

Isotopic Detection of Aminosugars with Glutamine A Novel Quantitative Method for Glycomics

Ron Orlando

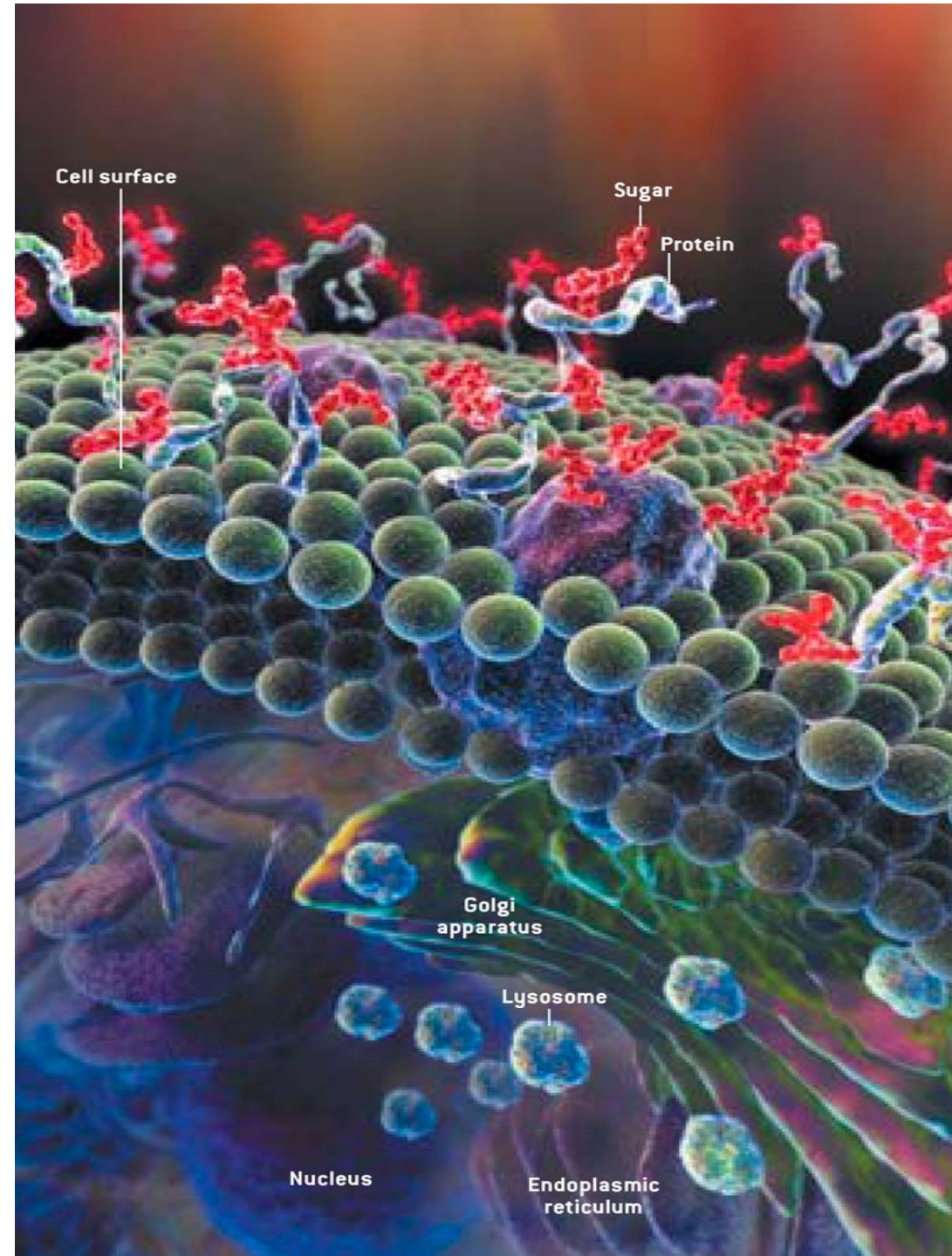
Complex Carbohydrate Research Center

University of Georgia

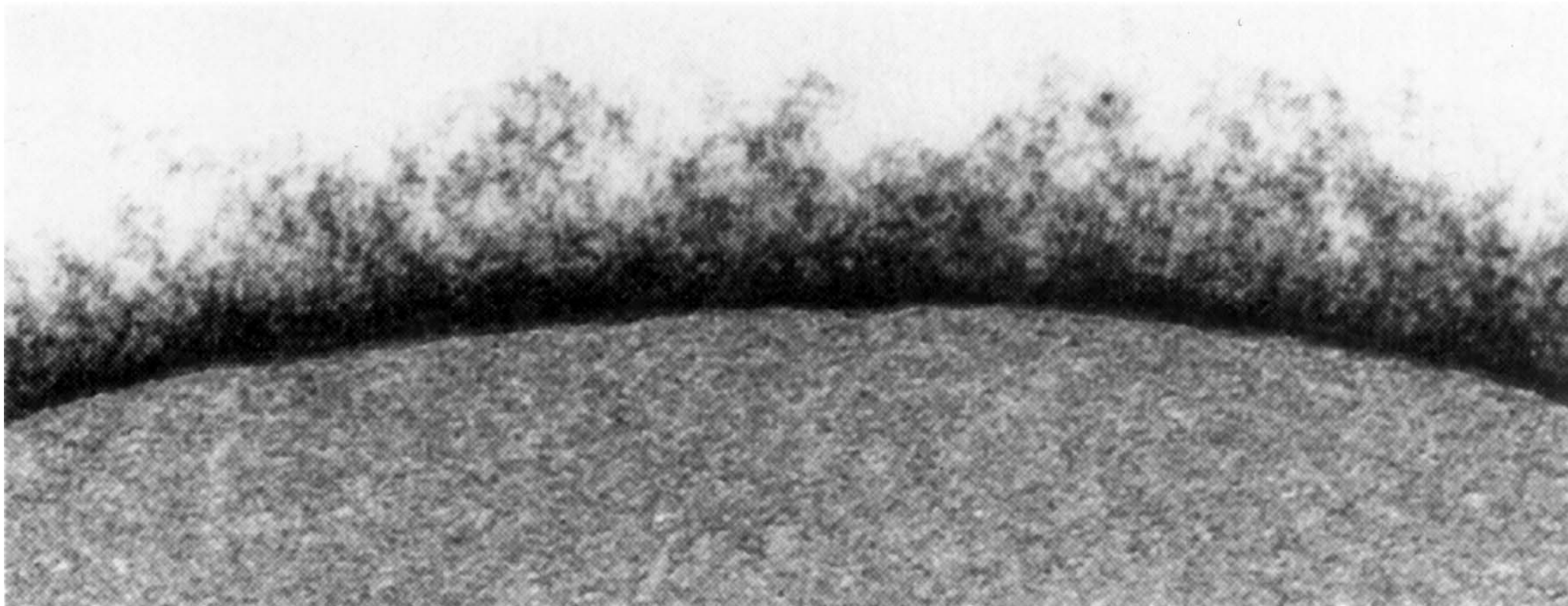
Athens, GA 30602



Glycoproteins constitute a large and heterogeneous class of glycoconjugates: most secreted or membrane bound/associate proteins are glycosylated



Extent of Cell Surface Glycosylation



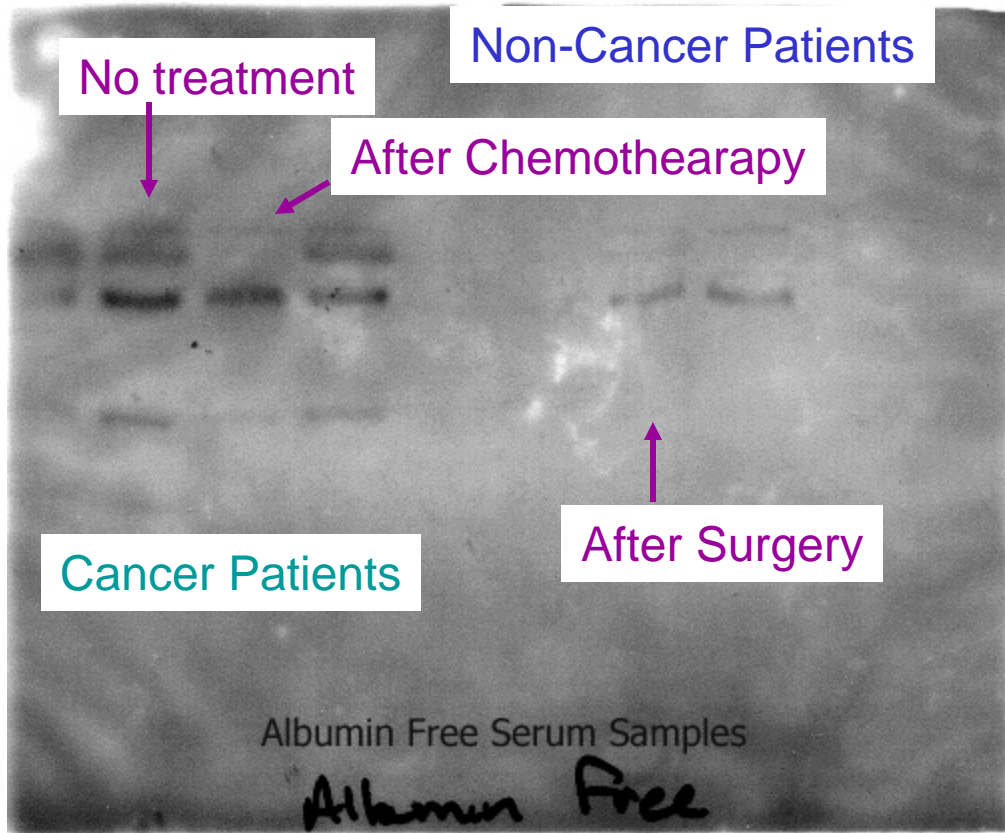
from S. Roseman, "Reflections on Glycobiology," J. Biol. Chem.
2001 276: 41527-41542

Importance of the Carbohydrate Chains attached to Glycoproteins

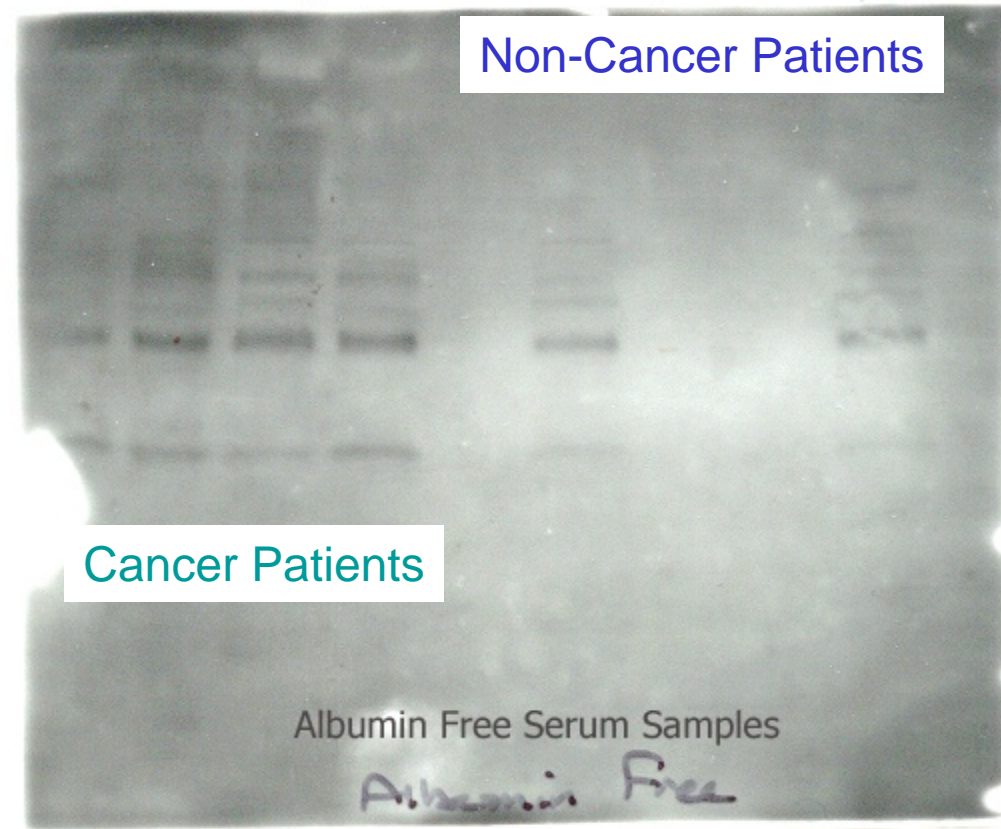
- 50-90% of proteins are glycosylated
- Often required for biological activity
- Required for proper protein folding
- Protect against proteolysis and thermal denaturation
- Participate in the immune response
- Change with condition of the cell/tissue

Glycosylation Changes associated with Ovarian Cancer Serum proteins

Lectin 1 - Fucose



Lectin 2 -Mannose



Glycomics

Proteomics
Identify Proteins
Quantify Expression

Glycomics
Identify Glycans
Quantify Expression

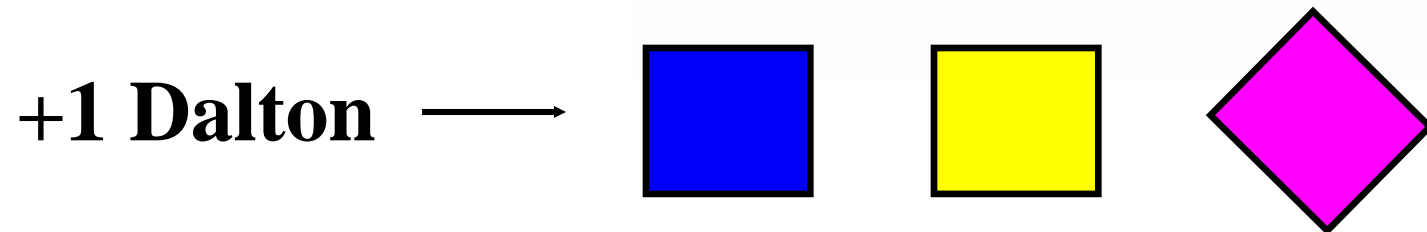
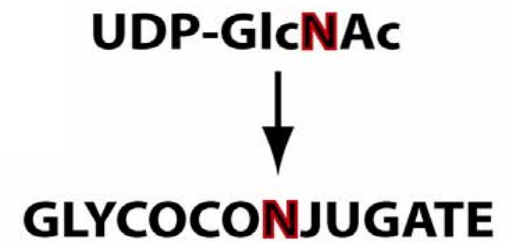
Glycoproteomics

Correlate Glycans to Glycosylation Sites
Identify Changes in Glycan Structures at Individual Sites
Determine Changes in Glycan Site Occupancy

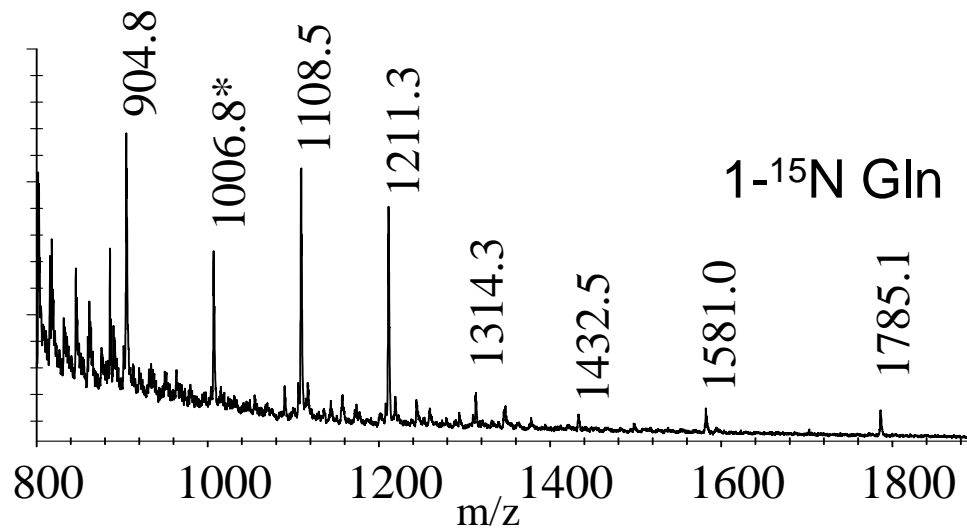
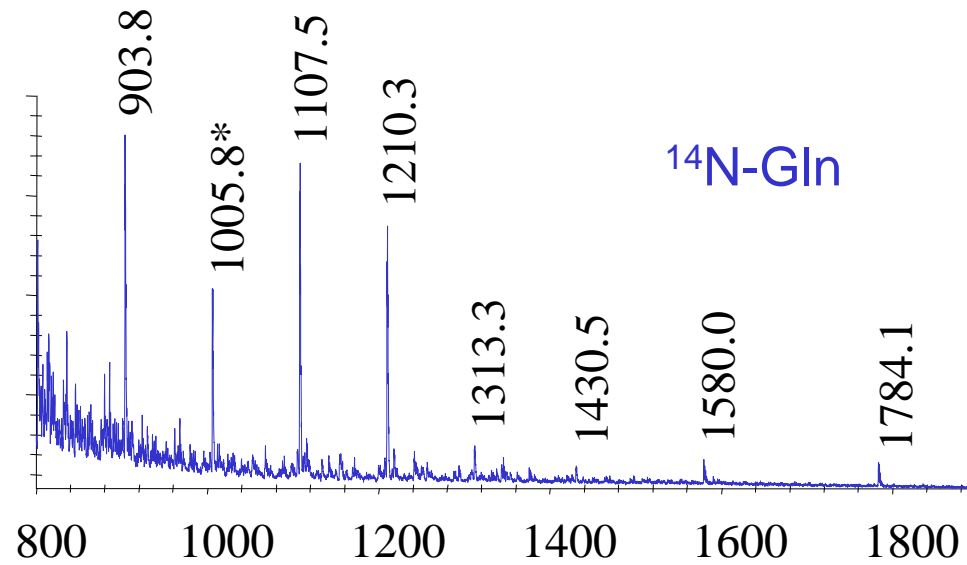
Methods for Quantitation – Proteomics/Glycomics

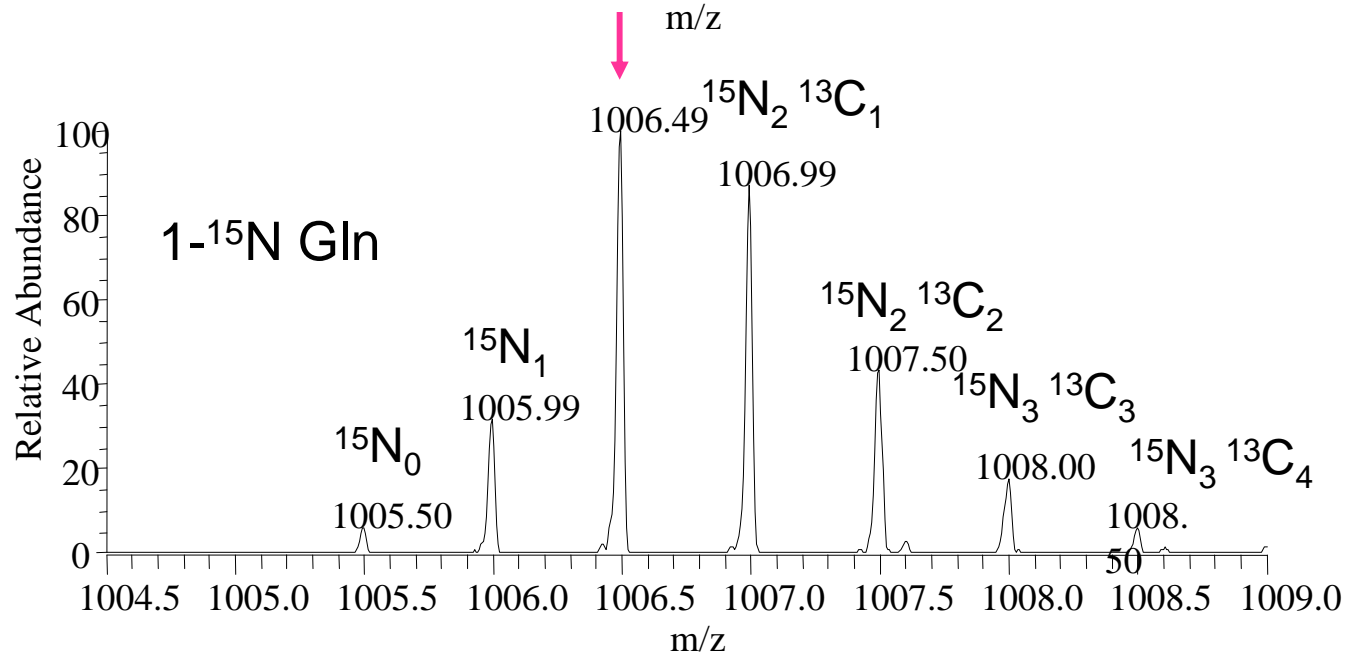
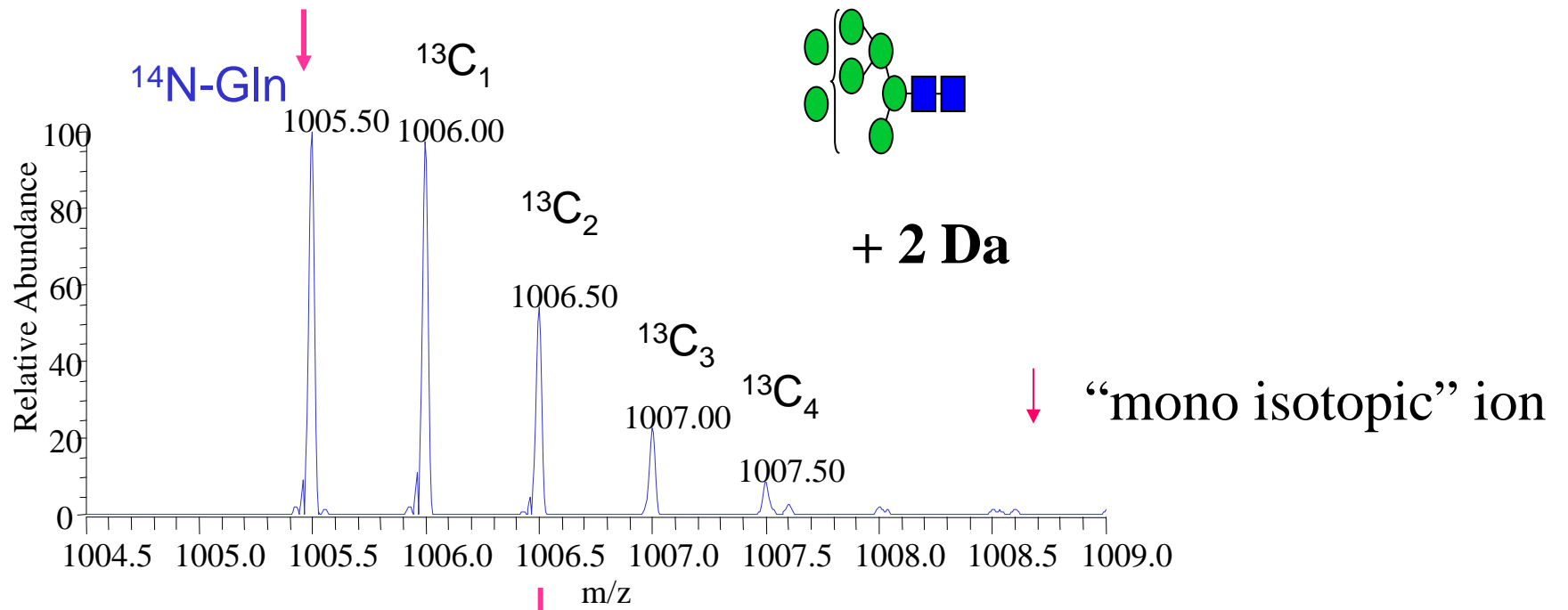
Label Free	AMT Spectral Counts	Total Ion Mapping
<i>in vitro</i> labeling	I-CAT, ^{18}O , etc. I-TRAQ	$^{12}\text{CH}_3$ - $^{13}\text{CH}_3$ QUIBL
<i>in vivo</i> labeling	SILAC	I-DAWG

IDA WG: Isotopic Detection of Aminosugars With Glutamine



^{15}N Incorporation into Glycoprotein Glycans, m ESCs





Labeling Efficiencies for a Variety of N-linked Glycans

# of Nitrogens	Composition	% ¹⁵ N Incorporation
2	(Hex) ₄ - (Man) ₃ (GlcNAc) ₂	98.1
	(Hex) ₅ - (Man) ₃ (GlcNAc) ₂	98.9
	(Hex) ₆ - (Man) ₃ (GlcNAc) ₂	98.7
4	(Hex) ₂ (HexNAc) ₂ (Deoxyhexose) ₁ - (Man) ₃ (GlcNAc) ₂	98.5
	(Hex) ₂ (HexNAc) ₂ (Deoxyhexose) ₂ - (Man) ₃ (GlcNAc) ₂	98.0
	(Hex) ₃ (HexNAc) ₂ (Deoxyhexose) ₁ - (Man) ₃ (GlcNAc) ₂	98.8
5	(Hex) ₁ (HexNAc) ₃ (Deoxyhexose) ₁ - (Man) ₃ (GlcNAc) ₂	100.3
	(Hex) ₂ (HexNAc) ₃ (Deoxyhexose) ₃ - (Man) ₃ (GlcNAc) ₂	100.6
	(Hex) ₁ (HexNAc) ₃ (Deoxyhexose) ₂ - (Man) ₃ (GlcNAc) ₂	100.3
Average		99.1

Conclusions

- IDAWG is introduced as an *in vivo* isotopic labeling strategy for quantitative - comparative glycomics/glycoproteomics.
- IDAWG is expected to be applicable to a variety of glycans
- Currently, the use of IDAWG in other cell lines, including those that are post mitotic, is being explored to optimize labeling times.
- IDAWG enables other studies, kinetics, pulse-chase, etc

The IDAWG approach is an easily applied and powerful new tool in the glycomics toolbox.

Acknowledgements

- Lei Cheng, Gerardo Alvarez-Manilla, James Atwood, Stephen Dalton, Kelley Moremen, Michael Tiemeyer, Lance Wells

- NIH

