Supplemental Information

Nanoparticle preparation.

Dextran-coated superparamagnetic iron oxide nanoparticles (SPIO) were obtained from a commercial source (Micromod) or prepared by the published method [1]. Briefly, 0.63 g of FeCl3.6H2O and 0.25 g FeCl2.4H2O were mixed with 4.5 g dextran (MW ~20,000, Sigma) in 10 mL of deionized (Millipore) water at room temperature. This acidic solution was neutralized by the dropwise addition of 1 mL concentrated aqueous ammonia under vigorous stirring and a steady purge of nitrogen, and it was then heated at ~70° C for 1 hr. After purification by centrifuge filtering column (100,000 MWCO, Millipore), nanoparticles with a size range of 50~80 nm were obtained by filtering the magnetic colloid through a 0.1 μm pore diameter membrane (Millipore) and separating them using a MACS® Midi magnetic separation column (Miltenyi Biotec).

To add amines on the nanoparticle surface, ~ 15 mg Fe of the above magnetic colloid or 5 mg of Micromod D-SPIO was reacted in 5 mL of strong base (5M aqueous NaOH solution) containing 1 mL of epichlorohydrin (Sigma) for 24 hrs, purified by dialysis for 24 hrs and filtered through the 0.1 μm pore diameter membrane. Then, 1 mL of concentrated ammonium hydroxide (30%, EM Science) was added to 1mL of epichlorohydrin-activated nanoparticles (~10mg Fe/mL). The mixture was agitated at room temperature overnight to perform the reaction. In order to remove excess ammonia ions, the reacted mixture was dialyzed against double-distilled water for 24 hours using dialysis cassette (10,000MW cutoff, Pierce). To further rinse the particles and change a buffer from water to phosphate buffered saline (PBS, pH 7.4), the colloid was trapped on the magnetic column (Miltenyi Biotec) and then rinsed with PBS three times. After the rinse, the colloid was eluted from the column with 1mL PBS.

Mass spectrometry.

Protein Identification using 1D LC/MS/MS: Five micrograms of total protein were reduced by 5mM final concentration of DTT and alkylated by final concentration

of 15mM iodoacetamide prior to tryptic digestion by trypsin at 37ºC overnight. Tryptic peptides were desalted and concentrated by C18 Zip Tip (Millipore, MA) and were dissolved in loading buffer A (2% Acetontrile in 0.1% formic acid in HPLC grade water). The automated Nano LC LTQ MS/MS (Thermo Scientific, Waltham, MA) were performed as described by Salvesen et al. [2] (using an Eksigent Nano 2D LC system, a switch valve, a C18 trap column (Agilent, Santa Clara, CA), and a capillary reversed phased column (10 cm in length, 75 mm id) packed with 5 mm, C18 AQUASIL resin with an integral spray tip (Picofrit, 15 mm tip, New Objective, Woburn, MA),using a linear gradient elution from buffer A (2% acetonitrile in H2O plus 0.1% formic acid) to 15% buffer A plus 85% buffer B (ACN plus 0.1% formic acid) in 45 min. The LC/MS run was operated in the data dependent mode. Data on the four strongest ions above an intensity of 50x10e4 were collected with dynamic exclusion enabled and the collision energy set at 35 %.)

Large Scale protein identification using 2D LC/MS/MS: 400 ug total Mouse plasma proteins, (from control and enriched samples), precipitated with acetone were re-suspended in PBS and were digested with trypsin as described in previous section. Tryptic peptides were desalted and concentrated using Waters Sep Pak Plus C18 cartridges. Peptides were dissolved in 83 ul of 95% solvent C/5% solvent D (Solvent C = 5% acetonitrile, 95% H2O, 0.1% formic acid/3% Solvent D = 25% acetonitrile, 75% water, 0.1% formic acid, 500 mM KCl). SCX (strong cation exchange) column was used to separate peptides on first dimension of separation using gradient: Start at 5% D; 5% D to 9% D at 1 min, 9% D to 20% D at 24 min; 20%D to 40% D at 34 min; 40% D to 100% D at 44 min; stay at 100%D for 1 min; back to 5%D in 1 more min; continue at 5%D for 4 more min. 80 ul of total 800 ul of SCX fractions 1-24 were used for a 180 min RP gradient, 2-5% B in 2 min, 5-35% B in 178 min, then up to 80% B for 6 min on the 15 cm RP column then back to 2%B for the final 6 min and the LTQ-Orbitrap which is coupled to our RP LC system was set to scan the precursors in the Orbitrap at a resolution of 60,000 and with a top5 data-dependent MS/MS method in the profile mode on the precursors and centroid mode on the MS/MS

spectra. The MS and MS/MS data were submitted to Sorcerer 2 for protein identification against the ipi.MOUSE database with a precursor mass tolerance of 10.0 ppm.

Protein Identification and data analysis

The MS/MS spectra were analyzed by Sorcerer 2 (Sage-N Research Inc.) with SEQUEST (v.27, rev. 11) as the search program for peptide/protein identification. SEQUEST was set up to search the target-decoy ipi.MOUSE.v3.14 database containing protein sequences using trypsin as the digestion enzyme with the allowance of up to 2 missed cleavages and precursor mass tolerance of 1.5 amu. A molecular mass of 57 Da was added to all cysteines to account for carboxyamidomethylation in case of alkylation of cysteines. Differential search includes 16 Da for methonine oxidation; serine, threonine and tyrosine phosphorylation (79.9 Da), lysine ubiquitination with GG tag (114Da) and LRGG tag (382Da) to identify certain modifications. The search results were viewed, sorted, filtered, and statically analyzed by using comprehensive proteomics data analysis software, Peptide/Protein prophet (ISB). In a qualitative and quantitative proteomics study, it is critical to minimize false positive identifications by MS/MS. In this study, we used the following two search criteria. First, the minimum trans-proteomic pipeline (TPP) probability score for proteins and peptides are both 0.99 and 0.9, respectively, to assure very low error (much less than 1% and false positive rate of 0.02 or less) with reasonably good sensitivity. Second, we set up threshold of cross correlation (Xcorr) scores set for filtered peptides to1.5, 2.0, and 2.5 for 1, 2, and 3 charged fully digested peptides, respectively. The relative abundance of each identified proteins in different samples were analyzed by QTools, our In-house developed open source tool for automated differential peptide/protein spectral counting alalysis.http://sr.burnham.org/sr/homepage/proteomics/links.html

Knockout mice

C3 deficient (JAX strain B6.129S4-C3tm1Crr/J) and immunoglobulin-deficient (B6.129S2-Igh-6tm1Cgn/J) mice were generously provided by Dr. Robert

Rickert from the Burnham Institute. Histidine-proline rich glycoprotein deficient mice [4] and alpha-2HS-glycoprotein (fetuin-A) deficient mice were generously provided for experiments by the laboratory of Dr. Willi Jahnen-Dechent, RWTH Aachen University Clinic in Germany. High molecular weight kininogen deficient mice [5] were from the laboratory of Dr. Keith McCrae from Case Western Reserve University in Cleveland. Mice homozygous for both mannose-binding lectin MBL-A and MBL-C targeted mutations (strain B6.129S4-Mbl1tm1Kata Mbl2tm1Kata/J) were purchased from the Jackson Laboratory. Mice lacking fibrinogen, plasma fibronectin, or vitronectin were from the laboratories that established the knockouts [6], and previously used in the Ruoslahti laboratory.

Legends for supplemental figures and tables

Supplemental Table 1. Protein hits that were identified by washing nanoparticles and eluting the bound plasma proteins.

Supplemental files 2A-B. Comparison of proteins that were identified after 2D separation of plasma proteins from control plasma sample (black font) and enriched plasma sample (red font). The corresponding IPI, number of unique peptide identified, N instance (unique peptide spec count), coverage and protein probability are shown. The filter was set to 0 FDR (False Discovery Rate). Files A and B are replicates 1 and 2, respectively.

Supplemental files 3A-B. Comparison of protein spectral counts in control (grey column) and enriched (blue column) sample, and the calculated ratio (orange column). The spectral data from all the SCX fractions was compiled and summarized using QTools software. Each row shows IPI, gene identification and ontology, localization and spectral count. Files A and B are replicates 1 and 2.

Supplemental Figure 1. Raw mass spectrometry data for some of the proteins identified.

References

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