Supporting Information

Quantitative Capillary Zone Electrophoresis-Mass Spectrometry Reveals the N-glycome Developmental Plan during Vertebrate Embryogenesis

Yanyan Qu¹,³, Kyle M. Dubiak¹, Elizabeth H. Peuchen¹,⁴, Matthew M. Champion¹, Zhenbin Zhang¹, Alex S. Hebert², Sarah Wright¹, Joshua J. Coon², Paul W. Huber¹, Norman J. Dovichi¹*

1- Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA
2- Genome Center of Wisconsin, Department of Chemistry, University of Wisconsin, Madison, WI 53706, USA
3- Current affiliation: Astrazenca, Gaithersburg, MD 20878
4- Current affiliation: Pfizer, McPherson, KS 67460, USA

Corresponding author E-mail address: ndovichi@nd.edu
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Figure S1. A) Extracted ion electropherograms (EIEs) intensity of predominant aminoxylTMT\(^{15}\)-glycans having overwhelmed even the highest sialylated structure in stage 8 embryos, an intermediate time point of our chosen embryonic sequence. α-glucose oligomers were unexpected to dominate the spectrum, summing to 84% of the total signals; oligomannosidic and paucimannosidic N-glycans accounted for 15%; sialylated species were relative two orders of magnitude lower abundance compared to that of the most abundant oligomannose Man6, and even three orders of magnitude compared to glucose4. B) Hydrophilic-mode solid phase extraction (SPE) fractionation of sixplexed *X. laevis* embryo glycome. The aim in downscaling the dominant mass suppression was partially achieved; highest abundant Glucose 3 and 4 grouped with paucimannose were separated; while oligomannose still dominated the rest of fractions; glycan compositions with the most of diversity were richened in 85% ACN fraction.
Figure S2. CID-MS/MS spectra of aminoxylabeled neutral complex N-glycans in X. laevis embryo by CZE-ESI-MS/MS. A) G46100 (1125.9795, 2+), supported the presence of Gal extension on LacNAc antennae; Isomers with different fucose localization were observed; B) G56100 (818.6818, 3+), suggested the preference of bisecting biantennary structure with Gal extended LacNAc antennae; tri-antennary isomer might exist; and C) G56300 (916.0534, 3+), indicated the presence of Lewis antigens; tri-antennary isomer might exist. Glycan compositions were represented as G (HexNAc; Hex; Fuc; NeuAc; and phosphate).
Figure S3. CID-MS/MS spectra of aminoxylabeled sialylated composition G45010 (1117.4613, 2+) in X. laevis embryo by CZE-ESI-MS/MS. Possible isomers with different sialylated antennae pattern were observed (as suggested by the diagnostic ions of both m/z 698.2615 (1+, HexNAC$_2$NeuAc) and m/z 657.2349 (1+ HexHexNAC$_1$NeuAc$_1$)). Glycan compositions were represented as G (HexNAC; Hex; Fuc; NeuAc; phosphate).
Figure S4. CID-MS/MS spectra of aminoxyTMT\(^6\) labeled mannose-6-phosphate (M6P) N-glycans from *X. laevis* embryo by CZE-ESI-MS/MS. A) mono-phosphorylated high-mannose-type, G26001 (889.8510, 2+); and B) di-phosphorylated high-mannose-type capped with HexNAc, G37002 (1112.4087, 2+). Glycan compositions were represented as G (HexNAc; Hex; Fuc; NeuAc; phosphate).
Figure S5. HCD-MS/MS spectra of different aminoxyTMT labeled glycans derived from six *Xenopus laevis* embryo stages with TMT reporter ions zoomed-in.
Figure S6. Quantitative changes of individual neutral complex N-glycan composition with different degree of fucosylation. (*, p-value<0.05)

Figure S7. Lectin blots of *Xenopus laevis* embryo lysates at different stages. A) AAL; and B) UEA-I. The band at migration range labeled as 1 and 2 were excised from the membrane, digested, and analyzed by UPLC-MS/MS.
Figure S8. Formerly glycosylated peptides/proteins distribute different expression in early and late stage X. laevis embryos. A) Venn diagrams of N-glycoprotein species and N-glycosylation sites identified; and B) top 15 abundant N-glycosylation identified in early and late stage. Gene symbol and de-N-glycopeptide sequence were included.
Figure S9. Gene ontology (GO) enrichment analysis of N-glycoprotein species identified from stage 1 (left) and stage 41 (right) *X. laevis* embryos. GO categories searched include A) biological processes and molecular function; (B) cell component. *, enrichment significance $p$-value < 0.05; **, $p$-value < 0.01.