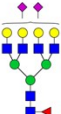
Supplementary Figure 3. Proteomics results of LPS- and fatty acids-treated microglia showing under- and overexpressed proteins related to N-glycosylation. Diagrams used showcasing N-glycan precursor synthesis and attachment of N-glycan precursor were lifted from *Essentials of Glycobiology* (Varki et al., 2022). ALG5= Asparagine-Linked Glycosylation 5 Homolog; OST=Oligosaccharyltransferase, RPN1/2= Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1/2; STT3A=Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3A

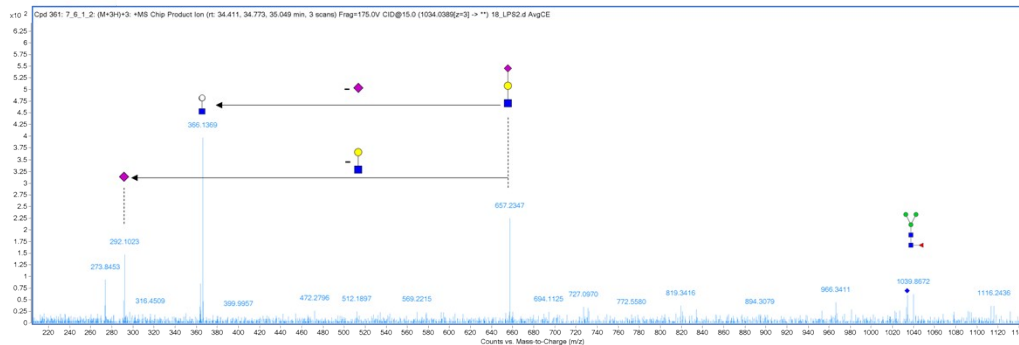
a  Microglia (HMC3)
MS/MS of 9200
Precursor ion m/z 942.33(z=2+)



b  Microglia (HMC3)
MS/MS of 7612, terminal fucose
Precursor ion m/z 1034.04 (z=3+)

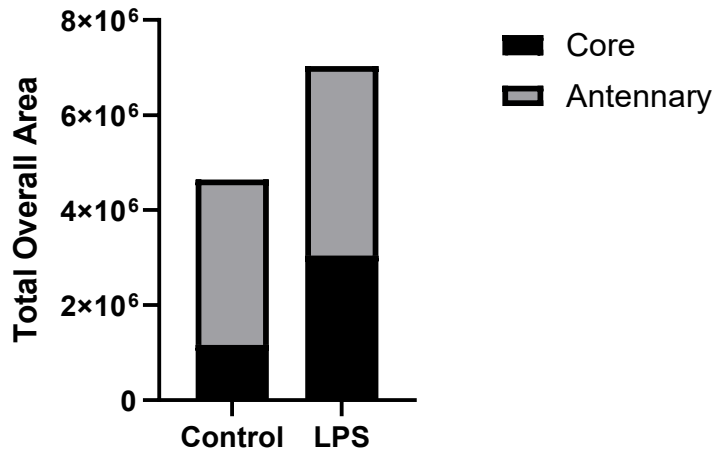


c  Microglia (HMC3)
MS/MS of 7612, core fucose
Precursor ion m/z 1034.04 (z=3+)

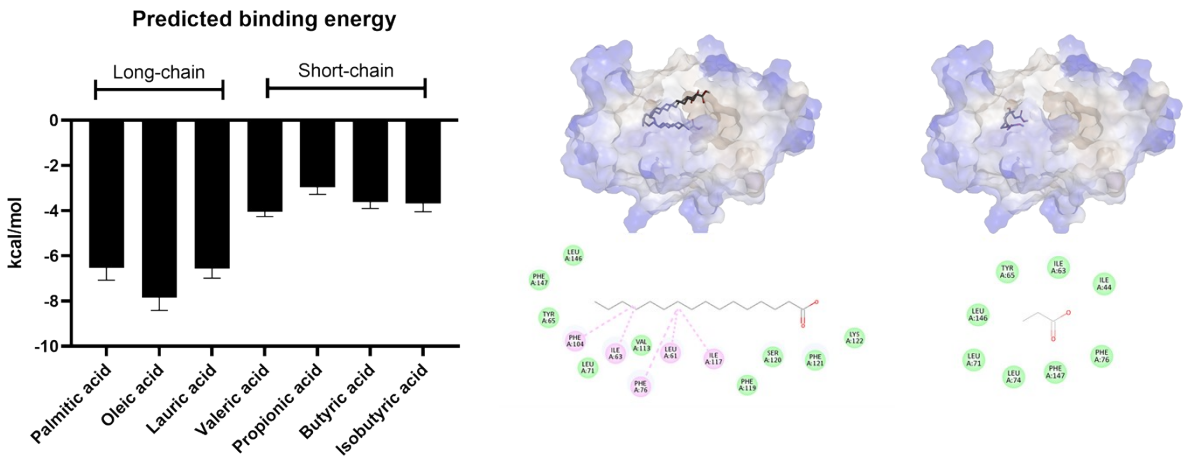


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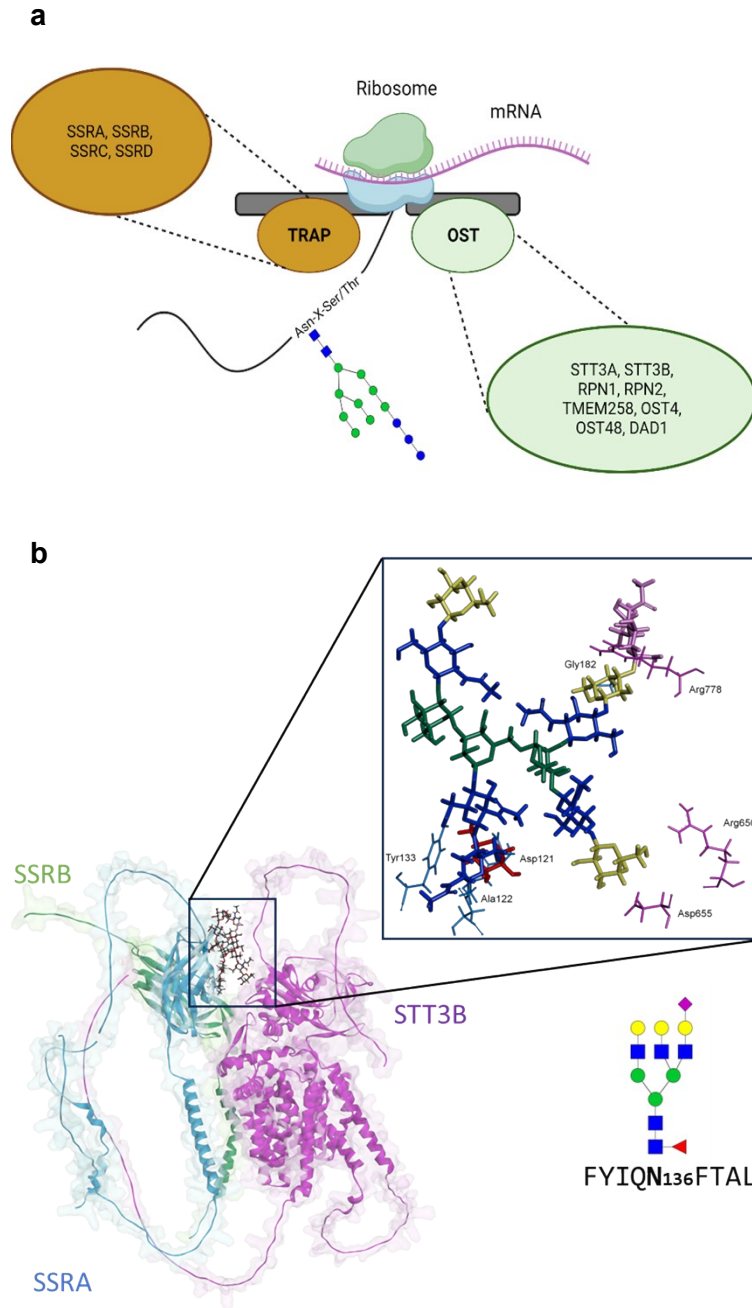
ry Figure 4. Tandem MS/MS spectra of select differentially expressed N-glycans affected by LPS in microglial cells.



Supplementary Figure 5. Observed Fucose Position in Sialofucosylated N-glycan, Hex7HexNAc6Fuc1Sia1. Determination of core vs antennary was done based on relative retention time (where Hex5HexNAc4Fuc1Sia1 with the highest peak area was used as the retention time marker).



Supplementary Figure 6. *In silico* calculations and models of binding between MD-2 protein and dietary (palmitic, oleic, lauric) and gut microbiota-derived (valeric, propionic, butyric, isobutyric) fatty acids. MD-2= myeloid differentiation factor 2



Supplementary Figure 7. Interaction between TRAP complex and OST complex. (7a)

TRAP and OST are in proximity which suggests possible interaction between the 2 protein complexes. (7b) *In silico* approach shows sialofucosylation is critical in the binding between SSRA and STT3B, catalytic subunit of the oligosaccharyl transferase (OST) complex that catalyzes the first step in protein N-glycosylation.