Electronic Supplementary Information (ESI)

Digitized single scattering nanoparticles for probing molecular binding

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1. Experimental section

1.1 Apparatus

The absorption of AuNPs colloid solution was measured with UV-3600 UV-Vis-NIR Spectrophotometer (Shimazdu, Japan). The size and shape of AuNPs were observed through S-4800 scanning electron microscope (Hitachi, Japan). Dark-field light scattering images were obtained through BX51 optical microscope (Olympus, Japan) equipped with dark-field condenser (U-DCW, 1.2-1.4) and DP72 single chip true-color CCD camera (Olympus, Japan), which was controlled by IPE software (MediaCybernetics, USA). The scattering lights from AuNPs were collected by a 100× object lens (adjustable numerical aperture from 0.6 to 1.3) and photographed by a 2070 × 1548 pixel true-color digital CCD camera. The acquired images were all 24-bit TIFF/BMP picture files.

1.2 Reagents

Chloroauric acid tetrahydrate (HAuCl₄•4H₂O) was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). 3-Mercaptotrimethoxysilane (MPTMS), Cysteamine (Cys), 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide (EDC), N-hydroxysuccinimide (NHS), biotin, and avidin were purchased from Sigma-Aldrich.
(Missouri, USA). Citrate trisodium, Ethanol, 1-Butanol, 1-Octanol and DMSO are analytical grade for use without further purification. PBS buffer (pH, 7.4) was used for molecular binding reaction.

1.3 Synthesis of gold nanoparticles

Gold nanoparticles were synthesized according to a traditional method by reducing HAUCl₄ with sodium citrate¹. To a clean cone-shaped bottle previously washed with freshly prepared aqua regia, 50 mL of HAUCl₄ solution (0.25 m mol/L) was added and brought to boiling. Then, 0.4 mL of citrate trisodium (50.0 m mol/L) was added under vigorous stirring. The mixture was kept boiling and refluxed for 20 minutes, and then cool down to room temperature under continuous stirring.

1.4 Dark-field light scattering imaging of AuNPs bathed in different solvents

To observe the scattering features of AuNPs bathed in different solvents, we employed a homemade device, which is consisted of slide glass and cover glass to produce a simulative flow cell. Dark-field light scattering images of AuNPs were taken by depositing AuNPs to the bottom of the cell in water, ethanol, 1-butanol, ethylene glycol (EG), and DMSO, respectively. Their refractive indexes (RIs) are 1.333, 1.362, 1.3993, 1.4318 and 1.479, respectively. To calculate the RI sensitivities, the linearity between the scattering intensity ratio (I/I₀) and the solvent RI (n) was fitted. The slopes of the linear lines represent the RI sensitivities.

1.5 Preparation of biotinylated AuNPs

According to previous reports²⁻⁴, the preparation of biotinylated AuNPs was carried out as following procedures: 1) glass slides were cleaned with freshly prepared aqua regia for 60 min; 2) the cleaned glass slides were modified with MPTMS by incubating with isopropyl alcohol solution containing 2.0 m mol/L MPTMS for 12 h to present thiols (–SH) on glass surface; 3) AuNPs were immobilized on the glass slide surface through Au–S bonds by dipping thiolated glass slide into gold colloid solution for 12 h; 4) to make gold
nanoparticles with –NH₂ groups that can be conjugated with biotin via EDC/NHS, glass slides were inserted into ethanol solution containing 5.0 mmol/L cysteamine for 12 h; 5) the immobilized AuNPs was incubated in ethanol solution containing 2.0 mmol/L biotin, 10 mmol/L EDC and 5.0 mmol/L NHS for about 24 h to make AuNPs surface with biotin.

1.6 Recording avidin-biotin binding

Biotinylated AuNPs attached glass slide was dried with N₂ and conglutinated with two cover glasses to produce a cell. Then, the biotinylated AuNPs were equilibrated with PBS buffer solution for 10 min. After that, PBS buffer containing different concentrations of avidin was added and incubated with biotinylated AuNPs at room temperature (25 °C). To record the molecular binding events, dark-field light scattering images were taken with designed time as indicated in the text.

1.7 Data analysis

The calculation of scattering intensity of single plasmonic nanoparticle was carried out through Image-Pro Plus (IPP) software. In the dark-field light scattering images, one light spot represents a single nanoparticle. The scattering intensity of an AuNP was calculated by summing all pixels of a scattering light spot, and the intensity of one pixel is the average color value of RGB channels. All RGB channels have a certain integer color values ranging from 0 to 255 for 24-bit true-color in computing.

References

2. Additional Figures

Fig. S1. Characterization of the prepared AuNPs. (A) Absorption spectrum and (B) scanning electron microscopy image of AuNPs. The prepared AuNPs had characterized extinction band at 532 nm and an average diameter of about 54 nm.

Fig. S2. Plot depicting the linear relationship between the scattering intensity ratio \( I/I_0 \) and the solvent RI \( n \) of the same nanoparticles in Fig. 2B with the exposure time of (A) 200 ms and (B) 300 ms. \( I \), the scattering intensity of single AuNPs bathed in different solvents; \( I_0 \), the scattering intensity of AuNPs bathed in water. The error bars are calculated from the measurements of 11 particles.
**Fig. S3.** Plot depicting the linear relationship between the scattering intensity ratio ($I/I_0$) and the solvent RI ($n$) of the five nanoparticles in Fig. 2A. The corresponding linear equation of each particle is listed right.

![Graph showing the linear relationship between scattering intensity ratio and solvent RI](image)

**Fig. S4.** (A) Dark-field light scattering images of AuNPs bathed in water, ethanol, 1-butanol, ethylene glycol (EG) and DMSO (from left to right). (B) the scattering intensity change of red nanoparticles with RI. The scattering light of the red nanoparticle disappeared gradually as the RI increased, indicating the decrease of the scattering light intensity. This result was further validated by the calculation of the intensity.

![Scattering images and bar graph](image)
**Fig. S5.** Real time measurements of the RI changes ($\Delta n$) of local medium of single biotinylated AuNPs before and after exposure to different avidin concentrations.

**Fig. S6.** Control experiments. Time-dependent scattering intensity ratio ($I/I_0$) of non-biotinylated AuNPs incubation with avidin (0.1 $\mu$mol/L), biotinylated AuNPs incubation with biotin-saturated avidin (0.1 $\mu$mol/L), and biotinylated AuNPs interacting with bovine serum albumin (BSA) (0.1 $\mu$mol/L). $I$, the scattering light intensity of single AuNPs at different time; $I_0$, the scattering intensity of AuNPs bathed in PBS buffer.