Published in final edited form as:

Proteomics. 2014 May ; 14(10): 1158–1164. doi:10.1002/pmic.201300381.

Optimizing Capillary Electrophoresis for Top-Down Proteomics of 30–80 kDa Proteins

Yihan Li, Philip D. Compton, John C. Tran, Ioanna Ntai, and Neil L. Kelleher

Departments of Chemistry and Molecular Biosciences, and the Proteomics Center of Excellence
Northwestern University Evanston, IL 60208, USA

Abstract

The direct analysis of intact proteins via mass spectrometry offers compelling advantages in comparison to alternative methods due to the direct and unambiguous identification and characterization of protein sequences it provides. The inability to efficiently analyze proteins in the ‘middle mass range’, defined here as proteins from 30–80 kDa, in a robust fashion has limited the adoption of these “top-down” methods. Largely a result of poor liquid chromatographic performance, the limitations in this mass range may be addressed by alternative separations that replace chromatography. Herein, the short migration times of capillary zone electrophoresis-electrospray ionization-tandem mass spectrometry (CZE-ESI-MS/MS) have been extended to size-sorted whole proteins in complex mixtures from *Pseudomonas aeruginosa* PA01. An electrokinetically pumped nanospray interface, a coated capillary and a stacking method for on-column sample concentration were developed to achieve high loading capacity and separation resolution. We achieved full width at half maximum of 8–16 seconds for model proteins up to 29 kDa and identified 30 proteins in the mass range of 30–80 kDa from *Pseudomonas aeruginosa* PA01 whole cell lysate. These results suggest that CZE-ESI-MS/MS is capable of identifying proteins in the middle mass range in top-down proteomics.

Keywords

CZE-ESI-MS/MS; *Pseudomonas aeruginosa* PA01; top-down proteomics

1 Introduction

While the advantages of the direct analysis of intact proteins have long been known, it has taken dramatic improvements in front-end separations paired with improved instrumentation to encourage wide-spread adoption of the approach. [1] In a top-down workflow recently introduced by this group, [2–4] intact protein mixtures are first subjected to solution isoelectric focusing (sIEF). The resultant pI sorted proteins are then further fractionated by gel-eluted liquid fraction entrapment electrophoresis (GELFrEE) based on molecular weight. Until recently, the final stage of separation had employed reversed-phase liquid

Correspondence: Professor Neil L. Kelleher, Departments of Chemistry and Molecular Biosciences, and the Proteomics Center of Excellence, Northwestern University, Evanston, IL 60208, USA, n-kelleher@northwestern.edu, Fax: 847-467-3276.

The authors have declared no conflict of interest.

chromatography (RPLC) with electrospray ionization (ESI) compatible solvents. Identifying more than 1000 unique proteins and over 3,000 proteoforms from human cells, these methods demonstrate the routine and high throughput identification of intact proteins.

The protein identifications in these studies are dominated by species under 30 kDa. Largely, this bias is due to inefficient front-end separations as well as increased difficulty of protein detection at high mass. [5] Focusing on separations, proteins can interact irreversibly with RPLC resins, causing poor chromatographic resolution and recovery [6], especially proteins above 30 kDa. While considerable efforts have been made to improve separation resolution and protein recovery from RPLC [3], there is a dearth of literature that details investigations of alternative separation techniques to RPLC for high mass proteins.

Capillary zone electrophoresis (CZE), the simplest separation mode in capillary electrophoresis (CE), is an exciting alternative to RPLC for top-down proteomics. The use of CZE offers several important advantages; the low flow rate generated from electroosmotic flow (EOF) is well-matched to ESI, it does not require resins and it provides fast and efficient separations.[7] Further, when compared to capillary isoelectric focusing (cIEF) and capillary sieving electrophoresis (CSE), CZE generates lower background noise, due to the absence of ampholytes or background matrix which can cause ion suppression and contamination when coupled to mass spectrometry.

A rich literary history of CZE-ESI-MS studies of intact proteins has continued to grow in the last two decades as designs for CE-ESI-MS interfaces have evolved and mass spectrometry techniques have improved. Smith et al. pioneered this area when they evaluated a house-built system coupled by a coaxial sheath-flow interface that separated and detected a polypeptide and five model proteins (2 to 29 kDa) in a quadrupole mass spectrometer. [8] Valaskovic et al. added to this work when they employed a coaxial sheath-flow interface with a gold-coated spray tip integral to the separation capillary to couple CZE to a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer. With this design, they reported high resolution and accurate mass measurements of model proteins (8 to 29 kDa) and identification of carbonic anhydrase (29 kDa) from human red blood cells. [9] Moini et al. pushed the limits of sensitivity with a sheathless interface incorporating a split-flow setup when they analyzed the ribosomal proteome of *Escherichia coli*, a protein mixture of moderate complexity. They detected 55 *Escherichia. coli* ribosomal proteins (5 to 30 kDa) with post-translational modifications in a quadrupole ion trap, using only 3.4 ng of ribosomal proteins. However, none of these proteins were identified. [10]

Other than the conventional coaxial sheath-flow interface and the sheathless interface, the Dovichi Laboratory developed an electrokinetically pumped sheath-flow electrospray interface. [11] The interface has proven to be as sensitive as LC-ESI-MS in bottom-up proteomics studies over the past two years. [11–16] This CE-ESI-MS system was recently applied to top-down proteomics and baseline-separated four model proteins and five impurities with a limit of detection of 800 amol for bovine serum albumin (BSA, 66 kDa). Importantly, identification was achieved only for three of these model proteins and one impurity (12 to 24 kDa) in an LTQ-Orbitrap Velos mass spectrometer. [17]

Here, we applied this interface to couple CZE with a Q-Exactive mass spectrometer as the final stage of separation in a top-down proteomics platform. Intact proteins from *Pseudomonas aeruginosa* PA01 cell lysate were fractionated by GELFrEE and fractions containing proteins above 30 kDa were analyzed by CZE-ESI-MS to evaluate this platform. To accommodate the low sample concentration caused by poor protein solubility, we applied a stacking method using organic solvent in CZE-ESI-MS analysis to improve sample loading amounts. [18] The separation capillary was dynamically coated by a cationic polymer, poly-arginine (PA), to prevent protein adsorption on the inner wall of the capillary. [19, 20]

2 Materials and methods

2.1 Materials

All reagents were purchased from Sigma Aldrich (St. Louis, MO), unless stated. Water and methanol were purchased from Honeywell, Burdick & Jackson (Muskegon, MI). Fused silica capillary was purchased from Polymicro Technologies (Phoenix, AZ). GELFrEE 8100 Fractionation system, 8% cartridge, loading and separation buffers were purchased from Expedeon (San Diego, CA). Halt protease and phosphatase inhibitor cocktail was purchased from Thermo Pierce (Rockford, IL).

2.2 Methods

2.2.1 Preparation of *Pseudomonas aeruginosa* PA01—*Pseudomonas aeruginosa* PA01 cells were cultured and harvested at mid-log phase. The cells were lysed in 15mM tris-HCl containing 4% SDS, 1mM DTT, 1 mM sodium butyrate, and Halt protease and phosphatase inhibitor cocktail (Thermo Pierce). The cell lysate was aliquoted and stored at -80°C until use.

2.2.2 GELFrEE Separation—Protein sample (400 μg) was precipitated with three volumes of acetone followed by suspension in 150 μL of loading buffer. Protein fractionation was performed using the GELFrEE 8100 Fractionation System using an 8% cartridge. 12 fractions were collected and subsequently visualized on SDS-PAGE slab gels, loading 10 μL of each GELFrEE fraction followed by silver staining.

GELFrEE fractions 3 to 12 were precipitated using methanol/chloroform/water as previously described for the removal of SDS. [3] The fractions were then suspended in 5 μL of 0.1% formic acid. The SDS removal of each fraction was done on the same day as the CZE-ESI-MS/MS analysis to prevent protein degradation that occurs with longer exposure to air and MS compatible solvents. [21]

2.2.3 CZE-ESI-MS—Separation voltage was provided by a Spellman CZE 1000R high-voltage power supply while the electrospray voltage was provided by a Bio-Rad POWER PAC 3000 power supply. Electrospray was generated using an electrokinetically pumped sheath flow through a nanospray emitter with a 2–3 μm opening. [11] Voltage programming was controlled by LabView software. The separation capillary (id 50 μm , od 150 μm , length 30.0 cm) was coated with poly-arginine (PA, 0.005% in water, molecular weight > 70,000) by flushing the capillary with PA for 5 minutes at 5 psi. The capillary was filled with 0.1%

formic acid after coating. 240 nL of stacking buffer (60% isopropanol, 0.8% formic acid) and 80 nL of each GELFrEE fraction were injected hydrodynamically in sequence. The injection end of the capillary was immersed in 0.4% formic acid. The separation was driven by -2 kV applied to the capillary for 30 minutes while the electrospray voltage (1.5 kV) was applied to the sheath liquid reservoir. The sheath liquid contained 50% methanol in 0.4% formic acid. Each fraction was analyzed in triplicate. After each analysis, the capillary was flushed by ammonium hydroxide (10% in water) for 5 minutes at 5 psi. Injections were done manually in this work.

The estimation of injection volumes was done via experimentation. Coomassie brilliant blue was suspended in 0.1% formic acid and stacking buffer, and injected into the capillary separately. The color of Coomassie brilliant blue could be seen clearly in the capillary even with low concentration (0.02%), thereby minimally impacting the viscosity of the buffers. The length of the loaded solution plug could be measured manually. The injection volume was calculated from the length and ID of the capillary with high confidence in accuracy.

Data were collected with a Q-Exactive mass spectrometer (Thermo Fisher). The S-lens RF level was set at 70 and the ion transfer tube temperature was set at 320°C. The source fragmentation voltage was 15 V. MS¹ data were collected using the Orbitrap mass analyzer (10 microscans, 7,500 resolving power at m/z 200) with a scan range of m/z 500–2000. Fragmentation was achieved using higher-energy collisional dissociation (HCD) with normalized collision energy of 25. Data-dependent fragmentation was performed using a top two MS² acquisition strategy with a 50 m/z isolation window. Each scan included 5 microscans at 60,000 resolving power at m/z 200. Dynamic exclusion was enabled with a repeat count of 2, an exclusion duration of 45s and a repeat duration of 10s.

2.2.4 Data analysis—Intact precursor and fragment masses were determined using in-house software (cRAWler), which utilizes Xtract (Thermo/Fisher) to determine monoisotopic neutral masses from precursor and fragment ion spectra and kDecon [22] to generate average masses from precursor data. The lists of precursor and fragment masses were analyzed with a distributed version of ProSightPC 3.0. The *Pseudomonas aeruginosa* PA01 database was built from the 2012_08 UniProt release. It can be downloaded from <ftp://prosightftp.gsX1gON@prosightpc.northwestern.edu/Pseudomonas>. Each precursor ion was searched against the database using a 100,000 Da window and fragment ions were matched using an absolute mass search logic with a tolerance of 10 ppm. [23] When a precursor mass could not be determined, the entire database was searched. At least 4 matched fragment ions and an Evalue lower than $1E^{-4}$ were required for protein identification.

3 Results and discussion

3.1 Optimized CZE method

The direct insertion of CZE into a top-down workflow comes with several challenges. First, the loading volume of CZE is typically limited to lower than 1% of the capillary volume by separation resolution, resulting in less than 6 nL of sample loaded in a 30 cm long capillary (id 50 μ m, od 150 μ m). With increasing molecular weight, protein solubility generally limits

concentrations and, by extension, the amount of protein loaded. Under these conditions, the utility of CZE for intact proteins would be limited.

Adjusting for this limitation, the loading volume in this work was increased to 80 nL, or approximately 15% of the capillary volume. Increased loading capacity was accomplished with a stacking method that was developed based on the work published by Shihabi in 2002. The mechanism of this stacking method is not completely clear. [18] The increased loading capacity provided by this stacking method functions with a dynamic capillary coating to increase the overall sensitivity of CZE analyses.

Protein loss caused by intact protein adsorption on the inner wall of capillary has been an issue for CZE analysis. [24] Dynamic capillary coatings (cationic, anionic and neutral) have been studied and reported in the literature as an easy and efficient way to reduce protein adsorption. [19] A cationic dynamic coating, poly-arginine (PA), was applied here because intact proteins are positively charged under acidic separation conditions. The columbic interaction between the coating and the proteins prevents protein adsorption to the capillary wall. Importantly, no significant interference of PA was observed in either model protein or GELFrEE fraction analyses. The capillary was flushed with ammonium hydroxide (10% in water) between runs to wash off the PA coating, preventing any carryover during sample analysis.

3.2 Analysis of model proteins

The CZE-ESI-MS system was first evaluated by separating a mixture of four standard proteins, ubiquitin (8 kDa, 0.5 ng), myoglobin (17 kDa, 5 ng), trypsinogen (24 kDa, 8 ng), carbonic anhydrase (29 kDa, 8 ng) and a contaminant protein, superoxide dismutase (16 kDa). Protein peaks in Figure 2 were extracted by the most abundant charge state of each protein with a mass tolerance 0.05 amu: ubiquitin (m/z 1224.42), myoglobin (m/z 771.59), trypsinogen (m/z 1999.43), carbonic anhydrase (m/z 854.75) and superoxide dismutase (m/z 1040.50). Peak widths at 50% peak height were as narrow as 8 s for trypsinogen, 12 s for myoglobin, carbonic anhydrase and superoxide dismutase, and 16 s for ubiquitin. Five proteins were baseline-separated except a partial overlap of carbonic anhydrase and myoglobin. The total separation window was about 4 min and peak capacity is estimated to be 7.

3.3 CZE based top-down platform and protein identification in complex samples

The CZE-ESI-MS platform (Fig. 1) was further evaluated using samples derived from a GELFrEE separation of a whole cell lysate. The GELFrEE separation was visualized on a SDS-PAGE gel (Fig. 3) to assess separation quality and to determine the mass range of each fraction. The majority of proteins lower than 30 kDa eluted in the first two fractions, although some overlap between fractions occurs naturally and abundant small proteins were present in later fractions. This work emphasizes the analysis of proteins above 30 kDa, so only fractions 3 to 12 were analyzed by CZE-ESI-MS/MS.

Proteins in a GELFrEE fraction were separated within 30 min and the resulting separation windows were approximately 11 to 15 min. One analysis of fraction 5 is presented in Figure 3 as a representative result of CZE separation. Three proteins (31, 37 and 43 kDa) were

identified with high confidence (E value $7\text{E}-38$, $5\text{E}-17$ and $3\text{E}-66$) and their charge state distributions are illustrated in Figure 3. The HCD fragmentation of Elongation factor Tu protein (43 kDa) is depicted in Figure 4, with 32 matched fragment ions obtained within 10 ppm error.

In total, this work identified 65 proteins from 30 CZE-ESI-MS/MS analyses with 30 of the identified proteins having molecular weights in excess of 30 kDa (S-Table 1–27). The intact mass of 16 proteins in the mass range from 30 to 50 kDa could be manually validated by MS^1 data. The peak widths at 50% peak height of 12 proteins were as narrow as 24 to 57 seconds (S-Table 1 in supplemental material) while the peak widths at 50% peak height of two abundant proteins were 84 and 78 seconds (43 kDa and 57 kDa). Protein peak at 50% peak height was estimated by selected ion electropherograms of the abundant charge states in MS^1 data. The selected ion electropherogram of B-type flagellin, a 49 kDa protein identified in fraction 8, is illustrated in Figure 5, which was extracted by 9 charge states (m/z 1466.30, 1510.69, 1557.88, 1608.12, 1661.70, 1718.92, 1780.34, 1846.24 and 1917.18) with a mass tolerance 0.05 amu. The protein peak was approximately 24 s wide at 50% peak height. Fraction 8 contained proteins in the mass range of 48 to 60 kDa (S-Table 2 in supplemental material), which in general have wide charge state distributions in MS^1 data. Noise peaks from 14 to 19 min in the electropherogram were selected in Figure 5A due to similar m/z of charge states of other proteins in the analyses.

Addressing two limitations of the current platform could further increase the number of proteins identified in future studies. First, further improvements in the loading capacity of CZE would aid in detection of lower abundance proteins. GELFrEE fractions for RPLC-based top-down workflows are typically suspended in 25 μL of loading buffer (5 μL for CZE in this work) after SDS removal and 10 μL of the resultant sample is loaded onto an RPLC column. With 5 times more concentrated GELFrEE fractions and an 80 nL loading volume, the identifications in this study were achieved with only 1/25 the typical loading amount of RPLC. Second, the dynamic range problem associated with all whole cell lysate proteomic studies applies here. Incorporating more dimensions of separation would almost certainly result in more identifications.

3.4 Reproducibility

The number of proteins identified in each analysis in the mass range of 30 to 80 kDa is listed in S-Table 2. The number of shared identifications in fraction 4 to fraction 9 was 50% of the total identifications, which is reasonable protein identification reproducibility compared to RPLC based top-down proteomics studies. [4]

The four major peaks in the total ion electropherogram of Fraction 3 (S-Figure 1 in supplemental material) were used to evaluate the reproducibility of CZE separation. The migration times of the four major peaks were reproducible and the relative standard deviations (RSDs) of migration times were less than 3% for each peak (S-Table 28 in supplemental material). The intensities of the four peaks were also reasonably consistent between runs (RSDs <30%, S-Table 29). This indicates CZE separation with this stacking method is reproducible analyzing intact proteins from complex proteome.

4 Concluding remarks

We demonstrated in this work the utility of CZE-ESI-MS/MS for top-down proteomics in the mass range of 30 to 80 kDa. CZE-ESI-MS/MS identified 30 proteins in this mass range from *Pseudomonas aeruginosa* PA01 whole cell lysate and further development will make it a viable final stage of front-end separation in top-down proteomics platforms. Further, it is worthwhile to highlight that this work is the first study that presents a large number of protein identifications from a complex sample in a CZE based top-down analysis. As sample preparation, capillary coating materials, separation conditions, electrospray conditions and mass spectrometer operating parameters improve, CZE will become an important alternative to RPLC for the analysis of the high mass proteome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Norman J. Dovichi in Department of Chemistry and Biochemistry in University of Notre Dame, Ryan T. Fellers in Proteomics center of excellence in Northwestern University, Kenneth R. Durbin, Owen S. Skinner in Kelleher Lab in Northwestern University, and Dr. Adam D. Catherman in Protein analytical department in Genentech Inc. for their help with this project. This work was supported by the National Institute of General Medical Sciences and National Institute of Drug Abuse of the National Institutes of Health under award numbers R01 GM067193 and DA018310, and the National Science Foundation under award number ABI-1062432 to Indiana University. Additional support was provided by the Chicago Biomedical Consortium with support from The Searle Funds at The Chicago Community Trust, and the Robert H. Lurie Comprehensive Cancer Center. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation, the National Center for Genome Analysis Support, or Indiana University.

Abbreviations

GELFrEE	gel-eluted liquid fraction entrapment electrophoresis
HCD	higher-energy collisional dissociation
PA	poly-arginine
sIEF	solution isoelectric focusing

References

1. Smith LM, Kelleher NL, Linial M, Goodlett D, et al. Proteoform: a single term describing protein complexity. *Nat Methods*. 2013; 10:186–187. [PubMed: 23443629]
2. Tran JC, Zamdborg L, Ahlf DR, Lee JE, et al. Mapping intact protein isoforms in discovery mode using top-down proteomics. *Nature*. 2011; 480:254–258. [PubMed: 22037311]
3. Vellaichamy A, Tran JC, Catherman AD, Lee JE, et al. Size-sorting combined with improved nanocapillary liquid chromatography-mass spectrometry for identification of intact proteins up to 80 kDa. *Anal Chem*. 2010; 82:1234–1244. [PubMed: 20073486]
4. Kellie JF, Catherman AD, Durbin KR, Tran JC, et al. Robust analysis of the yeast proteome under 50 kDa by molecular-mass-based fractionation and top-down mass spectrometry. *Anal Chem*. 2012; 84:209–215. [PubMed: 22103811]
5. Compton PD, Zamdborg L, Thomas PM, Kelleher NL. On the scalability and requirements of whole protein mass spectrometry. *Anal Chem*. 2011; 83:6868–6874. [PubMed: 21744800]

6. Sadek PC, Carr PW, Bowers LD, Haddad LC. A radiochemical study of irreversible adsorption of proteins on reversed-phase chromatographic packing materials. *Anal BioChem.* 1986; 153:359–371. [PubMed: 3010763]
7. Geiger M, Hogerton AL, Bowser MT. Capillary electrophoresis. *Anal Chem.* 2012; 84:577–596. [PubMed: 22148626]
8. Smith RD, Wahl JH, Goodlett DR, Hofstadler SA. Capillary electrophoresis/mass spectrometry. *Anal Chem.* 1993; 65:574A–584A.
9. Valaskovic GA, Kelleher NL, McLafferty FW. Attomole protein characterization by capillary electrophoresis-mass spectrometry. *Science.* 1996; 273:1199–1202. [PubMed: 8703047]
10. Moini M, Huang H. Application of capillary electrophoresis/electrospray ionization-mass spectrometry to subcellular proteomics of *Escherichia coli* ribosomal proteins. *Electrophoresis.* 2004; 25:1981–1987. [PubMed: 15237397]
11. Wojcik R, Dada OO, Sadilek M, Dovichi NJ. Simplified capillary electrophoresis nanospray sheath-flow interface for high efficiency and sensitive peptide analysis. *Rapid Commun Mass Spectrom.* 2010; 24:2554–2560. [PubMed: 20740530]
12. Wojcik R, Li Y, Maccoss MJ, Dovichi NJ. Capillary electrophoresis with Orbitrap-Velos mass spectrometry detection. *Talanta.* 2012; 88:324–329. [PubMed: 22265506]
13. Li Y, Champion MM, Sun L, Champion PA, et al. Capillary zone electrophoresis-electrospray ionization-tandem mass spectrometry as an alternative proteomics platform to ultraperformance liquid chromatography-electrospray ionization-tandem mass spectrometry for samples of intermediate complexity. *Anal Chem.* 2012; 84:1617–1622. [PubMed: 22182061]
14. Li Y, Wojcik R, Dovichi NJ, Champion MM. Quantitative multiple reaction monitoring of peptide abundance introduced via a capillary zone electrophoresis-electrospray interface. *Anal Chem.* 2012; 84:6116–6121. [PubMed: 22690842]
15. Sun L, Zhu G, Li Y, Wojcik R, et al. CZE-ESI-MS/MS system for analysis of subnanogram amounts of tryptic digests of a cellular homogenate. *Proteomics.* 2012; 12:3013–3019. [PubMed: 22888077]
16. Zhu G, Sun L, Yan X, Dovichi NJ. Single-Shot Proteomics Using Capillary Zone Electrophoresis-Electrospray Ionization-Tandem Mass Spectrometry with Production of More than 1 250 *Escherichia coli* Peptide Identifications in a 50 min Separation. *Anal Chem.* 2013; 85:2569–2573. [PubMed: 23394296]
17. Sun L, Knierman MD, Zhu G, Dovichi NJ. Fast top-down intact protein characterization with capillary zone electrophoresis-electrospray ionization tandem mass spectrometry. *Anal Chem.* 2013; 85:5989–5995. [PubMed: 23692435]
18. Shihabi ZK. Transient pseudo-isotachopheresis for sample concentration in capillary electrophoresis. *Electrophoresis.* 2002; 23:1612–1617. [PubMed: 12179979]
19. Horvath J, Dolnik V. Polymer wall coatings for capillary electrophoresis. *Electrophoresis.* 2001; 22:644–655. [PubMed: 11296918]
20. Chiu RW, Jimenez JC, Monnig CA. High molecular weight poly arginine as a capillary coating for separation of cationic proteins by capillary electrophoresis. *Anal Chim Acta.* 1994; 307:193–201.
21. Tipton JD, Tran JC, Catherman AD, Ahlf DR, et al. Nano-LC FTICR tandem mass spectrometry for top-down proteomics: routine baseline unit mass resolution of whole cell lysate proteins up to 72 kDa. *Anal Chem.* 2012; 84:2111–2117. [PubMed: 22356091]
22. Durbin KR, Tran JC, Zamdborg L, Sweet SMM, et al. Intact mass detection, interpretation, and visualization to automate top-down proteomics on a large scale. *Proteomics.* 2010; 10:3589–3597. [PubMed: 20848673]
23. LeDuc RD, Kelleher NL. Using ProSight PTM and related tools for targeted protein identification and characterization with high mass accuracy tandem MS data. *Curr Protoc Bioinformatics.* 2007; 19:13.16.11–13.16.28.
24. Righetti PG, Sebastiano R, Citterio A. Capillary electrophoresis and isoelectric focusing in peptide and protein analysis. *Proteomics.* 2013; 13:325–340. [PubMed: 23180512]

Figure 1. Schematic diagram of the top-down platform utilizing CZE as the final stage of front-end separations.

Figure 2.
Selected ion electropherograms of five model proteins analyzed by CZE-ESI-MS.

I - Electron transfer flavoprotein subunit alpha, E value 7E-38;

II - Chain [27-373] in leucine-, isoleucine-, valine-, threonine-, and alanine-binding protein, E value 5E-17;

III - Elongation factor Tu, E value 3E-66.

Figure 3.

Representative data for CZE-ESI-MS/MS analysis of a single GELFrEE fraction containing proteins in the mass range of 33 to 43 kDa. GELFrEE separation is shown in the slab gel image. The separation of proteins is shown in the base peak electropherogram and MS¹ spectra of three proteins are shown along with protein identifications.

Figure 4. HCD fragmentation of a 43 kDa protein (Elongation factor Tu). A: Single-scan fragmentation spectra of the $[M + 39H]^{39+}$ charge state; B: Sequence and fragmentation patterns observed with HCD.

Figure 5.

Selected ion electropherogram of a 49 kDa protein (B-type flagellin). A: Selected ion electropherogram of charge states from $[M + 34H]^{34+}$ to $[M + 26H]^{26+}$; B: MS¹ spectra summed from 12.9 to 13.8 min.