Results of the 2010 Glycoprotein Research Group's (gPRG) study on Quantitative Glycoprotein Analysis

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Objectives

Identify the major N-linked glycans detected in three similar glycoprotein samples

Quantify the relative differences in the distribution of the N-linked glycans

Study Design

Problem: We wanted a sample set with known glycan changes. It is impossible to produce a single glycoprotein with known changes in N-linked glycosylation.

Answer: Use a mixture of glycoproteins that have unique glycans, change the glycoprotein ratios, and thus change the glycan ratios in a known manner.

Study Design – II

Glycoproteins selected:

Ovalbuman (Ov) – high mannose and hybrid glycans

Asialo-fetuin (AF) – bi- and tri-antennary complex glycans without sialic acids (no NANA or NGNA)

α1 acid glycoprotein (α1) – complex glycans all terminated with sialic acids

Human – all NANA

Bovine – NGNA and NANA

Study Design – III

Glycoprotein Mixtures: (ratios W:W:W:W)

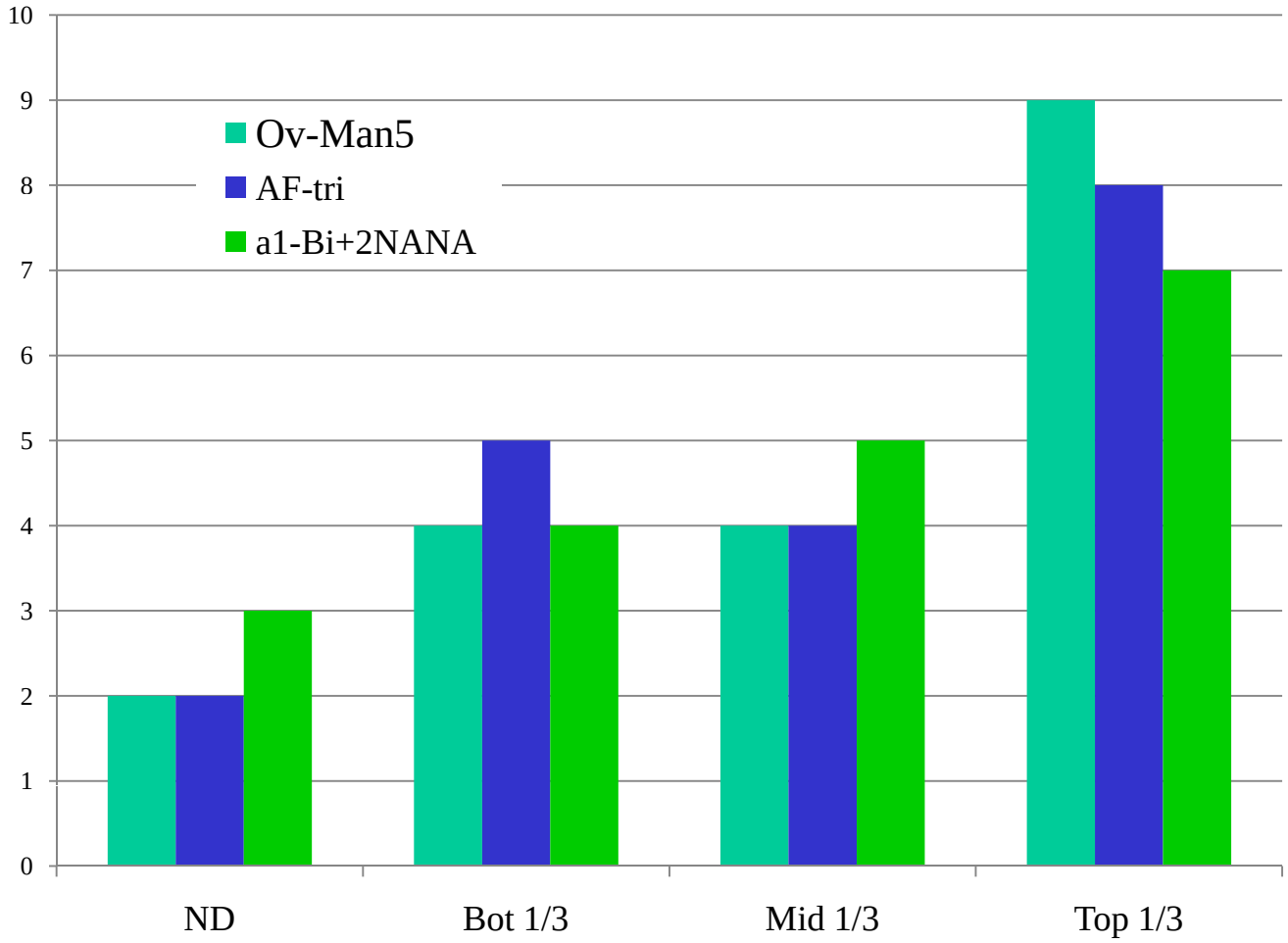
	OV	:	AF	:	α1H	:	α1B
GPRG-1	20	:	1	:	4	:	0
GPRG-2	20	:	0.3	:	4	:	0
GPRG-3	20	:	2	:	0	:	4

500 μg of each sample sent out

Double blind study

Study Design – III

Glycans from each sample were readily detectable by most labs



General Results

35 – Samples requested --- 19 – Data submissions

7 ABRF Members – 12 Non-members
10 Academic – 7 Industry – 2 Vendors
14 North America – 4 Asia – 1 Europe
10 Core labs

Most (18 of 19) labs released glycans with PNGase F

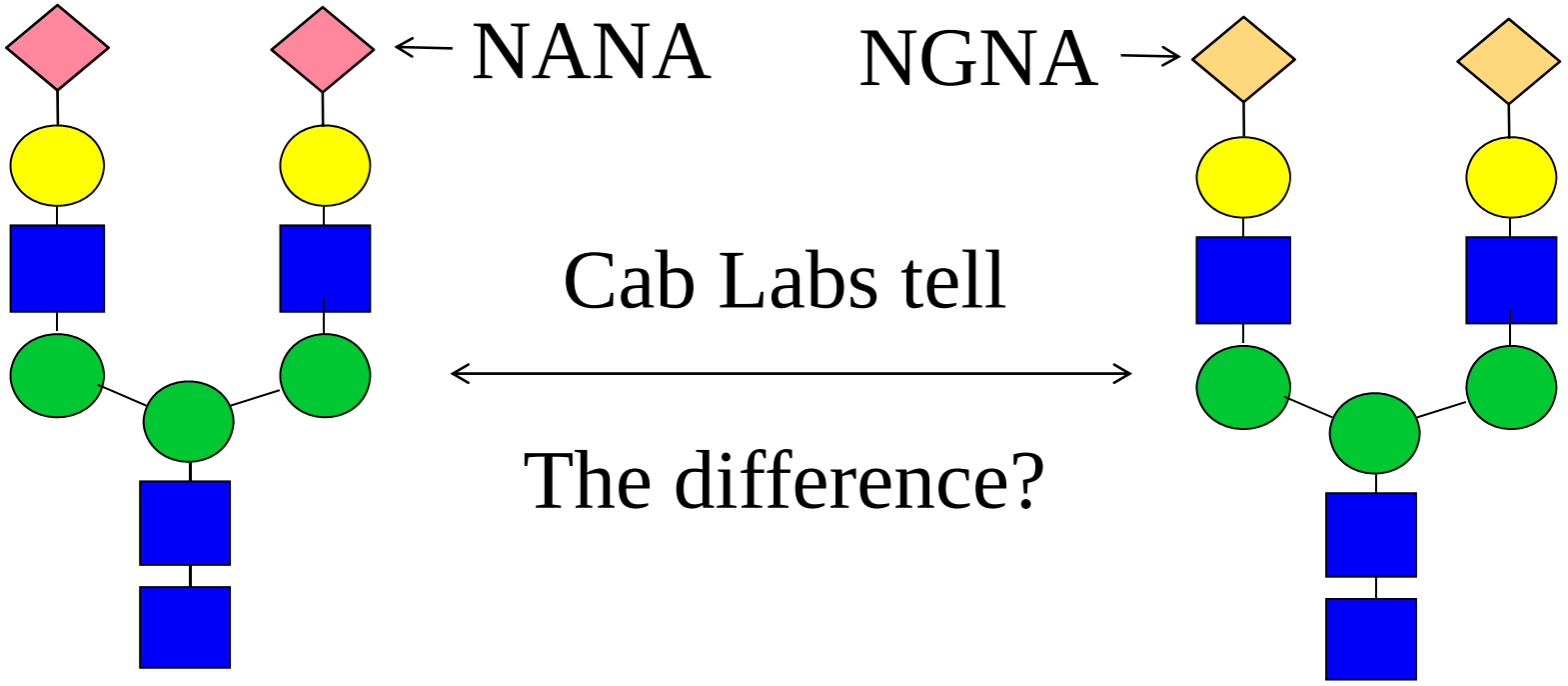
All labs analyzed with MS
10 MALDI – 7 LC/MS – 2 ESI

Identification NGNA – I

Sialic Acids typically cap complex glycan?

humans exclusively have NANA

other mammals have NANA and NGNA



NGNA only present on α1 Bovine (only in GPRG-3)

Identification NGNA – II

8 **Not detected** – 11 detected

Not Detected Trends:

6 LC-MS (from total of 7 LC-MS)

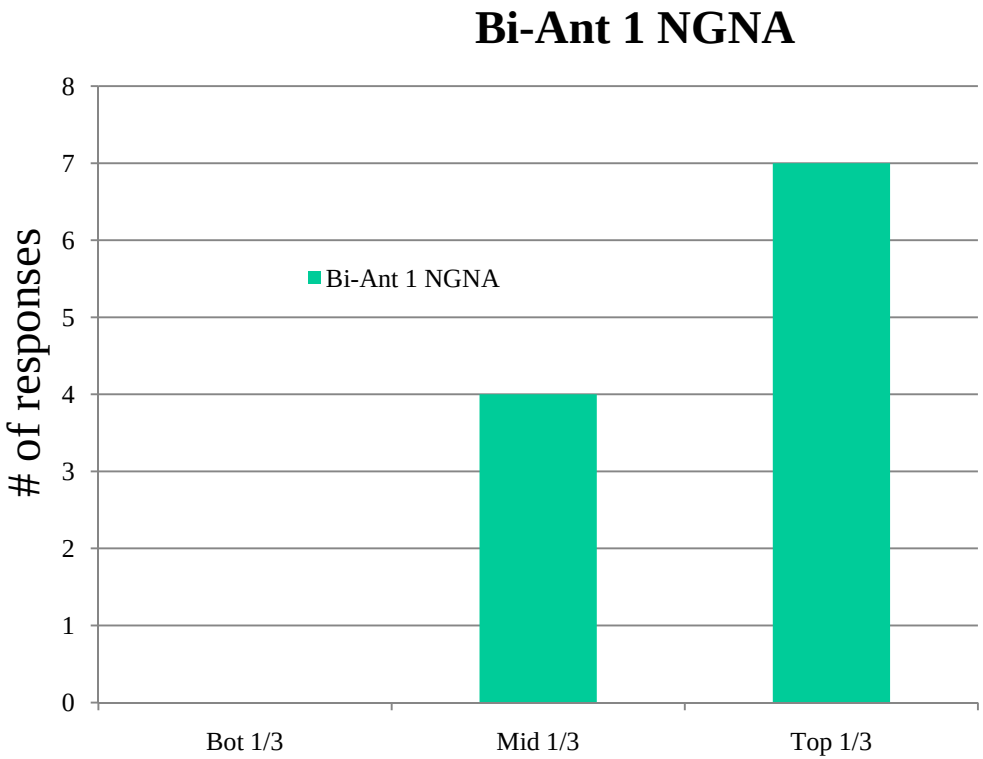
2 MALDI (from 10 total MALDI)

4 of 4 labs that used a reductive amination tag did not detect NGNA (3 LC + 1 MALDI used this approach)

5 of 7 industrial labs did not detect NGNA

Identification NGNA – III

8 Not detected – **11 detected**

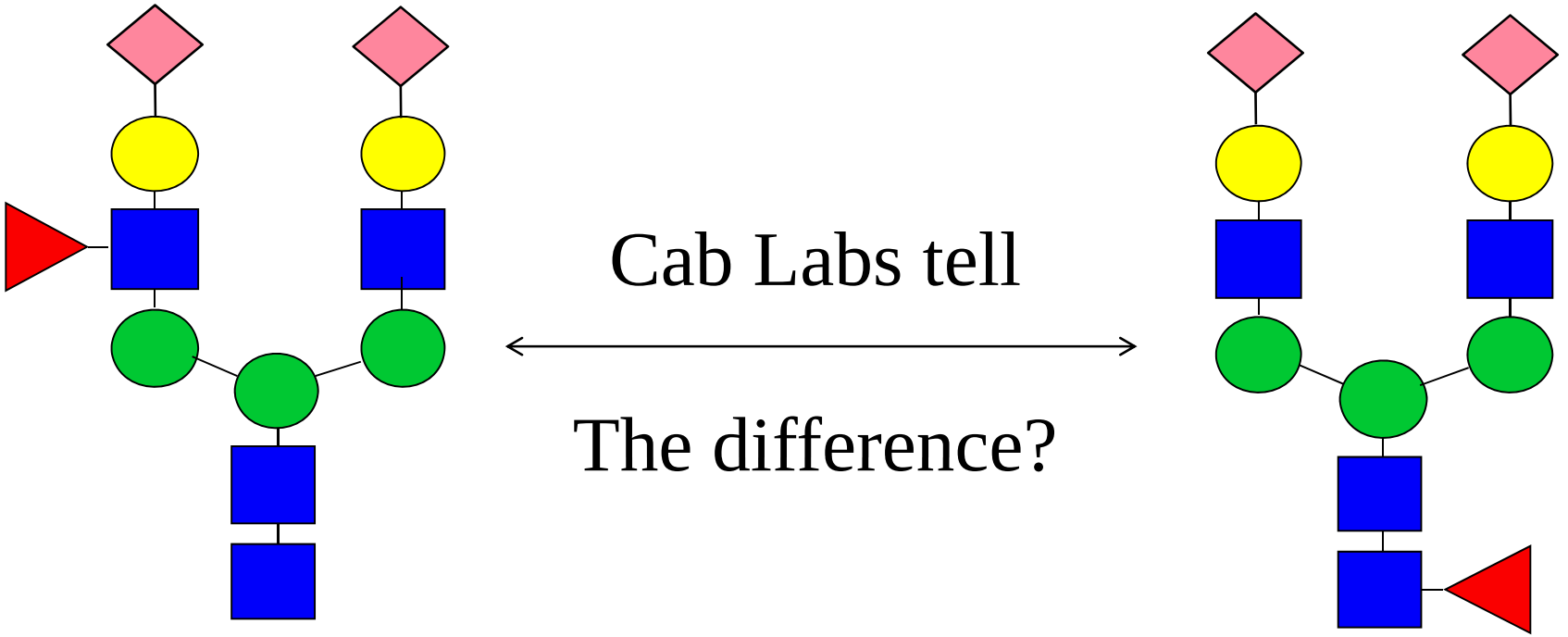


Detected Trends:
Labs that detected NGNA claimed it was a very abundant glycan.

Most labs that detected NGNA permethylated the sample.

Fucosylation – I

α1 Human has low levels of Antenna Complex Glycans



Present GPRG 1+2

Not Present

Fucosylation – II

18 of 19 labs identified fucosylated glycans despite low level

14 labs **incorrectly** identified this as core fucosylation

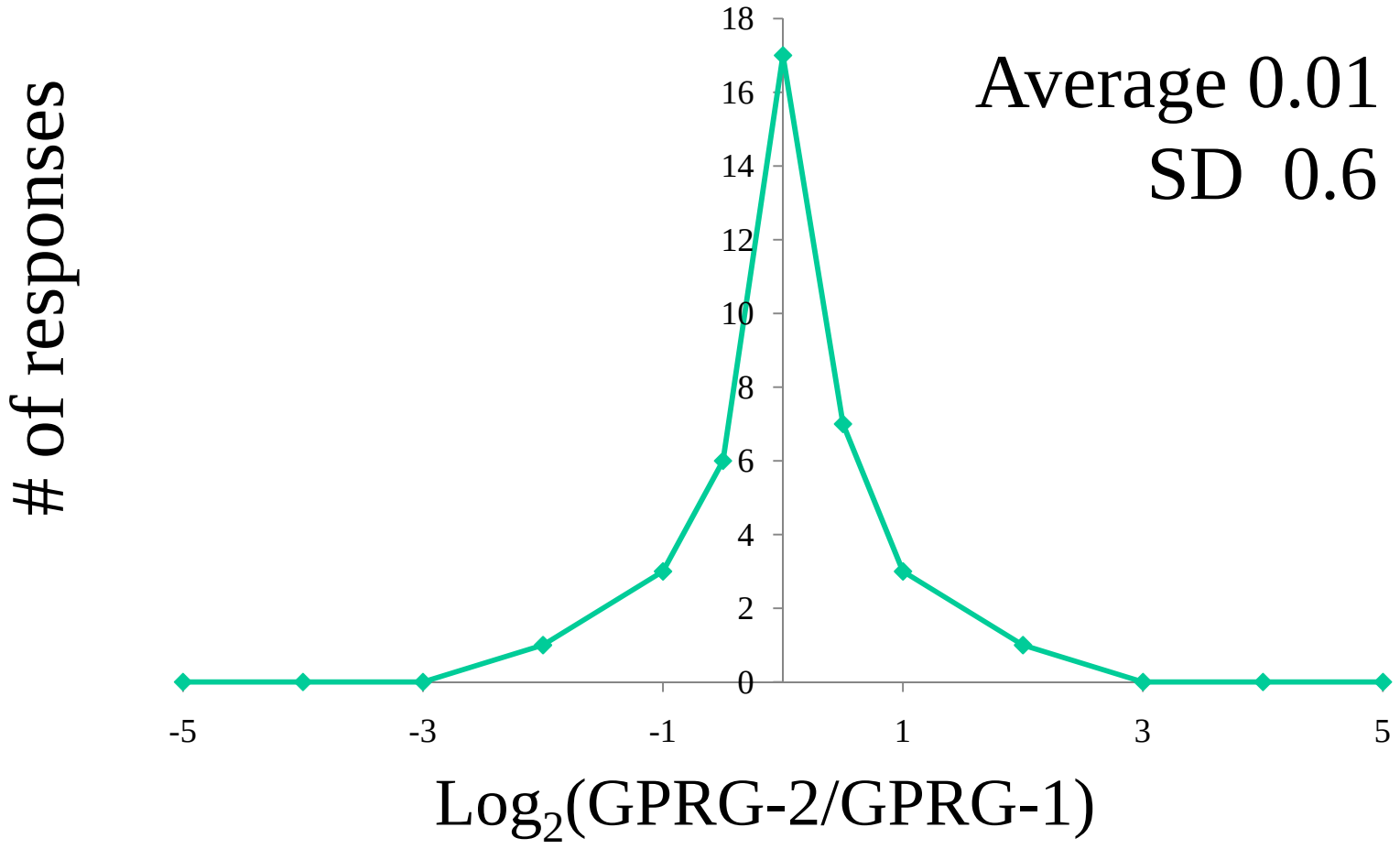
Incorrect ID Trends:

All labs (9 of 9) using MS (not MS^2) mis-ID site of fucose

All of the labs using software (5 of 5) for interpreting MS/MS data assigned fucose to the incorrect site

Quantification – Glycans that do not change

Each sample contained the same amount of Ov.
Responses for Man_{5+6} for GPRG-2+3 vs GPRG-1



Quantification – I

The amount of bi- and tri-antennary complex glycans without sialic acids changed between samples

Relative expression changes (relative to GPRG-1)

GPRG-2 down 3x – $\log_2(-1.5)$

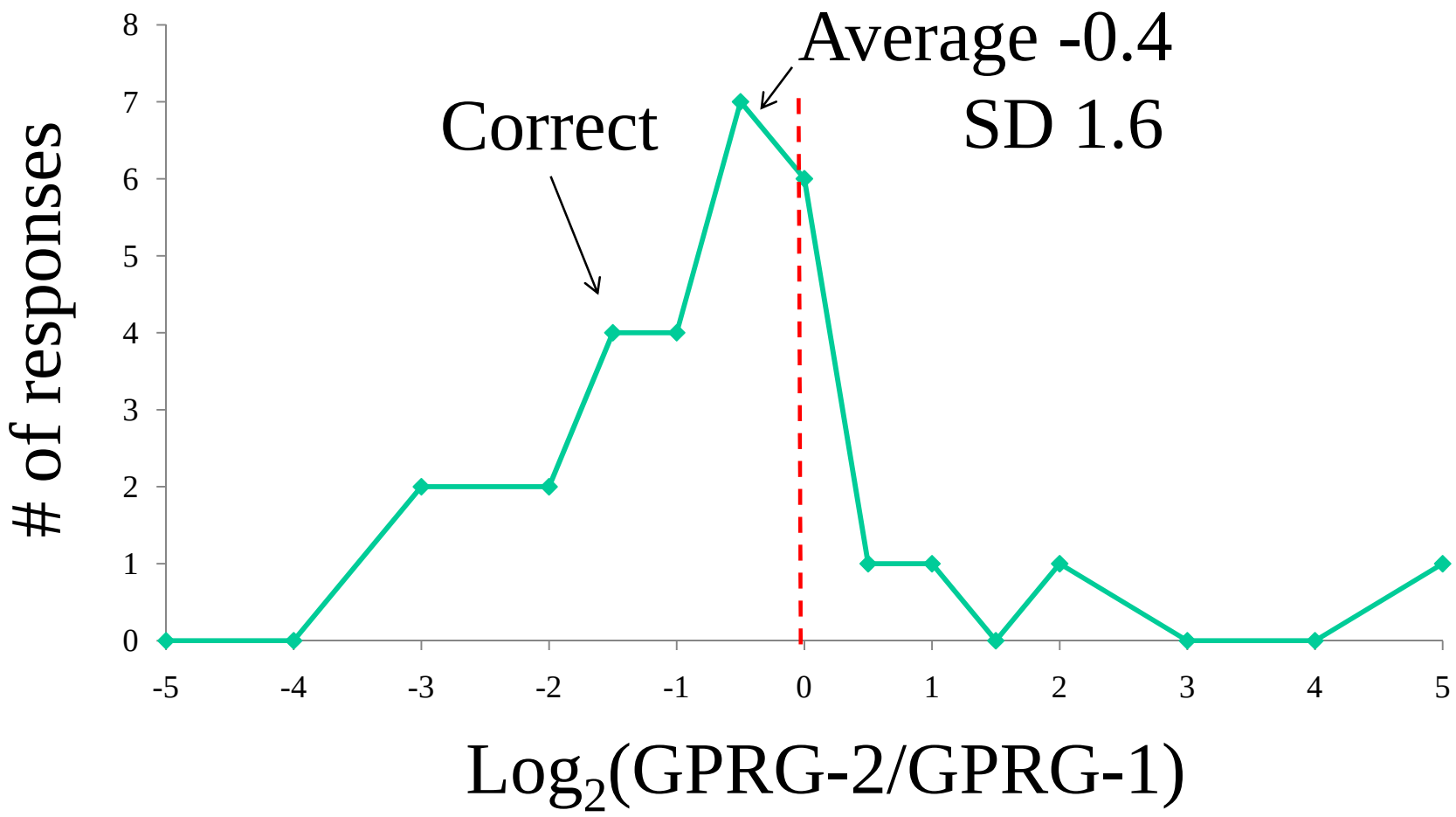
Next closest options down 4x or 2x

GPRG-3 up 2x – $\log_2(1)$

Next closest options up 0.5x or 3x

Quantification – II

GPRG-2 vs GPRG-1, “down regulated”

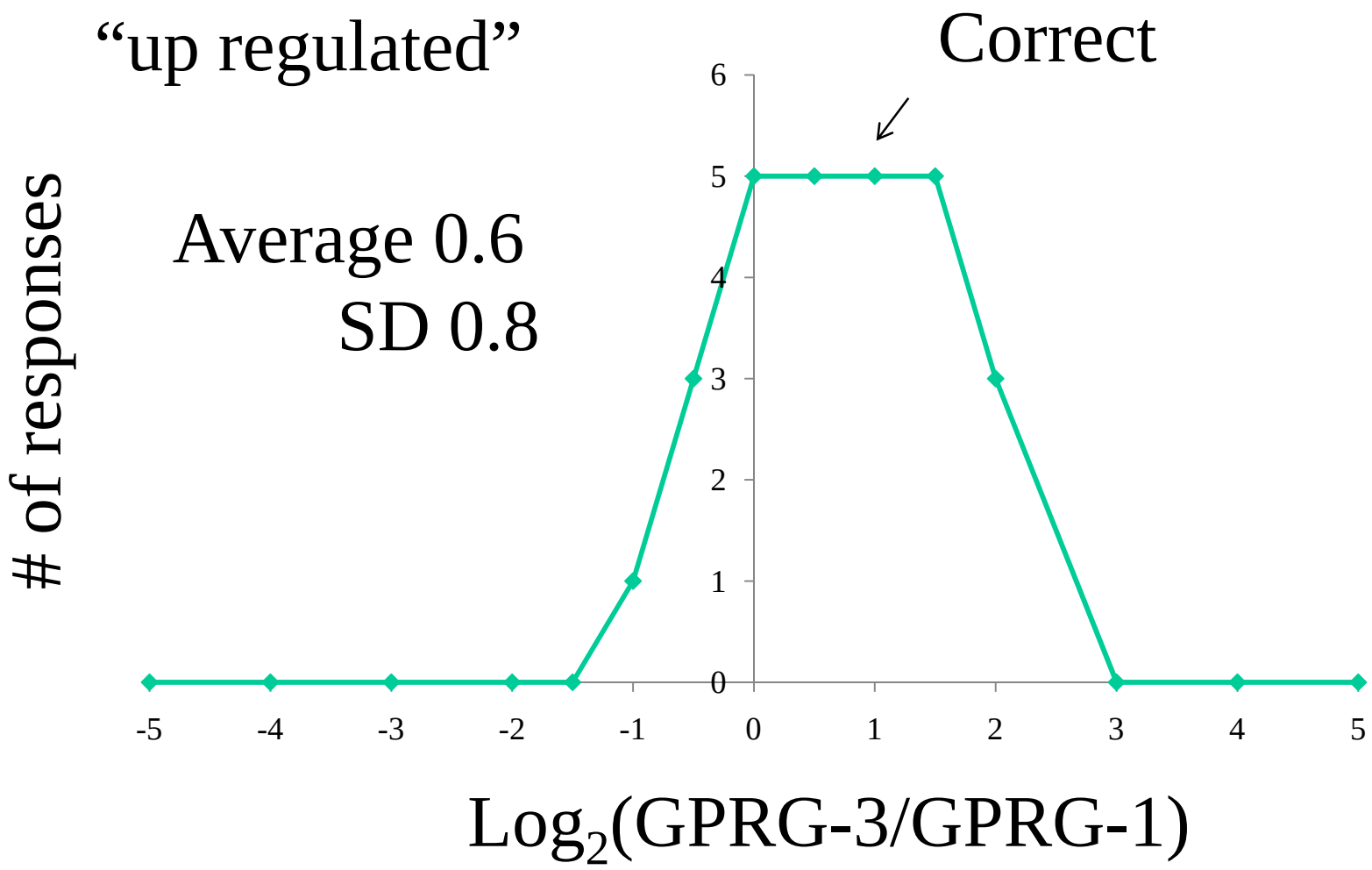


Quantification – III

GPRG-3 vs GPRG-1,
"up regulated"

Correct

Average 0.6
SD 0.8



Quantification – IV

No labs were able to correctly identify 3 or 4 of the changes in glycan composition

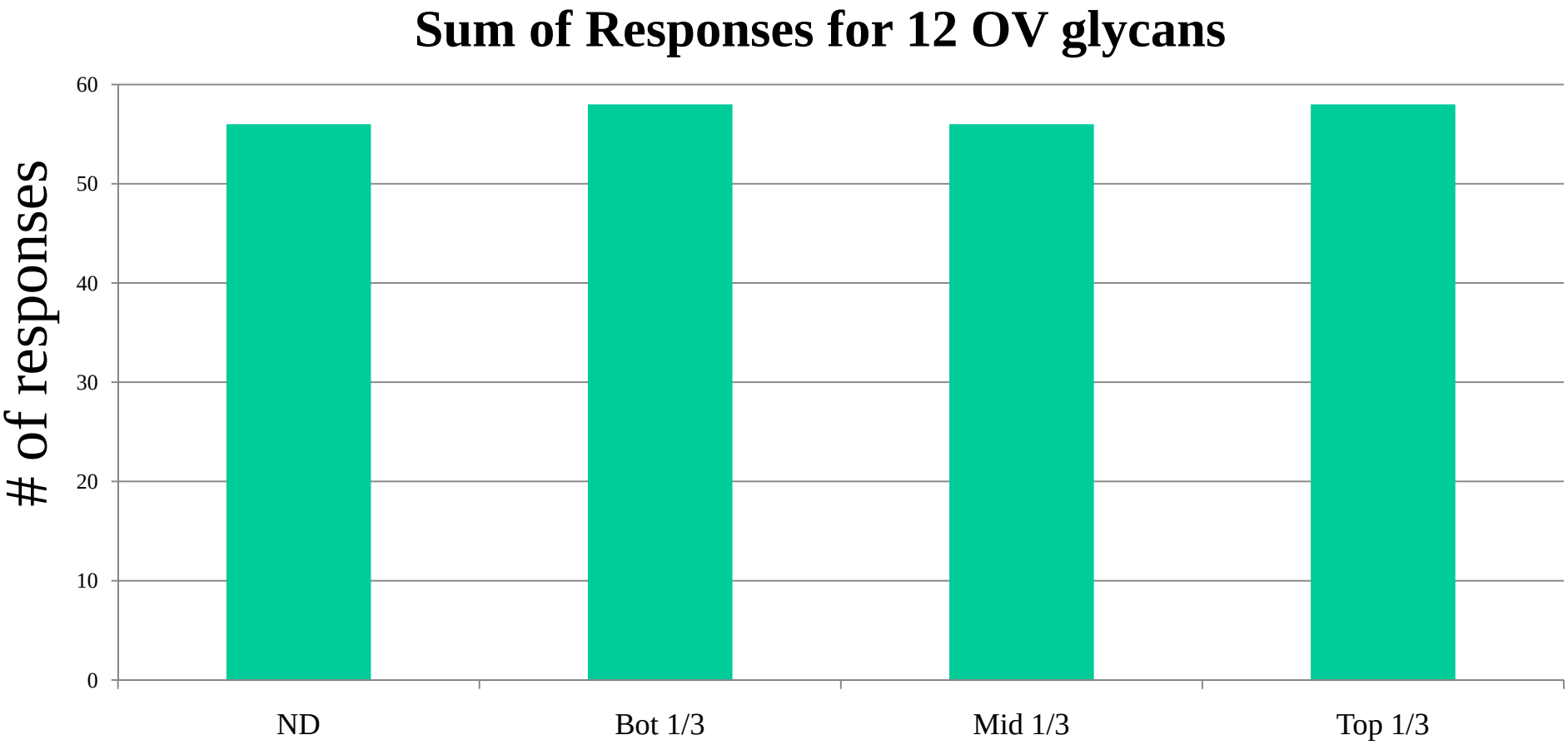
1 lab identified 2 of the 4 glycan changes correctly

7 labs identified 1 of the 4 glycan changes correctly

11 labs did not identify any of the glycan changes correctly

Quantification – V

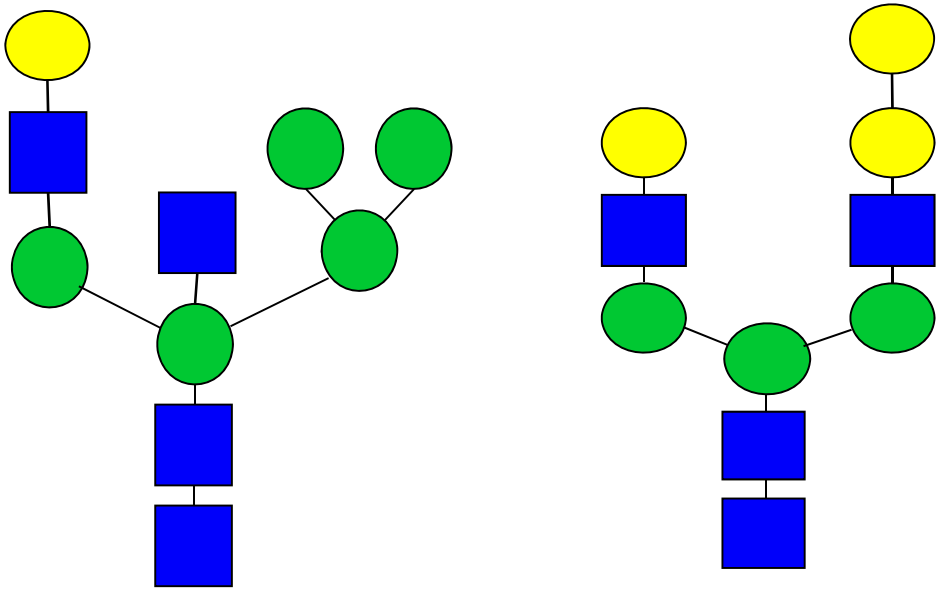
Inter-laboratory Reproducibility?



Conclusions

There is lots of room for improvement.
We have not even gotten to the hard problems, like quantitating individual glycans present in isomeric mixtures.

Hopefully the gPRG can help facilitate this process.



The gPRG needs new committee members! If you would like to join, contact me.

Acknowledgments

Angela Crawford – Sigma for providing the glycoproteins

Art Nuccio – CCRC/UGA for making the mixtures

Members of the gPRG

Rod Keck

Joe Zaia

Wolfgang Egge-Jacobsen

Karen Jonsher – EB liason