Received: 11 August 2017 | Revised: 27 February 2018 | Accepted: 10 March 2018

DOI: 10.1002/rcm.8116

RESEARCH ARTICLE

WILEY Rapid Communications in Mass Spectrometry

On-line capillary electrophoresis/laser-induced fluorescence/mass spectrometry analysis of glycans labeled with Teal™ fluorescent dye using an electrokinetic sheath liquid pump-based nanospray ion source

Shaheer Khan[1] | Jenkuei Liu[1] | Zoltan Szabo[1] | Baburaj Kunnummal[1] | Xiaorui Han[2] | Yilan Ouyang[2] | Robert J. Linhardt[2] | Qiangwei Xia[3]

[1] Pharma Analytics, BioProduction Division, Thermo Fisher Scientific, 180 Oyster Point Blvd, South San Francisco, CA 94080, USA

[2] Rensselaer Polytechnic Institute, Biotech 4005, 110 8th Street, Troy, NY 12180, USA

[3] CMP Scientific Corp, 760 Parkside Ave, STE 211, Brooklyn, NY 11226, USA

Correspondence
Q.W. Xia, CMP Scientific Corp., 760 Parkside Ave, STE 211, Brooklyn, NY 11226, USA.
Email: james.xia@cmpscientific.com

Present address
B. Kunnummal, SCIEX, 1201 Radio Road, Redwood City, CA 94065, USA.

Rationale: N-linked glycan analysis of recombinant therapeutic proteins, such as monoclonal antibodies, Fc-fusion proteins, and antibody-drug conjugates, provides valuable information regarding protein therapeutics glycosylation profile. Both qualitative identification and quantitative analysis of N-linked glycans on recombinant therapeutic proteins are critical analytical tasks in the biopharma industry during the development of a biotherapeutic.

Methods: Currently, such analyses are mainly carried out using capillary electrophoresis/laser-induced fluorescence (CE/LIF), liquid chromatography/fluorescence (LC/FLR), and liquid chromatography/fluorescence/mass spectrometry (LC/FLR/MS) technologies. N-linked glycans are first released from glycoproteins by enzymatic digestion, then labeled with fluorescence dyes for subsequent CE or LC separation, and LIF or MS detection. Here we present an on-line CE/LIF/MS N-glycan analysis workflow that incorporates the fluorescent Teal™ dye and an electrokinetic pump-based nanospray sheath liquid capillary electrophoresis/mass spectrometry (CE/MS) ion source.

Results: Electrophoresis running buffer systems using ammonium acetate and ammonium hydroxide were developed for the negative ion mode CE/MS analysis of fluorescence-labeled N-linked glycans. Results show that on-line CE/LIF/MS analysis can be readily achieved using this versatile CE/MS ion source on common CE/MS instrument platforms.

Conclusions: This on-line CE/LIF/MS method using Teal™ fluorescent dye and electrokinetic pump-based nanospray sheath liquid CE/MS coupling technology holds promise for on-line quantitation and identification of N-linked glycans on recombinant therapeutic proteins.

1 | INTRODUCTION

N-linked glycans are a family of oligosaccharides that are attached to the asparagine residues of a protein.[1] Most recombinant therapeutic proteins are glycoproteins with N-linked glycans.[2-4] Given the structural diversity of N-linked glycans, both qualitative identification and relative quantitation of the glycan species are important for therapeutic protein quality assessment.[5-8] Because of the lack of a fluorophore for laser-induced fluorescence (LIF) detection and charged groups for mass spectrometry (MS) identification, N-linked glycan analysis is currently carried out by releasing the glycans from the proteins using PNGase-F enzyme digestion, followed by labeling with a fluorescence dye. It has been previously reported that adding a fluorescence tag greatly facilitates both chromatographic and electrophoretic separation

of the N-linked glycans.[9-14] Therefore, these fluorescence dyes are usually optimized not only for sufficient fluorescence quantum yield, but also for high separation efficiency and strong MS response. Two main workflows have been developed over the past two decades for the analysis of these fluorescence-labeled N-linked glycans: one is capillary electrophoresis/laser-induced fluorescence (CE/LIF); the other is liquid chromatography/fluorescence mass spectrometry (LC/FLR-MS). Considering the high separation efficiency of CE, it is highly desirable to have the CE instrument equipped with either MS detection (CE/MS[11,12]) or on-line laser-induced fluorescence and mass spectrometry detection (CE/LIF/MS[13-15]) capabilities. However, despite the success of many CE/LIF assays, CE/MS and on-line CE/LIF/MS technologies have been challenging because of difficulties in CE/MS coupling and challenges in method development.[13-15] With the surge in recombinant therapeutic protein development, CE/MS and on-line CE/LIF/MS analyses of fluorescence-labeled N-linked glycans have become increasingly important.

Taking into consideration the needs for MS identification, we recently developed a multi-capillary CE system and a fluorescence dye (the Teal™ dye) workflow to allow for rapid fluorescence labeling of N-linked glycans, high glycan separation efficiency, high fluorescence quantum yield, and strong negative ion mode MS response brought by the three sulfonate groups on this novel fluorescence dye. The current study explores the first use of a newly developed electrokinetically pumped nanospray ion source[16-18] for the CE/MS and CE/LIF/MS analysis of the fluorescence-labeled N-linked glycans.

2 | EXPERIMENTAL

2.1 | Materials

Glycan standards were purchased from V-Labs, Inc. (Covington, LA, USA) and Prozyme (Hayward, CA, USA). Sample preparation of glycan standards and Teal™ and APTS labeling reactions were performed as described in the GlycanAssure™ user manual.[19] For NIST-mAb (RM8671) glycan release and labeling, 10 µL NIST-mAb (10 µg/µL) was deglycosylated, glycans were recovered by magnetic beads and labeled with Teal™ as described earlier,[20] except an additional magnetic bead purification step was needed to remove the excess dye that was required to achieve maximum labeling. Teal-labeled glycans from four separate deglycosylation reactions were labeled, purified by magnetic beads, dried in vacuum and diluted with 30 µL deionized water to provide the sample for CE/MS analysis. Ammonium acetate (LC/MS grade), ammonium hydroxide (LC/MS grade), and methanol (LC/MS grade) were purchased from Sigma Aldrich.

2.2 | Purification by high-performance liquid chromatography (HPLC)

The glycan-dye reaction mixture was suspended in acetonitrile/water (75:25 v/v) and purified by HPLC (HP1100; Agilent) equipped with an on-line fluorescence detection system. The chromatography was carried out on a XBridge® Glycan BEH Amide column (4.6 Å, 3.5 µm particle size, 100 × 4.6 mm; Waters) using a gradient from 75% acetonitrile to 50% acetonitrile in water at flow rate of 0.75 mL/min over 45 min. The column temperature was maintained at 50°C. The fractions corresponding to product were collected and dried in vacuum and diluted with water to a desired concentration. The concentration of the formulated glycan-dye conjugate was confirmed by UV/VIS spectroscopy.

2.3 | CE/MS analysis

The CE separation was performed on a Beckman Coulter P/ACE MDQ capillary electrophoresis unit and an EMASS-II CE-MS ion source (CMP Scientific Corp., Brooklyn, NY, USA). Either an ammonium acetate based buffer or an ammonium hydroxide based buffer system was used as background electrolyte. The sheath liquid was either 5 mM ammonium acetate, 80% acetonitrile, or 10 mM ammonium bicarbonate, 99% methanol. The sample was injected under 5–7 psi pressure for 5–7 s. A separation voltage of 30 kV under normal polarity was used. The electrospray voltage at the CE/MS interface was −1.7 to −2.0 kV. The spray emitter was 1.0 mm O.D. × 0.75 mm I.D., borosilicate glass with a 15-µm tip and the distance from the emitter tip to the mass spectrometer was adjusted to 2–4 mm with the help of a microscope.

A LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) was coupled online to the CE system. The MS analysis was carried out in negative ion mode. The capillary voltage was −35 V. The capillary temperature was 250°C and the tube lens voltage was −100 V. The Fourier transform (FT) MS resolution was 30,000 and all other parameters were set as default.

2.4 | CE/LIF/MS analysis

The CE separation was performed on a Beckman Coulter P/ACE MDQ CE system equipped with laser-induced fluorescence (LIF) detection and an EMASS-II CE/MS ion source (CMP Scientific Corp., Brooklyn, NY, USA). The CE/LIF cartridge that comes with the P/ACE MDQ was used to support the separation capillary installation. The excitation laser was 488 nm and the emission wavelength was set at 520 nm.

2.5 | CE/LIF/MS capillary

The separation capillaries used for the on-line CE/LIF/MS analysis were manufactured by CMP Scientific Corp. (Brooklyn, NY, USA). The capillary I.D., capillary length, the distance from the capillary inlet to the LIF detection window, and the distance from the LIF detection window to the mass spectrometer were optimized for separation and alignment between the LIF channel and mass spectrometry data.

2.6 | Data analysis

The MS data analysis was performed using Xcalibur software (Thermo Fisher Scientific). The laser-induced fluorescence data analysis was performed on Beckman Coulter's 32 Karat™ software.

3 | RESULTS AND DISCUSSION

For simplicity, throughout this paper we use prefix of either "Teal-" or "APTS-" to indicate that the glycans under discussion are labeled with one of the dyes, whereas non-labeled glycans are referred to using their regular glycan symbols.

3.1 | APTS and Teal™ dyes

One commonly used fluorescence dye, aminopyrene trisulfonic acid (APTS), has been routinely used in the biopharmaceutical industry for the characterization of N-linked glycans released from recombinant therapeutic proteins.[11-14] We recently developed a non-reductive amination fluorescence dye, the Teal™ dye, which reacts with reducing sugars to form stable products. The structures of APTS and Teal™ contain three sulfonic acids. The APTS and Teal™ dyes, in their free forms, have excitation max wavelengths of 423–430 and 465–466 nm, respectively. After being conjugated with glycans, the excitation wavelengths of both dyes are close to each other: ~450 nm for conjugated APTS; ~466 nm for conjugated Teal™. The emission wavelengths of both conjugated dyes are also similar: ~515 nm for conjugated APTS; ~520 nm for conjugated Teal™. The fact that the Teal™ dye shows very similar excitation and emission wavelengths to those of the APTS dye is valuable because one can analyze Teal™ dye labeled glycans on existing CE/LIF instruments that were designed for the APTS glycan labeling chemistry.

3.2 | CE/MS configuration

Although high reactivity and separation efficiency of the Teal™ dye in CE/LIF assays have been reported earlier,[20] MS detection of Teal-labeled glycans is highly desirable to generate accurate identification and minimize peak assignment ambiguity. The CE/MS method that we adopted for the analysis of Teal-labeled glycans uses an electrokinetically pumped nanospray sheath liquid CE/MS interface design (Figure S1, supporting information).[16-18,21-23] The analytes are injected into the separation capillary from the capillary inlet, which is placed inside the CE system. The capillary outlet was, instead of being placed inside the CE unit, taken out and placed through a CE/MS interface tee, pointing towards the mass spectrometer. In order to minimize sheath liquid dilution to the analytes, and maximize detection sensitivity, a few millimeters of the outlet end were etched from an O.D. of ~360 μm to ~150 μm. A borosilicate glass electrospray emitter was placed coaxially to cap the capillary outlet. A sheath liquid vial was housed inside and connected through plastic tubing to the side of the CE/MS interface tee. An external power supply provided electrospray voltage, which is wired to the side of the ions source, and passed onto the spray emitter through an electrode placed inside the sheath liquid vial (Figure S1, supporting information). The electrospray voltage that was applied onto the spray emitter generates electroosmotic flow, which delivers a steady nanoflow of sheath liquid. When analytes migrate out of the capillary, the surrounding sheath liquid entrains the molecules and brings them into the mass spectrometer. We have demonstrated in earlier studies the use of this nanoflow sheath liquid CE/MS ion source for both positive and negative ion mode MS analysis of highly sulfated oligosaccharides.[18]

3.3 | CE/MS using ammonium acetate buffer

The Teal-G2 glycan (Figure 1) was used in initial experiments. After method development, 10 mM ammonium acetate with 40% acetonitrile was found to work well as background electrolyte (BGE) for negative mode CE/MS analysis (data not shown). However, it was observed that the 40% acetonitrile buffer rapidly evaporated, degrading run-to-run reproducibility. The 40% acetonitrile was replaced with 20% acetonitrile and 20% isopropanol to increase the background electrolyte viscosity and to reduce evaporation. This buffer composition of 10 mM ammonium acetate, 20% acetonitrile, 20% isopropanol, greatly enhanced the CE/MS performance. On a 70-cm bare fused-silica capillary with an I.D. of 50 μm, within 12 min the Teal-G2 glycan came out as a ~20 s wide peak, showing as doubly (*m/z* 1102.78) and triply (*m/z* 734.85) charged species in the mass spectrum.

The BGE system was then tested with a mixture of Teal-Man6 and Teal-A2F (Figure 2). The two Teal-labeled glycans were baseline separated by more than 1 min. In addition, the salt peak migrated much earlier than the glycan peaks. This shows good on-line desalting and separation efficiency using this ammonium acetate based BGE. Further testing of three repeat injections using a mixture of Teal-Man6 and Teal-A2F (Figure S2, supporting information) showed excellent migration time reproducibility.

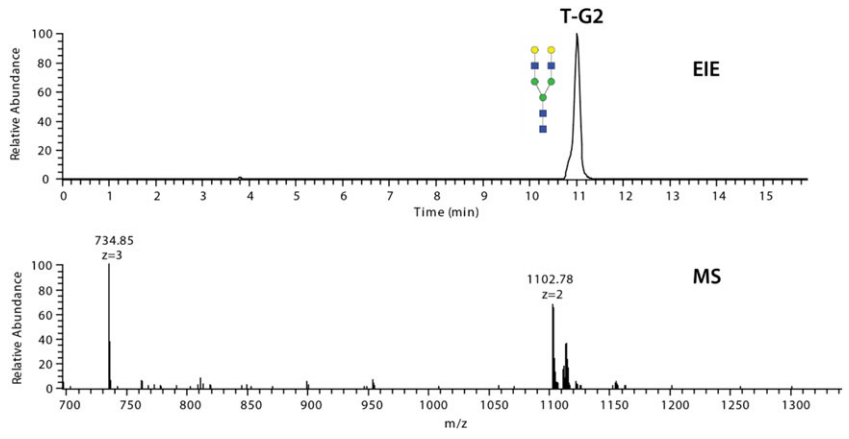

FIGURE 1 Extracted ion electropherogram (*m/z* 900–1000) of Teal-G2 using ammonium acetate based BGE. Separation capillary: 360 μm O.D., outlet O.D. etched to 150 μm, 50 μm I.D., 70 cm bare fused silica; background electrolyte: 10 mM ammonium acetate, 20% acetonitrile, 20% isopropanol; sheath liquid: 5 mM ammonium acetate, 80% acetonitrile; injection; 5 psi, 5 s; separation: 30 kV, normal polarity; electrospray: −1.7 kV [Color figure can be viewed at wileyonlinelibrary.com]

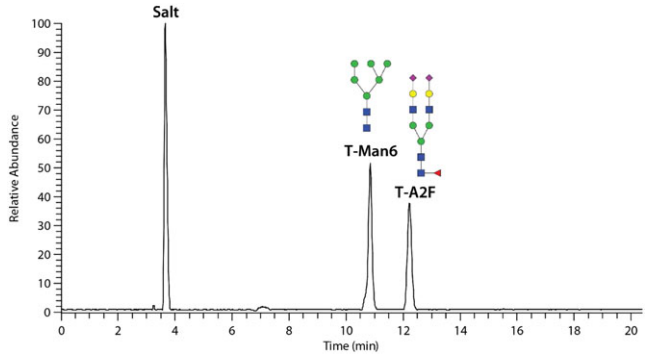

FIGURE 2 Extracted ion electropherogram (*m/z* 900–1000) of Teal-Man6 and Teal-A2F using ammonium acetate based BGE. Separation capillary: 360 μm O.D., outlet O.D. thinned to 150 μm, 50 μm I.D., 70 cm bare fused silica; background electrolyte: 10 mM ammonium acetate, 20% acetonitrile, 20% isopropanol; sheath liquid: 5 mM ammonium acetate, 80% acetonitrile; injection; 7 psi, 7 s; separation: 30 kV, normal polarity; electrospray: −1.7 kV [Color figure can be viewed at wileyonlinelibrary.com]

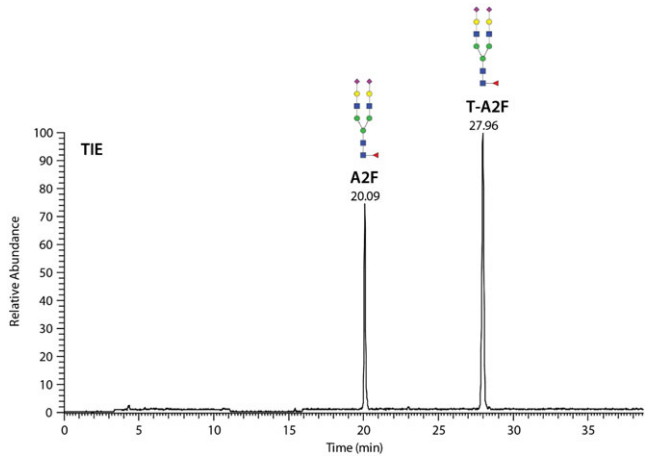

FIGURE 3 Ammonium hydroxide buffer system for Teal-labeled N-glycan CE-MS analysis. Total ion electropherogram of A2F and Teal-A2F using ammonium acetate based BGE. Separation capillary: 360 μm O.D., outlet O.D. thinned to 150 μm, 75 μm I.D., 100 cm bare fused silica; background electrolyte: 0.5 N ammonium hydroxide, 50% methanol; sheath liquid: 10 mM ammonium bicarbonate, 99% methanol; Injection: 1 psi, 5 s; BGE plug injection: 1 psi, 5 s; separation: 30 kV, normal polarity; electrospray: −1.9 kV [Color figure can be viewed at wileyonlinelibrary.com]

3.4 | CE/MS using ammonium hydroxide buffer

Although the ammonium acetate based background electrolyte and sheath liquid buffer systems performed well, later it was observed that the bare fused-silica capillary surface needed to be regenerated frequently (data not shown). An ammonium hydroxide buffer system was explored as the background electrolyte that can facilitate regeneration of the bare fused-silica capillary surface during analysis. Initial efforts were spent on testing a previously reported alkaline BGE system.[13] However, soon it was realized that the BGE of 0.7 M ammonia, 0.1 M ε-aminocaprioic acid, 70% methanol as previously reported generated very high mass spectrometry background, preventing effective glycan identification. Taking into consideration that the previously reported CE/MS work was carried out using a much higher sheath liquid flow than that on the EMASS-II ion source,[13,18,21-23] it was decided to remove ε-aminocaprioic acid from the formulation, and only use ammonium hydroxide and

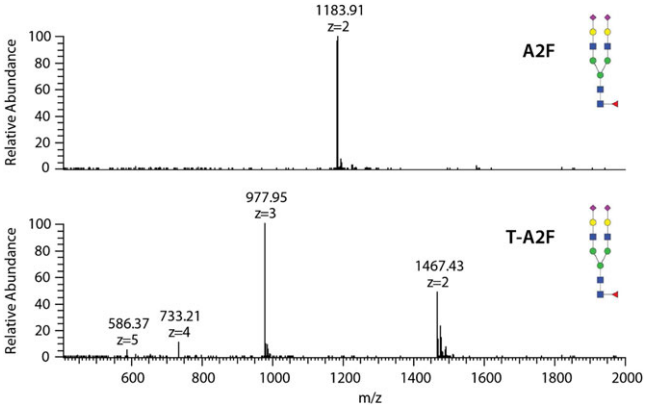

FIGURE 4 Teal™ label adds up to three additional negative charges to the glycan. Mass spectra of (top) unlabeled A2F and (bottom) Teal-labeled A2F [Color figure can be viewed at wileyonlinelibrary.com]

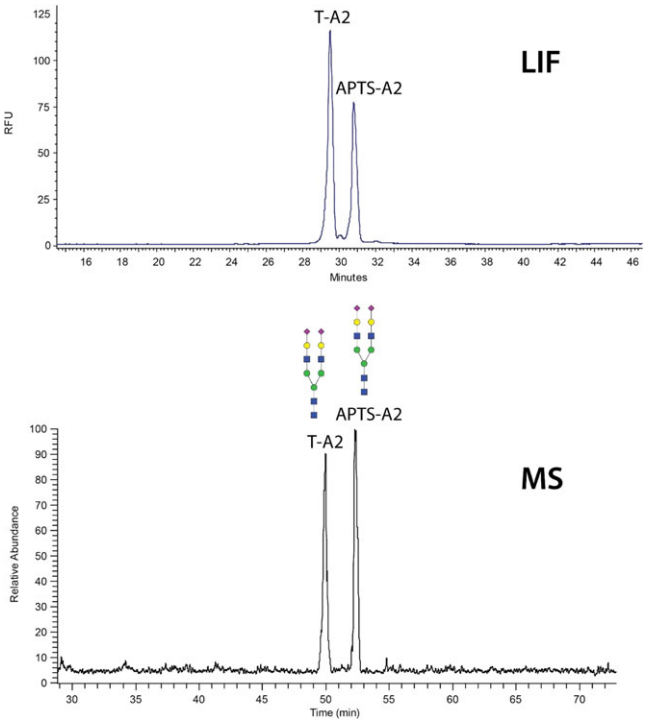

FIGURE 5 Comparison of LIF and MS responses of Teal- and APTS-labeled A2 glycans using the online CE/LIF/MS system: (top) LIF signal of Teal- and APTS-labeled A2 glycans; (bottom) MS signal of Teal- and APTS-labeled A2 glycans. Sample: 0.7 pmol/μL of Teal- and APTS-labeled A2 glycan mixture; separation capillary: 360 μm O.D., outlet O.D. thinned to 150 μm, 75 μm I.D., 115 cm bare fused silica, 70 cm from inlet to LIF detector; background electrolyte: 0.7 N ammonium hydroxide, 70% methanol; sheath liquid: 10 mM ammonium bicarbonate, 99% methanol; Injection: 1.5 psi, 5 s; BGE plug injection: 1.5 psi, 5 s; separation: 30 kV, normal polarity; electrospray: −1.9 kV [Color figure can be viewed at wileyonlinelibrary.com]

methanol in the BGE buffer. Figure 3 shows the CE/MS result of a mixture of Teal-A2F and unlabeled A2F using 0.5 M ammonium hydroxide, 50% methanol. The migration time of the native A2F glycan is 20 min, whereas the Teal-A2F migrated at 28 min. This wide separation window is highly desirable, as it provides ample room for detecting minor species in complex mixtures. The mass spectra of the unlabeled A2F and Teal-A2F (Figure 4) showed that the Teal™ dye added three additional negative charges to A2F: native A2F only exhibited the doubly charged form (*m/z* 1183.91), whereas Teal-A2F was detected with up to five negative charges (*m/z* 586.37).

3.5 | On-line CE/LIF/MS analysis

Despite the availability of many high-resolution and high mass accuracy mass spectrometers, simultaneous LIF detection in addition to MS detection is often highly desirable for the analysis of fluorescence-labeled N-glycans.[13-15] There are a number of reasons for this: first, these fluorescent dyes were designed and optimized for CE/LIF analysis; second, from a biopharmaceutical researcher's point of view, it is always important to have an orthogonal technology to cross-validate the findings from one technology – optical detectors are fully orthogonal to mass spectrometers, thus are valuable in validation; third, unlike unwieldy and expensive mass spectrometers, LIF detectors are inexpensive and universal; fourth, LIF detectors can offer a much wider linear dynamic range than high mass accuracy mass spectrometers – so that researchers are more likely to rely on the LIF channel for quantitation; fifth, LIF detection signals can often be converted to provide absolute quantitation, which is valuable in many circumstances.

We intended to build a CE/LIF/MS workflow with minimum modification on commonly available instruments. Figure S3 (supporting information) shows the successful setup using a commercially available capillary cartridge: we purposely extended the capillary after the LIF detection window, so that the capillary outlet can be inserted into the CE/MS interface tee on the EMASS-II ion source. Such a capillary is depicted in Figure S3 (supporting information): the entire length of this capillary is 115 cm, of which 70 cm is from the inlet to the LIF detection window, the other 45 cm allows for the capillary outlet to reach the mass spectrometer through the CE/MS ion source.

Teal™ and APTS dyes were compared using this CE/LIF/MS system (Figure 5). A mixture of Teal-A2 and APTS-A2 was analyzed: the Teal-A2 and APTS-A2 glycans passed through the LIF detection window at 29.5 and 31 min, respectively (Figure 5A); about 20 min later, they reached

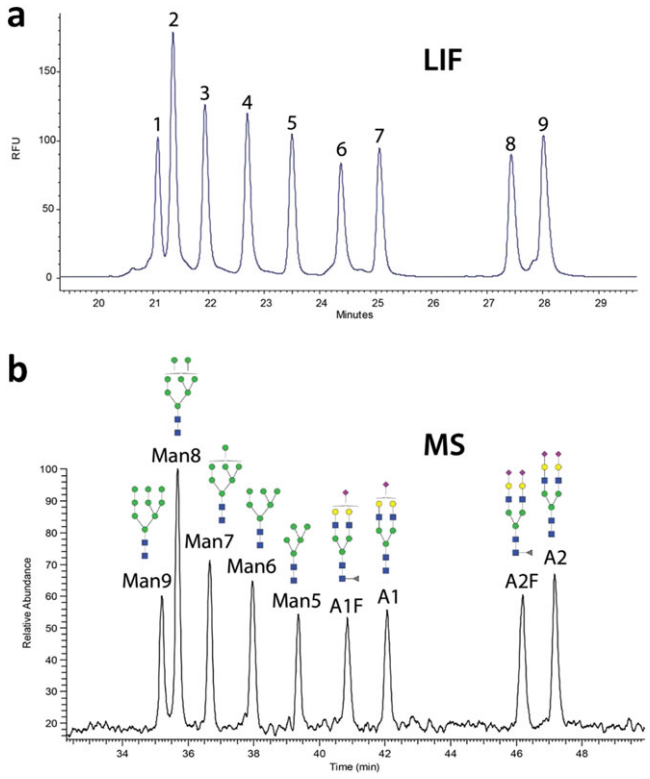

FIGURE 6 CE-LIF-MS of Teal-labeled high mannose and acidic N-glycans: (A) LIF signal of Teal-labeled high mannose and acidic glycans; (B) MS signal of Teal-labeled high mannose and acidic glycans. Sample: mixture of 1.1 pmol/μL of each Teal-labeled glycan; separation capillary: 360 μm O.D., outlet O.D. thinned to 150 μm, 75 μm I.D., 115 cm bare fused silica, 70 cm from inlet to LIF detector; background electrolyte: 0.7 N ammonium hydroxide, 70% methanol; sheath liquid: 10 mM ammonium bicarbonate, 99% methanol; Injection: 0.3 psi, 5 s; BGE plug injection: 0.3 psi, 5 s; separation: 30 kV, normal polarity; electrospray: −1.9 kV [Color figure can be viewed at wileyonlinelibrary.com]

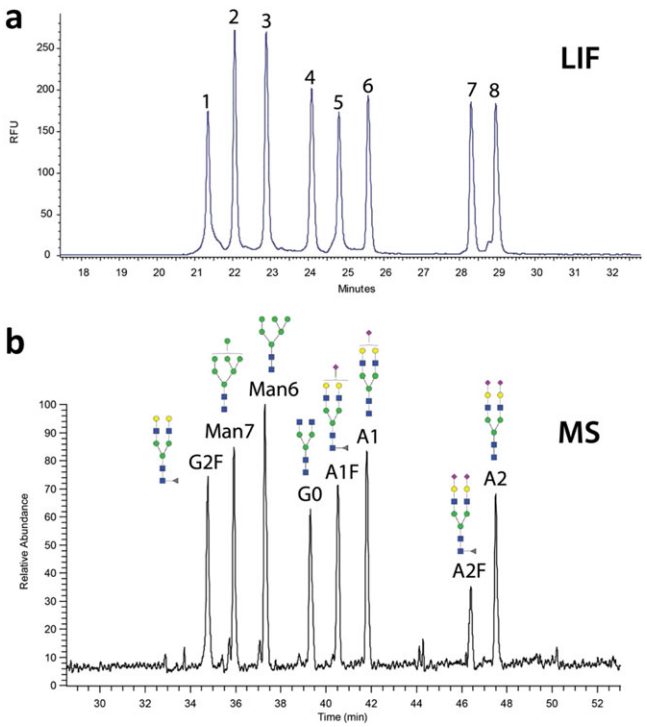

FIGURE 7 CE-LIF-MS of Teal-labeled common N-glycans: (A) LIF signal of Teal-labeled common glycans; (B) MS signal of Teal-labeled common glycans. Sample: mixture of 1.25 pmol/μL of each Teal-labeled glycan; separation capillary: 360 μm O.D., outlet O.D. thinned to 150 μm, 75 μm I.D., 115 cm bare fused silica, 70 cm from inlet to LIF detector; background electrolyte: 0.7 N ammonium hydroxide, 70% methanol; sheath liquid: 10 mM ammonium bicarbonate, 99% methanol; Injection: 0.5 psi, 5 s; BGE plug injection: 0.5 psi, 5 s; separation: 30 kV, normal polarity; electrospray: −1.9 kV [Color figure can be viewed at wileyonlinelibrary.com]

the mass spectrometer, one after the other (Figure 5B). Teal-A2 glycan showed ~67% higher peak intensity than APTS-A2 in the LIF channel, whereas Teal-A2 showed similar peak height to APTS-A2 in the mass spectrum. The complex impact on the electrospray ionization of glycans by incorporation of a highly charged group has been thoroughly studied and reported by Muddiman's group.[24] It is therefore important to test the MS response of the Teal-labeled glycans, and to compare that with the signal of APTS-labeled glycans. This result suggests that the Teal™ dye is fully compatible with this CE/LIF/MS system, and provides comparable LIF and MS detection sensitivity to the APTS dye.

3.6 | On-line CE/LIF/MS of complex glycan mixture

Two complex glycan mixtures were tested using this on-line CE/LIF/MS method. One set contains a series of high mannose and sialic acid glycans (Figure 6); the other set contains a number of N-glycans that are commonly observed on recombinant therapeutic monoclonal antibodies (Figure 7). In both sample sets, all of the Teal-labeled glycans were baseline separated. In addition, the LIF peaks are well aligned with the MS peaks. The stacked view of LIF and MS data (Figures 6 and 7) clearly demonstrates that one-to-one peak assignment from LIF to MS data can be unambiguously performed, allowing for simultaneous quantification by LIF and identification by MS. Finally, the N-glycans released from NISTmAb were Teal-labeled and analyzed by CE/LIF/MS and peaks corresponding to the expected oligosaccharide components were detected (Figure S4, supporting information).

4 | CONCLUSIONS

Using two background electrolyte buffer systems, we demonstrated that a novel fluorescent dye is compatible with mass spectrometry for the analysis of N-glycans. Analysis on an on-line CE/LIF/MS system that we have developed showed that this novel dye generated a similar level of detection sensitivity to the APTS dye. Because of its higher reactivity, this dye has the advantage of needing much less in quantity to label glycans than that of APTS dye – this may be critical for future CE/LIF/MS method development. Usually, fluorescent dyes come with lots of salt – the more labeling reagents that need to be used, the more sample cleaning is needed for subsequent MS analysis. Furthermore, the successful development of various CE/MS and CE/LIF/MS methods for N-glycan analysis clearly demonstrates the versatility of this electrokinetic sheath liquid pump-based nanospray CE/MS technology and its potential in therapeutic protein characterization.

ACKNOWLEDGEMENTS

The authors would like to thank colleagues at CMP Scientific Corp. and Thermo Fisher Scientific for valuable discussions regarding conditions for negative mode electrospray ionization. Special thanks to Prof. Norman Dovichi and his research group at the University of Notre Dame for sharing their insights for negative ion mode electrospray buffer conditions.

ORCID

Qiangwei Xia http://orcid.org/0000-0003-3617-1808

REFERENCES

1. Roy J. Glycosylation of antibody therapeutics: Optimization for purpose. *Methods Mol Biol*. 2009;483:223-238. https://doi.org/10.1007/978-1-59745-407-0_13
2. Walsh G, Jefferis R. Post-translational modifications in the context of therapeutic proteins. *Nat Biotechnol*. 2006;24(10):1241-1252. https://doi.org/10.1038/nbt1252
3. Jefferis R. Posttranslational modifications and the immunogenicity of biotherapeutics. *J Immunol Res*. 2016;1-15. https://doi.org/10.1155/2016/5358272
4. Beck A, ed. *Glycosylation Engineering of Biopharmaceuticals*. Vol. 988. Totowa, NJ: Humana Press; 2013:81-91. doi:https://doi.org/10.1007/978-1-62703-327-5
5. Damen CWN, Chen W, Chakraborty AB, et al. Electrospray ionization quadrupole ion-mobility time-of-flight mass spectrometry as a tool to distinguish the lot-to-lot heterogeneity in N-glycosylation profile of the therapeutic monoclonal antibody trastuzumab. *J Am Soc Mass Spectrom*. 2009;20(11):2021-2033. https://doi.org/10.1016/j.jasms.2009.07.017
6. Kawasaki N, Itoh S, Hashii N, et al. The significance of glycosylation analysis in development of biopharmaceuticals. *Biol Pharm Bull*. 2009;32(5):796-800. https://doi.org/10.1248/bpb.32.765
7. Lingg N, Zhang P, Song Z, Bardor M. The sweet tooth of biopharmaceuticals: Importance of recombinant protein glycosylation analysis. *Biotechnol J*. 2012;7(12):1462-1472. https://doi.org/10.1002/biot.201200078
8. Zhang YJ, Luo L, Desai DD. Overview on biotherapeutic proteins: impact on bioanalysis. *Bioanalysis*. 2015;8(1):1-9. https://doi.org/10.4155/bio.15.224
9. Chiesa C, O'Neill RA. Capillary zone electrophoresis of oligosaccharides derivatized with various aminonaphthalene sulfonic acids. *Electrophoresis*. 1994;15(1):1132-1140. https://doi.org/10.1002/elps.11501501171
10. Zieske LR, Fu D, Khan SH, O'Neill RA. Multi-dimensional mapping of pyridylamine-labeled N-linked oligosaccharides by capillary electrophoresis. *J Chromatogr A*. 1996;720(1):395-407. https://doi.org/10.1016/0021-9673(95)00346-0
11. Zaia J. Capillary electrophoresis-mass spectrometry of carbohydrates. *Methods Mol Biol*. 2013;984:13-25. https://doi.org/10.1007/978-1-62703-296-4_2
12. Mittermayr S, Bones J, Guttman A. Unraveling the Glyco-Puzzle: Glycan structure identification by capillary electrophoresis. *Anal Chem*. 2013;85(9):4228-4238. https://doi.org/10.1021/ac4006099
13. Bunz S-C, Cutillo F, Neusüß C. Analysis of native and APTS-labeled N-glycans by capillary electrophoresis/time-of-flight mass spectrometry. *Anal Bioanal Chem*. 2013;405(25):8277-8284. https://doi.org/10.1007/s00216-013-7231-4
14. Bunz S-C, Rapp E, Neusüss C. Capillary electrophoresis/mass spectrometry of APTS-labeled glycans for the identification of unknown glycan species in capillary electrophoresis/laser-induced fluorescence systems. *Anal Chem*. 2013;85(21):10218-10224. https://doi.org/10.1021/ac401930j
15. Gennaro LA, Salas-Solano O. On-line CE–LIF–MS technology for the direct characterization of N-linked glycans from therapeutic antibodies. *Anal Chem*. 2008;80(10):3838-3845. https://doi.org/10.1021/ac800152h
16. Han M, Rock BM, Pearson JT, Rock DA. Intact mass analysis of monoclonal antibodies by capillary electrophoresis–mass spectrometry. *J Chromatogr B*. 2016;1011:24-32. https://doi.org/10.1016/j.jchromb.2015.12.045
17. Sun X, Lin L, Liu X, et al. Capillary electrophoresis–mass spectrometry for the analysis of heparin oligosaccharides and low molecular weight heparin. *Anal Chem*. 2016;88(3):1937-1943. https://doi.org/10.1021/acs.analchem.5b04405

18. Lin L, Liu X, Zhang F, et al. Analysis of heparin oligosaccharides by capillary electrophoresis–negative-ion electrospray ionization mass spectrometry. *Anal Bioanal Chem*. 2017;409(2):411-420. https://doi.org/10.1007/s00216-016-9662-1
19. Thermo Fisher Publication Number 100038224. Available: https://tools.thermofisher.com/content/sfs/manuals/100038224_GlycanAssureSystem_QR.pdf.
20. Feng H, Li P, Rui G, et al. Multiplexing N-glycan analysis by DNA analyzer. *Electrophoresis*. 2017;38(13–14):1788-1799. https://doi.org/10.1002/elps.201600404
21. Sarver SA, Schiavone NM, Arceo J, et al. Capillary electrophoresis coupled to negative mode electrospray ionization-mass spectrometry using an electrokinetically-pumped nanospray interface with primary amines grafted to the interior of a glass emitter. *Talanta*. 2017;165:522-525. https://doi.org/10.1016/jtalanta201701.002
22. Peuchen EH, Zhu G, Sun L, Dovichi NJ. Evaluation of a commercial electro-kinetically pumped sheath-flow nanospray interface coupled to an automated capillary zone electrophoresis system. *Anal Bioanal Chem*. 2017;409(7):1789-1795. https://doi.org/10.1007/s00216-016-0122-8
23. Sun L, Zhu G, Zhang Z, Mou S, Dovichi NJ. Third-generation electrokinetically pumped sheath-flow nanospray interface with improved stability and sensitivity for automated capillary zone electrophoresis–mass spectrometry analysis of complex proteome digests. *J Proteome Res*. 2015;14(5):2312-2321. https://doi.org/10.1021/acs.jproteome.5b00100
24. Walker SH, Papas BN, Comins DL, Muddiman DC. Interplay of permanent charge and hydrophobicity in the electrospray ionization of glycans. *Anal Chem*. 2010;82(15):6636-6642. https://doi.org/10.1021/ac101227a

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Khan S, Liu J, Szabo Z, et al. On-line capillary electrophoresis/laser-induced fluorescence/mass spectrometry analysis of glycans labeled with Teal fluorescent dye using an electrokinetic sheath liquid pump-based nanospray ion source. *Rapid Commun Mass Spectrom*. 2018;32:882–888. https://doi.org/10.1002/rcm.8116