Electronic Supplementary Material (ESI) for Analytical Methods

<Supporting information>

Multiplexed Quantification of Surface-Bound Proteins on Gold Nanoparticles

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1. Materials and Methods

1) Materials

AuNPs (40 nm in diameter) were prepared by using the previously explicated method.[1] Sodium borohydride, sodium citrate, cobalt chloride, ammonium acetate, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), ammonium bicarbonate, calcium chloride, dimethyl sulfoxide (DMSO), and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen tetrachloroaurate(III) hydrate ($HAuCl_4$) was purchased from Kojima Chemicals Co., Ltd. (Sayama-shi, Japan). Bovine serum albumin (BSA) was purchased from SERVA (Heidelberg, Germany). Ethanol was purchased from Merck (Darmstadt, Germany). Acetonitrile (ACN) was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). Trypsin was purchased from Promega (Madison, WI, USA). The isotope-labeled internal standards, YEEHLYER* and LGEYGFQNALIV*R were custom-synthesized from AnyGen Co., Ltd. (Gwangju, Korea). α-Cyano-4-hydroxycinnamic acid (CHCA) was purchased from Bruker Daltonics (Leipzig, Germany).

2) Methods

Preparation of carboxylic acid-presenting AuNPs. 1 mL of as-made AuNPs (1.1 nM) was washed with tri-distilled water by centrifugation three times and incubated with a mixed solution of tri(ethylene glycol)-terminated alkanethiol (100 μM in ethanol) and carboxylic acid penta(ethylene glycol)-terminated alkanethiol (100 μM in ethanol) in a ratio of 7 : 3 for 12 hr. The resulting AuNPs were washed with absolute ethanol using centrifugation at 14000 rpm for 3 min three times and stored at 4 ℃.

Immobilization of multi-proteins on AuNPs. 1 mL of acid-presenting AuNPs (1.1 nM in ethanol) was treated with 350 μL of N-hydroxysuccinimide (NHS) (10 mg/mL in DMSO) and 150 μL of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (20 mg/mL in DMSO) for 2 hr, and washed with DMSO and phosphate buffer (PB) by centrifugation at 14000 rpm for 3 min. 1 mL of a mixture of GST and BSA in various ratios of 3 : 7, 5 : 5 and 7 : 3 in various total concentrations ranging from 10 μg/mL to 1000 μg/mL in PB was incubated with the above NHS-activated AuNPs for 1 hr at 4 ℃. The AuNPs were then washed with distilled water by centrifugation at 14000 rpm for 3 min three times and stored at 4 ℃.

On-particle digestion and in-solution digestion. For on-particle digestion, surface bound multi-proteins on AuNPs were treated with trypsin solution (2 ng/μL in 20 mM ammonium bicarbonate, 0.5 mM calcium chloride, and 10% acetonitrile) and incubated for 12 hr at 37 ℃. The isotope-labeled internal standards YEEHLYER* (5 μL of 0.8 μM) and LGEYGFQNALIV*R (5 μL of 2 μM) were added to the tryptic digested mixture and thoroughly mixed by pipetting. The mixture was centrifuged at 14000 rpm for 3 min, and then the supernatant was collected. 1 μL of the supernatant was analyzed by MALDI-TOF MS using CHCA (1 mg/150 μL in 50% acetonitrile, 0.1% TFA in distilled water) as a matrix.

For in-solution digestion, 5 µL of trypsin solution (2 ng/µL in 20 mM ammonium bicarbonate, 0.5 mM calcium chloride, and 10% acetonitrile) was applied to proteins. The mixture was incubated at 37 ℃ for 12 hr, and then 1 µL of mixture was analyzed by MALDI-TOF MS using CHCA (1 mg/150 µL in 50% acetonitrile, 0.1% TFA in distilled water) as a matrix.

MALDI-TOF MS analysis. Mass analysis was performed using an Autoflex III MALDI-TOF mass spectrometer (Bruker Daltonics) equipped with a smartbeam laser as an ionization source. All of the spectra were acquired with a 19 kV accelerating voltage, a 100 Hz repetition rate, and a positive mode with an average of ~700 shots. The identification of the resulting peptides was obtained by Biotools program from Bruker.

Quantification of proteins. After digestion of proteins on gold nanoparticles (1 mL of 1.1 nM, 1.1 pmole, 6.62 x 10^{11} particles), the isotope-labeled internal standards YEEHLYER* (5 µL of 0.8 µM, 4 pmole) and LGEYGFQNALIV*R (5 µL of 2 µM, 10 pmole) were added. The mixture was analyzed by MALDI TOF MS as described above. The comparison of mass intensities between RP and IS afforded total number of proteins on 1.1 pmole of nanoparticles. Division of total number of proteins by 6.62 x 10^{11} gave the number of proteins per particle. The standard deviations were obtained by three independent experiments.

2. Additional data

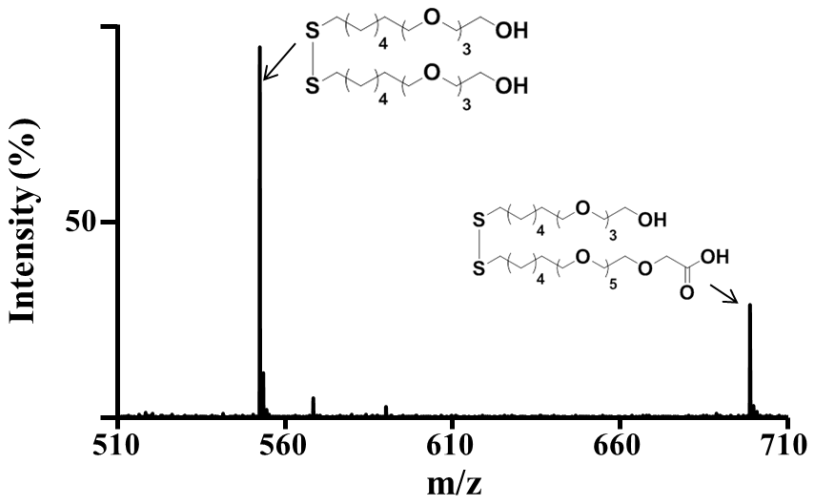

Figure S1. Verification of carboxylic acid presenting-AuNPs by MALDI-TOF MS. MS analysis gave two major peaks at 552.8 $[M + Na]^+$ and m/z 698.8 $[M + Na]^+$ corresponding to the tri(ethylene glycol) containing disulfide and the carboxylic acid containing disulfide, respectively.

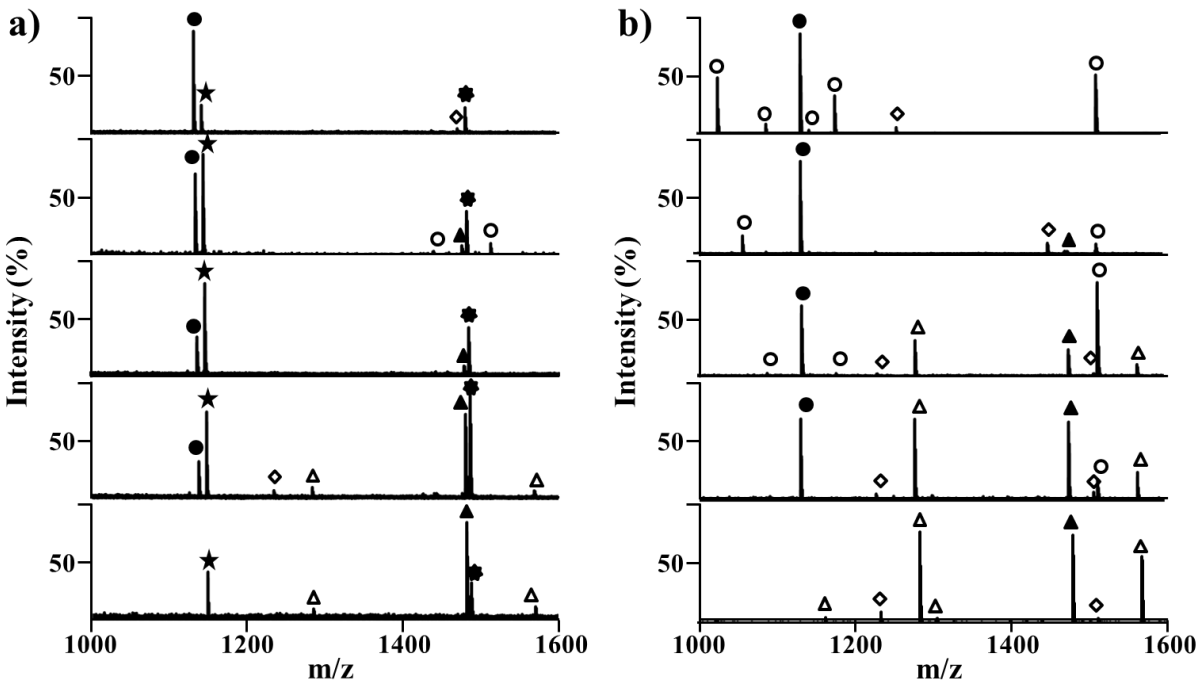

Figure S2. Mass spectra of a) on-particle digestion and b) in-solution digestion of a mixture of GST and BSA in the presence of ISs at various ratios (from top to bottom, GST : BSA = 10 : 0, 7 : 3, 5 : 5, 3 : 7, and 0 : 10). The peaks at m/z 1137.5 (●) and 1478.8 (▲) correspond to the reference peptide for GST and BSA, respectively. The peaks at m/z 1148.2 (★) and 1484.8 (✹) correspond to the ISs for GST and BSA, respectively. (○: peptides from digested GST, △: peptides from digested BSA, ◇: peptides from auto-digested trypsin.)

Table S1. Peptide fragments of on-particle and in-solution digested GST, BSA, and auto-digested trypsin.

Sequence of digested peptide of GST	**On-particle**	**In-solution**
LTQSMAIIR (Mw.1031)		√
MSPILGYWK (Mw.1093)	√	√
YEEHLYER (Mw.1138)	√	√
YGVSRIAYSK(Mw.1142)		√
RIEAIPQIDK (Mw.1181)		√
AEISMLEGAVLDIR (Mw.1515)	√	√
Sequence of digested peptide of BSA	**On-particle**	**In-solution**
HPEYAVSVLLR (Mw.1282)	√	√
HLVDEPQNLIK (Mw.1305)	√	√
RHPEYAVSVLLR (Mw.1438)		√
LGEYGFQNALIVR (Mw.1478)	√	√
DAFLGSFLYEYSR (Mw.1566)	√	√

Trypsin auto-digestion	**On-particle**	**In-solution**
YPSINPRECR (Mw.1233)		√
GISPPTRYPPIGTR (MW. 1510)		√

Reference

1. A. M. Schwartzberg, T. Y. Olson, C. E. Talley and J. Z. Zhang, *J. Phys. Chem. B*, 2006, **110**, 19935-19944