DIRECT PROTEIN 2D-LC MALDI WITH ON-TARGET DIGESTION FOR HIGH-THROUGHPUT PROTEOMIC ANALYSES

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Introduction:
Large-scale proteomic analyses necessitate high-throughput sample preparation techniques. However, highly complex mixtures require multi-dimensional fractionation prior to MS analysis to maximize the yield of useful MS data. This geometrically expands sample numbers, dramatically intensifying processing load, commonly involves dilution (*e.g.*, HPLC), often demanding sample concentration, and typically requires multiple steps of sample handling, including transfer to different reaction vessels. These steps are time consuming, lead to sample losses and potential contamination. We have explored the use of a novel, simple, inexpensive (non-robotic) 96-well array technology, the BD™ MALDI Concentrator[1], to conduct one-pot on-target sample preparation for MALDI-MS analysis. We have applied this technology with deposition from 1D and 2D protein LC direct-to-target for peptide mapping by MALDI-TOF MS.

Methods:
Protein standards were digested in-solution on-target/in-well using the BD Biosciences MALDI Concentrator™, dried under vacuum and co-crystallized with matrix under differing conditions. 1D and 2D-HPLC fractionation of protein mixtures was conducted with a Beckman PF2D™ system. Fractions were collected directly into the wells of the BD device, and optimized conditions were used to concentrate, digest in-solution on-target/in-well, and co-crystallize the samples with matrix. MALDI mass spectra were obtained with a Bruker Reflex IV™ MALDI-TOF MS. Results were compared, with fractionated protein mixtures and peptide standards that had been digested, concentrated and co-crystallized with matrix by conventional methods. Results were further compared with LC fraction collection into 96 well plates and with 1D SDS-PAGE followed by in-gel digestion of proteins.

Results:
Application of the BD MALDI Concentrator is shown schematically in Figures 1 and 2. One-pot on-target/in-well digestion, concentration and sample/matrix co-crystallization under optimized solvent conditions readily yielded MS analyses with minimal sample loss from 1 pmol protein standards and as little as 10 fmol of peptide standards from up to 200 µl starting solution. This amounted to good recovery of MS signal from picomolar protein and sub picomolar peptide concentrations. This methodology was expanded to analyze protein mixtures separated by 1D and 2D RP-protein-LC. An example of a standard protein mixture separated by non-porous RP-LC is shown in Figure 3. Fractions were collected and subjected to in-well digestion and analyzed by MALDI-TOF MS. Each fraction which contained protein produced good quality MALDI data (Figure 3b.), significant enough for automated database search and interpretation. An example of the search results for the fraction corresponding to myoglobin (~75% coverage) is shown in 3c. (search performed on the new Boson University Protein Identification server)[2]. Results using the BD Concentrator compared well with in-gel digestion of protein standards separated via SDS-PAGE and with standards separated by 1D and 2D RP-protein-LC. While the resolution of the current RP-LC separation is less than that obtained with 1D SDS-PAGE, the ease and degree of recovery is enhanced as is the ability to automate the system. Direct application of this technology is anticipated in the future for the 2D-protein-LC analysis of human serum/plasma samples which are currently analyzed after collection into 96 well plates prior to MALDI TOF MS analysis[3]. The coupling of 1D and 2D-protein-LC to MALDI-TOF MS through the collection of LC fractions directly into the 96-well array concentrator enabled rapid, high-throughput protein fractionation, digestion, peptide matrix co-crystallization, and MALDI-TOF MS analyses with minimal sample handling.

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Figure 1. Sample preparation in a 96 well BD Bioscience MALDI Concentrator™.

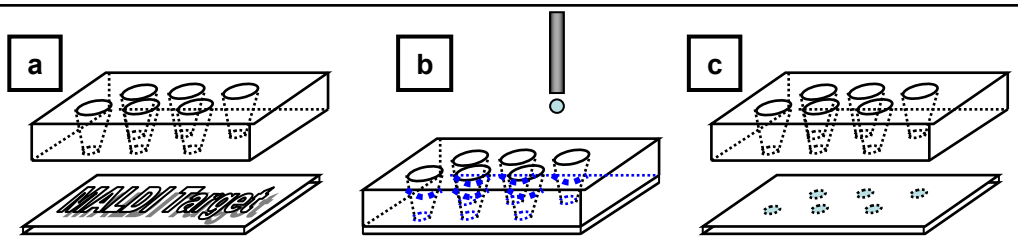

Figure 2. a) MALDI target + BD MALDI concentrator, b) fraction collection from LC, c) after processing and addition of matrix: ready for MALDI-TOF MS.

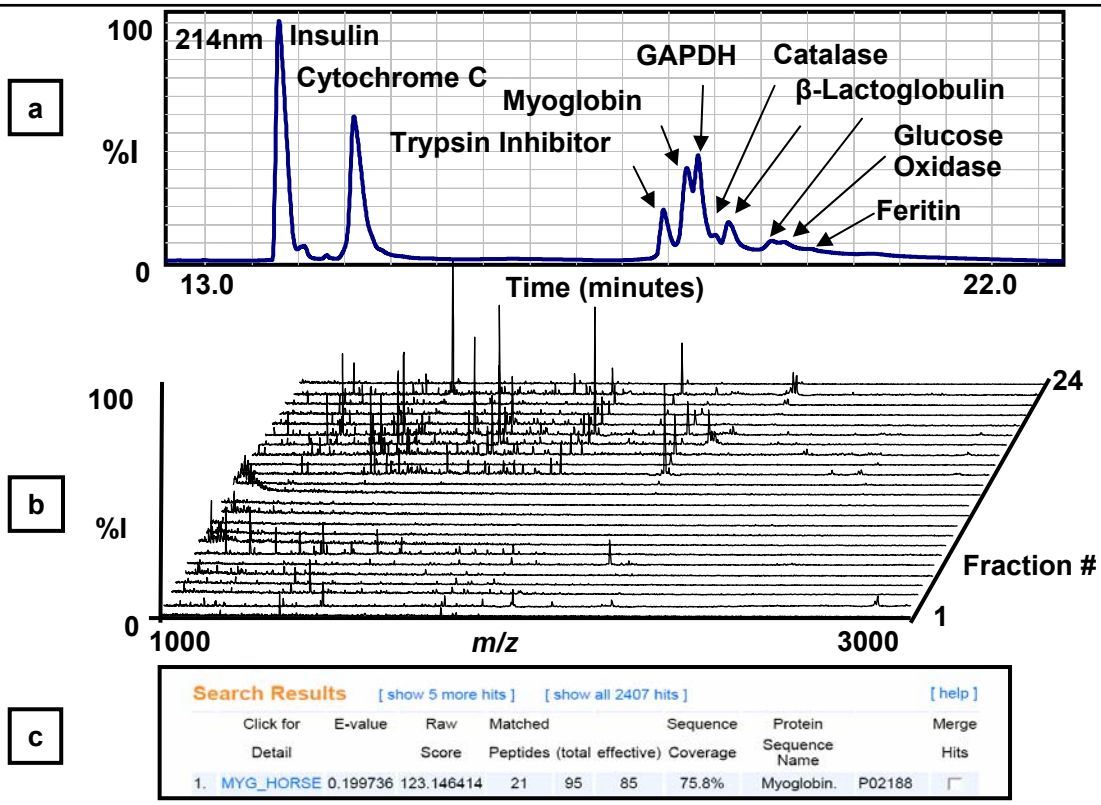

Figure 3. a) RP-HPLC of a protein mixture, b) MALDI-TOF MS of fractions colleted and processed using the BD MALDI concentrator, c) database search results from BUPID[2] for the fraction corresponding to myoglobin.

References:
1. Chen, K., ASMS 2005, TP09 (#161). 2. Tong, W., ASMS 2005, WP21 (#388). 3. Dauly, C., ASMS 2005, TP29 (#492).