3 Dimensional MALDI Plates employing collimated-hole structures used to coupling high capacity, high flow separations to MALDI-TOF analysis for top down proteomics

Poster Number MP 565

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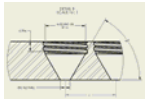
INTRODUCTION

-Collimated-hole structures are used to construct 3 dimensional MALDI plates[1]
- Individual holes filled with monolithic chromatography media
  - -Styrene/divinylbenzene and Butyl and Stearyl methacrylate for reversed phase (RP) capture
  - -Glycidyl methacrylate[2] and vinyl azalactone[3] co-polymers for immobilized enzyme plates

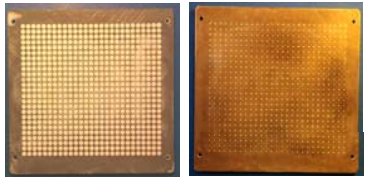
-3D plates are envisioned to enable high capacity (1mg) loading and high flow rate chromatography (200µL/min - 1mL/min) directly to MALDI-MS and MS-MS analysis
-Top down proteomic workflows employing serial and parallel digestion of sample presented

MATERIALS & METHODS

3D MALDI PLATES
-Current plates are constructed by machining holes into large format (4.875 x 5.000 x 0.125in) MALDI plates designed for Virgin Instruments mass spectrometer
  --although plates may be formatted to any dimensions
-Conical holes designed to maximize capacity for sample capture and minimize non-conductive polymer surface on analytical plate surface



-variety of polymers have been constructed for RP peptide capture using hardware designed for either UV and thermal initiation
-sample is loaded separately and sequentially into the individual holes on CHS plate but sample elution and washing (if necessary) takes place simultaneously
-sample are loaded through the analytical plate surface (small hole) and eluted in the opposite direction with matrix



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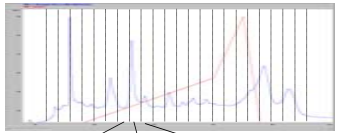
TOP DOWN PROTEOMICS WITH SERIAL DIGESTION

-2D LC used to separate protein sample
1) RP capture media in plate allows for high-resolution RP separation in 1st dimension

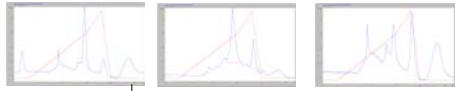
C4 separation of Muscle extract



2) Anion exchange 2nd dimension compatible in-line trypsin digestion column

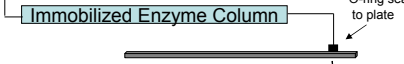
RP fractions further separated by Anion Ex.

3) Digested peptides captured directly onto MALDI plate



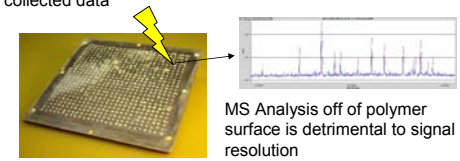
4)Sample washed and eluted to surface for MALDI analysis

5)Plate is dried and analyzed By MALDI MS



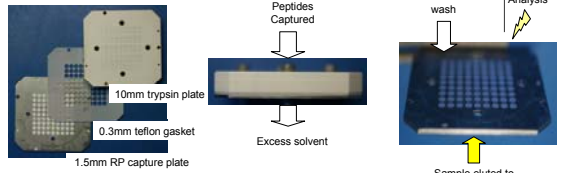
6) Resulting peptide used to ID protein by peptide mass fingerprinting or MS-MS analysis used for sequencing and database search

7) Trial experimentation has collected data from 500µg of collected in 30 1st dimension RP frxs Separated and digested in series and spotted on a single MALDI plate

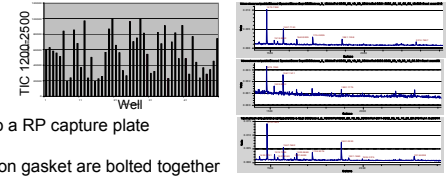


MS Analysis off of polymer surface is detrimental to signal resolution

TOP DOWN PROTEOMICS WITH PARALLEL DIGESTION



Parallel Digestion of 50 BSA SAMPLES



Individual samples or a single sample separated by 1 or more dimensions of chromatography may be digested in parallel using a CHS immobilized enzyme (IE) plate coupled to a RP capture plate

1)IE and RP plates with Teflon gasket are bolted together
2) Protein sample in passed through holes for digest and peptide capture
3) Plates are separated and peptides are eluted to RP plate surface for MS
4) Results show TIC (1200-2500) and low, median and high spectra from the parallel digestion of 50 BSA samples

References:

1) Hattan SJ, Vestal ML *Anal. Chem.*, 2008, *80* (23), pp 9115–9123
2) D. S. Peterson, T. Rohr, F. Svec, J. M. J. Fréchet, *Anal. Chem.*, 2002, 74, pp 4081-4088
3) G. T. Hermanson, A. K. Mallia, P. K. Smith, *Immobilized Affinity Ligand Techniques* Academic Press Inc.

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