Development of data-independent acquisition (DIA-MS) methods for glycan and RNA modification analysis

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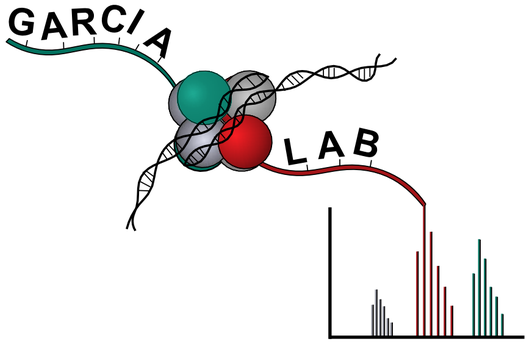

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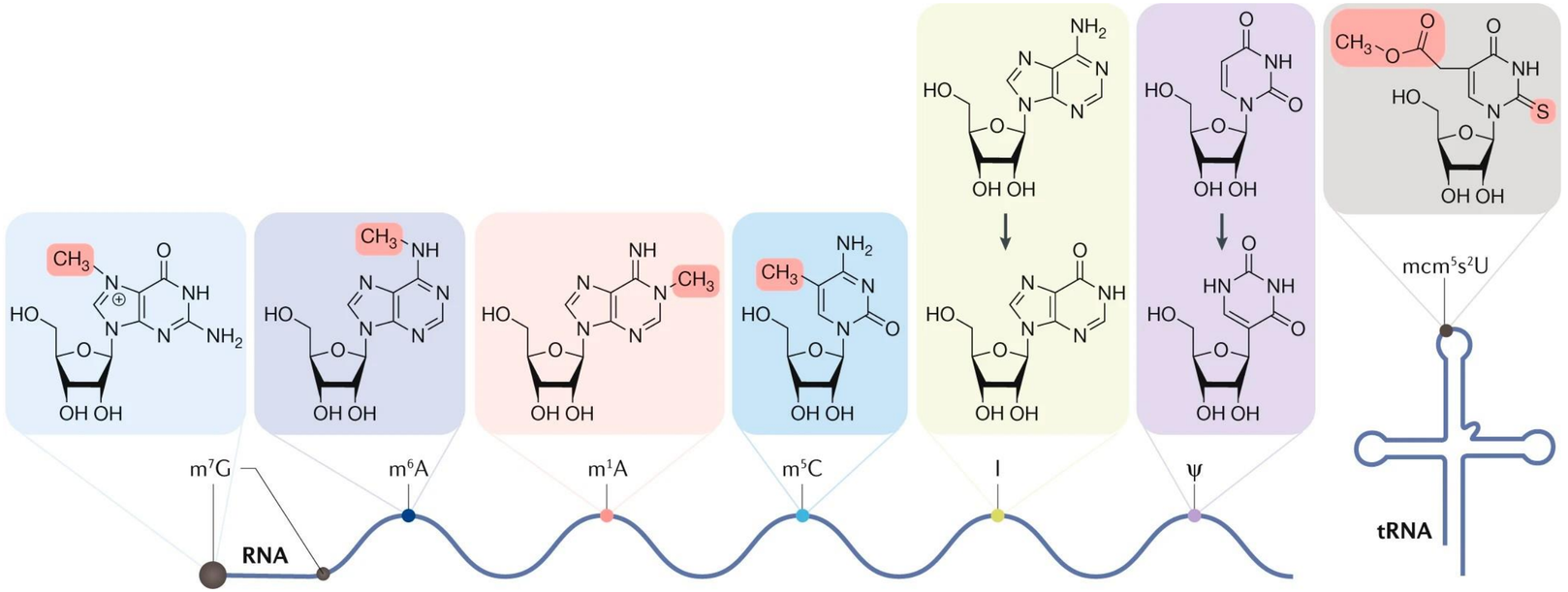
School of Medicine

RNA modifications are highly diverse and crucial

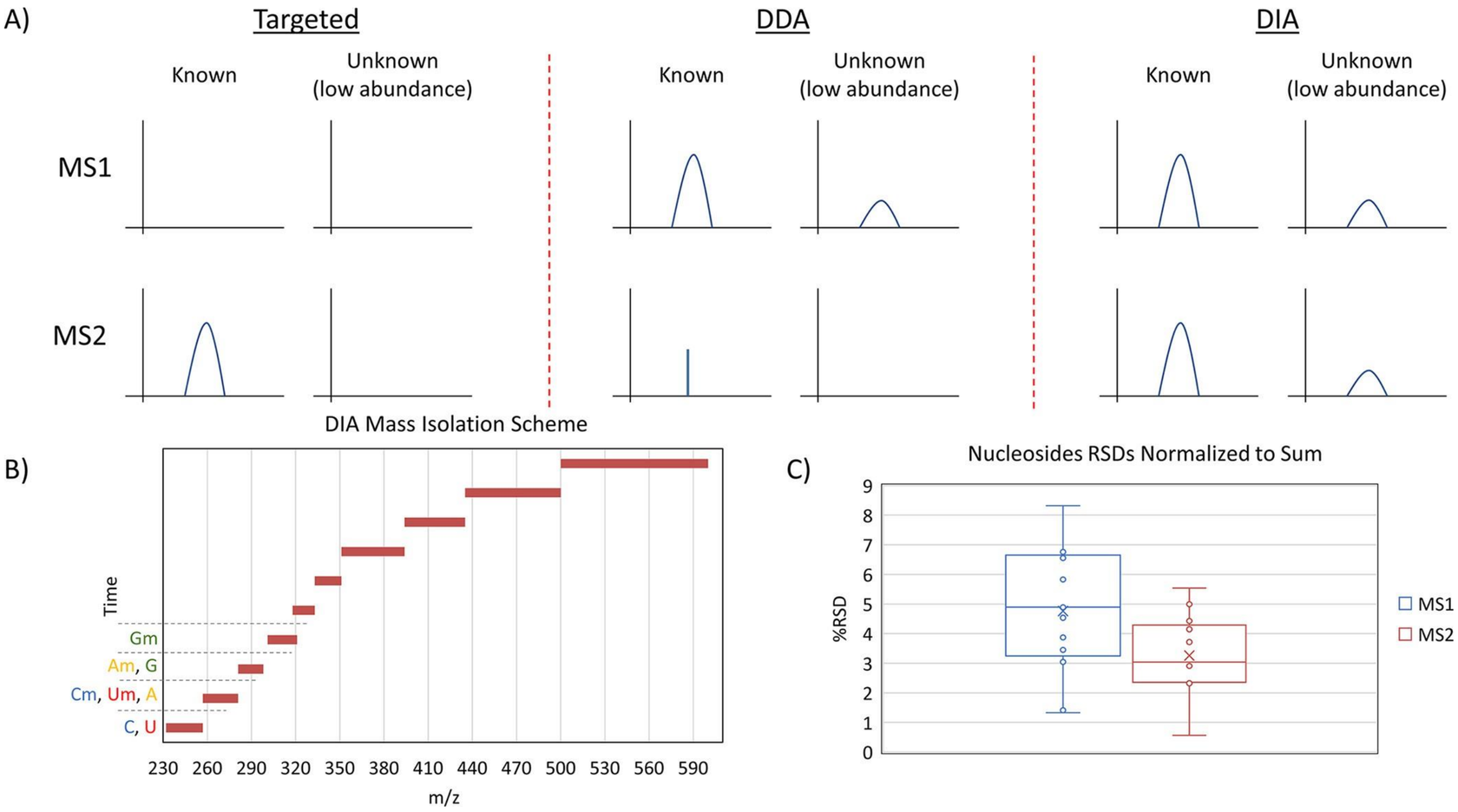


- Over 140 different types of modification have been shown to be present on RNA, and both coding and noncoding RNA species are modified, with ribosomal RNA and transfer RNA being the most heavily modified.
- RNA modifications are involved in many fundamental biological processes through modulating the RNA structure, RNA–protein interactions, and nucleic acid interactions.
- Understanding the function of these RNA modifications requires a reliable characterization and quantification.

Barbieri *et al.*, *Nat. Rev. Cancer*, **2020**

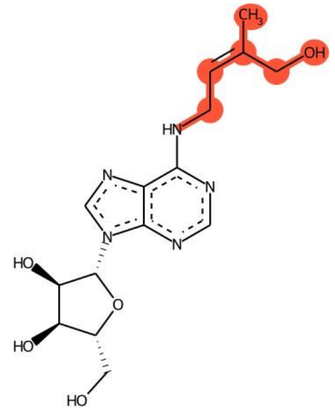
Data independent acquisition (DIA) for the detection of RNA Modifications

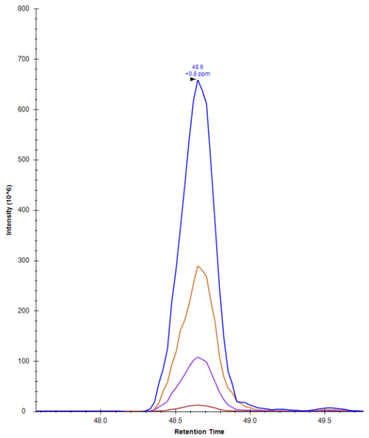
- MS-based methods have emerged as a powerful tool to analyze RNA modifications, and different modifications can be characterized in a single analysis.
- Targeted acquisition requires prior knowledge of analytes.
- DDA is not optimal for low abundant modifications.
- DIA allows for the detection of low abundant molecular species and MS2-based quantitation.



K. Janssen and **Y. Xie** *et al.*, *J. Am. Soc. Mass Spec.*, **2022**

io6A





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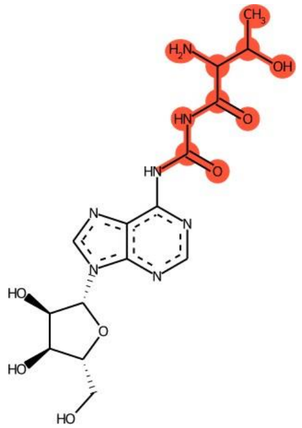
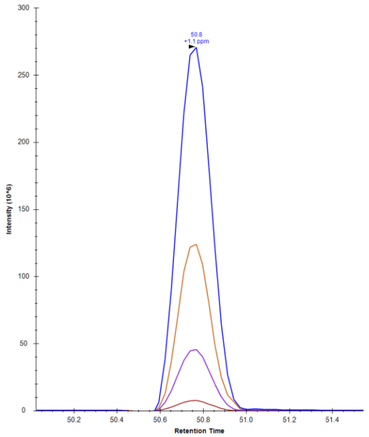
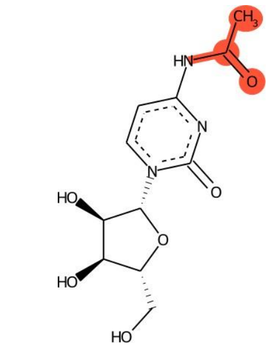
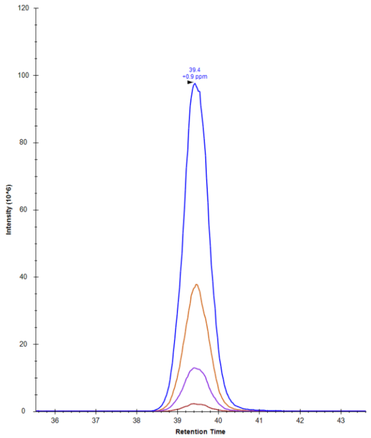


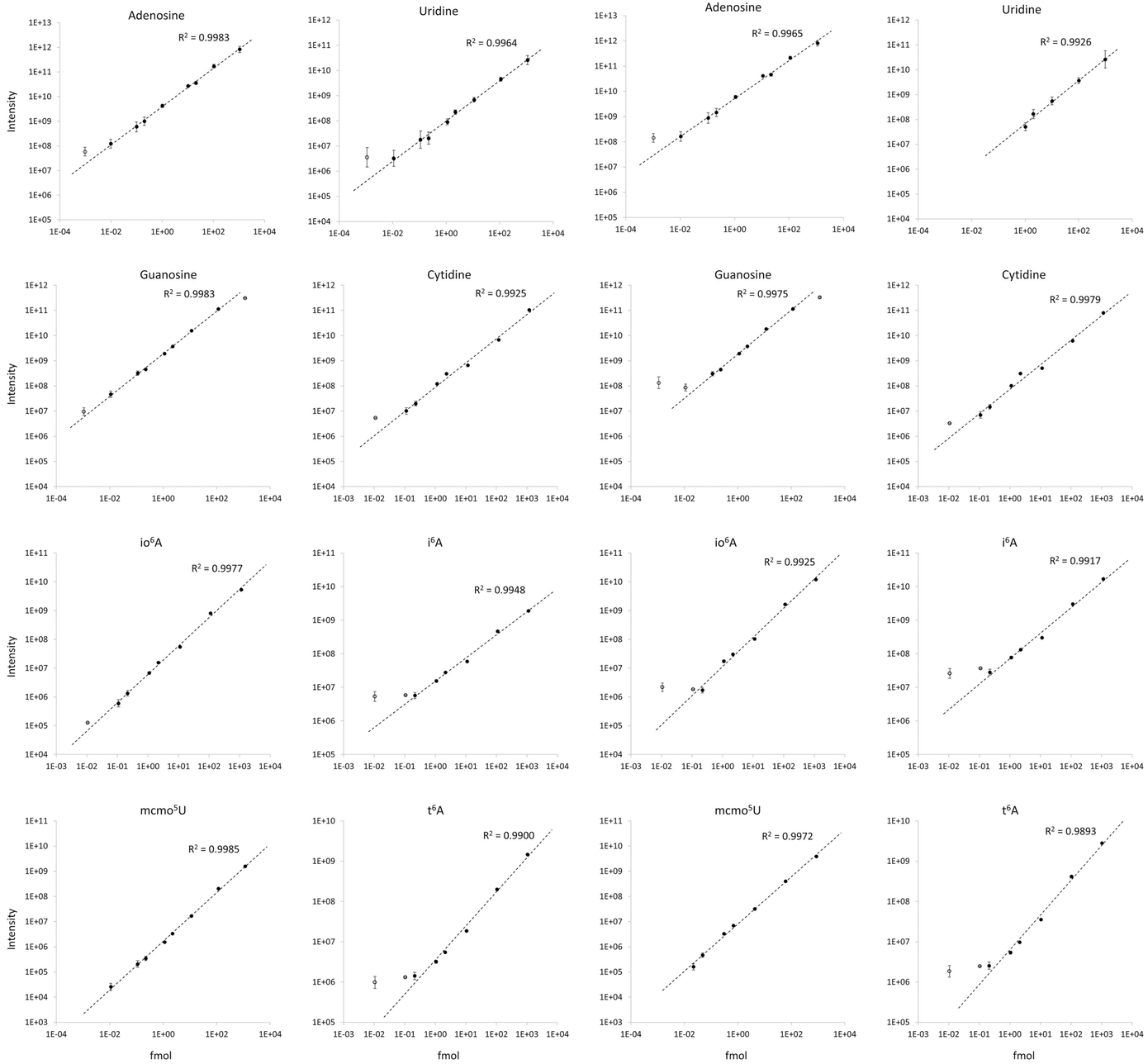


ac4C





MS2



MS1

The Skyline transition list is available at: *https://pubs.acs.org/doi/abs/10.1021/jasms.2c00065*

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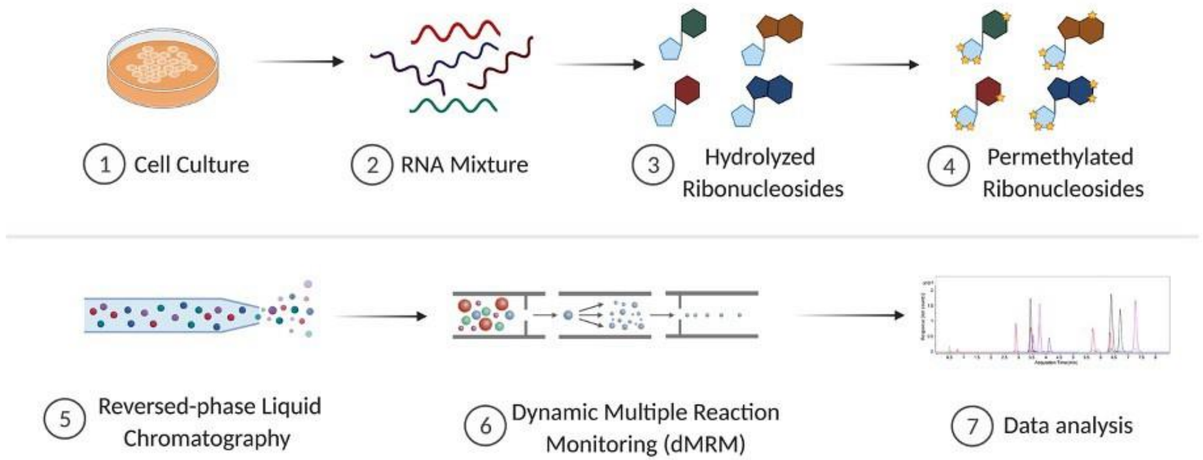
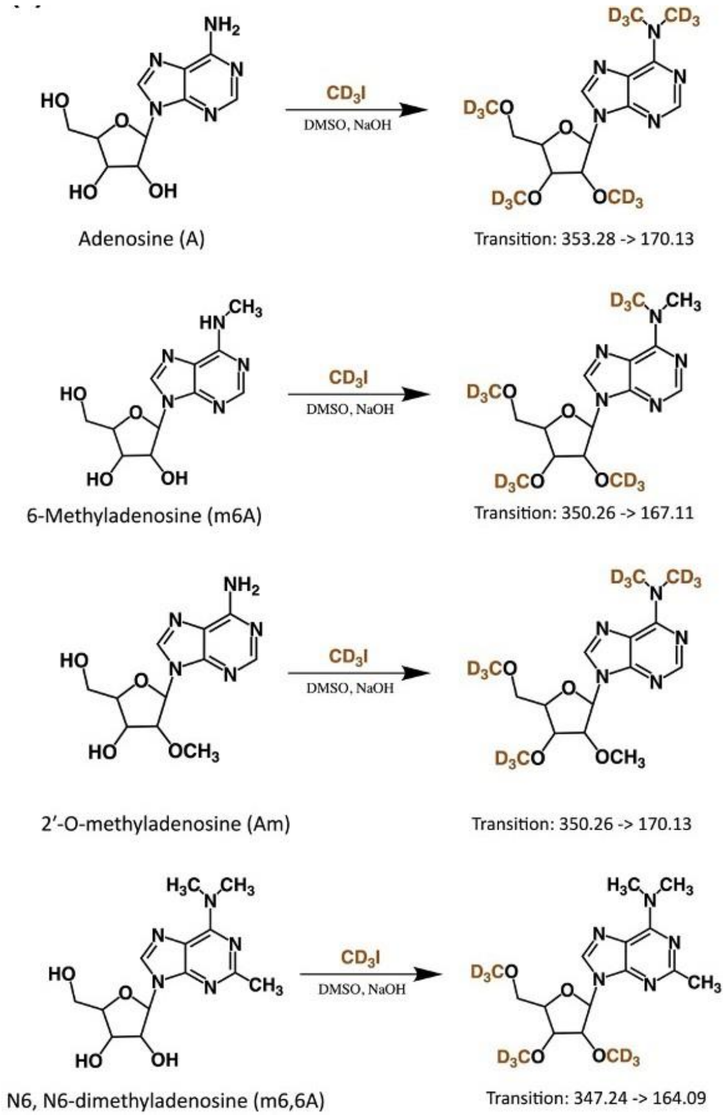
Permethylation provides enhanced characterization and quantification of RNA modifications



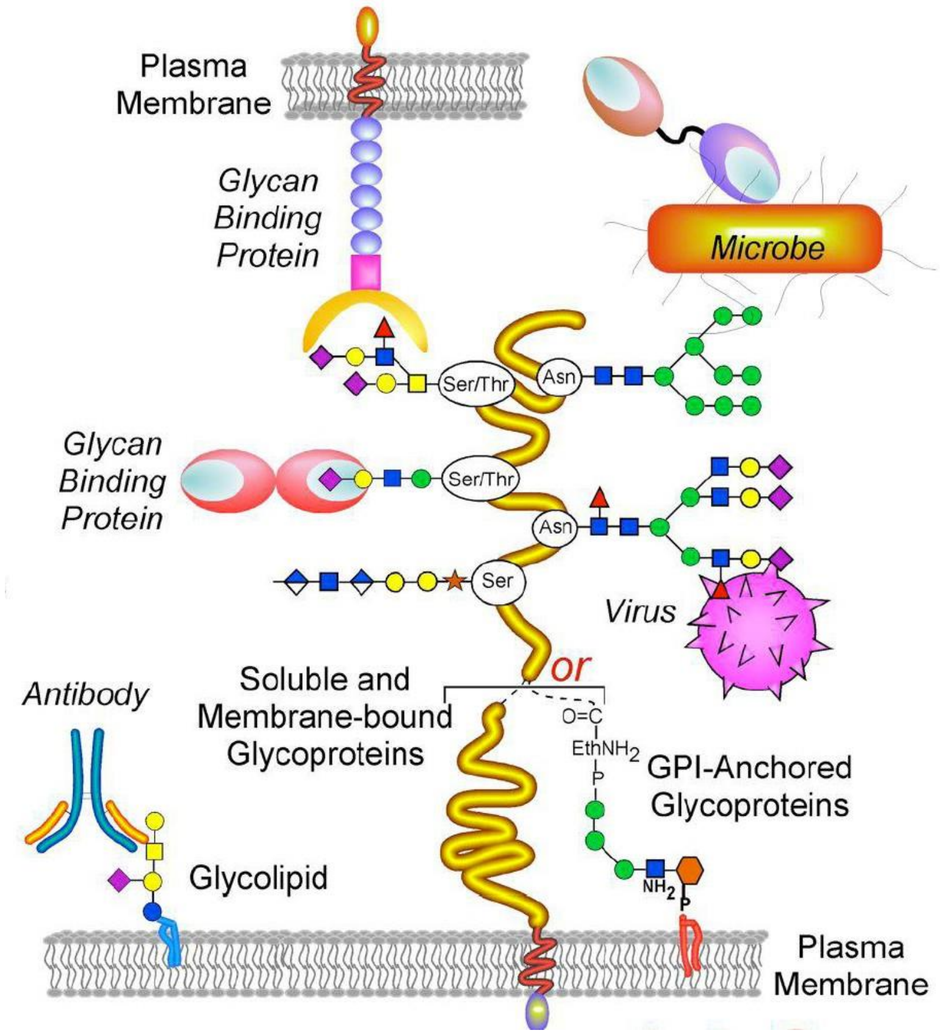
- The endogenous methylated ribonucleosides were distinguishable by different precursor and/or product ions with isotopically labeled iodomethane.
- The chemical reaction was highly predictable, this method could be easily extended to characterize currently unknown RNA modifications and DNA modifications.



Y. Xie *et al.*, *Anal. Chem.*, **2022**

Glycomics is critical for understanding biological functions

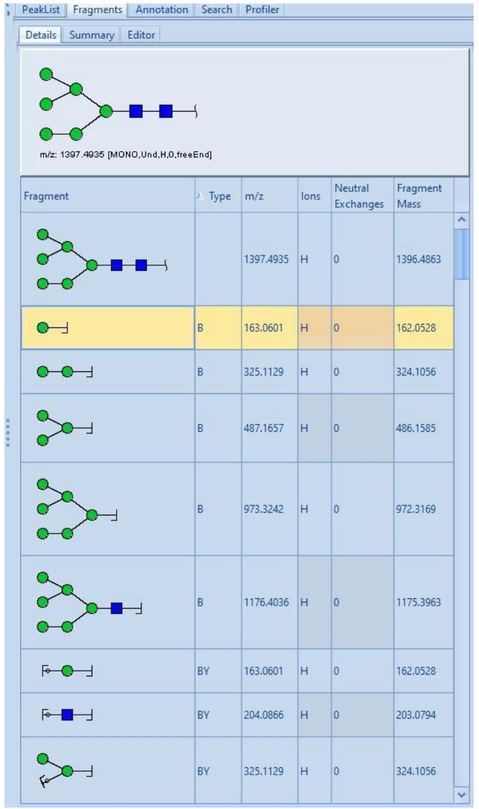
- Cell membranes are covered with a matrix of glycans on a scaffold of lipids and proteins.
- Glycosylation is critical for physiological and pathological cellular functions.
- Mass spectrometry has several important features that make it ideal for glycomic analysis.



More details about the glycanDIA method can be found in WP 207 Poster

Essentials of Glycobiology (2nd edition) **2008**

GlycanDIA data analysis workflow



Precursor Formula	Precursor Mass	Precursor Name	Precursor Type	Precursor Charge	Product Mass	Product Name	Product Charge
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	145.0495	Z-Hex1-B	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	163.0601	Hex1-B,Y-Hex1-B,Z-Hex1-C	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	181.0707	Hex1-C,Y-Hex1-C	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	186.0761	Z-HexNAc1-B	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	204.0866	exNAc1-B,Z-HexNAc1-C,Z-HexN	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	222.0972	Y-HexNAc1-C,Y-HexNAc1	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	307.1024	Z-Hex2-B	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	325.1129	Y-Hex2-B,Hex2-B,Z-Hex2-C	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	343.1235	Y-Hex2-C,Hex2-C	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	407.1660	Z-HexNAc2	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	425.1766	Y-HexNAc2	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	469.1552	Z-Hex3-B	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	487.1657	Y-Hex3-B,Z-Hex3-C,Hex3-B	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	505.1763	Y-Hex3-C,Hex3-C	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	551.2083	ZZ-Hex1HexNAc2	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	569.2188	YZ-Hex1HexNAc2	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	587.2294	YY-Hex1HexNAc2	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	631.2080	Z-Hex4-B	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	649.2186	Y-Hex4-B,Z-Hex4-C	[M+H]
C52H88O41N2	1396.4863	6_2_0_0	HM	[M+H]	667.2291	Y-Hex4-C	[M+H]
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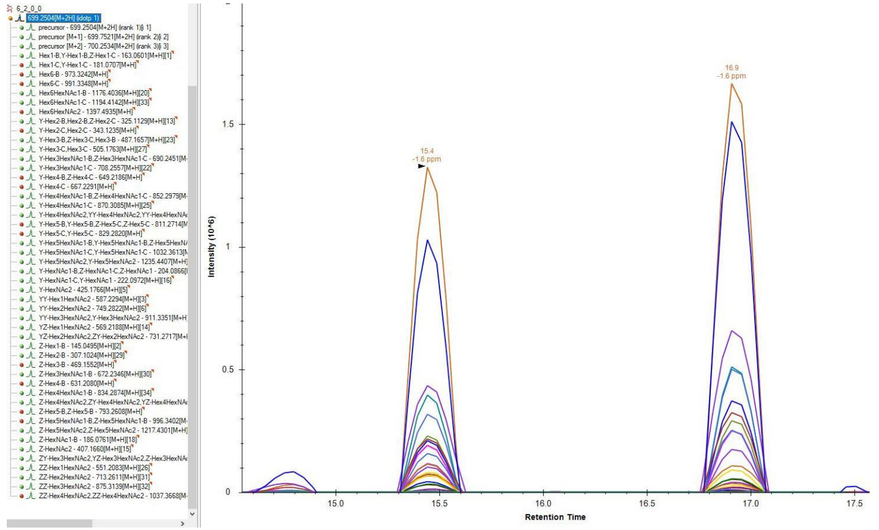
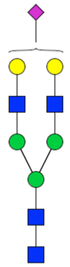


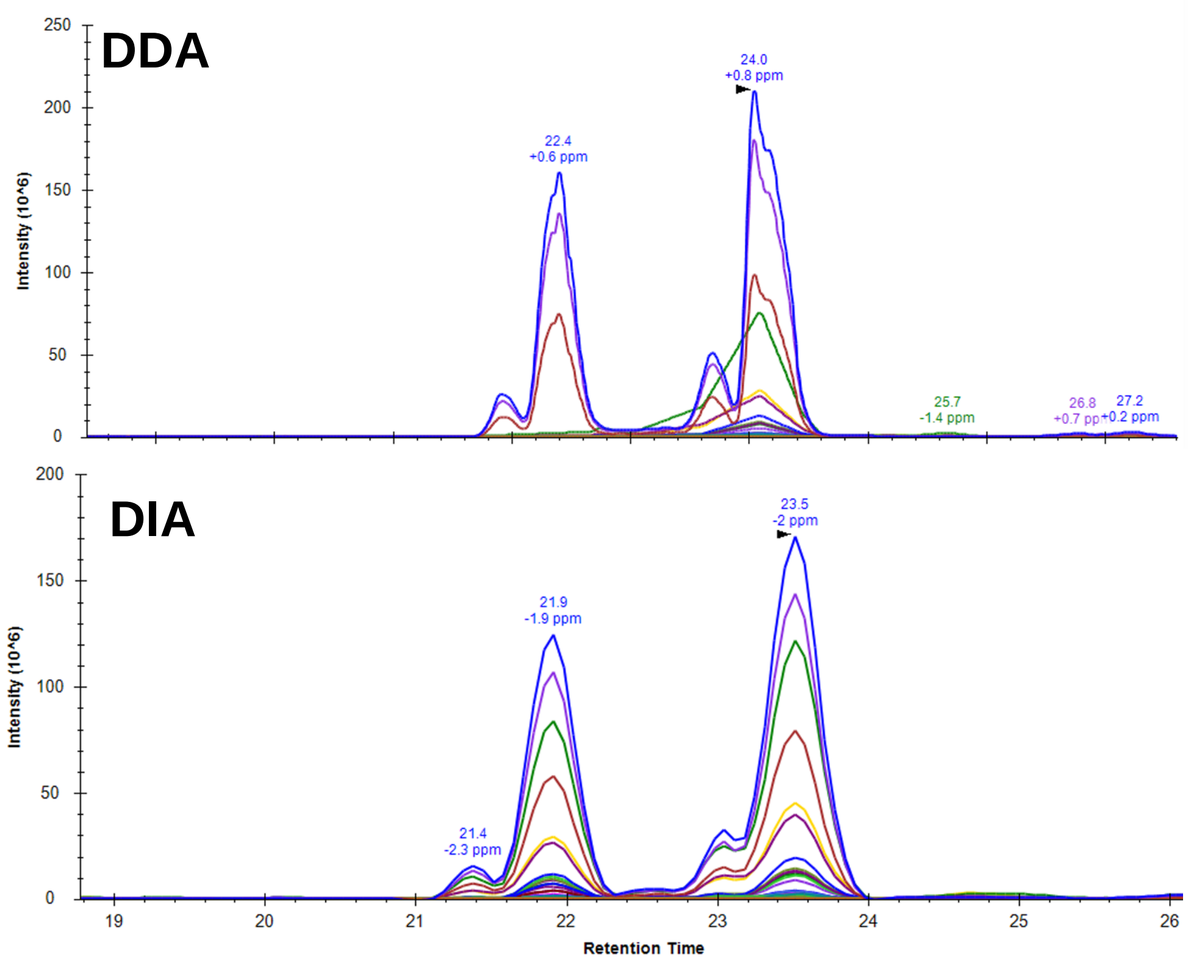
(a) Predict glycan fragmentation using GlycoWorkbench

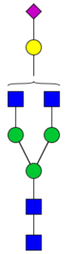
(b) Build transition list for different glycans

(c) Identification and Quantification using Skyline

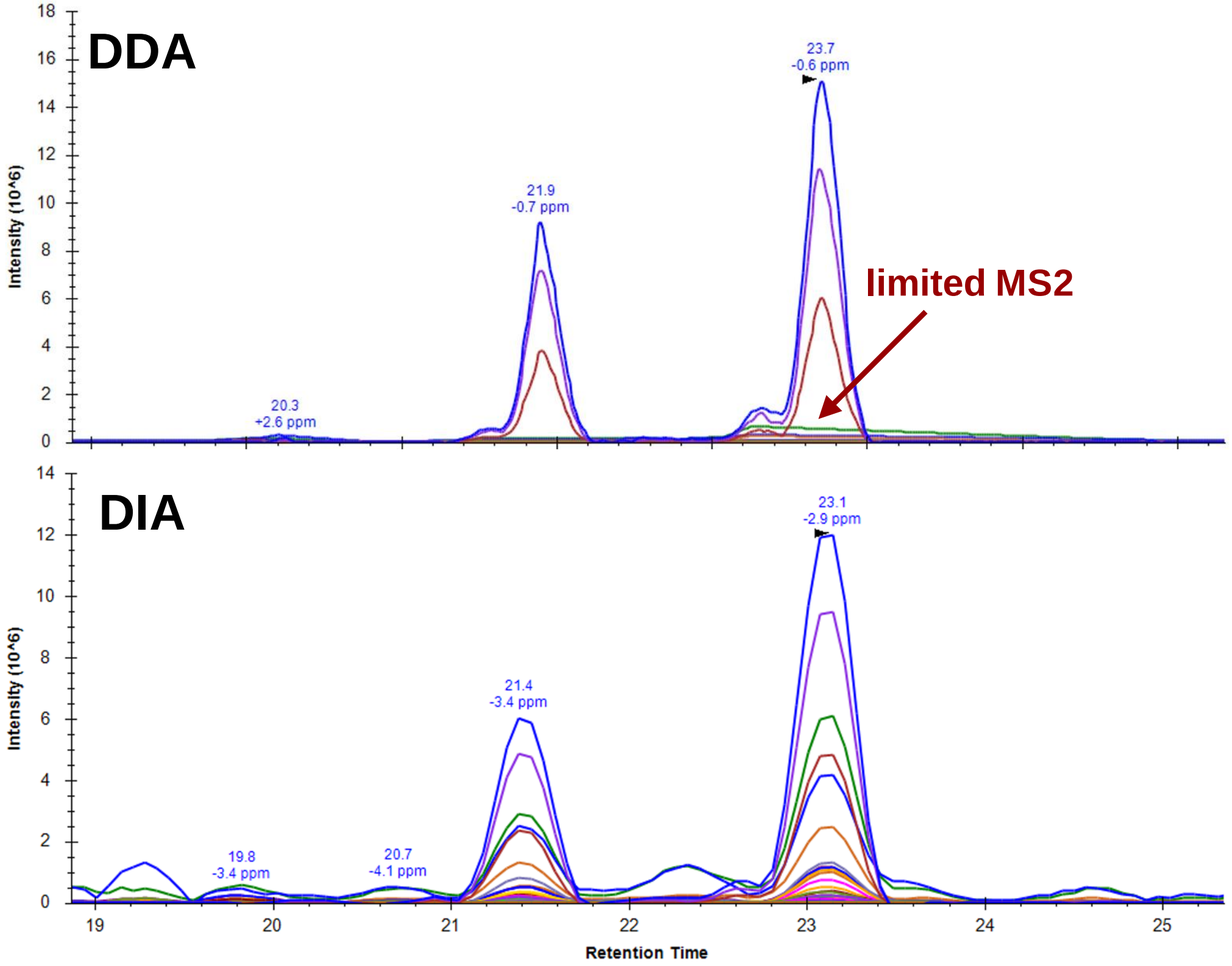


High abundant 5401 glycan





Low abundant 4401 glycan



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