



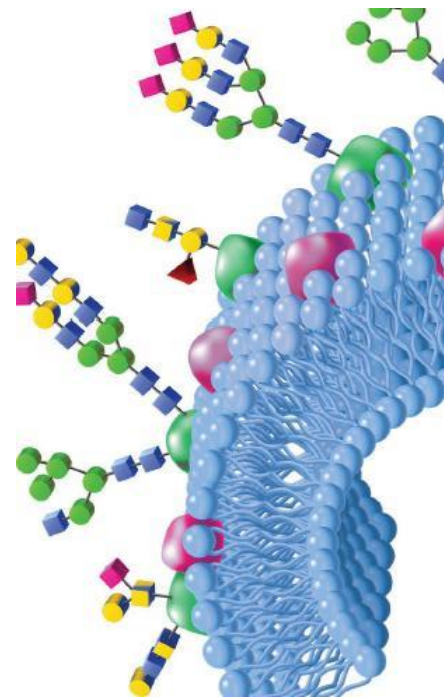
# **GlycanAssure™: The First Fully Integrated High Throughput and High Resolution Glycan Analysis Platform**

**Irina Rakotondramasy**  
Field Application Specialist

The world leader in serving science

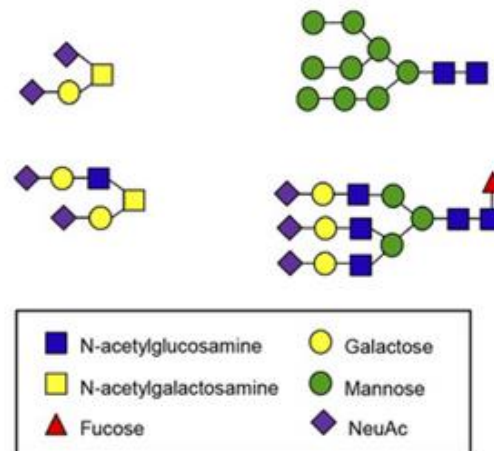
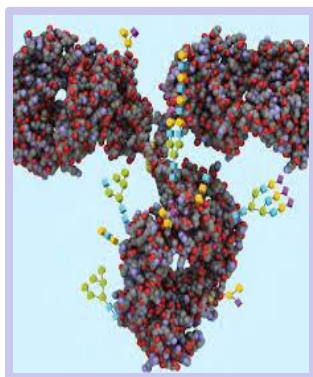
# Outline

- Glycosylation: Glycan Diversity and roles
- Glycan analysis challenges
- The Applied Biosystems™ **GlycanAssure**™ Glycan Analysis and Quantitation System
  - Sample Preparation
  - Instrumentation
  - Software
- GlycanAssure representative data



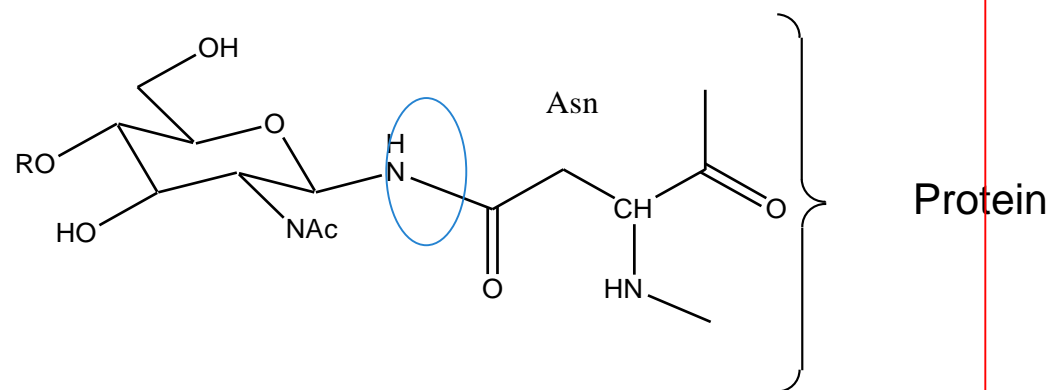
# Glycosylation – Controlling Where Sugars Go

- Glycosylation: attachment of glycans to proteins
- More than 50% of human proteins are glycosylated
- Glycoproteins biosynthesis
  - Glycans are not templated
  - Glycan structure is determined by sequential glycosyltransferase action
- Glycosylation is heterogeneous
  - Structures of sugars attached
    - ➔ 9 most common monosaccharides which are found in human glycome
  - Sites to which they are attached

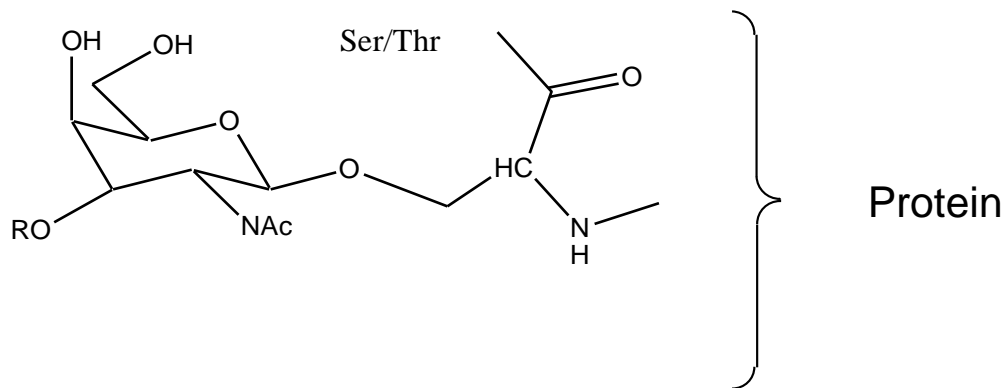


# Diversity of Glycans

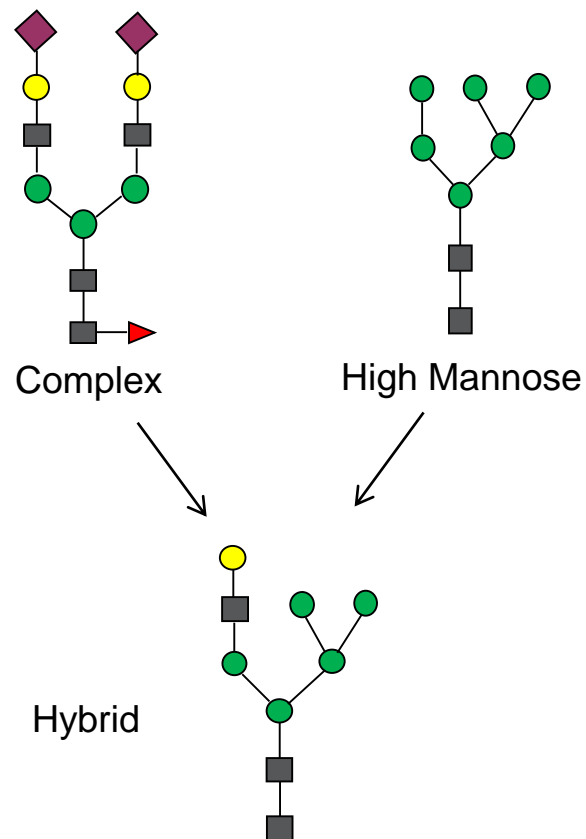
## N-Glycan



## O-Glycan



## Three General Types



# Many Conditions Affecting Humans Involve Glycans

## Pregnancy

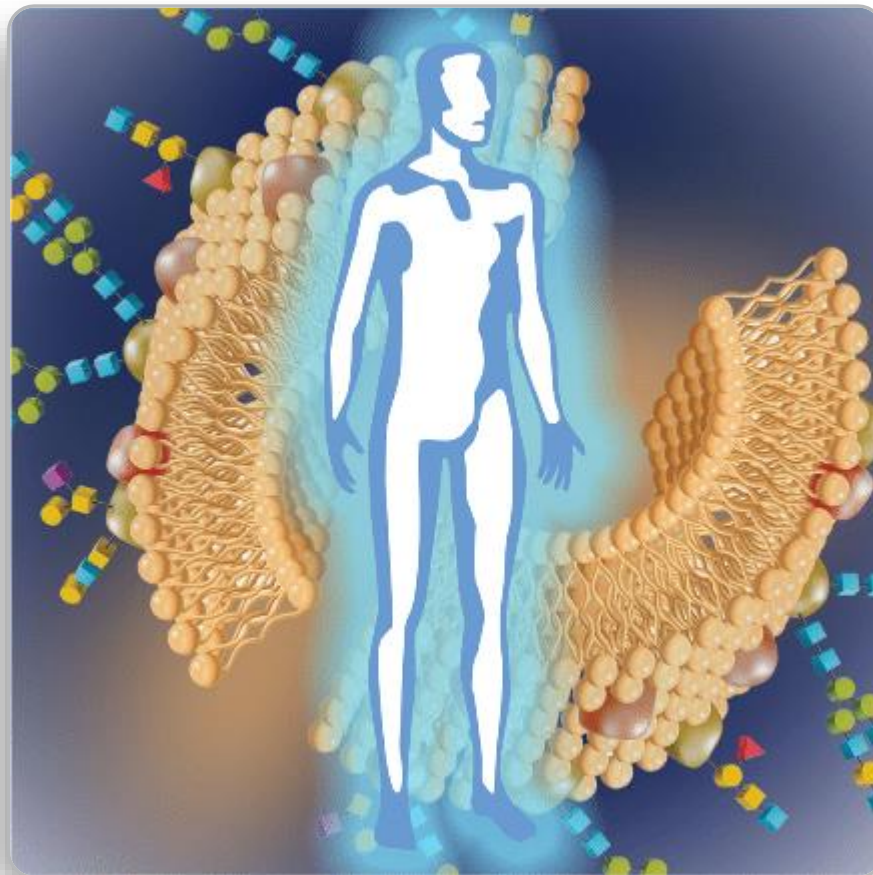
N-glycosylation of human serum transcortin and thyroxine-binding globulin

## Aging

Outer arm galactosylation of human serum IgG

## Alcoholism

Increase in relative number of serum asialo-transferrin glycoforms



## Cancer

Unique oligosaccharides  
Such as CA 125 (Ovarian)  
CA 19-9 (Colon)

## Liver Disease

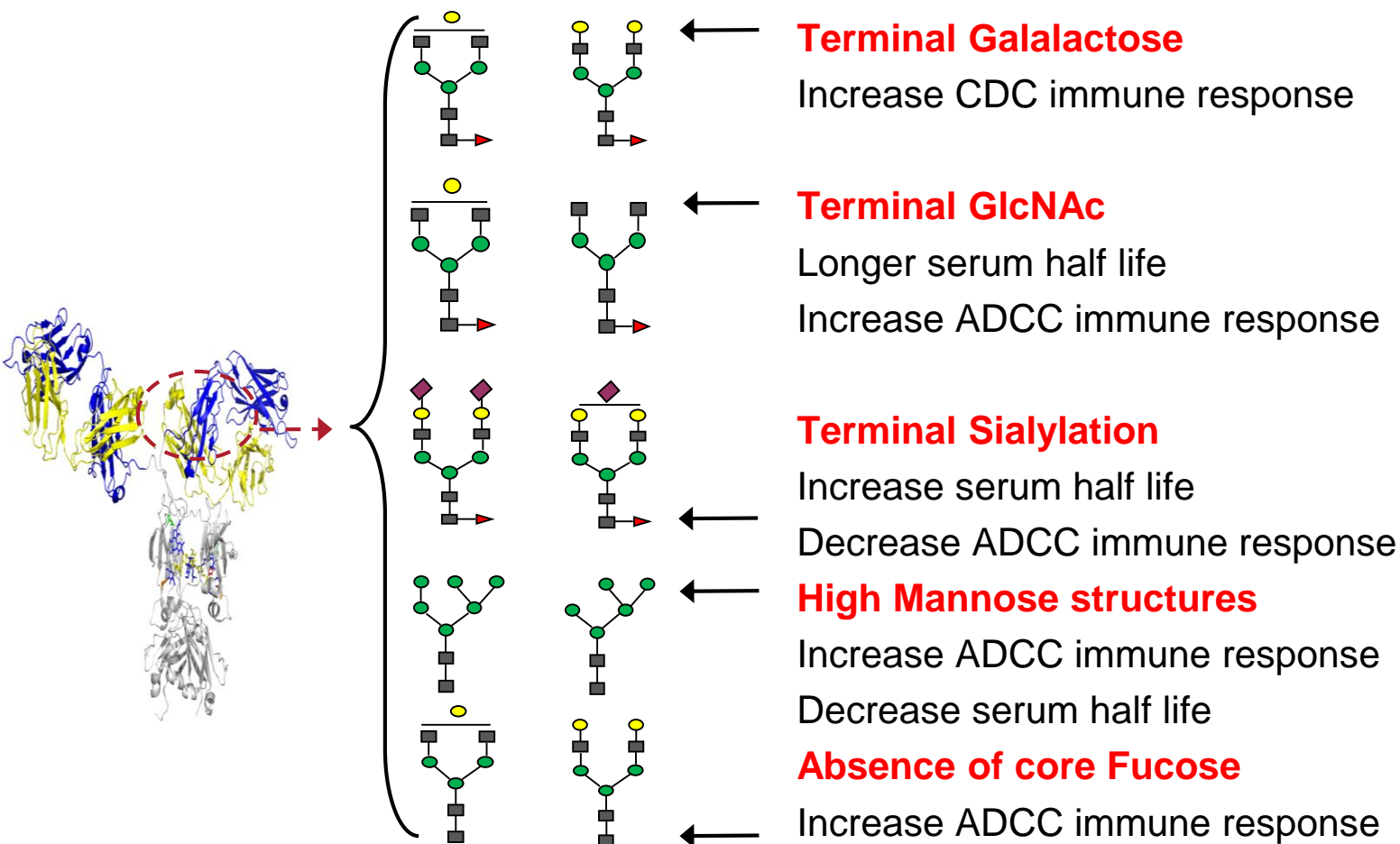
Increase in number of  
sialic acid residues  
on fibrinogens

## Tuberculosis

agalactosyl IgG

Ref: Raymond Dwek et al., *Ann. Rev. Biochem.*, 57 (1988) 785-838

# Biotherapeutics: Glycosylation a Critical Quality Attribute



Raju et al, *Current Opinion in Immunology*, 2008, 20; 471

**Protein function is tightly linked  
to glycosylation structure**

**ADCC:** Antibody-dependent Cell-mediated Cytotoxicity  
**CDC:** Complement-Dependent Cytotoxicity



# Current Regulatory Guidelines

Ensures glycans/glycosylation is well characterized and monitored in biotherapeutics

- FDA (USA) and EMA (Europe) define the regulatory requirements
    - On the development, production, characterization and specifications for monoclonal antibodies and related products ‘glycan structures should be characterized, and particular attention should be paid to their degree of of mannosylation, galatosylation, fucosylation and sialylation’
- European Medicines Agency  
EMA/CHMP/BWP/157653/2007 (2009)
- As monoclonal antibody molecules come off patent, biosimilars will start entering the market.
    - FDA (USA) and EMA (Europe) consider the degree of glycosylation to be a critical factor in determining the degree of “similarity” to the original approved drug

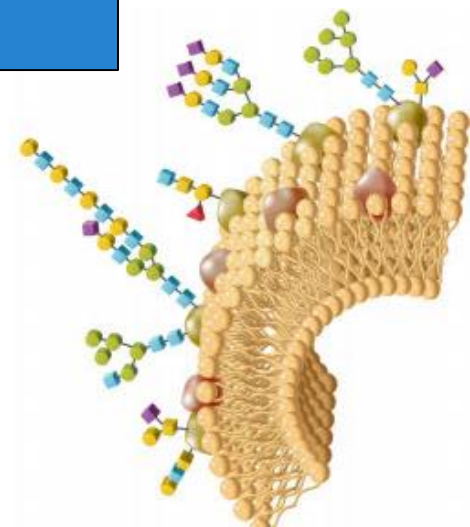
# Glycan Diversity Poses an Analytical Challenge

Oligomer	Composition	Peptide/Oligo	Oligosaccharide
Dimer	AA / AB	1 / 2	11 / 20
Trimer	AAA / ABC	1 / 6	120 / 720
Tetramer	AAAA / ABCD	1 / 24	1,424 / 34,560
Pentamer	AAAAA / ABCDE	1 / 120	17,872 / 2,144,640

Ref: Richard Schmidt, *Angew. Chem. Int. Ed. Engl.*, 25 (1986) 212-235

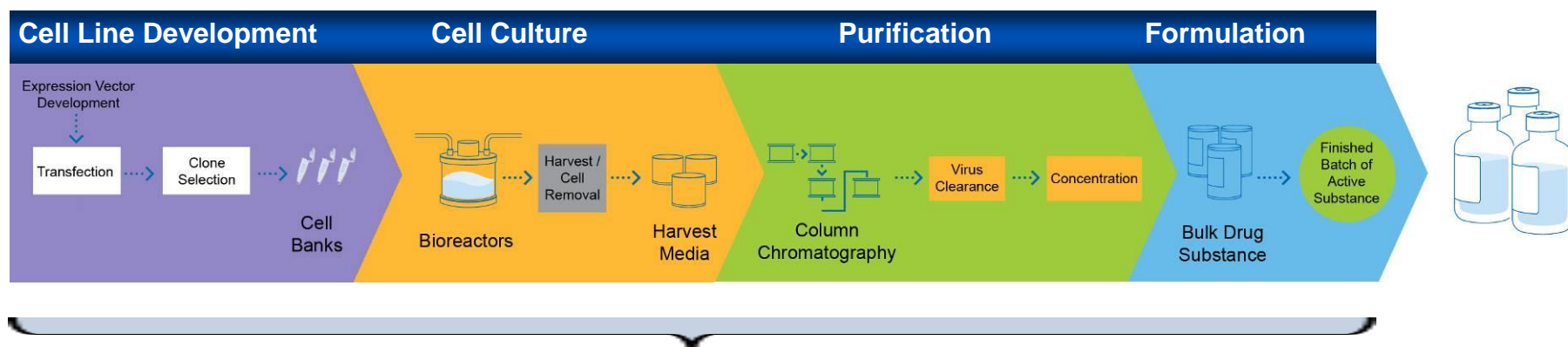
Number of Possible  
Isomers

- Complex, diversified structures
- No chromophore / fluorophore
- Mostly not charged



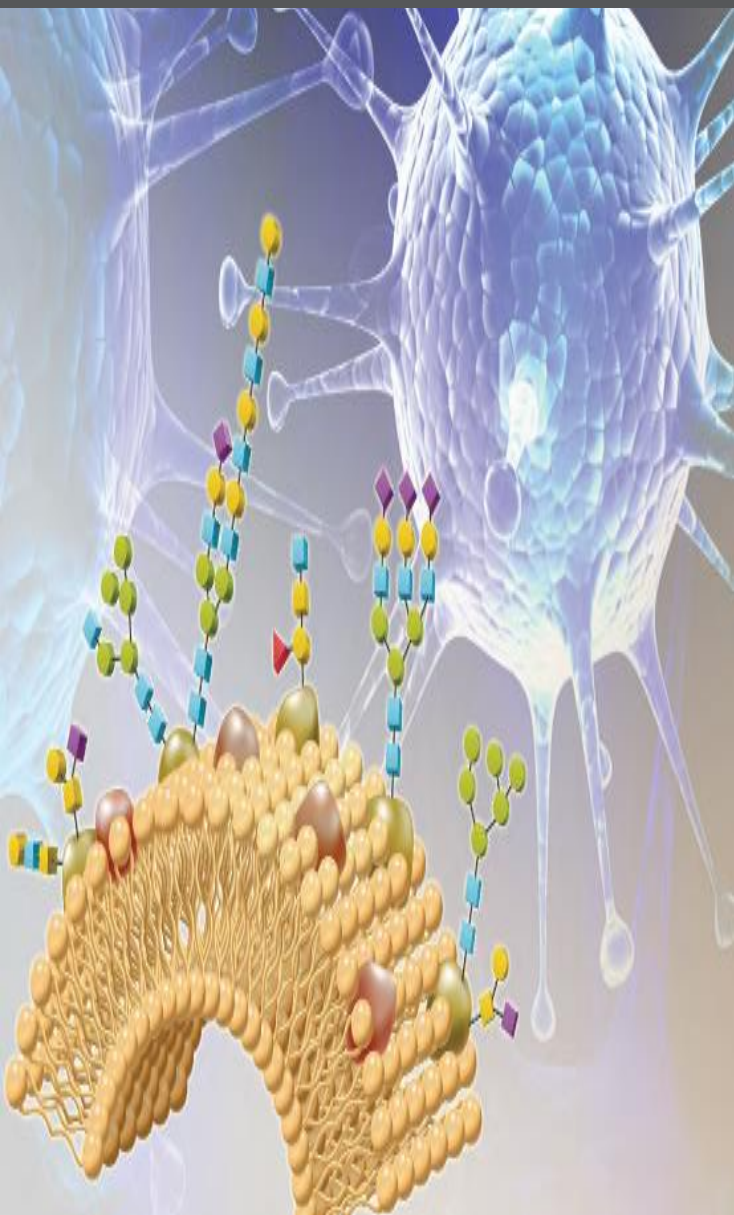


# At which stage Glycan Analysis is done?



Glycan analysis is done in every step of biopharmaceutical workflow

# Glycan Analytic Challenges and Holistic Workflow Solutions



- Analytical methods for glycan analysis:
  - Structural characterization: NMR & MS
  - HPLC
    - ✓ HPAE/PAD - Normal phase
    - ✓ HILIC (HPLC and UPLC)
  - Capillary Electrophoresis
  - Other Methods: LC-MS, CE-MS, CESI-MS
- Need a suite of tools/software offering complete glycan analysis, designed to address challenges such as:
  - **High-throughput**
  - **High resolution**
  - **High Sensitivity**
  - **Faster Speed**

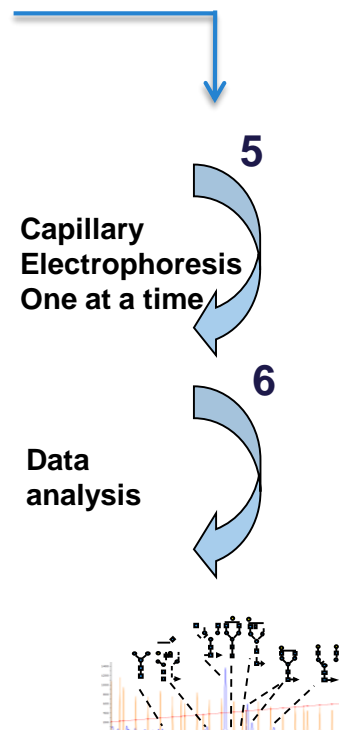
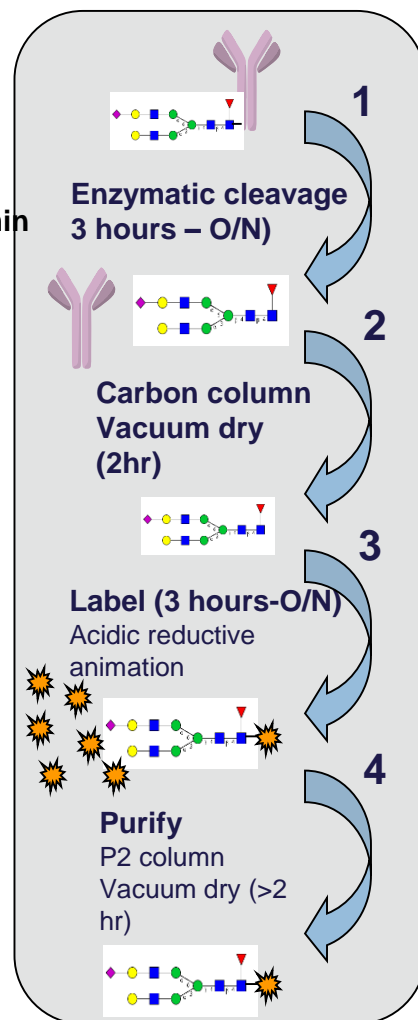
# Long and Labor Intensive Traditional Protocols

- Denaturation of glycoprotein**
- SDS & DTT at 90C for 10 min
  - NP40 and PNGaseF

**Vacuum drying required  
for effective dye labeling**

**APTS dye (excess)  
toxic NaCNBH<sub>3</sub> as reductant**

**Removal of excess dye to  
increase sensitivity  
(Vacuum dry)**



**Total Time: 1– 3 days**

# Applied Biosystem GlycanAssure™ System

**First Fully Integrated System Combining  
Throughput & Data Quality**



APTS Kit



Turquoise Kit



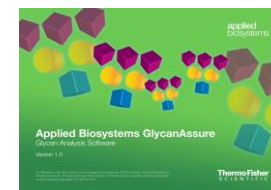
Teal Kit

Flexible sample preparation kit

**Kit is flexible to run 1 - 96 samples  
at a time with 1 year shelf life**



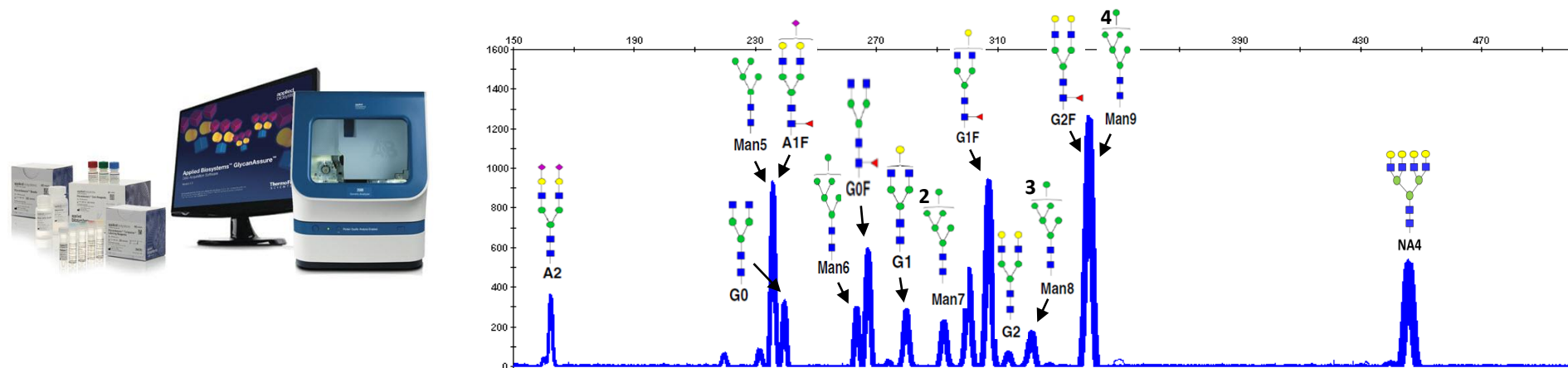
**3500 & 3500XL Genetic Analyzer for  
Protein Quality Analysis**



**Data Acquisition & Data Analysis  
Software**

# GycanAssure Key Features

## First Fully Integrated Glycan Analysis System



### Feature

### Benefit

**Superior performance**

Resolution of sialylated glycans, structural isomers, fucose species, high-mannose species

**Ease of use**

Magnetic bead-based sample preparation using less labor (reduced pipetting and hands-on time); no use of sodium cyanoborohydride; and no vacuum centrifugation steps

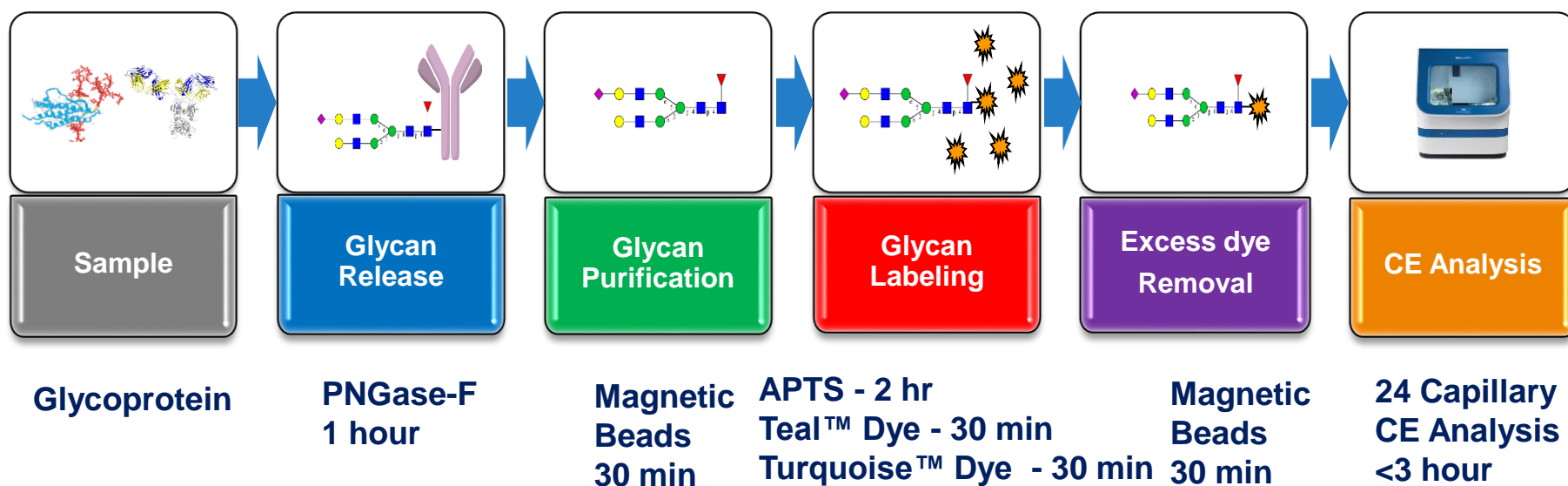
**Sensitivity and selectivity**

Multiple fluorescent dyes for superior sensitivity and selectivity—traditional APTS and the novel Teal™ and Turquoise™ dyes for glycan labeling

**Higher throughput**

Sample preparation and analysis of 96 samples in 7–9 hours

# GlycanAssure Workflow



- No vacuum centrifugation steps
- No use of Sodium cyanoborohydride

***Hands on Time < 3hr***

***Sample Prep to Data: 7 - 9hrs\* (96 samples)***

*\*Depending on dye*



## Applied Biosystems™ 3500xL Protein Quality Analyzer

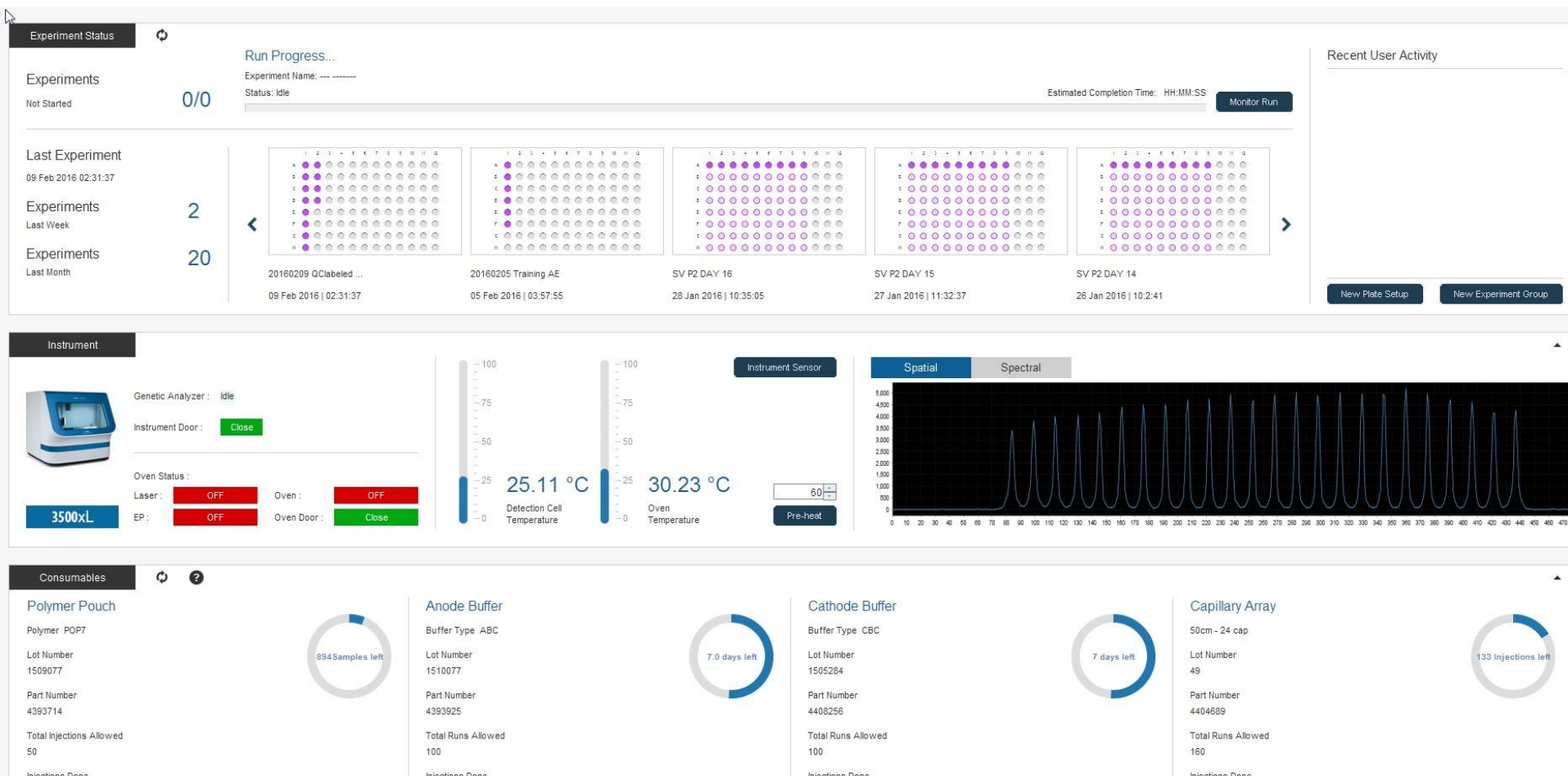
- ✓ Industry “gold standard” CE instrument
- ✓ Parallel analysis of 24 samples in 45 min
- ✓ **< 2 min/sample run time**
- ✓ Multi-color capability
- ✓ Calibration across capillaries using **internal standard**
- ✓ RFID-tagged capillary array & CE consumables for automated tracking



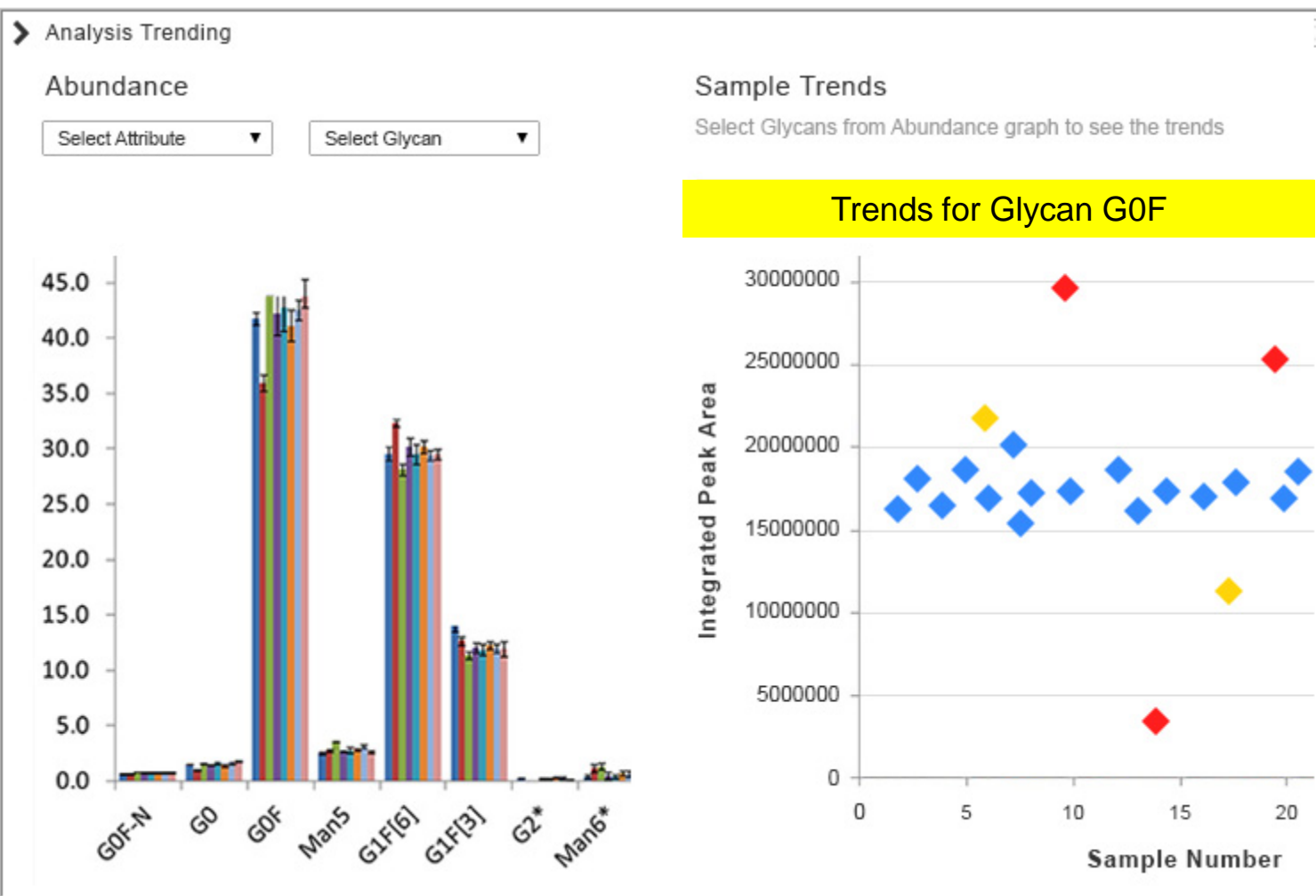
24-capillary  
array (50 cm)

**No compromise in data quality**

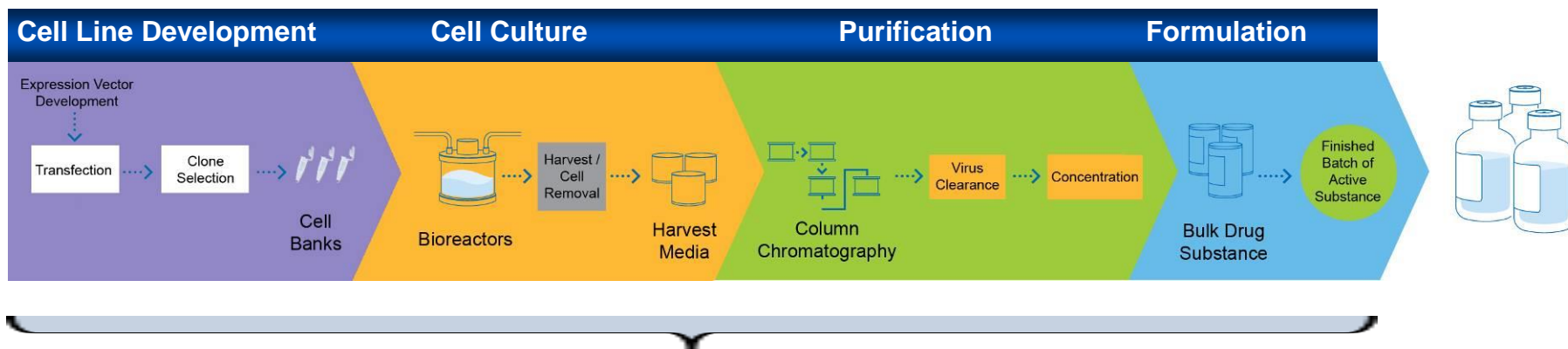
# GlycanAssure : Data Acquisition Software



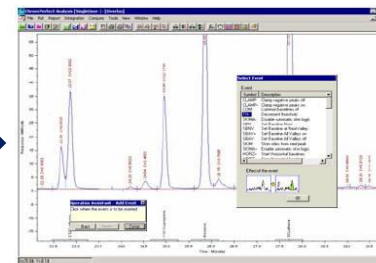
# GlycanAssure : Data Analysis Software



# Role of GlycanAssure in Analytical Tool Kit



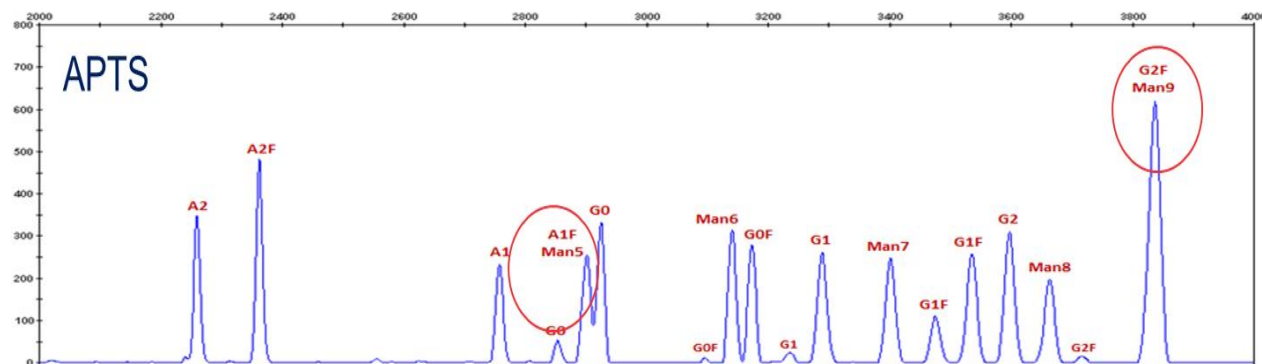
Glycan analysis is done in every step of biopharmaceutical workflow



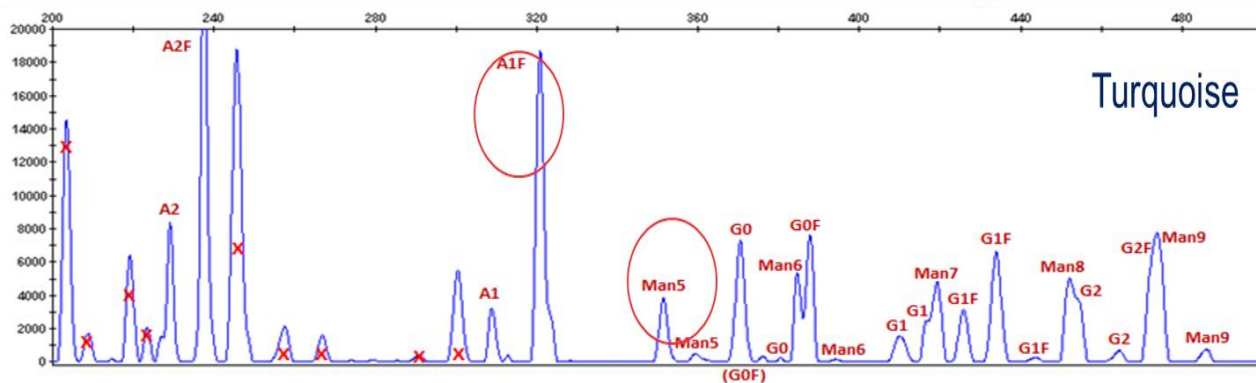
**GlycanAssure can be used at every stage of biotherapeutic drug development**

# GlycanAssure – Multiple Dyes for Sensitivity and Selectivity

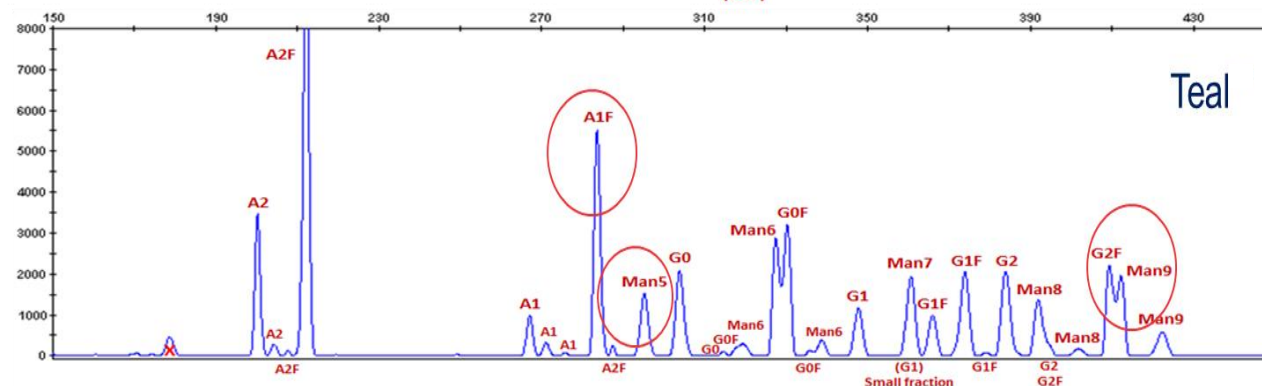
Traditional,  
industry-  
standard  
dye



Detection  
and  
quantitation  
of glycans at  
very  
low levels



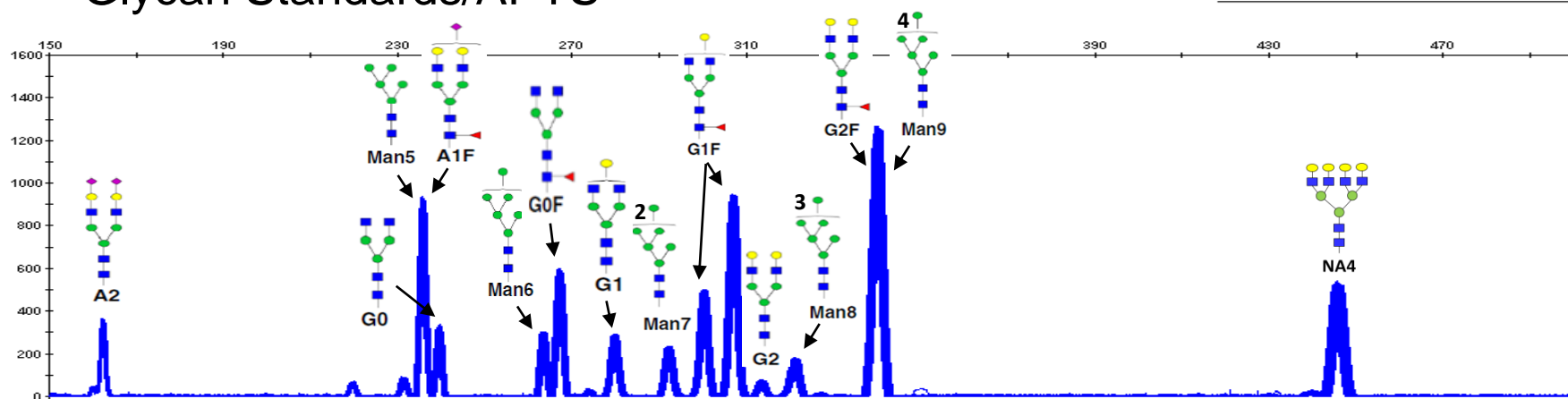
High  
separation  
efficiency for  
identification  
of more  
glycans



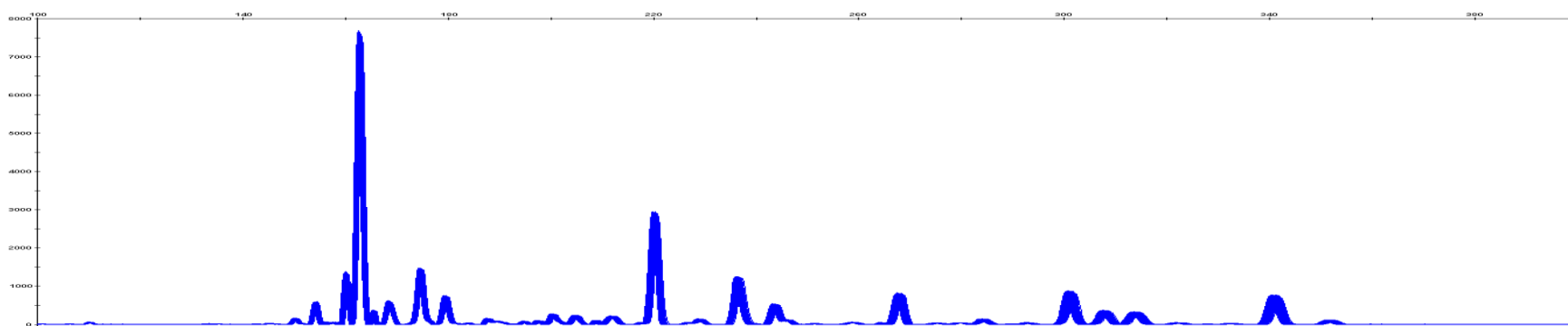
## Overlay of 288 Injections

	1	2	3	4	5	6	7	8	9	10	11	12
A	●	●	●	●	●	●	●	●	●	●	●	●
B	●	●	●	●	●	●	●	●	●	●	●	●
C	●	●	●	●	●	●	●	●	●	●	●	●
D	●	●	●	●	●	●	●	●	●	●	●	●
E	●	●	●	●	●	●	●	●	●	●	●	●
F	●	●	●	●	●	●	●	●	●	●	●	●
G	●	●	●	●	●	●	●	●	●	●	●	●
H	●	●	●	●	●	●	●	●	●	●	●	●

### Glycan Standards/APTS



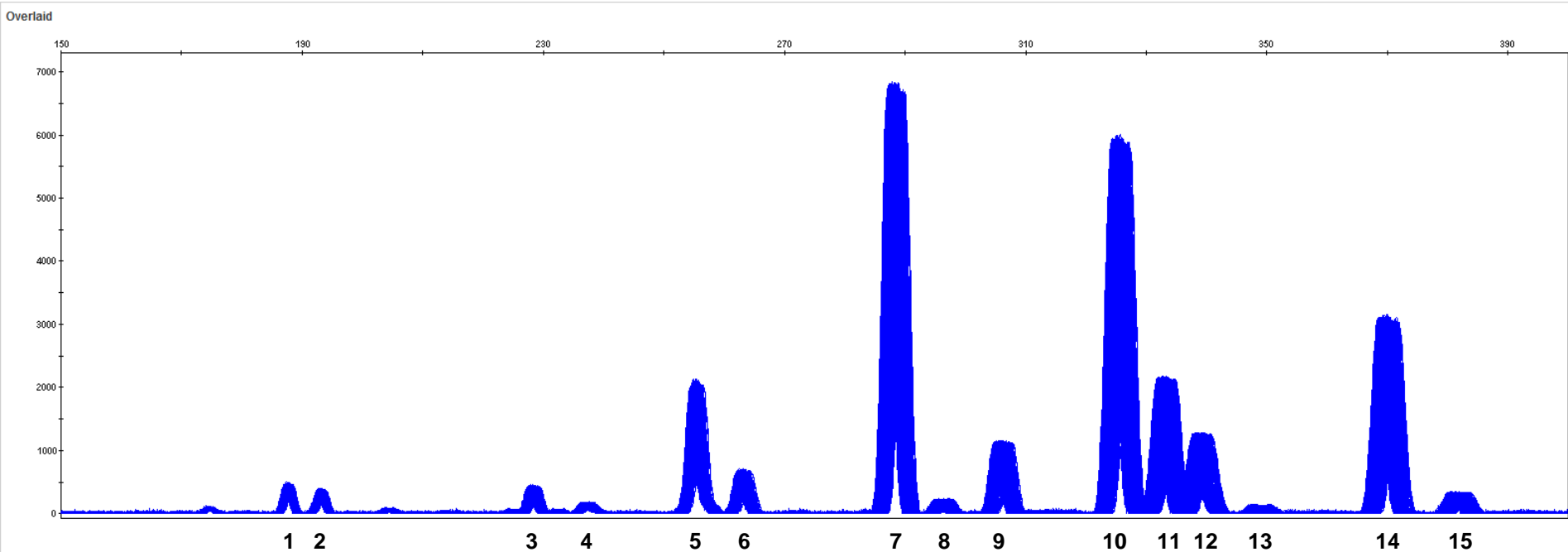
### Plasma Glycans/APTS





# Reproducibility

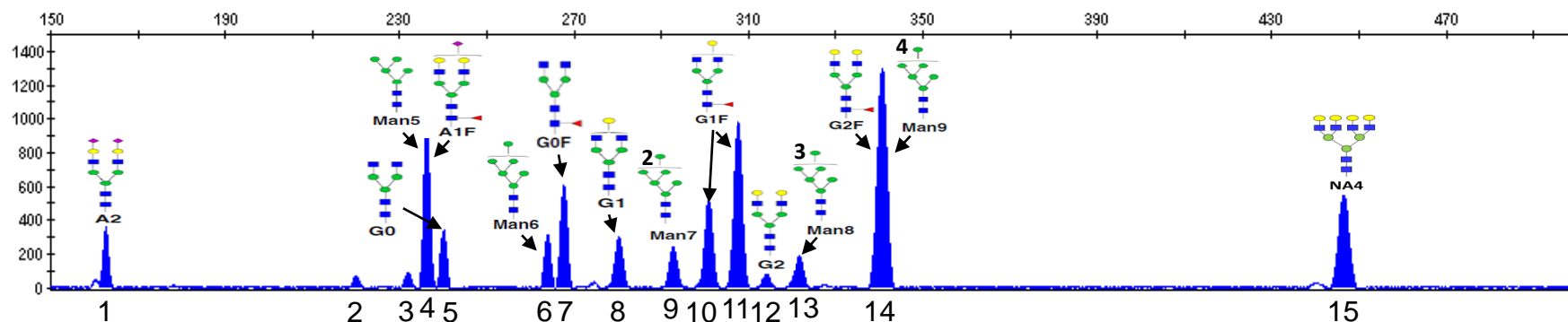
## 300 Injections of IgG sample / APTS



APTS	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
AVERAGE	1.23	1.01	1.27	0.65	7.24	2.37	26.32	0.82	4.32	24.68	8.89	5.63	0.53	13.56	1.48
SD	0.04	0.05	0.03	0.04	0.14	0.05	0.24	0.06	0.36	0.2	0.09	0.33	0.06	0.13	0.15
CV%	3.25	4.95	2.36	6.15	1.93	2.11	0.91	7.32	8.33	0.81	1.01	5.86	11.32	0.96	10.14

# Injection Repeatability

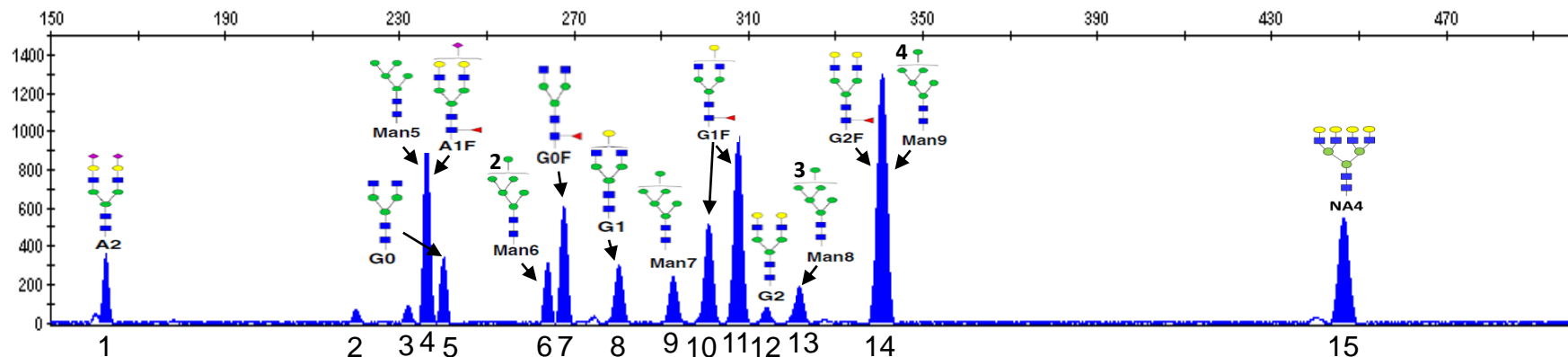
- Each injection has samples from 24 capillaries



Injection	1	2	3	4	5	6	7	8	9	10	11	12	Average of 12 injections	CV%
Peak	Average Relative	Average Relative	Average Relative	Average Relative	Average Relative	Average Relative	Average Relative	Average Relative	Average Relative	Average Relative	Average Relative	Average Relative		
1	3.5%	3.5%	3.5%	3.5%	3.5%	3.5%	3.5%	3.5%	3.5%	3.5%	3.4%	3.4%	3.5%	0.7%
2	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	0.8%	1.2%
3	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	0.8%
4	11.1%	11.1%	11.1%	11.0%	11.0%	10.9%	11.0%	10.9%	10.9%	10.9%	10.9%	10.9%	11.0%	0.6%
5	3.6%	3.7%	3.7%	3.6%	3.7%	3.6%	3.6%	3.6%	3.6%	3.6%	3.6%	3.6%	3.6%	0.8%
6	3.6%	3.7%	3.6%	3.6%	3.7%	3.6%	3.6%	3.7%	3.7%	3.7%	3.6%	3.6%	3.6%	0.4%
7	7.9%	7.9%	7.9%	7.9%	7.9%	7.9%	7.9%	7.9%	7.9%	7.9%	7.9%	7.9%	7.9%	0.4%
8	4.4%	4.4%	4.4%	4.4%	4.4%	4.4%	4.4%	4.4%	4.4%	4.4%	4.4%	4.4%	4.4%	0.4%
9	3.6%	3.6%	3.6%	3.6%	3.6%	3.6%	3.6%	3.6%	3.6%	3.6%	3.6%	3.6%	3.6%	0.3%
10	7.9%	7.9%	7.9%	7.9%	7.9%	8.0%	8.0%	8.0%	8.0%	8.0%	8.0%	8.0%	8.0%	0.5%
11	15.1%	15.1%	15.1%	15.1%	15.1%	15.2%	15.2%	15.2%	15.2%	15.2%	15.2%	15.2%	15.1%	0.3%
12	1.0%	1.0%	1.0%	1.0%	1.1%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.3%
13	3.0%	3.0%	3.0%	3.0%	3.0%	3.0%	3.0%	3.0%	3.0%	3.0%	3.0%	3.0%	3.0%	0.5%
14	23.3%	23.3%	23.3%	23.3%	23.3%	23.3%	23.3%	23.3%	23.4%	23.4%	23.3%	23.4%	23.4%	0.2%
15	10.1%	10.1%	10.1%	10.1%	10.1%	10.1%	10.0%	10.1%	10.0%	10.0%	10.1%	10.1%	10.1%	0.4%
Total	126192	118457	119091	118978	117111	117493	117763	117667	117203	117266	117481	117613	118526	2.1%

Tight relative quantities among repetitive injections (from 24 caps)

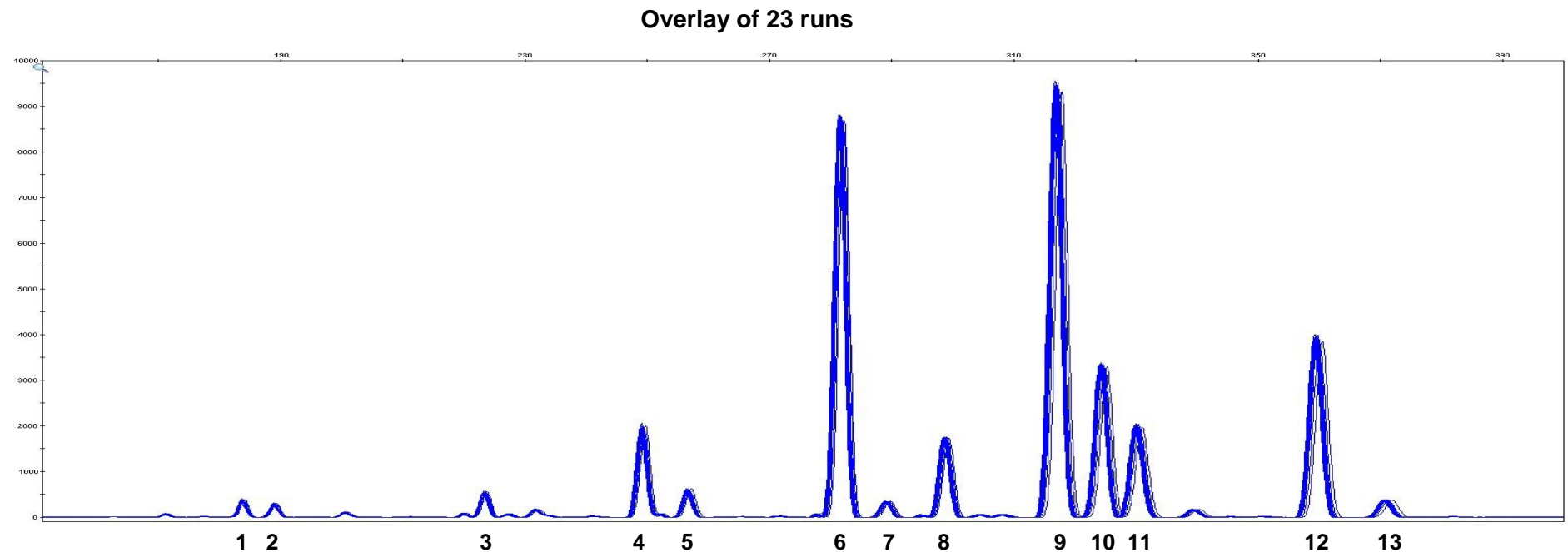
# Variability Across Instruments & Arrays (APTS)



	Peak	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 <sup>st</sup> 3500 32 Inj	Average	3.4%	0.8%	1.0%	10.5%	3.3%	3.6%	8.4%	4.4%	3.7%	8.0%	15.3%	1.0%	3.0%	23.5%	10.2%
	CV%	2.8%	2.2%	2.3%	1.1%	2.0%	2.8%	9.2%	1.1%	0.9%	0.5%	0.9%	3.6%	1.5%	1.0%	1.3%
2 <sup>nd</sup> 3500xl 24 Inj	Average	3.3%	0.8%	1.1%	9.7%	2.8%	3.7%	8.1%	4.5%	3.7%	8.2%	15.7%	1.0%	3.1%	24.0%	10.4%
	CV%	2.0%	1.9%	1.8%	1.3%	2.3%	1.7%	0.9%	0.6%	0.7%	0.7%	0.4%	3.3%	0.8%	0.4%	1.2%
3 <sup>rd</sup> 3500xl 24 Inj	Average	3.5%	0.8%	1.0%	11.1%	3.6%	3.6%	7.9%	4.4%	3.6%	7.9%	15.1%	1.0%	3.0%	23.3%	10.1%
	CV%	1.3%	3.0%	3.2%	1.2%	2.3%	1.7%	0.9%	1.1%	1.2%	0.7%	0.6%	6.1%	1.2%	0.7%	1.7%
3500xl Cap 1*	Average	3.5%	0.8%	1.0%	11.1%	3.7%	3.7%	7.9%	4.4%	3.6%	7.9%	15.1%	1.0%	3.0%	23.3%	10.0%
	CV%	1.5%	3.8%	2.7%	0.9%	1.9%	1.3%	0.6%	1.0%	1.1%	0.7%	0.3%	3.0%	1.1%	0.5%	0.9%
3500xl Cap 2*	Average	3.4%	0.8%	1.1%	10.9%	3.6%	3.8%	7.9%	4.4%	3.6%	7.9%	15.2%	1.1%	3.0%	23.3%	10.1%
	CV%	1.2%	2.4%	2.2%	0.9%	1.9%	1.8%	1.0%	0.9%	0.7%	0.5%	0.5%	1.8%	0.9%	0.4%	1.3%
3500xl Cap 3*	Average	3.4%	0.8%	1.1%	11.0%	3.6%	3.8%	8.0%	4.4%	3.6%	7.9%	15.2%	1.1%	3.0%	23.3%	9.9%
	CV%	1.5%	3.8%	2.1%	1.2%	2.3%	1.2%	0.6%	0.6%	0.8%	0.6%	0.3%	2.8%	1.2%	0.5%	0.7%

\*Tight relative quantities among repetitive injections (12 Injections)

# Sample Stability of APTS labeled IgG Glycans



CE RUN – Day One

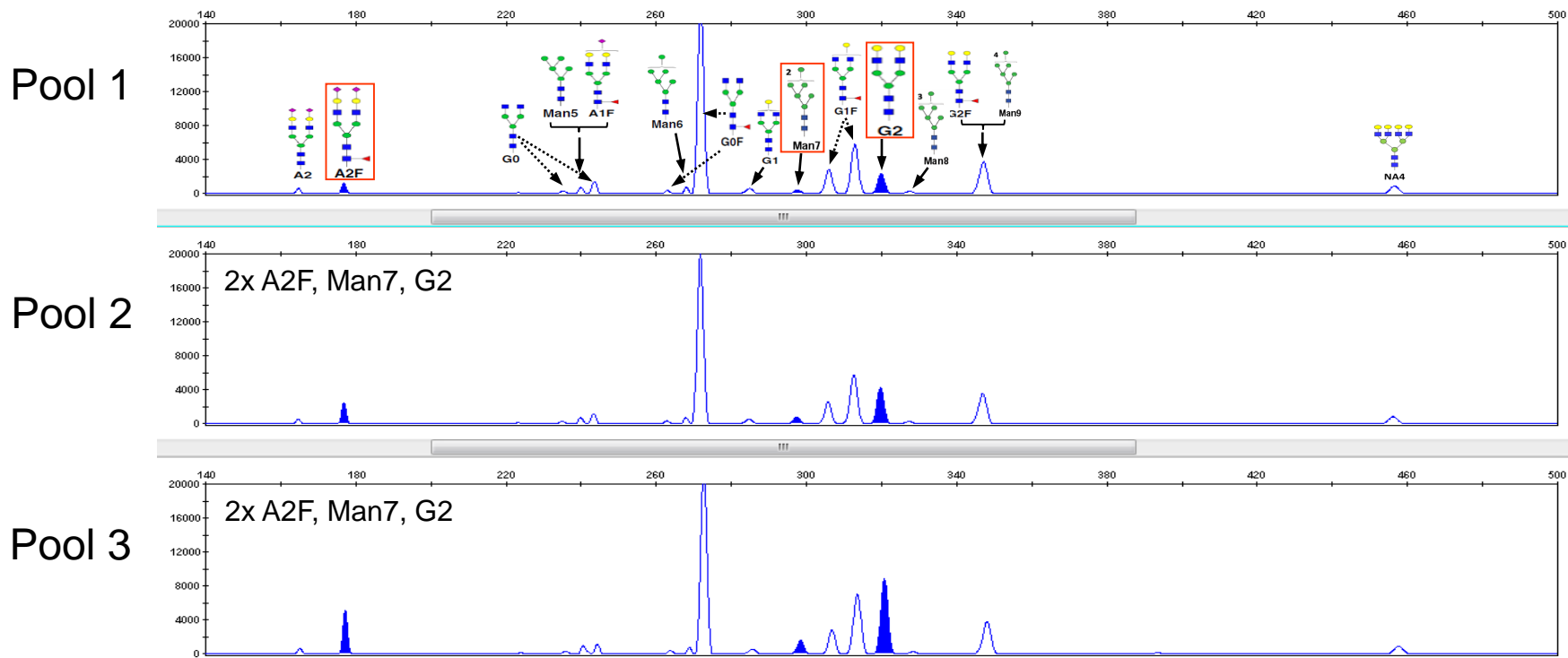
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13
Average	0.74	0.58	1.17	4.87	1.47	25.26	0.90	4.97	29.18	10.25	6.52	12.86	1.21
SD	0.05	0.04	0.05	0.15	0.06	0.20	0.05	0.03	0.27	0.09	0.06	0.15	0.03
% CV	6.44	6.14	3.98	3.13	3.99	0.77	5.11	0.63	0.92	0.92	0.92	1.13	2.75

CE RUN – 7 months later

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13
AVERAGE	0.72	0.57	1.22	4.86	1.58	25.73	0.96	5.02	29.26	10.3	6.55	12.63	1.22
SD	0.02	0.02	0.02	0.14	0.03	0.84	0.06	0.17	0.97	0.34	0.18	0.08	0.06
CV%	2.78	3.51	1.64	2.88	1.90	3.26	6.25	3.39	3.32	3.30	2.75	0.63	4.92

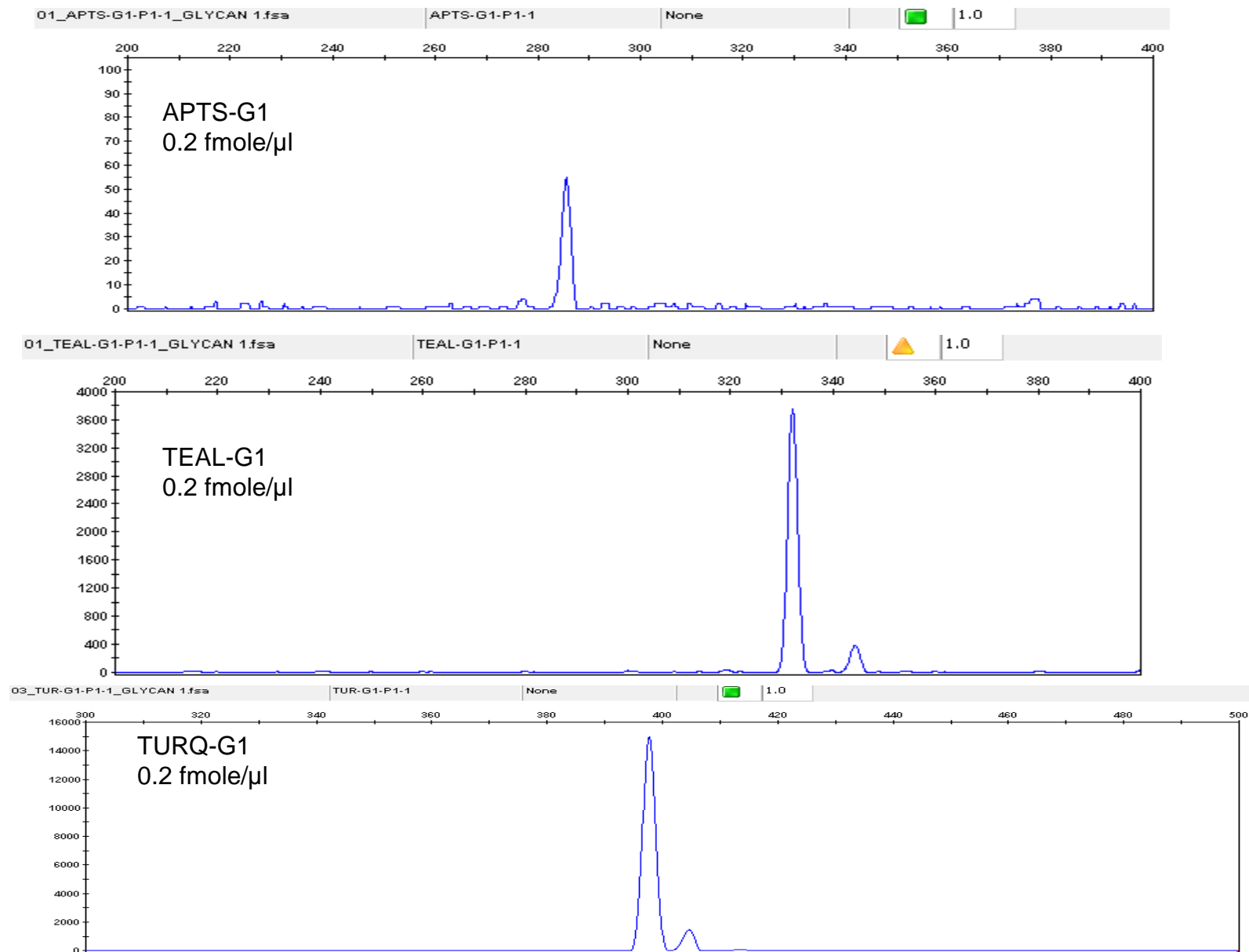
# Spike Recovery

- 15 diverse types of purified glycans
- Labeled with APTS, purified
- Mixed to create large difference in concentrations (50% - <1%)
- A2F (sialylated), Man7 (high mannose), and G2 (complex) glycans were increased 2X serially



Increased signals of three specific glycans while the rest remain constant

# Sensitivity





# Take Home Message

- **Easy sample prep**
  - ✓ Magnetic bead based sample prep
  - ✓ Hands-on-time <3 hrs for 96 samples
  - ✓ No use of Sod. Cyanoborohydride or vacuum centrifugation steps
- **Throughput**
  - ✓ Sample prep & data of 96 samples in 7-9hrs
- **Resolution**
  - ✓ Sialyated glycans, Fucose species, High Mannose species
  - ✓ Structural isomers
- **Dye Labeling Flexibility**
- **Sensitivity**
  - ✓ Analyze as little as 1µg of IgG
- **Low Cost**
  - ✓ Robust instrument and capillaries with low running cost
- **Integration**
  - ✓ Full sample prep, hardware & software solution
- **Support**
  - ✓ Full FAS support in every stages of method's implementation



# Acknowledgements

Jenkuei Liu  
Bharti Solanki-Nand  
Brian Evans  
Peter Bell  
Baburaj Kunnummal  
Dominic Caseñas  
Parita Ghia  
Michelle Yannetti



**Thank you**

For more information, please visit [thermofisher.com/glycanassure](https://thermofisher.com/glycanassure)

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The world leader in serving science

# Introducing Gibco™ GlycanTune™ A+, B+, C+ Total Feeds

## Complete feeds to dial in the glycosylation profile targeted

### What are they?

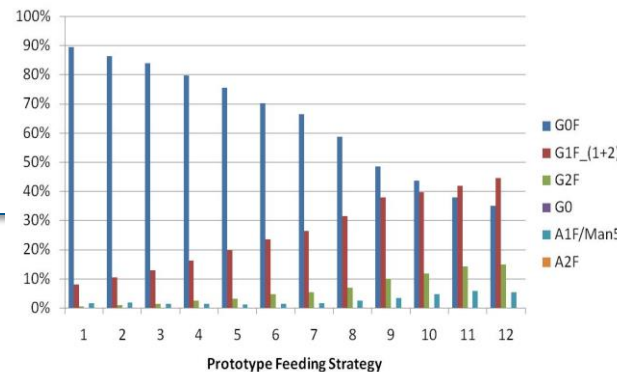
Complete feeds that:

- Match original EfficientFeed™ A+, B+, C+ supplements
- Include glycan shifting components
- pH neutral
- chemically defined
- animal origin-free



### What do they do?

- Shifts glycan profile to more G1, G2 from G0
- 2x-3x flexibility to reduce feeding volume or add more
- Reconstitute easily with just add water reconstitution
- Store at room temperature up to 30 days post hydration



***All the benefits of super-concentrating EfficientFeed+ products with the advantage of glycan targeting added***