Native proteomics by capillary zone electrophoresis-mass spectrometry

Qianyi Wang[1,#], Qianjie Wang[1,2,3,#], Zihao Qi[4,#], William Moeller[4], Vicki H Wysocki,[4,5,*] Liangliang Sun[1,*]

[1]Department of Chemistry, Michigan State University, 578 S Shaw Lane, East Lansing, Michigan 48824, United States

[2]Plant Resilience Institute, Michigan State University, East Lansing, Michigan, USA

[3]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan, USA

[4]Department of Chemistry and Biochemistry, The Ohio State University, Columbus, Ohio 43210, United States

[5]Resource for Native MS Guided Structural Biology, The Ohio State University, Columbus, Ohio 43210, United States

[#] Those three authors contributed equally to this work.

* Corresponding author.

Vicki H Wysocki, Email: wysocki.11@osu.edu

Liangliang Sun, Email: lsun@chemistry.msu.edu

Abstract

Native proteomics aims to measure endogenous proteoforms and protein complexes under a near physiological condition using native mass spectrometry (nMS) coupled with liquid-phase separation techniques. Native proteomics should provide the most accurate bird's-eye view of proteome dynamics within cells, which is fundamental for understanding almost all biological processes. nMS has been widely employed to characterize well-purified protein complexes. However, there are only very few trials of utilizing nMS to measure proteoforms and protein complexes in a complex sample (i.e., a whole cell lysate), and those studies are either too time and labor-consuming or only able to detect small proteoforms or protein complexes. Here, we pioneer the native proteomics measurement of large proteoforms or protein complexes up to 400 kDa from a complex proteome via online coupling of native capillary zone electrophoresis (nCZE) to an ultra-high mass range Orbitrap mass spectrometer (UHMR). The nCZE-MS technique enabled the measurement of a 115-kDa standard protein complex while consuming only about 100 pg of protein material, indicating the extremely high sensitivity of the technique. nCZE-MS analysis of an *E.coli* cell lysate detected 76 and 21 proteoforms or protein complexes in a mass range of 30-400 kDa and over 110 kDa, respectively, in a single run while consuming only 50-ng protein material. The mass distribution of detected proteoforms or protein complexes agreed well with that from mass photometry measurement. This work represents a technical breakthrough of native proteomics for measuring complex proteomes, suggesting that nCZE-MS might be developed as a central technique for native proteomics.

Keywords: Native proteomics; Capillary zone electrophoresis; Native mass spectrometry; Protein complex; Mass photometry

INTRODUCTION

Proteins regulate cellular processes by their diverse proteoforms[1,2] and the various protein complexes via non-covalent protein-protein interactions, protein-ligand bindings, and protein-DNA/RNA interactions.[3] Native mass spectrometry (nMS) provides critical insights into the structures, functions, and dynamics of proteoforms and protein complexes near physiological conditions.[4–7] Native MS has been widely employed to study well-purified proteoforms and protein complexes with low complexity through either direct infusion[8-13] or coupling with online/offline native separation methods, including size-exclusion chromatography (SEC),[14-18] ion-exchange chromatography (IEX),[19,20] hydrophobic interaction chromatography (HIC),[21,22] and capillary zone electrophoresis (CZE).[23]

Native proteomics aims to measure endogenous proteoforms and protein complexes under a near physiological condition on a proteome scale and it requires highly efficient separation techniques for protein complexes prior to nMS.[24] The first native proteomics study coupled off-line IEX or native gel-eluted liquid fractionation with direct infusion nMS for the characterization of protein complexes in mouse heart and human cancer cell lines, identifying 125 endogenous complexes from about 600 fractions.[25] More recently, direct infusion nMS was employed to measure protein complexes from a human heart tissue lysate using a Fourier-transform ion cyclotron resonance (FTICR) mass spectrometer with the identification of a handful of protein complexes about 30 kDa or smaller.[26] Native CZE-MS (nCZE-MS) has high separation efficiency and high detection sensitivity for protein complexes and has been applied to analyzing low-complexity protein samples, i.e., monoclonal antibodies,[27] large protein complexes like GroEL (near 1MDa),[28-30] ribosomes,[31] and nucleosomes.[32] Native SEC fractionation and online nCZE-MS analysis of an *E. coli* cell lysate identified 23 protein complexes smaller than 30 kDa, representing the first native proteomics study of a complex proteome using online liquid-phase separation-MS.[33] However, those native proteomics studies are either too time and labor-consuming or only able to detect small proteoforms/protein complexes from complex proteomes.

In this study, we developed a high-throughput nCZE-MS technique for native proteomics measurement of large proteoforms and protein complexes up to 400 kDa from complex samples, *i.e.,* an *E. coli* cell lysate. The nCZE-MS technique is based on the online coupling of nCZE to an ultra-high mass range (UHMR) Orbitrap mass spectrometer. We first evaluated the nCZE-MS technique using a standard protein complex mixture. Then, we employed the technique to measure endogenous proteoforms and protein complexes in *E. coli* cells. We also compared our nCZE-MS data with mass photometry[34] results in terms of mass distribution of *E. coli* proteoforms and protein complexes.

RESULTS AND DISCUSSION

Herein, we demonstrate the coupling of CZE to a UHMR Orbitrap mass spectrometer for the native proteomics of a complex sample, *i.e.,* an *E. coli* cell lysate, **Figure 1**. Briefly, the cultured *E. coli* cells (Top10 strain) were lysed in a Dulbecco's phosphate-buffered saline (dPBS) buffer containing complete protease inhibitors and

phosphatase inhibitors. The cell lysate was then buffer-exchanged on a spin column (Biorad P6) to a buffer containing 20 mM ammonium acetate (AmAc, pH ~7.0) by gel filtration, followed by nCZE-MS analysis. The online nCZE-MS was assembled by coupling a Sciex CESI-8000 Plus CE autosampler to a Thermo Fisher Scientific Q-Exactive UHMR mass spectrometer through a commercialized electrokinetically pumped sheath flow CE-MS interface (EMASS-II, CMP Scientific).[35,36] A 1-meter-long linear polyacrylamide (LPA) coated capillary (50-μm i.d., 360-μm o.d.) was used for the CZE separation and the LPA coating was employed to reduce the protein non-specific adsorption onto the capillary inner wall. The background electrolyte (BGE) for CZE was 25 mM AmAc (pH ~7.0), and the sheath buffer for electrospray ionization (ESI) was 10 mM AmAc (pH ~7.0). Only roughly a 50-ng aliquot of the *E. coli* sample was consumed in a single nCZE-MS run. Raw MS data were averaged every 30 seconds manually, followed by manual mass deconvolution and check using either UniDec[37] or ESIprot.[38] The detailed experimental procedure is described in the **Supporting Information**.

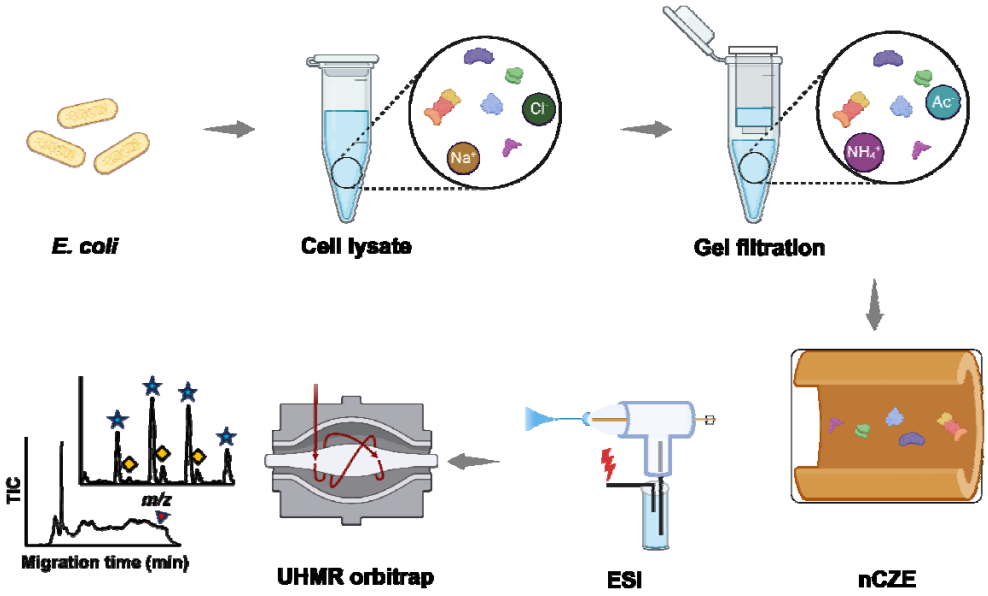

Figure 1. Flow chart of nCZE-ESI-MS for native proteomics of an *E. coli* cell lysate. The figure is created using the BioRender and is used here with permission.

We first investigated the sensitivity of the nCZE-ESI-UHMR platform for measuring protein complexes using a mixture of standard proteins and protein complexes, **Figure S1**. Strong signals were observed for streptavidin (SA, 53 kDa), carbonic anhydrase (CA, 29 kDa), C-reactive protein (CRP, 115 kDa), and bovine serum albumin (BSA, 66 kDa) in the original sample with a total protein injection of about 600 femtomoles. After sample dilution by a factor of 50, a clear CRP peak was still observed, even though only 100 pg of the protein complex (~800 attomole) was loaded, indicating the high sensitivity of the technique. **Figure S2A** shows one mass spectrum of three SA tetramers with masses as 53084.67 Da, 53216.07 Da, and 53347.97 Da. A 131-Da mass difference was observed between neighboring SA complexes, corresponding to N-terminal methionine variation on SA, which is consistent with the literature.[39] **Figure**

S2B shows a mass spectrum of CA-Zn(II) complex (29088.10 Da) [39,40] and another CA complex (29194.01 Da) with an additional 107-Da mass shift compared to the CA-Zn(II) complex. **Figures S2C** shows the mass spectra of pentameric CRP complex in the original sample. Based on De La Mora's prediction of the maximum (Rayleigh) charge 'Z_R' of a native protein during the ESI process ($Z_R = 0.0778*M^{0.5}$),[41,42] the max charge of CRP is around 26.4. The max charge states of CRP observed in the original and 50-time diluted samples are 27 and 26, matching well with the Z_R of native CRP. We observed slightly lower max charge states compared to the theoretical charge states for the SA tetramer, CA-Zn (II) complex, and BSA, **Figure S2D**. The data clearly demonstrate that intact protein complexes are maintained in native-like states during nCZE-ESI-UHMR measurements.

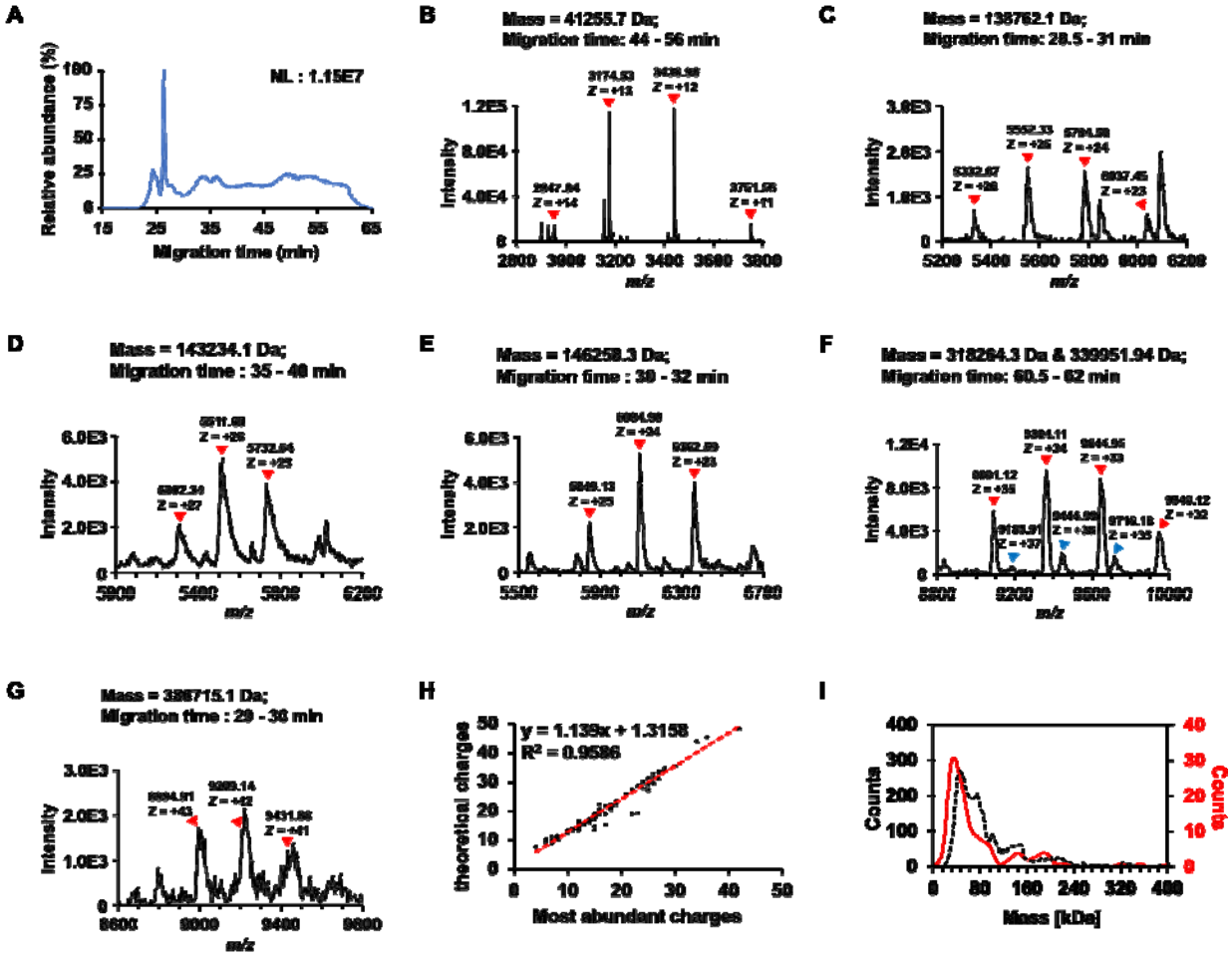

Figure 2. Summary of detected proteoforms or protein complexes from an *E. coli* cell lysate using nCZE-ESI-UHMR. (A) Representative electropherogram of nCZE-ESI-UHMR analyses of the *E. coli* cell lysate. (B)-(G) Mass spectra of six examples of large proteoforms/protein complexes detected. The charge states and deconvolved mass of each proteoform/protein complex are labelled. (H) Linear correlation between the most abundant charges and theoretical Rayleigh charges (Z_R) of all proteoforms/protein complexes detected in single-shot nCZE-UHMR. (I) Alignment of the mass distribution

of proteoforms/protein complexes in the *E. coli* cell lysate from mass photometry (black dash line) and nCZE-UHMR (red line) analyses.

The high sensitivity of nCZE-UHMR for the standard protein complexes motivated us to analyze an *E. coli* cell lysate. **Figure 2A** shows an example electropherogram of the sample from nCZE-MS. The proteoforms or protein complexes migrated out of the capillary in a time range of 20-65 minutes, allowing the mass spectrometer sufficient time for data acquisition (i.e., acquiring mass spectra and tandem mass spectra). In total, we detected 104 proteoforms or protein complexes in a mass range of 10-400 kDa after manual spectrum averaging and deconvolution. Information on the detected 104 proteoforms or protein complexes is listed in **Table S1**. **Figures 2B-2G** show the mass spectra of some examples larger than 40 kDa, i.e., ~41, 139, 143, 146, 318, 340, and 387 kDa. Those proteoforms or protein complexes show native-like and clear mass spectra. For example, **Figure 2F** shows two co-migrating proteoforms or protein complexes with masses ~318 and ~340 kDa. Their most-abundance charge states are +34 and +36, respectively. The largest proteoform or protein complex detected in this study is ~387 kDa, carrying around 42 charges (**Figure 2G**). Some additional examples are shown in **Figure S3**.

We further examined the correlation between the predicted Rayleigh charge (Z_R) from De La Mora's theory[41,42] and the experimental maximum charge state of detected proteoforms or protein complexes, **Figure 2H**. We used the most abundant charge state instead of the highest charge state for each proteoform/protein complex here to avoid potential variations introduced during manual determination of the highest charge state. We observed a strong linear correlation (R^2 = 0.96, slope of 1.14) between the experimental and predicted charge states. The slope indicates that the theoretical charges are slightly higher than the most abundant charges, suggesting the preservation of native states of the proteoforms or protein complexes in this experiment. We further employed mass photometry (MP) to measure the individual mass of proteoforms/protein complexes and their counts in the same *E. coli* cell lysate in a nearly physiological solution based on the quantification by light scattering.[34,43,44] The masses of proteoforms/protein complexes range from 10 kDa to 400 kDa according to the MP data, **Figure 2I** (black dashed line). Nearly 70% of the molecule counts (2366 of 3555) from the MP analysis are in the mass range of 30 to 95 kDa. Interestingly, the molecular mass distributions from the MP and nCZE-MS analysis agree reasonably well, **Figure 2I,** considering the low mass cutoff of MP. For example, the largest proteoform or protein complex detected by nCZE-MS is close to 400 kDa and 70% (73 out of 104) of the proteoforms/protein complexes from nCZE-MS are in the mass range of 32 to 96 kDa, **Table S1**. It has been demonstrated that nMS and MP can produce reasonably consistent mass assessment of large proteins or protein complexes and offer complementary information about the analytes.[45]

Our native proteomics study here is important because, for the first time, we can achieve a proteome-scale measurement of endogenous proteoforms and protein complexes in a complex biological sample under near physiological conditions by nMS with relatively high throughput. Over 100 endogenous intact proteoforms and protein complexes up to 400 kDa were detected from an *E. coli* cell lysate by online nCZE-MS in roughly 1-hour measurements with the consumption of 50-ng protein material. nCZE-MS can maintain the protein molecules from a complex cell lysate in close-to-native states during the measurement, evidenced by the strong linear correlation between the predicted Rayleigh charge (Z_R) and experimental most-abundance charge state of detected proteoforms or protein complexes, as well as the strong agreement in molecular mass distributions between the nCZE-MS and MP data.

The current study has two limitations. Firstly, we did not generate high-quality MS/MS data for the large proteoforms or protein complexes during the nCZE-MS run, impeding the identification of each protein. We will solve this issue by optimizing surface-induced dissociation or higher energy collisional dissociation (HCD) to achieve better fragmentation of large proteoforms or protein complexes in our future study. Second, the separations of large protein complexes by nCZE needs to be further improved regarding separation peak capacity and reproducibility. **Figure S4** shows the electropherograms of the *E. coli* cell lysate after triplicate nCZE-MS measurements. The separation peak capacity is limited, which may be due to the non-specific adsorption of proteins onto the capillary inner wall. The separation profiles have some significant changes after 45 min in the second and third runs compared to the first run, most likely due to changes at the capillary inner wall after the first run of the *E. coli* sample. We need to develop procedures to clean up the capillary inner wall between nCZE-MS runs[46] and improve the capillary inner wall coating through different chemistries, *e.g.,* carbohydrate-based neutral coating,[47] to reduce protein adsorption for better separation peak capacity and reproducibility.

CONCLUSIONS

We have demonstrated, for the first time, that nCZE coupled to an Orbitrap UHMR mass spectrometer is an effective and sensitive platform to measure large proteoforms or protein complexes up to 400 kDa from a complex proteome sample. This nCZE-MS technique enabled highly sensitive detection of standard protein complexes via consuming only pg amounts of protein material. The technique successfully detected over one hundred proteoforms or protein complexes from an *E. coli* cell lysate in a mass range of 10-400 kDa. With further improvements in gas-phase fragmentation and nCZE separation peak capacity and reproducibility, we envision that nCZE-orbitrap UHMR will become a powerful tool in native proteomics of complex proteome samples.

SUPPORTING INFORMATION

Experimental procedures, a list of proteoforms/protein complexes detected from nCZE-MS analysis of an *E. coli* cell lysate, electropherograms of a mixture of standard protein complexes, mass spectra of standard protein complexes, representative mass spectra of proteoforms/protein complexes detected from the *E. coli* sample, electropherograms of an *E. coli* cell lysate after triplicate analyses by nCZE-ESI-UHMR. (PDF)

Notes

The Wysocki lab collaborates with Thermo Fisher Scientific on separation protocols and improved dissociation in Orbitrap instruments.

Author contributions

Qianjie Wang prepared the *E. coli* cell lysate and did the sample preparation. Qianyi Wang did the data analysis. Qianyi Wang and Qianjie Wang wrote the draft of the manuscript. Qianyi Wang, Qianjie Wang, Zihao Qi, and William Moeller worked together for the native CZE-MS system setup and analysis of the prepared *E. coli* cell lysate. Zihao Qi generated the mass photometry data. Vicki H Wysocki and Liangliang Sun designed the experiments, oversaw the project, and edited the manuscript. All authors made comments on the manuscript.

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