

Electronic Supplementary Information

1. Experimental section:

1.1 Synthesis of gold nanoparticles

Gold nanoparticles were prepared under the protection of trisodium citrate according to the previous literature¹. First of all, 180 mL of 1 mM HAuCl₄ solution was added into a three-neck round-bottom flask with a condenser connected to one neck and brought to a vigorous boil under magnetic stirring. Then, 18 mL of 38.8 mM trisodium citrate was added quickly to the flask. After that, the solution was refluxed for another 15 min. After cooling to room temperature, the solution was filtered through 0.22 μm microporous membrane. The final gold colloid has a λ_{max} of 518 nm and the color is deep red. TEM was applied to study the size dispersion of the gold nanoparticles.

1.2 Preparation of gold layer on MALDI plate

The stainless-steel MALDI plate was washed under ultrasonic bath with ethanol, 0.1% formic acid and water sequentially, and dried at room temperature. According to Beer's Law and a molar absorptivity at 519 nm of 2.7×10^8 liter mol⁻¹·cm⁻¹, the concentration of gold colloid solution can be adjusted and in our experiment it was concentrated through centrifugation to be at 92 nM¹. Gold colloid solution was spotted on clean MALDI plate in an array format (2 μL for each spot) and dried naturally at room temperature. Fast drying is not recommended because the gold layer will be less uniform. The resulting gold-modified plate was subsequently heated at 200 °C for 2 hours and naturally cooled down for further modification.

1.3 Preparation of IBA-modified gold layer on MALDI plate

10 μM of SH-IBA (sequence: 5'-SH-(CH₂)₆-(ACAG₄TGTG₄)₂-3')² was treated with 2.5 mM TCEP at pH 5.0 in dark at room temperature for one hour. Then the as prepared IBA solution was spotted on the gold-modified MALDI target (2 μL for each spot) and reacted in dark overnight in a humid chamber. After reaction, the plate was washed with flowing water and dried at room temperature.

1.4 Insulin capture and MALDI-TOF analysis

Insulin was stored at -80°C in small aliquots. Insulin is easy to degrade under room temperature so in our experiment it was always freshly thawed before use. 2 μL of human insulin dissolved in buffer A (0.01M PBS buffer containing 1mg/mL HSA, pH 7.4) was dropped on IBA-modified gold layer on MALDI plate and incubated for an hour in humid chamber at room temperature. Then the MALDI plate was rinsed with buffer B (0.1% Tween 20 in water) and water using washing bottle for 1 min sequentially. 0.05 ng of porcine insulin was applied as the internal standard if required. After that, plate was dried in air and 2 μL of CHCA (4 mg/mL, 50% ACN, 0.1% TFA) was applied as the matrix for MALDI-TOF mass analysis.

For real sample analysis, human serum was thawed from -80°C and centrifuged at 4°C for 20 min to remove any insoluble component. Before analysis, serum samples were diluted 10 fold with PBS buffer (pH 7.4). Insulin was added to the diluted serum with a final concentration in the range of 5,000-20 ng/mL. Then the same experimental procedures as to those in standard solutions were adopted for insulin retrieval. The total protein concentration for 10-fold diluted serum is about 8 mg/mL as determined

by Bradford method.

MALDI-TOF mass spectrometry spectra were acquired on AB SCIEX TOF/TOF 5800 (Applied Biosystems) on linear positive ion mode. Sample was excited with Nd:YAG laser at 355 nm at a repetition rate of 400 Hz and acceleration voltage of 20 kV. Laser intensity was set at 5200, vertical scale was 0.1, input bandwidth was 20MHz and bin size was 0.5ns.

2. Results and Discussion

2.1 The amino acid sequences of human insulin and porcine insulin

| | Amino acid sequence |
|-------------------------|--|
| Human insulin A chain | GIVEQC CTSICS LYQLEN YCN |
| Porcine insulin A chain | GIVEQC CTSICS LYQLEN YCN |
| Human insulin B chain | FVNQHL CGSHLV EALYLV CGERGF FYTPK T |
| Porcine insulin B chain | FVNQHL CGSHLV EALYLV CGERGF FYTPK A |

2.2 TEM image of the as-synthesized gold nanoparticles

TEM was applied to investigate the size distribution of the gold nanoparticles synthesized as described in section 1.1. As shown in Fig.S1, gold nanoparticles is spherical, well dispersed, and have a uniform size distribution.

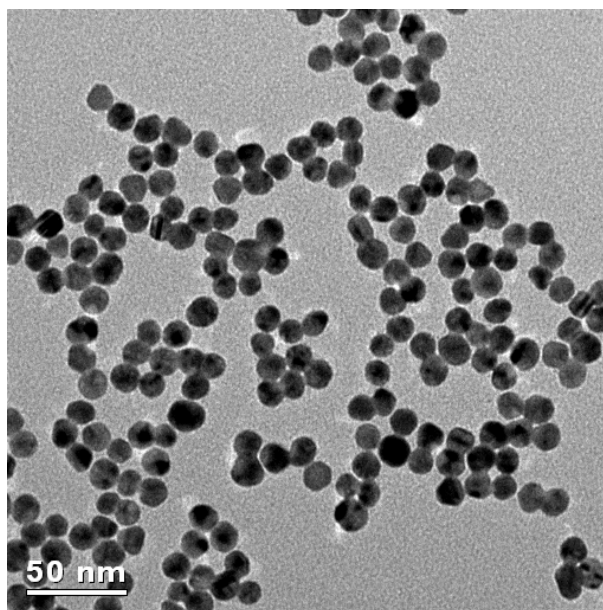
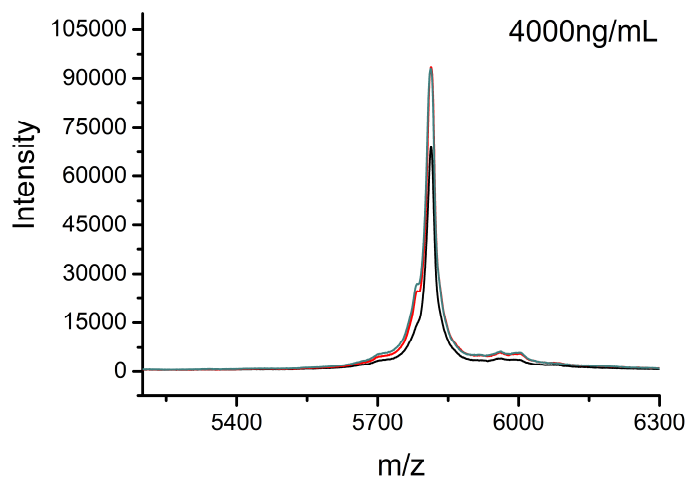
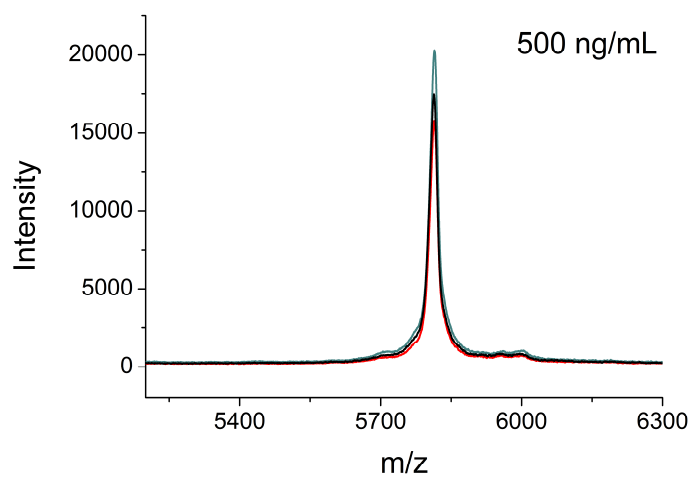
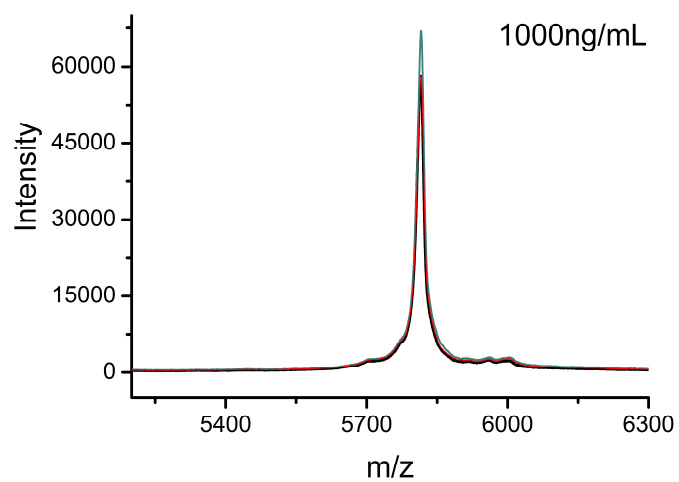
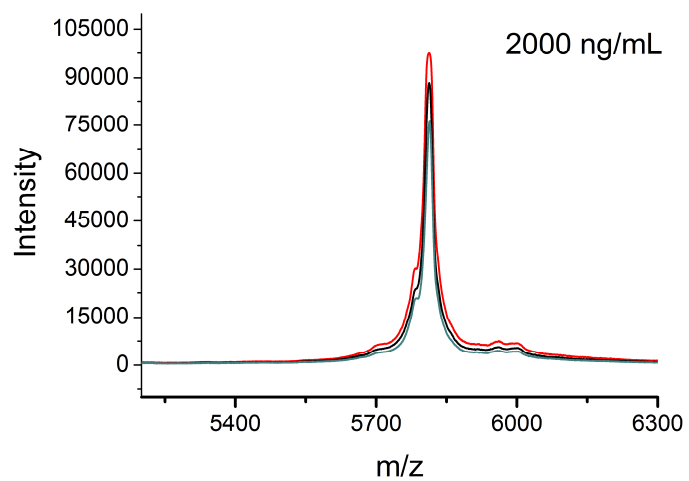


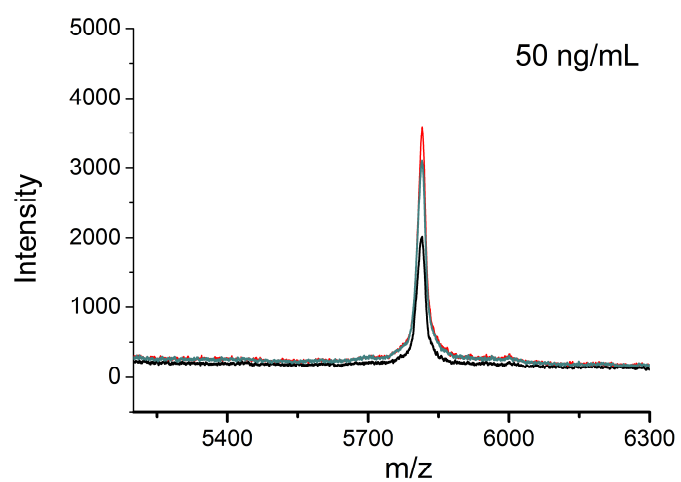
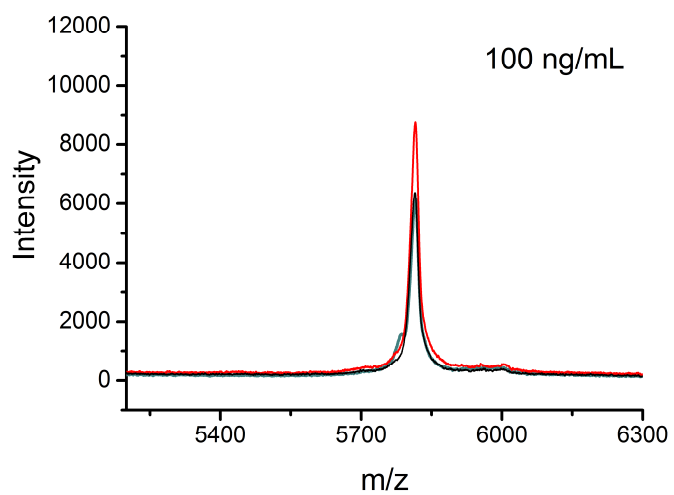
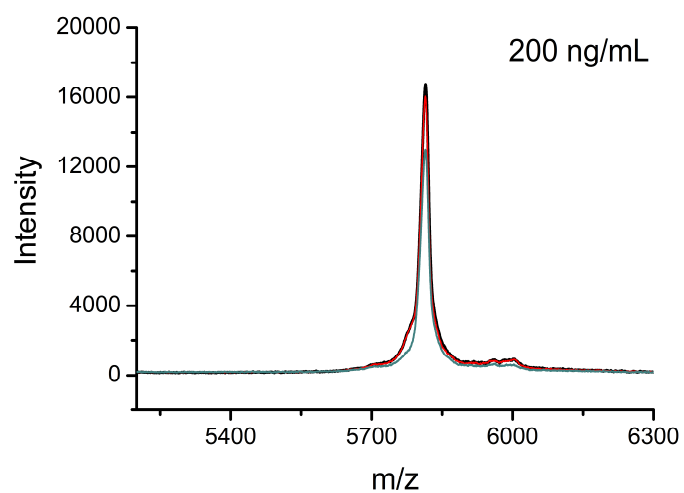
Fig.S1 TEM image of the gold nanoparticles

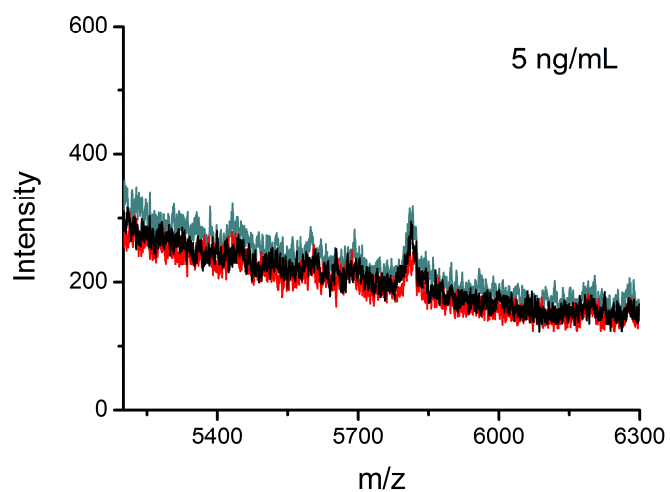
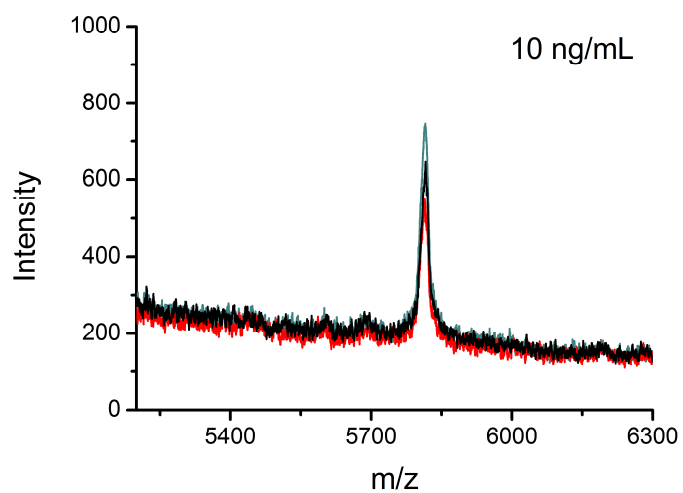
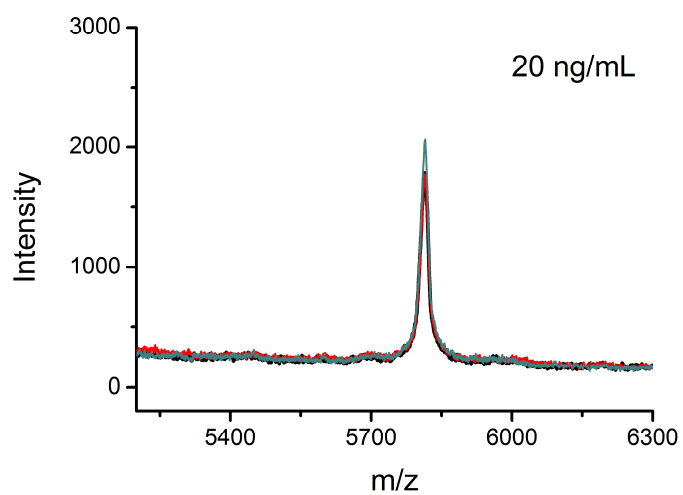
2.3 MALDI-MS spectra generated for calibration curve in standard solutions

All experiments were repeated for three times with the same dilution series of sample.





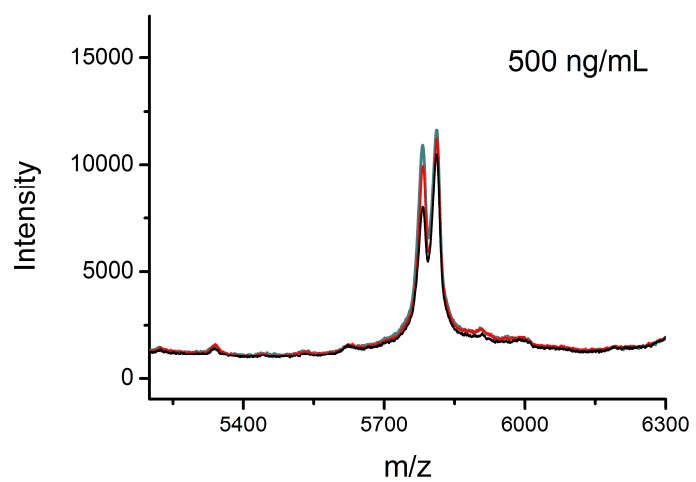
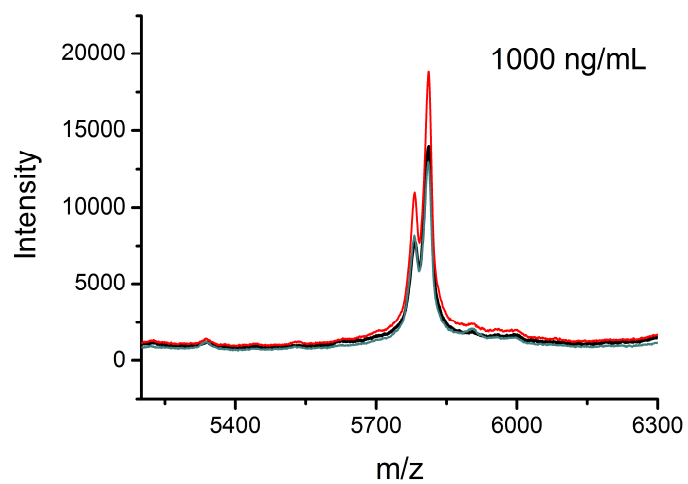
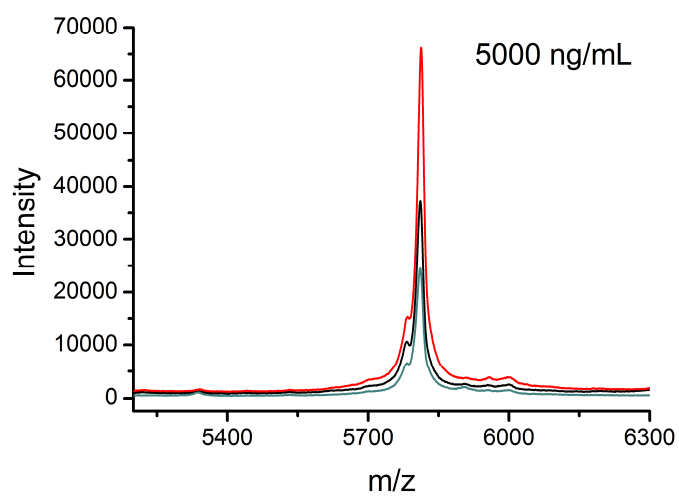


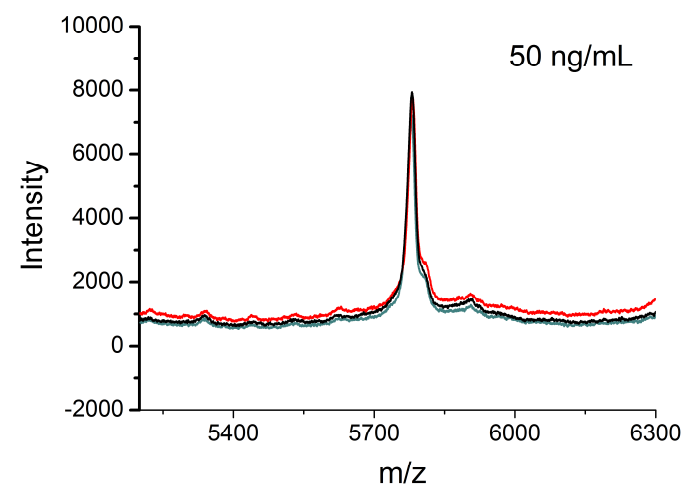
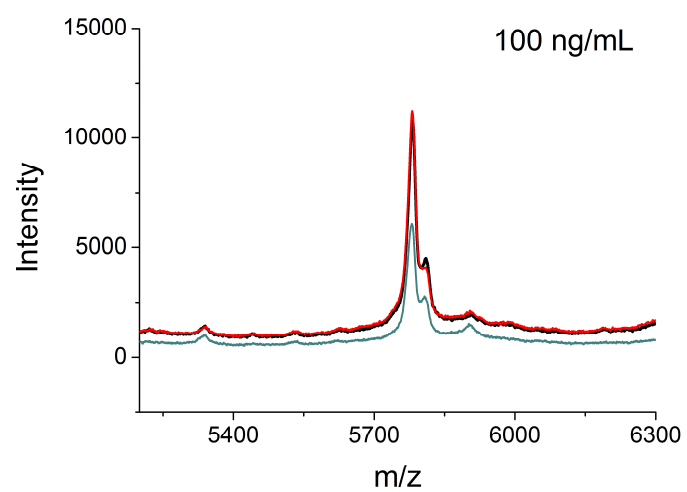
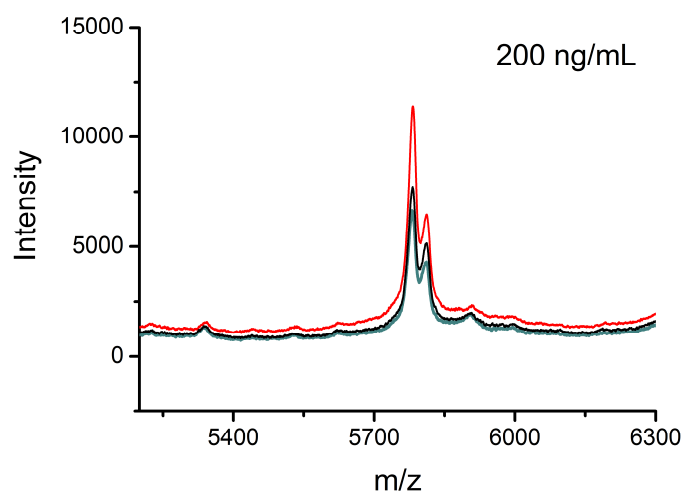


2.4 MALDI-MS spectra generated for calibration curve in serum sample

All experiments were repeated for three times with the same dilution series of

sample.





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References:

1. J. Liu and Y. Lu, *Nat. Protoc.*, 2006, **1**, 246-252.
2. A. C. Connor, K. A. Frederick, E. J. Morgan and L. B. McGown, *J. Am. Chem. Soc.*, 2006, **128**, 4986-4991.