AccuTOF™ LC/MS

# Identification of Replication Protein by Nanoelectrospray Time-of-Flight Mass Spectrometry

## Introduction

Nanoelectrospray (nanoESI) has become a powerful tool in bioanalytics and is now used as a routine analytical method[1]. The advantages of nanoelectrospray as compared to conventional electrospray (ESI) include very low flow rate and more tolerance toward salt contamination in the analyte solution[2]. Thus, a few µL of analyte solution suffice for extended mass spectrometric studies.

This applications report demonstrates the use of nanoESI for protein identification. A commercially available replication protein A3 is in-gel digested with trypsin and desalted with ZipTip $C_{18}$ tip. The analysis is performed using nanoESI coupled with the AccuTOF™ time-of-flight MS system to obtain the peptide fingerprint followed by a database search with ProFound[3] software.

## Experimental

The protein sample A3 was trypsin in-gel digested and extracted with the buffer solution containing 40 mM ammonium bicarbonate, 50% acetonitrile and 0.1 % TFA, and then dried using SpeedVac. The dried peptides were reconstituted with 0.1 % formic acid solution and desalted with ZipTip $C_{18}$ tip and eluted with 10 µL acetonitrile/0.1% formic acid solution (50/50). 2 µL of the eluent was applied to the spray needle (New Objective).

**MS Conditions**

| | | | |
|---|---|---|---|
| Source: | nanoESI | Ionization mode: | positive |
| Needle voltage: | 2000 V | Orifice 1 voltage: | 60 V |
| Orifice 2 voltage: | 4 V | Ring Lens voltage: | 9 V |
| Orifice 1 temp: | 100 °C | MCP voltage: | 2700 V |
| Curtain gas flow: | 0.5 unit | Auxiliary gas flow: | 0 unit |

## Results

Figure 1A shows the original mass spectrum of the tryptic digest protein A3 obtained with above nanoESI conditions. Many of the multiple charged peptides in the spectrum are apparent and have a good signal-to noise ratio. Figure 1B shows the deconvoluted mass spectrum obtained by using the software MagTran 1.0[4].

Figure 2 shows the results after database search with the software ProFound 4.10.5. Ten possible proteins are listed. Only one of them has a 100% probability and very high Z score (2.30) and peptide coverage (61%). The protein, therefore, was unambiguously identified as replication protein A3 with the molecular weight of 14 kD.

## Conclusions

Since the flow rate in nanoESI is very slow, only a very small amount of sample is required. Analyte suppression is also significantly reduced. NanoESI coupled with a high-resolution and high-sensitivity time-of-flight mass spectrometer makes it a powerful technique for peptide and protein identification.

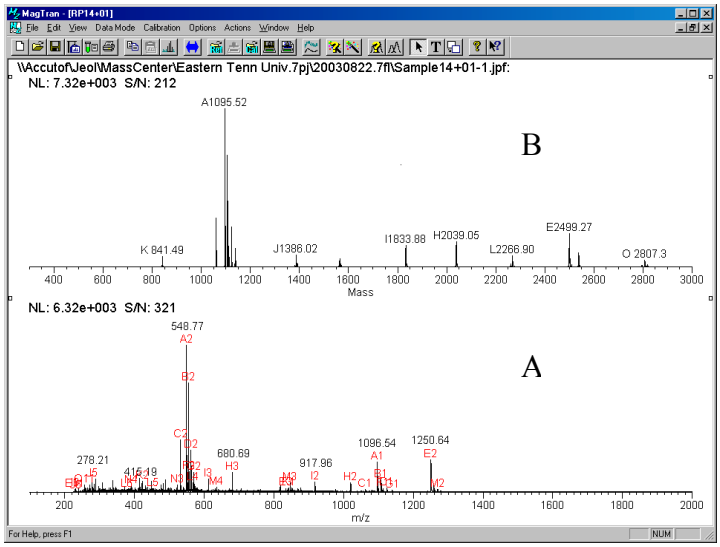


Fig 1. Mass spectrum of tryptic RP 14. (A) the original mass spectrum; (B) the deconvoluted mass spectrum.

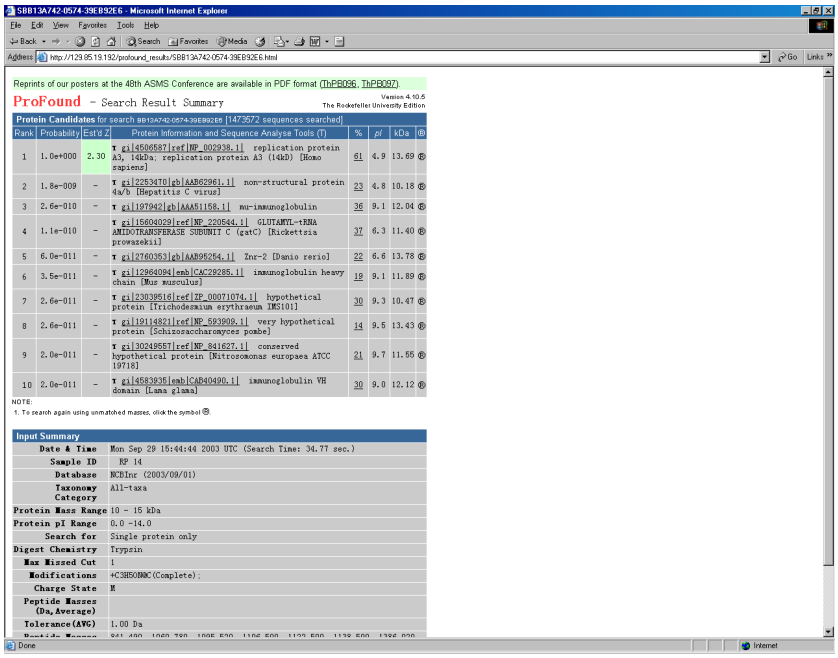


Fig 2. ProFound search result of peptide fingerprint mass spectrum in Fig 1B.

## Acknowledgements

We greatly acknowledge Mr. Mike Shell and Dr. Yue Zou at East Tennessee State University for providing the tryptic A3 protein samples.

## References

1. McLafferty, FW; Fridriksson, EK; Horn, DM; Lewis, MA and Zubarev, RA. Biomolecule Mass Spectrometry. Science 1999, 284, 1289-1290
2. Schmidt, A and Karas, M. Effect of different solution flow rates on analyte ion signals in nano-ESI MS, or: when does ESI turn into nano-ESI?. J Am Soc Mass Spectrum. 2003, 14, 492-500
3. Tang, C; Zhang, W; Fenyo, D and Chait, BT. Assessing the performance of different protein identification algorithms. 48$^{TH}$ ASMS Conference, 2000, 6.
4. Zhang, Z and Marshall, AG. A universal algorithm for fast and automated charge state deconvolution of electrospray mass-to-charge ratio spectra. J. Am. Soc. Mass Spectrom. 1998, 9, 225-233