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A Novel Nanoparticle-Coupled MALDI-TOF Approach for High Throughput Low Molecular Weight Biomarker Enrichment, Isolation, Preservation and Discovery

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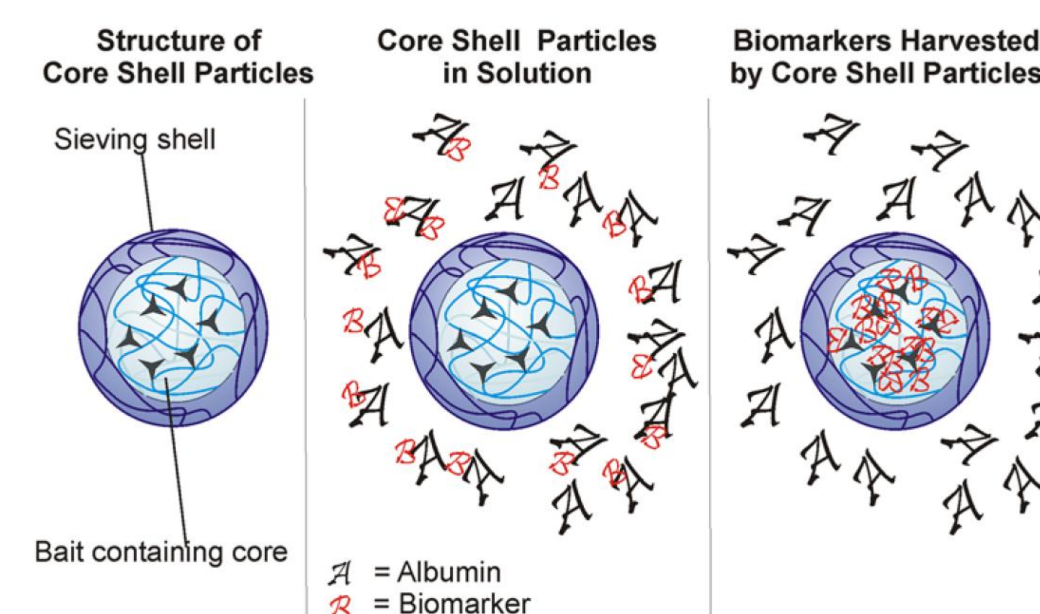
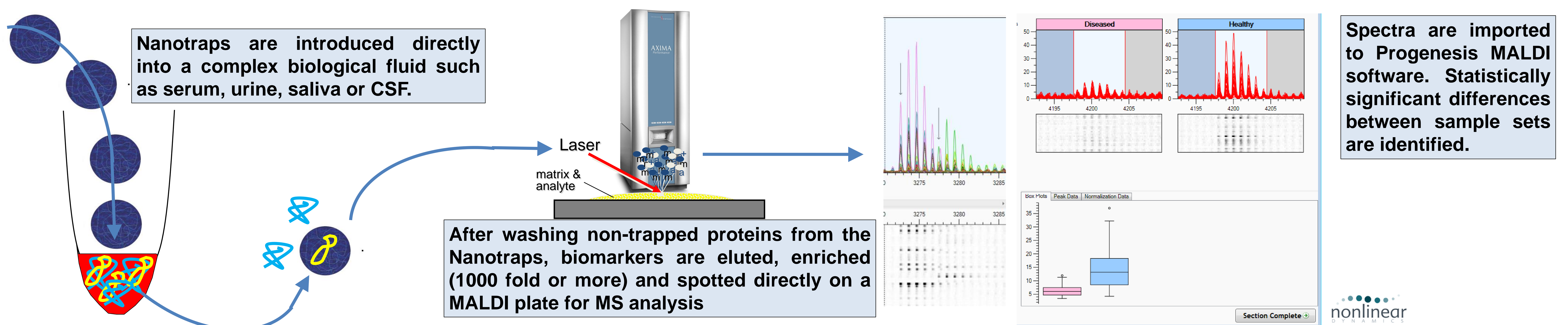
Each year Non Small Cell Lung Carcinoma (NSCLC) claims hundreds of thousands of lives worldwide. Prognosis for this disease is poor requiring a new collection of biomarkers to diagnose and treat patients. Low molecular weight (LMW) and peptidomic information have high potential for providing valuable biomarkers because this information traverses the endothelial barrier from tissue interstitum to circulation. These biomarkers are often masked in mass spectrometric (MS) analysis by highly abundant proteins, requiring intensive sample preparation techniques. A novel nanotechnology reagent, the Nanotrap®, a core-shell hydrogel nanoparticle, was developed to address many of the issues associated with the analysis of LMW biomarkers[1, 2, 3]. This presentation focuses on a Nanotrap biomarker discovery platform which combines the Nanotraps' ability to sequester, enrich and protect LMW biomarkers directly from complex biological fluids with the fast, highly sensitive Matrix Assisted Laser Desorption Ionization time of flight mass spectrometry (MALDI-TOF MS) analysis to screen samples for biomarkers. This presentation focuses on the benefits of Nanotraps and how to utilize the Nanotrap platform to identify differences between control and disease serum samples from lung cancer patients.

Nanotrap Sequestration

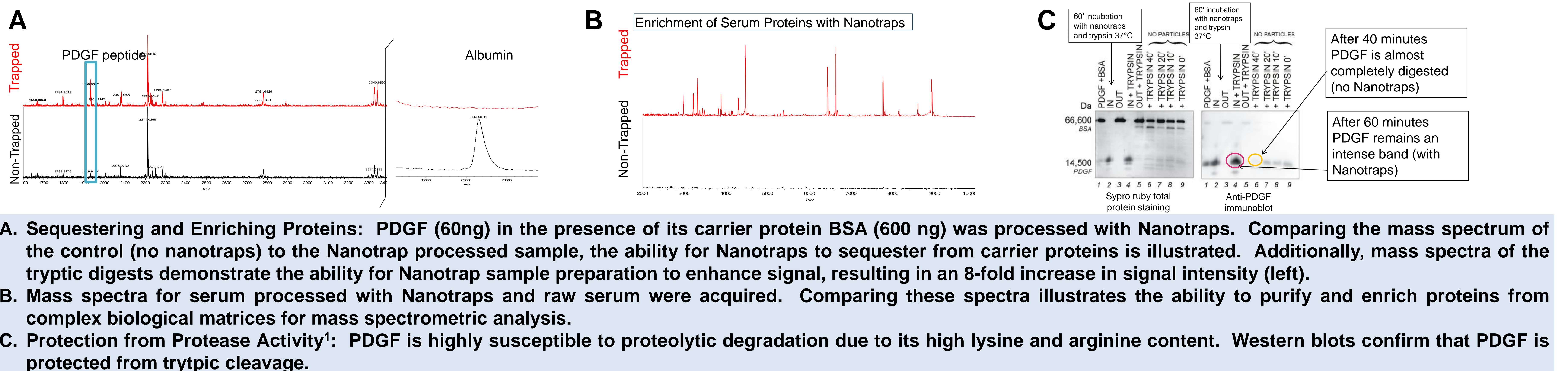


Core shell hydrogel nanoparticles, or Nanotraps result from the radical polymerization of N-isopropylacrylamide in the presence of a bait molecule such as acrylic acid or cibacron blue. By controlling the extent of crosslinking, very tight molecular weight cutoffs can be obtained. This results in a single step enrichment strategy that excludes high molecular weight carrier proteins and selectively and non-covalently binds proteins based on the characteristics of the bait. Once harvested directly from complex biological fluids, nanotraps have the added benefit of protecting biomarkers from protease activity.

The Nanotrap Biomarker Discovery Platform Workflow



Benefits of Nanotraps for Biomarker Discovery: Sequester, Enrich, Purify and Protect



A. Sequestering and Enriching Proteins: PDGF (60ng) in the presence of its carrier protein BSA (600 ng) was processed with Nanotraps. Comparing the mass spectrum of the control (no nanotraps) to the Nanotrap processed sample, the ability for Nanotraps to sequester from carrier proteins is illustrated. Additionally, mass spectra of the tryptic digests demonstrate the ability for Nanotrap sample preparation to enhance signal, resulting in an 8-fold increase in signal intensity (left).

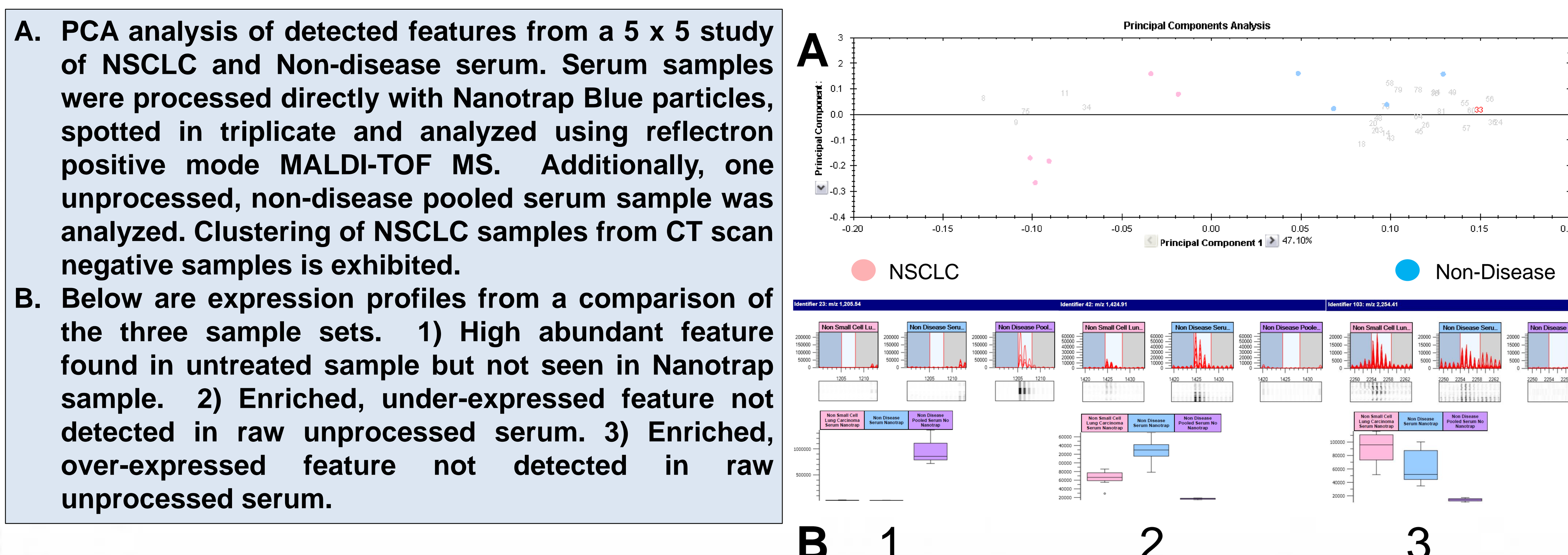
B. Mass spectra for serum processed with Nanotraps and raw serum were acquired. Comparing these spectra illustrates the ability to purify and enrich proteins from complex biological matrices for mass spectrometric analysis.

C. Protection from Protease Activity[1]: PDGF is highly susceptible to proteolytic degradation due to its high lysine and arginine content. Western blots confirm that PDGF is protected from trytpic cleavage.

Non Small Cell Lung Carcinoma Study

A. PCA analysis of detected features from a 5 x 5 study of NSCLC and Non-disease serum. Serum samples were processed directly with Nanotrap Blue particles, spotted in triplicate and analyzed using reflectron positive mode MALDI-TOF MS. Additionally, one unprocessed, non-disease pooled serum sample was analyzed. Clustering of NSCLC samples from CT scan negative samples is exhibited.

B. Below are expression profiles from a comparison of the three sample sets. 1) High abundant feature found in untreated sample but not seen in Nanotrap sample. 2) Enriched, under-expressed feature not detected in raw unprocessed serum. 3) Enriched, over-expressed feature not detected in raw unprocessed serum.



Summary

Nanotraps sequester, enrich, purify and protect LMW biomarkers. This method offers a fast, robust method for screening large sample sets. Its utility was demonstrated in a small scale lung cancer serum study that differentiated sample sets and identified features of interest that would not have been detected without Nanotrap enrichment.

Methods

PDGF sequestration experiment: All reagents purchased through Sigma Aldrich (Saint Louis, MO) unless otherwise noted. 100 uL of Nanotrap particles (CeresNano, Manassas, VA) were incubated with 1200 uL of solution containing PDGF-BSA (Cell Signaling Technology, Danver, MA) diluted in 1 mM citrate buffer pH=6, PDGF concentration (600 ng/mL, 60 ng/mL), BSA is present in 10 fold excess. Particles were washed with 1 mL of 18-MΩ-cm water twice and eluted with 100 ul 60 % acetonitrile, 2 % acetic acid. Eluates (100 ul) were collected and dried. Samples were digested using: Urea 2 M, DTT 5 mM, Iodoacetamide 15 mM, NH_4HCO_3 100 mM, Trypsin 1:100 (w/w) enzyme:total protein ratio. Samples were purified with Ziptip pipette tips and dried with a Speed-Vac. The dried samples were reconstituted in 20 µl 50% ACN, 50% DI H_2O, 0.1% TFA. 0.5 µl sample was spotted with 0.5 µl matrix (20 mg / ml Sinapinic Acid in 50% acetonitrile / 50% 18-MΩ-cm water) and dried for Matrix Assisted Laser Desorption Time of Flight Mass Spectrometry (MALDI-TOF MS) analysis, MALDI-TOF MS was performed using an AXIMA Performance (Shimadzu, Manchester U.K.) operating in linear positive mode with a 337 nm nitrogen laser firing at 50 Hz.
NSCLC Serum study: Human serum from five NSCLC (non small cell lung cancer) patients and five control individuals (CT scan negative) (150 µl each) was diluted with 300 µl Tris HCl and incubated with 300 µl CB (Cibacron Blue) containing particles. Particles were collected by centrifugation, washed with 300 µl water and eluted twice with 50 µl 70% acetonitrile, 10% Ammonium Hydroxide. Eluate from particles was diluted in α-cyano-4-hydroxycnnamic matrix and spotted in triplicate on a steel MALDI target. The samples were analyzed using an AXIMA Performance MALDI TOF-MS (Shimadzu Biotech, Kyoto Japan). Mass range analyzed was 1000-4000 kDa and the instrument was run in reflectron mode (power 75; profiles 100;shots 50; raster mode).

Reference:
1 Longo, C., et al., *Core-shell hydrogel particles harvest, concentrate and preserve labile low abundance biomarkers.* **PloS one, 2009. 4(3): p. e4763-4790.**
2 Rainczuk A., et al., *An Optimized procedure for the capture, fractionation and proteomic analysis of proteins using hydrogel nanoparticles.* **Proteomics, 2010, 10 p. 332-336**
3 Fredolini C., et al., *Investigation of the ovarian and prostate cancer peptidome for candidate early detection markers using a novel nanoparticle biomarker capture technology.* **AAPS J, 2010, Jun 12 (epub)**