Resolving N-glycan Isomers in the *Drosophila* Glycome via Tandem Mass Spectrometry

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ABSTRACT

Precise differentiation of N-glycan isomer remains a major analytical challenge due to the high diversity of possible structures. In this work, we present a label-free, de novo N-glycan linkage-analysis platform based on logically derived tandem mass spectrometry (LODES/MS^n). By exploiting the predictable collision-induced dissociation (CID) behavior of sodium-adducted glycans, our method interprets distinct cross-ring and glycosidic fragment signatures to resolve subtle structural differences. We demonstrate that characteristic mass losses (M–60, M–90, M–120, and M–(90+R)) reliably distinguish 1→3, 1→6, and branch-defining linkages at the reducing end. This enables unambiguous structural assignment without chemical derivatization, thereby improving analytical speed, sensitivity, and applicability to complex biological samples.

We applied this platform to systematically profile the N-glycome of *Drosophila melanogaster* across wild-type and multiple glycosylation-gene knockout lines, including MGAT1-KO, α-mannosidase-IIa-KO, and Fused Lobes-KO. Comparative analysis revealed clear genotype-dependent remodeling of high-mannose, hybrid, and complex N-glycans, allowing reconstruction or modify of species-specific biosynthetic pathways. Notably, we discovered an paucimannose structure, termed 1079B, whose formation cannot be explained by current canonical pathways—suggesting that there might exist previously unrecognized enzymatic routes in the *Drosophila* glycosylation network.

REFERENCE

1. Lin Y-T, Tsai M-H, Yen C-C, Chen J-L, Liew CY, Huan Y-C, et al. Structural diversity at the beginning of multicellular eukaryotic complex N-glycan biosynthesis. *ChemRxiv*. **(2025)**