



Identification and Characterization of Human Proteoforms by Top-Down LC-21 Tesla FT-ICR Mass Spectrometry

Anderson, L.C. (NHMFL); DeHart, C.J. (NHMFL/NU); Kaiser, N.K. (NHMFL); Fellers, R.T. (NU); Smith, D.F. (NHMFL); Greer, J.B. (NU); LeDuc, R.D. (NU); Blakney, G.T. (NHMFL); Thomas, P.M. (NU); Kelleher, N.L. (NU); Hendrickson, C.L. (NHMFL)

Introduction

Successful high-throughput characterization of intact proteins from complex biological samples by mass spectrometry (MS) requires an instrument capable of high mass resolving power, mass accuracy, sensitivity, and spectral acquisition rate. These limitations often necessitate the performance of hundreds of experiments to obtain reasonable coverage of the targeted proteome, which is still typically limited to molecular weights (MW) below 30 kDa. We demonstrate intact (top-down) proteomic analysis of human colorectal cancer cell lysate by liquid chromatography (LC)-MS on a 21 T FT-ICR mass spectrometer.

Experimental - There should be one blank line Arial, 10 pt. space before each heading.

Proteins from human colorectal cancer cells (DLD-1) were fractionated into discrete MW ranges and analyzed by on-line LC on the MagLab's 21 tesla Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer with the goal of maximum proteome coverage in the minimum possible time.

Results and Discussion

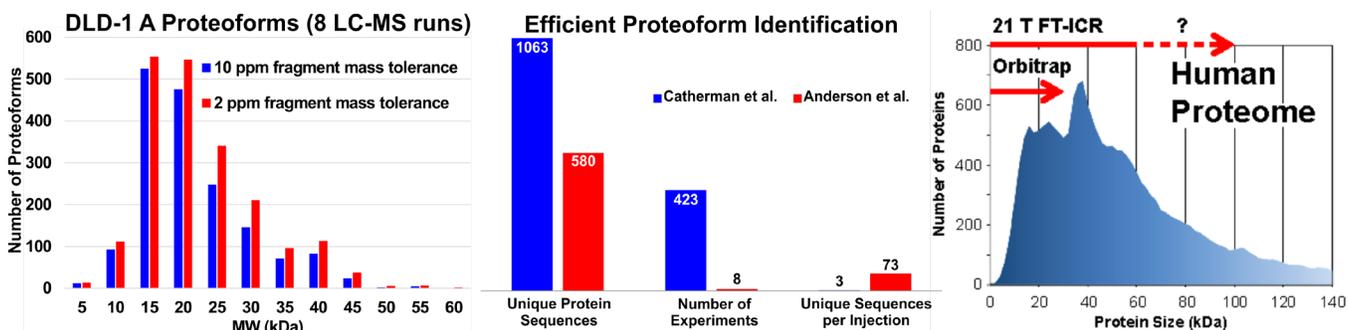


Figure 1: (Left) Number and MW of proteoforms identified at 10 and 2 ppm fragment mass tolerances from 8 LC-MS injections of DLD1 whole cell lysate. Higher mass accuracy reduces false discovery rate, enabling larger numbers of proteoforms to be identified. (Center) Comparison of the number of unique protein sequences identified between this study (Anderson et al.^[1]) and the largest previous top-down proteomic study (Catherman et al.^[2]). A nearly 25-fold improvement in efficiency was observed as the number of LC-MS experiments needed to achieve these results reveals that >50% the proteome coverage can be achieved in <2% of the time. (Right) At 21 T, proteins as large as 60 kDa are identified marking a 30 kDa improvement over commercial Orbitrap instrumentation, enabling characterization of more than half of the intact human proteome.

Conclusions

These experiments produced unparalleled results on the basis of number of identified proteins per total number of injections compared to previous studies. Improving throughput, sequence coverage, and the molecular weight range available to intact protein analysis by mass spectrometry will facilitate the discovery of potentially thousands of new proteoforms, which might have direct relevance in human disease.

Acknowledgements

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References

- [1] Anderson, L.C., *et al.*, *J. Proteome Res.*, **16**, 1087-1096 (2017).
- [2] Catherman, A.D., *et al.*, *Mol. Cell Proteomics.*, **12**, 3465-3473 (2013).