# Understanding Mammalian Metabolic Flux into N-glycans by Stable Isotopic Tracing Analysis

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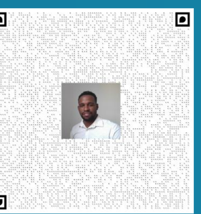


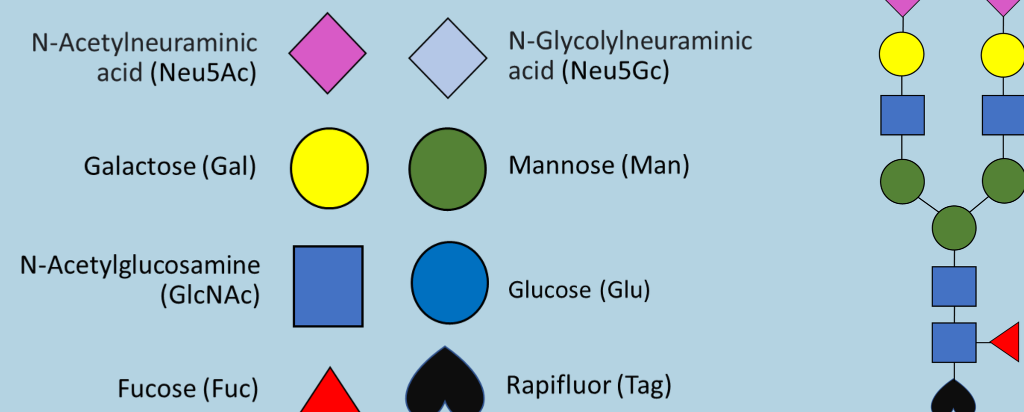

## Abstract

Congenital disorders of glycosylation (CDG) are one of the largest groups of inborn errors of metabolism with more than 170 types identified, most of which are under-studied. Among all the CDGs, PMM2-CDG is the most common and affects more than 50% of the CDG patients in the country. Recently, significant progress has been made in developing novel therapies for PMM2-CDG, including aldolase reductase inhibitors and mannose-1-phosphate based therapies. By increasing monosaccharide flux into N-linked glycoprotein biosynthesis, significant clinical improvement and developmental gains have been achieved in affected children on these therapies. This study aims to monitor the metabolic flux of uniformly labeled $^{15}N_2$-glutamine and $^{13}C_6$-glucose in human fibroblast cells and the whole-body tracing in mice of incorporated isotopically labeled N-glycans using in-depth high-resolution mass spectrometry (HRMS) methods.

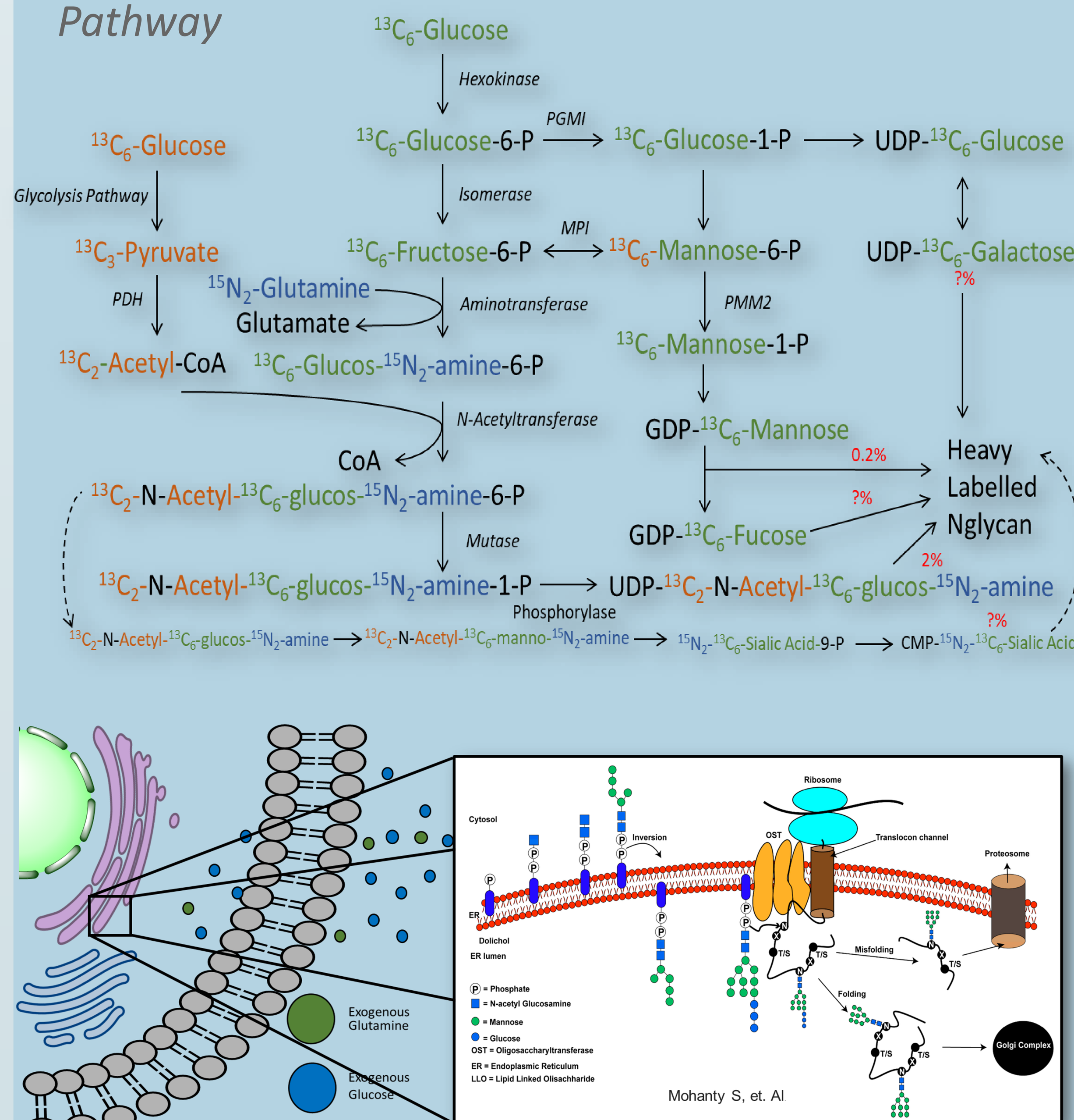
**PMM2-CDG (CDG-1a) Patients**: Childhood to Aldulthood. *Chang, I., et. Al.*

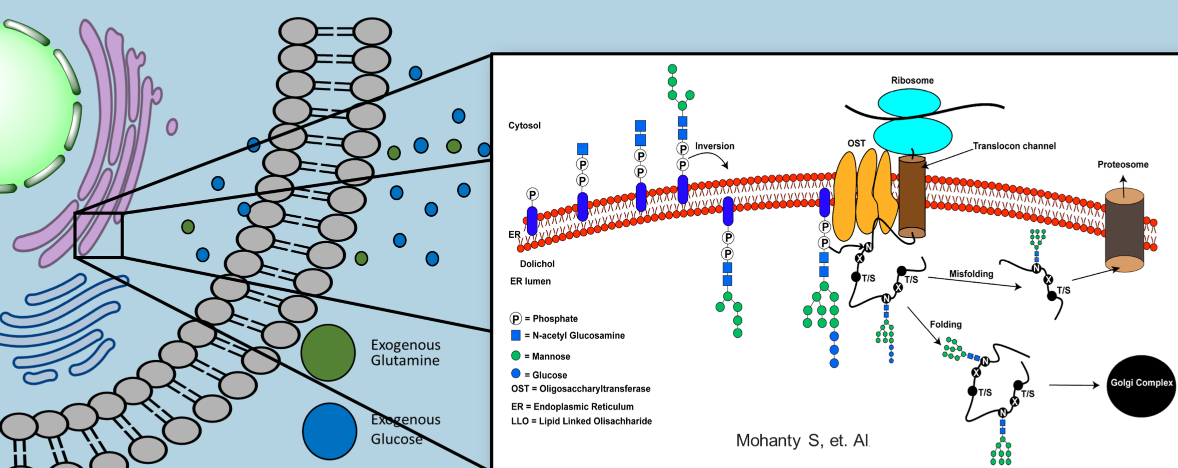
## Introduction

*N-Glycan Structure and Nomenclature.*



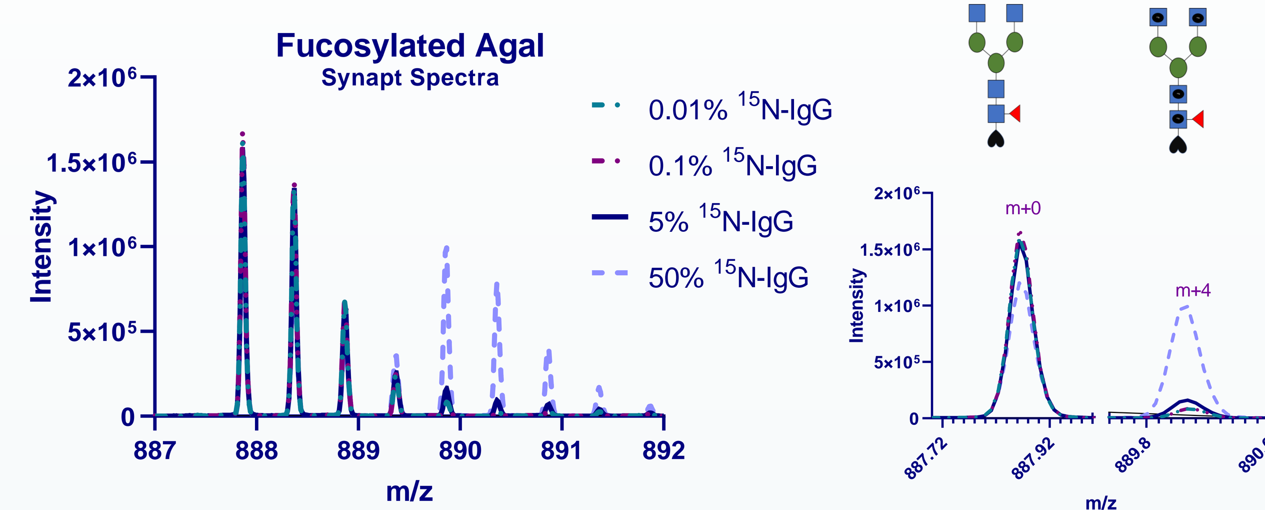

*Glucose and Glutamine Metabolic Flux to N-Glycan Pathway*



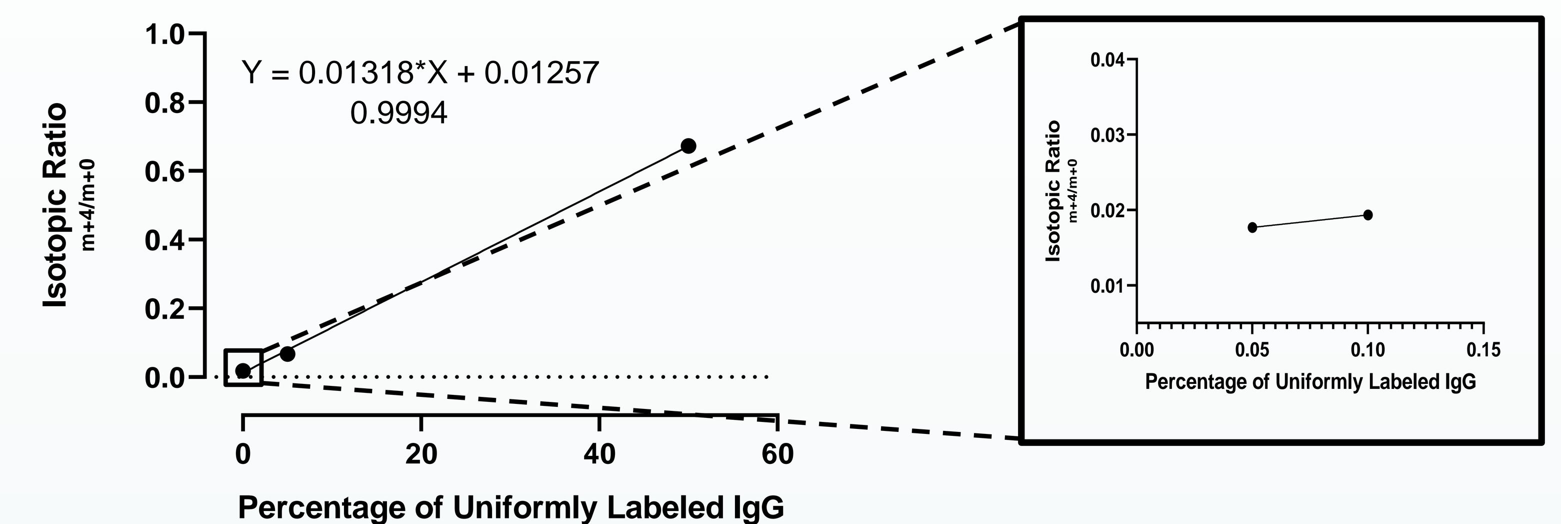



## Results

### *Quantification of heavy isotope $^{15}$N-Glycan in IgG*

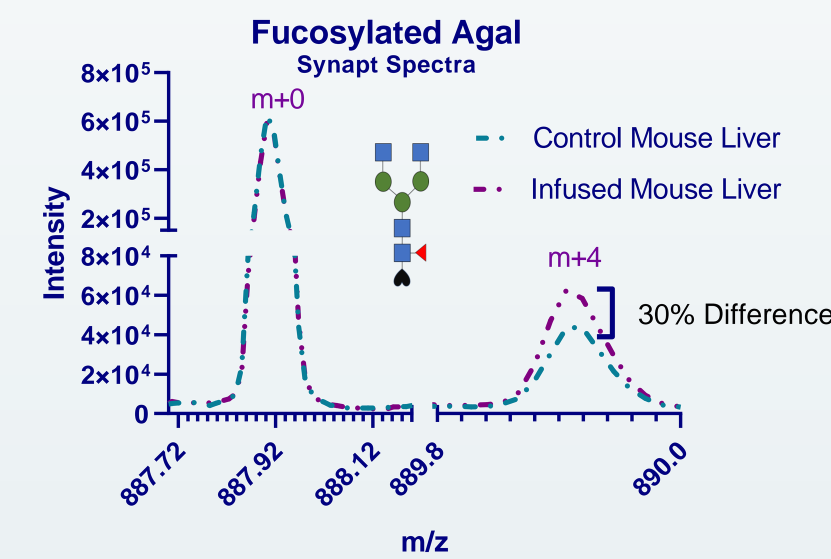



(Right) Overlay MS[1] spectra of Rapi-fluor tag Fucosylated-A-Gal N-Glycan mixture with labeled IgG and $^{15}$N-Labelled IgG to determine limit of detection of isotopically labeled N-glycan at a range of 0.01% to 50%. We determined that 0.1% mixture was the detection limit. (Left) Zoomed m+0 and m+4 isotopic peaks used for quantifying mixture.

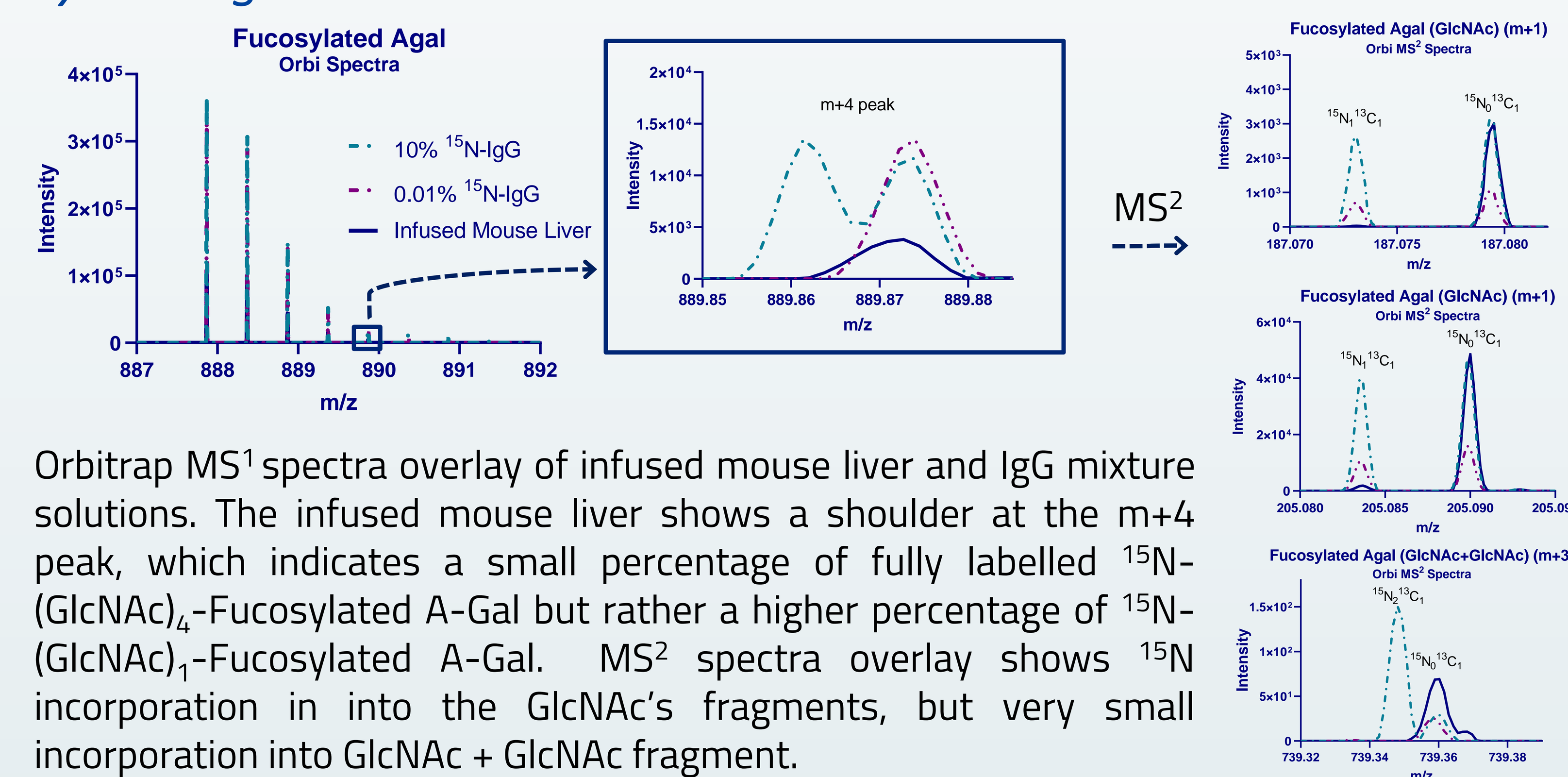



Standard curve of labelled Fucosylated-Agal by serially diluting 10ug/mL $^{15}$N-IgG N-glycan in 10 ug/mL IgG analyzed by Synapt G2-Si. The resolution and sensitivity allowed for large linear range that's use to estimate the amount of heavy labelled N-IgG N-Glycan. The linearity was shown to be 0.001ug/mL-10ug/mL (0.9994$r^2$) for $^{15}$N-Fucosylated A-Gal Glycan from IgG.

### *$^{15}$N-Glutamine whole-body tracing in mouse tissue*

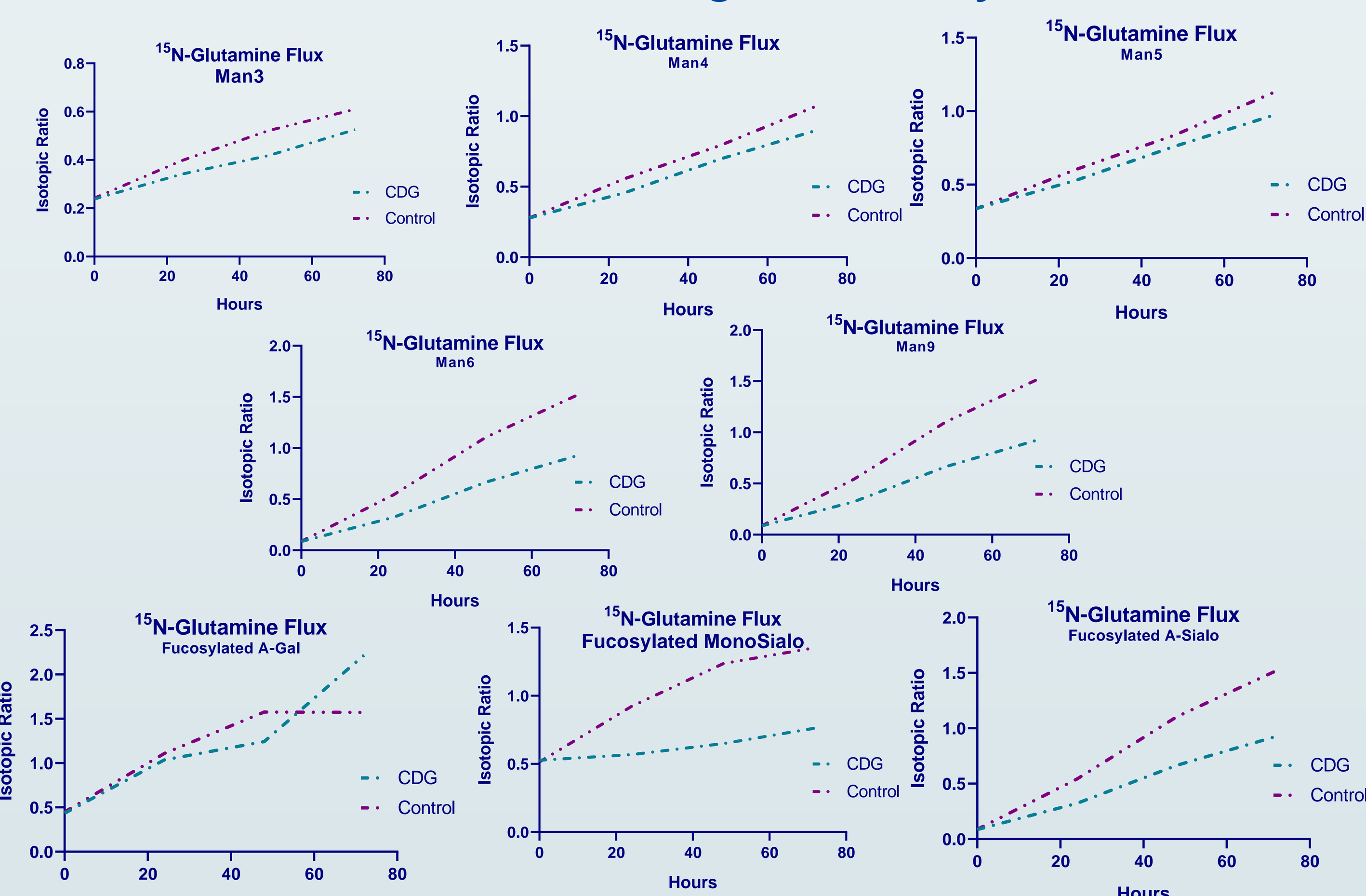



Synapt G2-Si MS[1] spectra overlay of infused mouse liver and wild-type mouse liver. Shows increase in the m+4 peak, which calculates to an increase of 5% of $^{15}$N-uniformally labelled Fucosylated A-Gal N-Glycan from a 4hr infusion $^{15}$N-Glutamine intravenously.



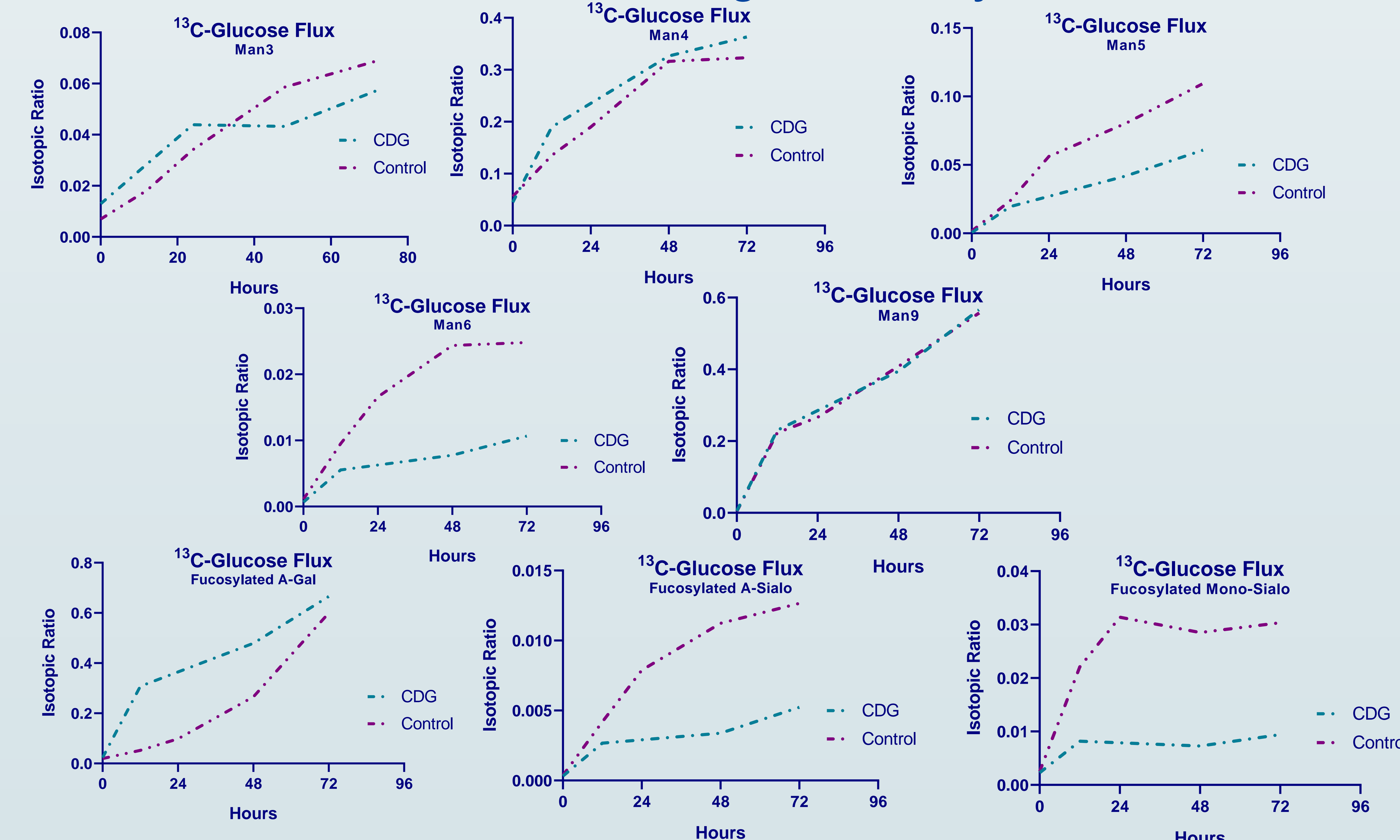

Orbitrap MS[1] spectra overlay of infused mouse liver and IgG mixture solutions. The infused mouse liver shows a shoulder at the m+4 peak, which indicates a small percentage of fully labelled $^{15}$N-$(GlcNAc)_4$-Fucosylated A-Gal but rather a higher percentage of $^{15}$N-$(GlcNAc)_1$-Fucosylated A-Gal. $MS^2$ spectra overlay shows $^{15}$N incorporation in into the GlcNAc's fragments, but very small incorporation into GlcNAc + GlcNAc fragment.

### *$^{15}$N-Glutamine tracing in human fibroblast*



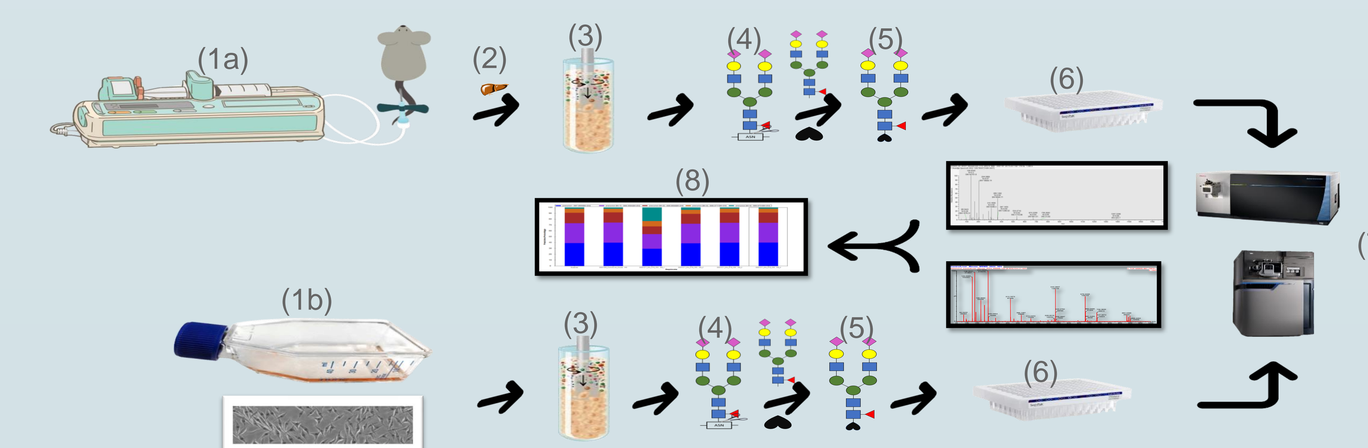

$^{15}$N-Glutamine flux for 24,48, and 72 hrs. into N-Glycan demonstrates impaired functionality in PMM2-CDG cell lines in comparison to normal fibroblast cells. Metabolic flux is increasingly distinguishable in complex and high mannose glycans in comparison to low mannose species.

### *$^{13}$C-Glucose tracing in human fibroblast*




$^{13}$C-Glucose flux for 12, 24, 48 and 72 hrs. into N-Glycan demonstrates similar impaired metabolic flux in PMM2-CDG cell lines of $^{15}$N-Glutamine. The data shows a profound difference with complex glycans and high mannose versus low mannose. The flux linearity differs from glutamine because the complex glucose metabolism, which affects a variety of monosaccharides at different stages of mammalian metabolic pathway into N-Glycans.

## Methodology




(1a) $^{15}N_2$-Glutamine Infusion. (1b) Human Fibroblast treated media. (2) Tissue Extraction. (3) Homogenizatinon & Cell Disruption. (4) PNGase. (5) Derivatization (6) Hilic Cleanup. (7) MS. (8) Data Analysis.

## Discussion

Our method provides an important paradigm to study mammalian metabolic flux into N-linked protein glycosylation both in vitro and in vivo.

Future direction would be to further characterize metabolic flux into each monosaccharide using $MS^n$ technology., also quantify metabolic flux in both additional mouse organ and in other CDG human fibroblast cell lines.