A Rapid Approach for Fabricating Functionalized Plates for Sample Purification and Mass Spectrometry Analysis: The Application of Hydrophobic Plate for Peptide/Protein Purification and Hydrophilic Plate for Glycopeptide Enrichment

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Supporting Information

a. Sample preparation by a C18 tip

A C18 tip (ZipTip, Millipore, USA) was prewetted with 100% ACN by aspirate and dispense step three times. The tip was then equilibrated by washing it twice with 0.1% TFA. A 10 μL volume of sample solution was aspirated into the preequilibrated tip, followed by washing with 10 μL of 0.1% TFA three times. A 10 μL volume of elution solution (80% ACN/0.1% TFA) was used to elute sample two times. The eluted sample solution was dried in a centrifugal concentrator (miVac Duo Concentrator; Genevac, NY, USA) and re-dissolved with 3 μL of 80% ACN/0.1% TFA solution, followed by a MALDI-TOF analysis.

b. High density lipoprotein (HDL) isolation from plasma

HDL was isolated from plasma, with a density between 1.063 and 1.21 g mL⁻¹, using ultracentrifugation in KBr-density gradient (Optima™ L-90K, Beckman Coulter, USA). All the reagents were purchased from Sigma. The cocktail of protease inhibitor (Roche), 1% Penicillin-Streptomycin-Neomycin mix (PSN, GIBCO), sodium azide (0.02% wt/vol), 10 mM Na₄P₂O₇, 1 mM Na₃VO₄, and 10 mM β-glycerophosphate was added immediately after the HDL collection to protect the plasma from in vitro oxidation and degradation. Purified HDLs were directly analyzed by on-target MALDI-TOF or dialyzed against degassed buffer A (20 mM Tris-HCl and 0.5 mM EDTA, pH 8.0) at 4 °C with three buffer A (3 L) changes in 24 h, followed by a MALDI-TOF analysis.

c. Protein digestion

HRP and BSA were individually dissolved in 50 mM of ammonium bicarbonate (ABC) and heated to 90 °C for 20 min. The denatured proteins were reduced with 10 mM DTT for 20 min at 56 °C, followed by the addition of 55 mM of IAA for 30 min, in the dark at 25 °C. Trypsin was added to the protein solution at an enzyme-to-substrate ratio of 1:50 (w/w) for 12 h at 37 °C.
For Bv-2 cell lysate containing 0.8M urea/0.005%SDS or 0.8M urea/0.05%SDS, the lysate proteins were dissolved in 50 mM of ammonium bicarbonate (ABC and heated to 37 °C for 60 min. The denatured proteins were reduced with 10 mM DTT for 20 min at 56 °C, followed by the addition of 50 mM of IAA for 60 min, in the dark at 25 °C. Trypsin was added to the protein solution at an enzyme-to-substrate ratio of 1:10 (w/w) for 12 h at 37 °C.
Figure S-1: Representative MALDI–TOF spectrum from 5 fm BSA digests with purification from (a) CP plate (b) C18 tip and (c) C18 magnetic nanoparticles. The S/N value was the average of four replicated measurements.
Figure S-2: Representative MALDI–TOF spectrum of the eluted BSA sample solution from CP plate. 5 fm BSA digests, 0.1% SDS sample solution was deposited on CP plate with different sample incubation time of (a) 1 min, (b) 6 min, and (c) 10 min.
Figure S-3: Representative MALDI–TOF spectrum from 5 fm BSA digests with (a) the addition of 200 mM urea after (b) CP plate and (c) C18 Tip sample preparation. The PMF score was the average value from 5 replicated measurements.
Figure S-4: Sample capacity evaluation of the CP-plate with ~2.8 mm i.d. spot arrays. The peptide (m/z 1479.8) purified from 0.05, 0.01, 0.5, 1, 5, 10, 50, and 100 µg of BSA digests using the CP-plate was mixed separately with 5 fm, 10 fm, 50 fm, 100 fm, 500 fm, 1 pm, 5 pm, and 10 pmol of ACTH peptide (as an internal standard, m/z 2465), and then subjected to MALDI-TOF analysis on a PDMS-coated plate. Y-axis: the peak ratio of the peptide peak (m/z 1479.8) to its corresponding ACTH peak signal (m/z 2465.2). X-axis: the loading amount of BSA digests. Each concentration was performed with three measurements with different C18 spots.
Figure S-5: MALDI-TOF-MS analysis of major apolipoproteins and their isoforms in HDL with dialysis desalting. A-I, apoAI (calculated mass: m/z 28078); A-I_{2+}+2add_{2+}, apoAI + two 98-Da adducts (calculated mass: m/z 14137.4); A-II, apoAII (calculated mass: m/z 17379.8); A-II', apoAII minus C-terminus-Gln (calculated mass: m/z 17253.7); A-II'', apoAII minus two C-terminus-Gln (calculated mass: m/z 17125.6); A-IImonomer, single chain apoAII (calculated mass: m/z 8809.9); C-I, apoCI (calculated mass: m/z 6630.6); C-I', apoCI minus N-terminus Thr-Pro (calculated mass: m/z 6432.4); C-II, apoCII (calculated mass: m/z 8204.1); proC-II, pro-apoCII (calculated mass: m/z 8914.9); C−III_{0}, apoCIII_{0} (calculated mass: m/z 8765.7); C−III_{1}, apoCIII_{1} (calculated mass: m/z 9421.3); C−III_{0}, Glyc', Galβl, 3GalNAc-O-apoCIII_{0} (calculated mass: m/z 9130.0); C−III_{2}, apoC−III_{2} (calculated mass: m/z 9712.6); C−III_{2}', ApoC−III_{2} minus C-terminus–Ala (calculated mass: m/z 9641.5); SAA4 (calculated mass: m/z 12863.2).
Table S-1: The detected glycopeptides of HRP from BSA (50 ng) and HRP (250 ng) tryptic digests mixture after HP plate enrichment. The N-glycosylation sites\textsuperscript{12,38} are marked with N\textsuperscript{#}. GlcNAc = N-acetylglucosamine, Fuc = fructose, Man = mannose, Xyl = xylose.

<table>
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<th>Peak</th>
<th>Observed m/z</th>
<th>Glycan composition</th>
<th>Amino acid sequence</th>
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<tr>
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