## **Glycomics Data Analysis Workflow**

Lists of candidate N- and O-glycan compositions known to be expressed in C. elegans were generated based previous reports (1-8). Glycan compositions known to be natively methylated in C. elegans were intentionally omitted in our targeted database as that information would be lost through permethylation. Phosphorylcholine-modified N-glycans were not considered in our study for simplicity. Glycan isomer abundances of a specific composition were summed as a single species. For each MS run, only scans after the 20 min mark (after column equilibration and sample loading) were considered. For each candidate glycan, MS/MS scans were identified where the precursor m/z was within 3 Da of the candidate m/z, considering charge states (z) of +1, +2, and +3. The background intensity of the precursors was calculated by first determining the max precursor intensity (maxPreInt) or all precursors matching a particular candidate glycan (numPrecursors), binning the precursor intensities to create an intensity distribution where the number of bins was equal to maxPreInt / numPrecursors, and then determining the value at the 15th percentile of the distribution to represent the background intensity (bgInt). All MS/MS scans with precursor intensity less than  $1.5 \times$  bgInt were discarded. The total ion count (TIC) for each glycan was calculated by summing the intensities from each peak in the assigned MS/MS scans in each MS run (glycanTIC).

A two-fold normalization method was utilized across the 35 runs for each sample type (*N*- and *O*-glycans) as follows. For each MS run, the sum of the TIC for all glycans was calculated (repSumTIC). The max replicate TIC sum (maxRepSumTIC) was determined for each Time Point. A normalization factor (repNormFactor) was determined for each replicate as repSumTIC / maxRepSumTIC for each Time Point. For the internal standard glycan (<sup>13</sup>C-permethylated isomaltopentaose, DP5), the intensity for each replicate was set to the maximum DP5 glycanTIC intensity over all replicates for each life cycle, under the assumption that an equal amount of that glycan is present in each replicate. For the first normalization, the glycan assignments for each replicate to calculate the normalized glycan TIC (glycanNormTIC). For the second normalization, the final glycan intensity value (glycanNormTICFinal) was calculated by dividing the normalized glycan TIC intensity by the standard normalized intensity (glycanNormTIC / stdNormTIC). Symbol and Text nomenclature for representation of glycan structures is displayed according to the Symbol Nomenclature for Glycans (SNFG) (9).

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