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Front end proteomic separations for top-down mass spectrometry analysis of low microgram samples

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Although the more widely used proteomic strategy has typically been peptide-based mass spectrometry (MS), the unambiguous characterization of protein sequence and post-translational modifications is usually better achieved through analyzing intact proteins. In the past, however, this use of top-down proteomics has not been achieved in a high-throughput format due to the under development in separations. With recent improvements in achieving high-throughput top-down proteomics with online MS detection and identification, custom front-end separations coupled to LC-LTQ-FTMS affords the opportunity to achieve unprecedented proteome coverage. With a record 1000 intact protein identification from the HeLa proteome and many more isoforms, some of which were correlated with DNA damage, we showed that top-down proteomics can now achieve a level of coverage approaching that of bottom-up proteomics. An enabling aspect to this achievement is the integration of the GELFrEE apparatus, a size based separation, into a four-dimensional separation platform. While the level of throughput is encouraging, high loading requirements limited the study to samples obtained from cell culture with low milligram quantities of proteins. In order to transition to the analysis of precious clinical samples, significantly lower sample requirements must be possible. Here, we couple a miniaturized GELFrEE apparatus to LC-LTQ-FTMS to separate protein mass differences down to 700 Da, while achieving similar proteome coverage with 10-20 fold less starting material. In addition, we demonstrate the ability to enrich proteins by 1-2 orders of magnitude by using a novel electrophoretic procedure. Finally, we show improved automation from sample loading, to separation to direct analysis using MS, which will prove crucial to expand the technology to non-expert users.

Biography

John C. Tran obtained his B.Sc. and Ph.D. in Chemistry from Dalhousie University, Canada. He obtained his Ph.D. in Prof. Alan Doucette's lab where he designed and developed biomolecule separation devices, including the patented GELFrEE apparatus, tailored for mass spectrometry. In 2008, he joined Prof. Neil Kelleher's lab as a post-doctoral associate, and was involved in technology developments that led to the largest top-down proteomics study to date. In 2010, he was a research associate in the Proteomics Center of Excellence (PCE) at Northwestern Universityand worked on improving workflows prior to mass spectrometric analysis for biological samples. In 2012, in addition to working in PCE and other companies as a consultant, he is performing research in the laboratory of Prof. Mike Moran at the University of Toronto.

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