Novel Fluorescent Labels for High Throughput N-glycan Analysis

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INTRODUCTION

Glycosylation is one of the key critical quality attributes of mAb based biotherapeutics. Glycosylation changes can impact biological drug's safety, efficacy, clearance and immunogenicity, making it necessary to accurately detect changes. Glycan profiling begins at cell line development and continues through process development and in certain cases drug substance release. Current glycan analysis methods involve laborious multistep sample preparation that takes anywhere from a day to multiple days for 96 samples, followed by single channel LC or CE separation. Here we report an integrated glycan solution that can generate data from 96 samples in 7-9 hrs; consisting of an easy magnetic bead based sample prep, 24 capillary array CE instrument and a glycan specific software for analysis.

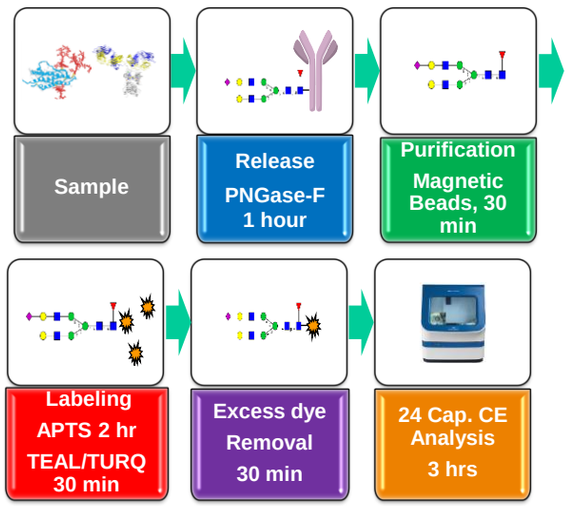

Figure 1. GlycanAssure™ Glycan Analysis Workflow – 3 hours of hands on time, 7-9 hours to process and analyze 96 samples with no vacuum centrifugation.

MATERIALS AND METHODS

All CE separations were performed using the 3500xL, a system configured with a 505 nm solid state laser and laser induced fluorescence detection (Applied Biosystems). 24 capillary arrays were used for separation of glycans. All other assay conditions were as described in the user guide for the Glycan Labeling and Analysis Kit (GlycanAssure™, Thermo Fisher Scientific, Publication Number MAN0014008). Glycan standards shown in the poster were obtained from V-Lab and Prozyme. The purified human serum IgG was obtained from Invitrogen (p/n 27102).

Experimental details for this work were as follow:

- Glycan separation polymer used: POP7
- Anode Buffer (p/n 4393927); Cathode buffer (p/n 4408256)
- Capillary length: total length = 61 cm, length to detector = 50 cm
- Capillary diameter: 50 µm I.D.
- Injection conditions: 1.6 kV for 24 sec.
- Run Voltage: 19.5 kV
- Capillary oven temperature: 60°C
- APTS EX 475nm EM 501nm; TEAL™ EX 466nm EM 505nm; TURQUOISE™ EX 493nm EM 520nm

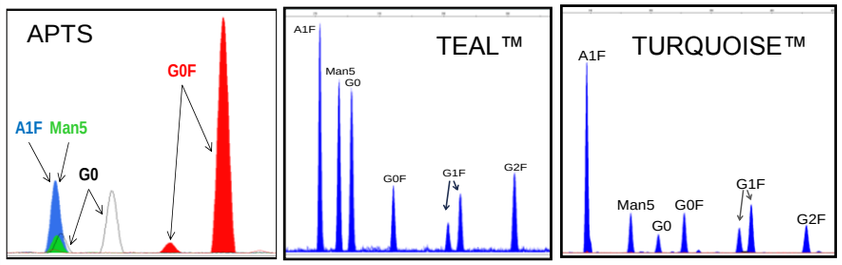

Figure 2. Separation of major glycans with APTS and proprietary dyes, TEAL and TURQUOISE. Glycans not resolved with conventional APTS, can be resolved with novel TEAL and TURQUOISE.

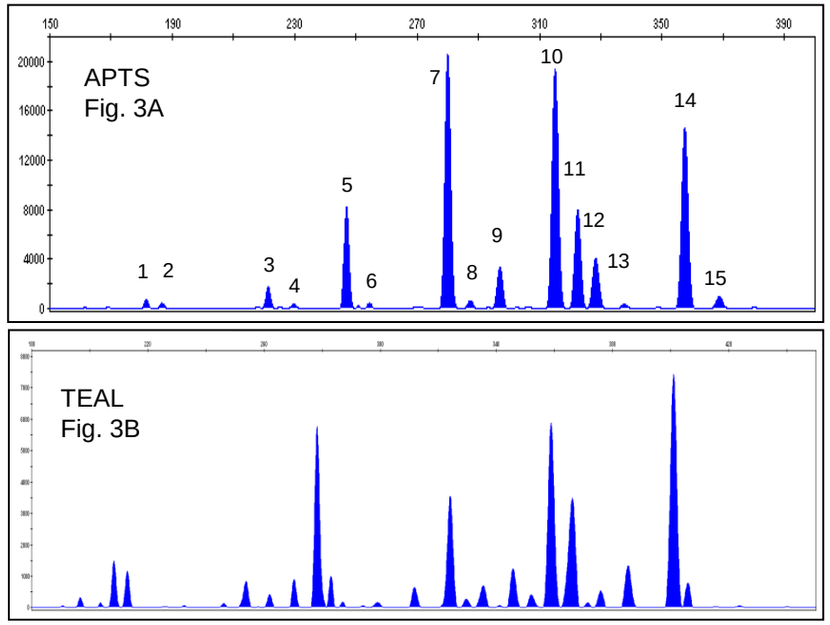

Figure 3. Separation of labeled N-glycans from human serum IgG. Proprietary dye TEAL (3B) resolves glycans better than conventional APTS (3A).

	10µg		50µg		100µg	
Peak #	%Area	%CV	%Area	%CV	%Area	%CV
1	0.90%	4.05%	0.72%	3.06%	0.64%	5.07%
2	0.61%	6.31%	0.45%	3.31%	0.39%	4.77%
3	1.64%	2.22%	1.69%	2.46%	1.66%	2.68%
4	0.42%	7.09%	0.42%	4.67%	0.38%	4.26%
5	8.54%	2.47%	8.31%	2.19%	8.18%	1.48%
6	0.76%	5.89%	0.55%	8.97%	0.42%	6.47%
7	22.51%	2.04%	23.07%	4.25%	23.71%	1.60%
8	0.63%	4.50%	0.71%	9.54%	0.71%	3.36%
9	3.73%	0.83%	3.82%	2.60%	3.85%	1.28%
10	23.52%	0.43%	23.33%	2.52%	23.84%	0.65%
11	9.98%	0.52%	10.16%	3.37%	9.91%	0.88%
12	5.37%	1.00%	5.46%	3.50%	5.42%	1.00%
13	0.43%	4.27%	0.44%	4.53%	0.43%	2.12%
14	19.58%	1.16%	19.53%	2.91%	19.20%	0.85%
15	1.38%	2.25%	1.33%	4.83%	1.27%	1.84%

Table 1. Consistent relative quantities of N-glycans from varying inputs of purified human serum IgG (Figure 3A). Eight independent sample preparations were performed for every input amount.

RESULTS AND DISCUSSION

The goal of the project was to develop novel fluorescent dyes to label glycans combined with high throughput separation method to quantitate complex glycan species associated with therapeutic glycoproteins. These glycans include structures with and without core fucose moiety, terminal galactose, terminal sialic acids, high mannose structures and several positional isomers. Cleaved glycans were purified using magnetic beads and labeled with fluorescent molecules APTS, TEAL, and TURQUOISE using optimized labeling conditions. Excess dye was removed using same magnetic beads used for glycan purification. CE separation was performed using optimized conditions.

Separation efficiencies of novel fluorescent dye labeled glycans were benchmarked against widely used APTS dye labeled glycans. In APTS dye labeled glycans we observed co-migration of Man5/A1F glycans. Both Teal and Turquoise labeled Man5/A1F glycans were baseline resolved (Figure 2). We attained the best resolution with TEAL where not only A1F/Man5 and Man9/G2F glycan pairs were resolved, but also positional isomers of A1, Man6, G1F, Man8 and Man9. (Data not shown). Proprietary dye TEAL resolves N-glycans from human serum IgG better than conventional APTS (Figure 3). Consistent relative quantities of glycans were obtained from varying inputs of human serum IgG in the range of 10 -100µg (Table 1).

Normalization with internal size standards co-migrating in every capillary, revealed highly reproducible separation of multiple glycan species (12 repeat injections across 24 capillaries, total 288 injections; Figure 4).

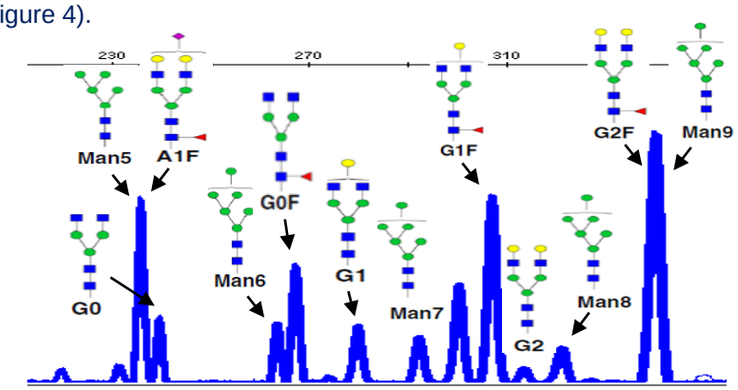

Figure 4. Overlay of 288 injections from 24-capillaries (3500xL) Glycan standards labeled with APTS

CONCLUSIONS

- **Streamlined Workflow:** Sample preparation workflow that includes new dyes, software and automatable purification capable of parallel processing of 96 samples and analysis on Applied Biosystems 3500xL CE system in less than 9 hours.
- **Resolution:** New dyes offer better resolution of key glycans
- **Precision:** High Run-to-run, capillary-to-capillary, and instrument-to-instrument reproducibility supports high throughput analysis of protein glycans.