CZE-nanoESI-SLIM-IMS-MS Platforms for Comprehensive, Ultrasensitive Proteome Analyses

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Overview

- Capillary zone electrophoresis (CZE) was coupled with ion mobility and mass spectrometry (IMS-MS) to evaluate advantages of the three dimensional measurements
- CZE-nanoESI-IMS-MS was used to address proteomic challenges including:
  - Ultra-sensitive and efficient separations
  - Enhancement of peak capacity
  - Separation & structural characterization of intact proteins in denatured and native conditions

Introduction

Progress towards comprehensive proteome characterization relies on the development of high resolution front end separation techniques, efficient ionization and improved ion transmission mass spectrometric analysis. In this work we couple CZE-nanoESI with various IMS platforms, including:

- a 1-m drift tube-IMS-MS
- a high charge capacity Structures for Lossless Ion Manipulation (SLIM-IMS) travelling wave (TW) device
- a high resolution SLIM-IMS TW device with a variable path length.

CZE coupled with the nanoESI sheath flow interface (see Figure 1A) allows for sensitive separations of peptides and intact proteins in various background electrolyte conditions.

Coupling CZE-nanoESI with IMS-MS provides opportunities to address the challenges of proteomic analyses, including enhancement of peak capacity and sensitivity, analysis of isomers, and solution phase/gas phase structural conformations.

Methods

- CZE separations were performed on an in-house constructed CE system.
- The CE-MS interface [1] employed a 15 µm i.d. tapered borosilicate glass emitter and an electrokinetically pumped sheath liquid with 300 µm between the etched capillary exit and the ESI emitter
- Experimental conditions used were as follows:
  - **CZE-ESI of 1 µM phosphopeptide isomers (Figure 2):** 30-cm long, 30 µm i.d., capillary with 200 mM formic acid as background electrolyte (BGE); 10 mM formic acid, 20% methanol as sheath liquid (SL); 19 kV separation,
  - **CZE-ESI for 10 µM BSA digest (Figure 3):** 28-cm long, 20 µm i.d. capillary with 100 mM acetic acid as BGE and 20 mM acetic acid, 20% methanol as SL; 28 kV separation
  - **CZE-ESI of intact proteins in acidic condition (Figure 4-A-C):** 45 cm long 30 µm i.d., capillaries, coated with 2 mg/mL Polybrene; with 100 mM formic acid as BGE; 10 mM formic acid, 20% methanol as SL; 19 kV separation
  - **Native CZE-ESI separations for intact proteins (Figure 4-D):** 35-cm long 20 µm i.d., capillary with 100 mM ammonium acetate at pH 7.1 as BGE; 10 mM ammonium acetate at pH 7.1 as SL; 15 kV separation

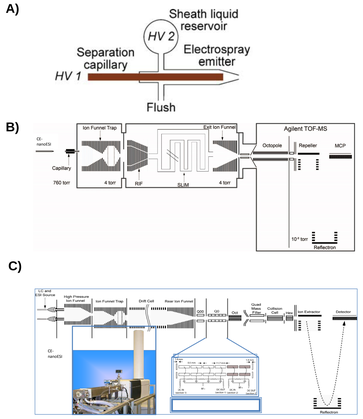

Figure 1: Schematics of the CZE-nanoESI interface and IMS-MS platforms:
A) CZE-nanoESI sheath flow interface [1];
B) Multi-pass SLIM-TW-IMS-TOF-MS [6];
C) DT-IMS-qTOF-MS.

Results

Enhancing peak capacity for peptide separations using extended path length SLIM-TW-IMS-TOF-MS

- Phosphopeptide isomers partially separate in both CZE and IMS dimensions (Figure 2).
- Extended path length (30.6 m) SLIM-IMS helps resolve co-eluting CZE species (Figure 2-D) and can be combined with parked CZE separations to enhance peak capacity

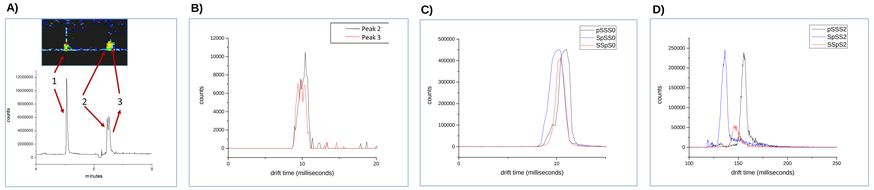

Figure 2: A) Total ion traces for CZE-MS and CZE-IMS-MS of APLSFRGSLPKSYVK (peak 1) and its singly phosphorylated counterparts (pSSS,sPSS and SSpS) (peaks 2,3). **B)** Representative IMS spectra of CE peaks 2 and 3. **C)** IMS spectra of infused phosphopeptide isomers generated with 1.25-m SLIM-IMS MS. **D)** IMS spectra of infused phosphopeptide isomers separated by the extended serpentine path SLIM-IMS-MS (30.6-m; 2 cycles).

Enhancing sensitivity of CZE peptide separations with a high charge capacity SLIM-TW-IMS-TOF-MS

- A high charge capacity prototype SLIM-TW-IMS-MS is compatible with fast CZE-nanoESI separations.

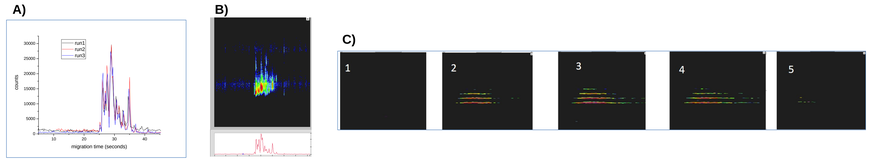

Figure 3: A) Total ion electropherograms (TIE) of the replicates of fast CZE-IMS-MS of 20 femtomoles of BSA tryptic digest, generated with a high charge capacity SLIM-IMS-MS platform. **B)** IMS spectrum corresponding to the TIE of BSA digest. **C)** Individual 0.25 second IMS frames of the CZE peak for the YICDNQDTISSK peptide $(M+H)^{2+}$.

Intact protein separations and structural characterization using CZE with DT-IMS-qTOF-MS

- CZE allows protein separation in native states (Figure 4) for correlation with IMS structures

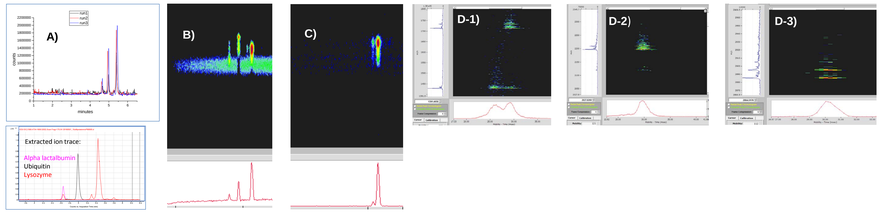

Figure 4: A) CZE-MS replicates for 10 µM intact proteins (Alpha Lactalbumin, Ubiquitin and Lysozyme) separated in acidic conditions. **B)** TIE and IMS spectrum for analysis of intact protein standards. **C)** Extracted CZE-IMS TIE for lysozyme. **D)** IMS spectra of intact proteins in native conditions: D-1 Ubiquitin, D-2 Carbonic Anhydrase, D-3 Myoglobin.

CE peak parking for longer analysis times

- NanoESI sheath flow CE-MS interface enables parking of the CE separation while maintaining a stable electrospray.

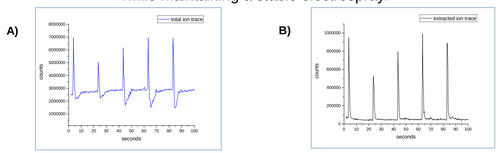

Figure 5: One second CE-MS separation steps (25kV CE voltage, 2kV ESI voltage) followed by 20 second CE voltage parking steps (2kV CE voltage, 2kV ESI voltage). Continuous infusion by CZE-TOF-MS of 5 µM peptide APLSFRGSLPKSYVK. **A)** Total ion trace **B)** Extracted ion trace of the $(M+H)^{3+}$ peptide ion.

Conclusions

- CZE, IMS and MS provide three separation dimensions which can be optimized for specific analytical problems
- CZE-IMS-MS enhances the peak capacity of both peptide and intact protein separations, provides a tool for solution versus gas phase conformations studies, and allows investigation into background electrolytes and pH conditions
- The electrokinetic sheath flow of the CZE-nanoESI interface supports stable electrospray even without the addition of organic solvent, allowing analysis of native solution proteins and complexes
- The increased charge capacity of SLIM devices helps alleviate low injection volume limitations of fast, high electric field CZE separations
- High resolution SLIM-IMS separations enhance CZE peak capacity and overcome quantitation limitations due to co-elution of the isomeric species
- CZE-nanoESI can be temporarily stopped without incurring sample losses or significant band broadening

Acknowledgements

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References

[1] Wojcik et al, *RCMS*. 2010; 24: 2554–2560
[2] Webb et al, *Anal. Chem.* 2014, 86, 9169−9176
[3] Hamid et al, *Anal. Chem.* 2015, 87, 11301−11308
[4] Garimella et al, *Analyst*, 2015, 140, 6845–6852
[5] TOD 4:10 pm: Smith et al; Ultrahigh IMS resolution using novel traveling wave approaches and long serpentine path length SLIM modules
[6] ThOH 8:50 am Webb, et al; Analysis of Protein Structural Changes with High Resolution Structures for Lossless Ion Manipulations (SLIM) Ion Mobility-Mass Spectrometry
[7] WOD 2:30 pm: Garimella et al; Fundamentals of Ion Dynamics in Structures for Lossless Ion Manipulations (SLIM)

Career Opportunities: For openings in the Integrative Omics Group at PNNL please visit http://omics.pnl.gov/careers

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