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Supplementary Information

for

Disulfide- and terminal alkyne-functionalized magnetic silica particles for enrichment of azido glycopeptides

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1. Experimental Section

1.1 Chemicals and reagents

$FeCl_3 \cdot 6H_2O$, sodium acetate, sodium citrate, sodium ascorbate, 28% $NH_3 \cdot H_2O$, triethylamine(TEA), KBr, $CuSO_4$, NaCl, $MnCl_2$, were bought from Sinopharm Chemical Reagent (Shanghai, China). Tetrahydrofuran (THF), ethanol, methanol and ethylene glycol were bought from Yangyuan Chemical (Changshu, China). 3-aminopropyltriethoxysilane (APTES), glutaric anhydride, 6-heptynoic acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, or EDC); were bought from Aladdin Reagent (Shanghai, China). Tetraethylorthosilicate (TEOS), cystamine dihydrochloride, NH_4HCO_3, trifluoroacetic acid (TFA), DL-Dithiothreitol solution (DTT, 1M), Iodoacetamide (IAA), Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA), sodium dodecyl sulfate (SDS), Triton X100 were bought from Sigma (St. Louis, MO). Tris(hydroxymethyl)aminomethane (Tris) and Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were bought from Bio Basic Inc. Bovine serum albumin (BSA) was bought from Pierece. Sequencing grade trypsin was bought from Promega.

2,5 Dihydroxybenzoic acid (DHB) was from Bruker Daltonics (Bremen, Germany).

HPLC grade acetonitrile (ACN) were from Merck. UDP-N-azidoacetylgalactosamine (UDP-GalNAz) was from by Shanghai Institute of Organic Chemistry,Chinese Academy of Sciences. Muc5AC (sequence: SAPTTSTTSAPTK; with a FAM modification on ν-amine) were purchased from Sawady Co. Ltd. Ultrapure water was from Milli-Q system (Millipore, Billerica, MA).

1.2 Fabrication of disulfide- and terminal alkyne functionalized magnetic silica particles

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(DA-MSP) (Scheme 1)

First, Fe_3O_4 cores were synthesized through a hydrothermo method[1]. Briefly, 3 g $FeCl_3 \cdot 6H_2O$, 4.8 g sodium acetate and 0.72 g sodium citrate were dissolved in 90 mL ethylene glycol by vigorous stirring and then the mixture were heated for 8 h at 200°C. After cooling to room temperature, the newly formed Fe_3O_4 cores were washed three times with ethanol and water, respectively. Then a layer of SiO_2 was deposited on the surface of the magnite core through a Stöber method. Briefly, the MSPs were dispersed in 100 mL H_2O, 300 mL 95% ethanol and 6 mL TEOS and 6 mL 28% $NH_3 \cdot H_2O$ were added. The mixture was stirred 24 h at R.T, then the microspheres were collected with the help of a magnet and washed 3 times with methanol and water, and dried in vacuum.

Then 4 sequential modification reactions were performed on the surface of the MSP:

1) The newly formed MSP were dispersed in 12 mL 16% APTES in toluene. The mixture were treated with ultrasound for 20 min then rocked 24 h at R.T. The microspheres were collected and washed by ethanol and THF. This step introduced amine on the surface, producing amine-MSPs.
2) The magnetic microspheres were dispersed in 20 mL THF, and then 3 mL pyridine 1.5 g glutaric anhydride were added. The mixture were stirred at R.T for 3 h. And the microspheres were collected and washed with methanol for 5 times.This step introduced carboxylic groups on the surface, making carboxyl-MSPs.
3) The magnetic microsphere were dispersed in 10 mL methanol. Then 300 μL TEA, 0.2 g EDC·HCl and 0.22 g cystamine·2HCl were added. The mixture was stirred at R.T for 5 h. The microspheres were collected and washed with methanol for 5 times. This step

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introduced disulfide linker, obtaining disulfide-MSPs.

4) The magnetic microspheres were dispersed in 12 mL methanol. And then 0.22 g EDC·HCl, 75 μL 6-Heptynoic acid and 200μL TEA were added to the mixture. The mixture were stirred at R.T overnight and the magnetic microsphere were washed with methanol 5 times, then dried in vacuum, stored at 4°C for furture use. This step introduced terminal alkyne, resulted in the desired DA-MSPs.

1.3 Characterizations

TEM images were taken with a he sections were observed in a Hitachi H600 TEM (Hitachi High-Technologies).Samples were first dispersed in ethanol and then collected using carbon-film-covered copper grids for analysis. FT-IR spectra were collected on Fourier Transform Infrared Spectrometer (EQUINOX 55, Bruker) spectrophotometer using KBr pellets. Magnetic characterization was carried out on a Physical Property Measurement System (PPMS-9T, Quantum Design). XPS experiments were carried out on a RBD upgraded PHI-5000C ESCA system (Perkin Elmer) with Mg Kα radiation (hν=1253.6 eV) or Al Kα radiation (hν=1486.6 eV).

1.4 Synthesis of a O-GalNAz Peptide as a standard azido glycopeptide

25 ng ppGalNAc -T2 (expressed and purified in house), 0.25 μL 10 mM UDP-GalNAz and 0.25μL 1 mM Muc5AC (sequence: SAPTTSTTSAPTK; with a FAM modification on ν-amine) were added to 10 μL 25 mM Tris-HCl (pH 7.4)，5 mM $MnCl_2$, 0.2% Triton X-100. Then the reaction was allowed to happen at 37 °C for 12 h. The reaction was terminated by heating at 95°C for 3 min. Then the mixture was separated by RP-HPLC (Shimadzu) on a

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C18 analytical column (COSMOSIL 5C18-AR-II, 4.6×250 mm). Fractions corresponding to products were collected and characterized with MALDI-TOF mass spectrometer (Bruker Autoflex) to verify the success of reaction and separation.

1.5 Protein Digestion

100 μg Bovine Serum Albumin (BSA) was disolved in 100 μL 25 mM NH_4HCO_3 and heated for 10 min at 100°C. Then 1 μL 1 M DTT was added, the mixture was heated at 37 °C for 1 h, then 6 μL 1 M IAA was added, the mixture was placed in dark for 2 h at R.T. Then 2 μL 1μg/μL trypsin was added. The mixture was heated at 37 °C overnight.

1.6 Enrichment of azido glycopeptides

In a typical procedure, 5 pmol standard peptide was incubated with about 0.5 mg magnetic microsphere and diluted by 200 μL 1% Triton, 10 mM PBS,7.8. Then 1 μL catalytic solution of 200 mM $CuSO_4$,20 mM TBTA, 400 mM sodium ascorbate (freshly prepared and mixed) was added to the mixture. The mixture was votexed at 1400 rpm for 30 min at R.T. Then the magnetic microspheres were washed twice with 200 μL 0.25 M NaCl, 0.5%SDS, 50%ACN, 25 mM Tris pH7.4; and 3 times with 200 μL 50% ACN. In the end, the microspheres were dispersed in 200 μL 20 mM DTT, 10 mM PBS, pH 7.5, 50% ACN and vortexed at 1400 rpm for 90 min at 37 °C to release the captured GalNAz peptide.

For mass spectrometry analysis, the captured peptides were released by 40 μL 5 mM TCEP, 10 mM PBS,pH7.5 at R.T for 45 min, and then 5 μL 2.5 mM TCEP, 50% ACN,10 mM PBS,7.5 at R.T for 15 min. The two fractions were combined and 2.5 μL 1 M IAA was added and the mixture was incubated at R.T for 30 min in darkness. The peptide solution was then

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desalted by C18 ziptips (Millipore), and eluted with 1.5 μL 50% ACN, 0.1%TFA.

1.7 MALDI-TOF MS Analysis

All the MALDI-TOF MS analysis was performed on a 5800 MALDI-TOFTOF (Applied Biosystems) in a reflector mode. Sample was prepared in a dried drop method. 1 μL sample was spotted on the MALDI well and after drying at R.T, 0.5μL 12 mg/mL DHB in 20%ACN, 0.1%TFA was spotted and dried at room temperature.

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2. Supporting Figures

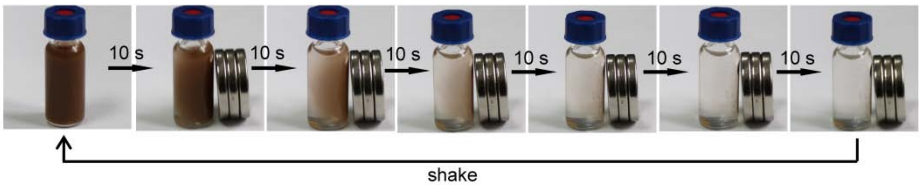

Figure S1. Dispersing and collection of the MSP microspheres in water with the help of a common magnet.

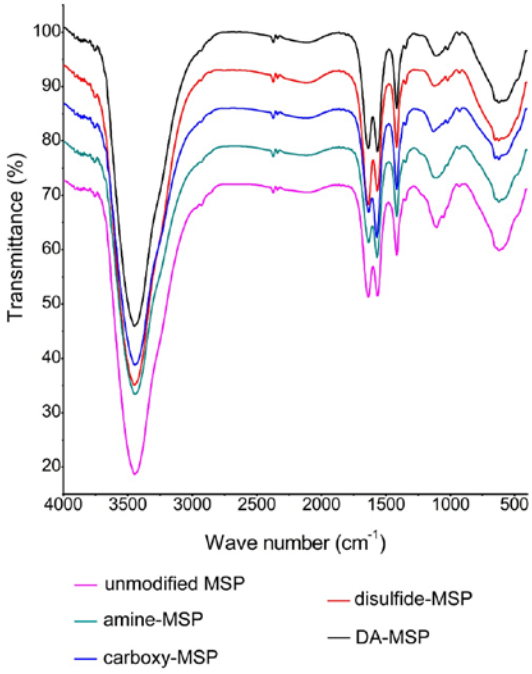

Figure S2. FT-IR analysis of the magnetic silica particles: unmodified MSP (pink); amine-MSP (dark cyan); carboxyl-MSP (blue); disulfide-MSP (red); and DA-MSP (black).

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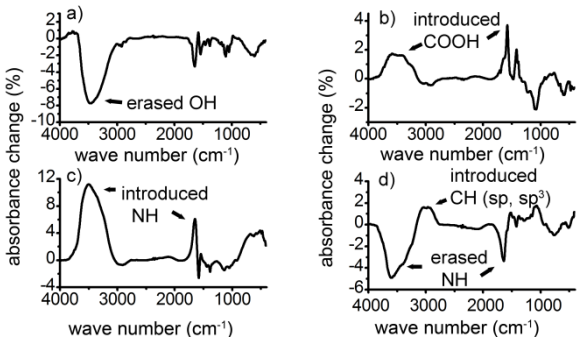

Fig. S3 Differential FT-IR spectra of the microspheres caused by the modification reactions. a) the absorbance changes of the microspheres caused by APTES-mediated silylation (producing amine-Fe_3O_4), in which the decrease at ~3500 cm^{-1} can be assigned to the reduction in the number of -OH groups; b) the absorbance changes caused by glutaric anhydride treatment (making carboxyl-MSP), as indicated by increases at ~1580 cm^{-1} and ~3500 cm^{-1}, which can both be assigned to the introduced carboxylic groups; c) the absorbance changes caused by linkage of cystamine (obtaining disulfide-MSP), as indicated by increases at ~1640 cm-1 and ~3500 cm^{-1}, which can be assigned to the introduced N-H group. d) the absorbance changes caused by linkage of 6-heptynoic acid (resulted in the desired DA-MSP), as indicated by increases at ~3030cm^{-1} and ~2960 cm^{-1}, which can be assigned to the introduced C-H bond (sp and sp3, respectively), while the decreases at ~3600 cm^{-1} and ~1640 cm^{-1} were assigned to the reduction in the number of NH groups.

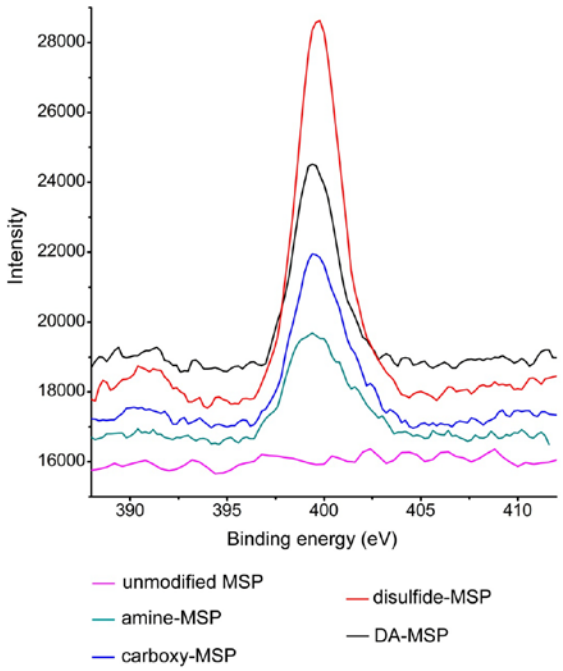

Figure. S4. N1s XPS spectra of the magnetic silica particles: unmodified MSP (pink); amine-MSP (dark cyan); carboxyl-MSP (blue); disulfide-MSP (red); and DA-MSP (black).

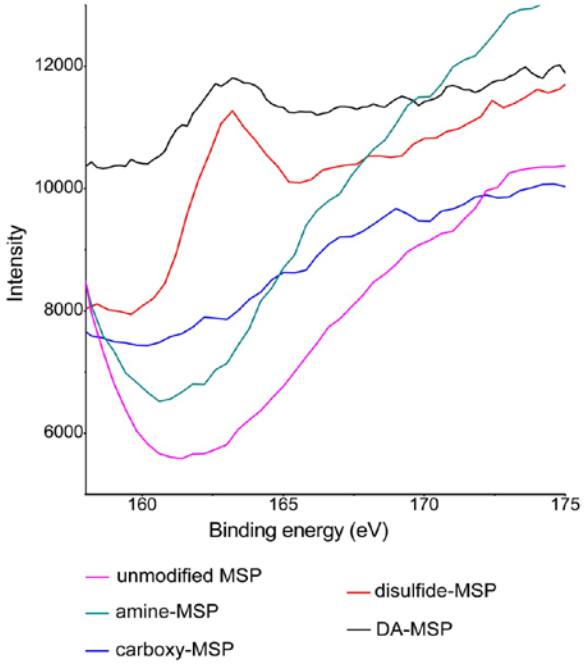

Figure. S5. S2p XPS spectra of the magnetic silica particles: unmodified MSP (pink); amine-MSP (dark cyan); carboxyl-MSP (blue); disulfide-MSP (red); and DA-MSP (black).

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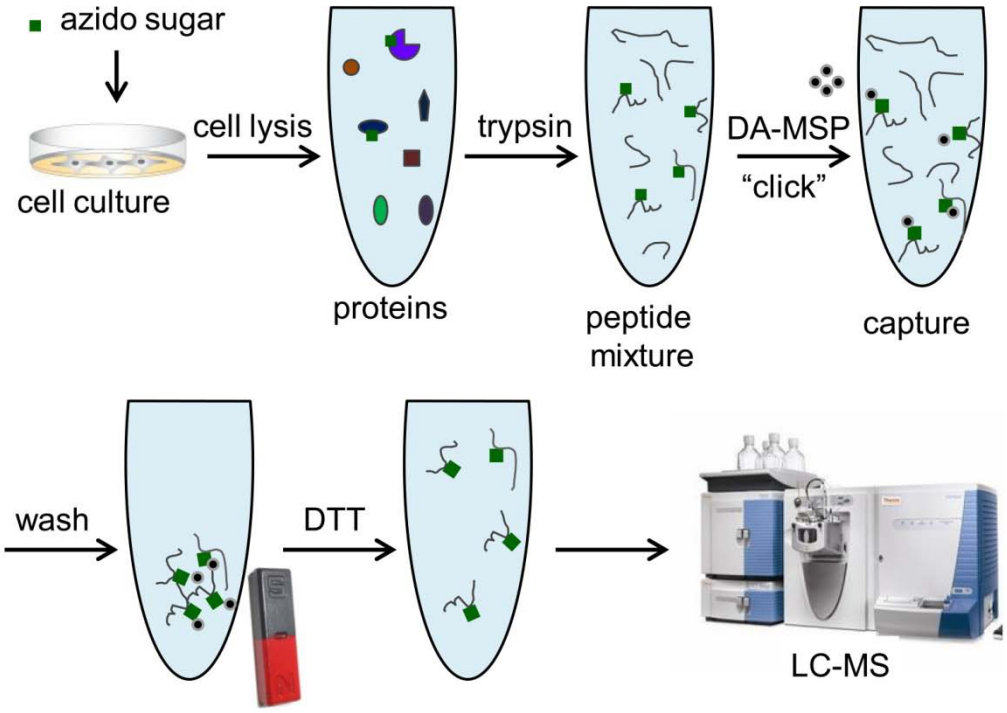

Scheme S1. Glycoproteomic analysis of azido sugar treated cells