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Electronic Supporting information (ESI)

Refractive index dependent real-time plasmonic nanoprobes on single silver nanocube for ultrasensitive detection of the lung cancer-associated miRNAs

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Table S1. Sequences of the nucleic acids

Name	Sequences (5-3)*
HS-ssDNA	TCAACATCAGTCTGATAAGCTATTTTTTTTTT-SH
miR-21	UAGCUUAUCAGACUGAUG UUGA
Single-base mismatch miRNA	UAGCUUAUCAGAC<u>C</u>GAUGUUGA
Random-miRNA	CUGACCUAUGAAUUGACAGCC

*The mutation base is indicated in underlined portion.

Experimental Section

Materials

Ag_2O (99.9%), NH_3•H_2O (28%), D-glucose (99%), and n-Hexadecyl-trimethylammonium bromide (HTAB, 98%) were purchased from Sigma-Aldrich, and used as received. HPLC-purified DNA and miRNAs were purchased from Takara Biotechnology Co. Ltd. (Dalian, China). The sequences of these DNA and miRNAs are listed in Table S1. The buffers were prepared using ultra-pure water (deionized with resistance >18 MΩ•cm).

Associated Content

Preparation of $[Ag(NH_3)_2]OH$ aqueous solution

Analytical grade Ag_2O (0.0116 g, 0.005 M) was dissolved in ca. 10mL of aqueous ammonia (0.02 M) in a dropwise manner under ultrasound (100 Hz, 1 min) until a clear colorless solution was obtained in a beaker.

Synthesis of 55 nm Silver Nanocubes

Silver Nanocubes (AgNCs) with an average diameter of 55 nm were prepared by 3 mL of freshly prepared $[Ag(NH_3)_2]OH$ (10 mM) solution was added into a Teflon-lined stainless steel autoclave of 22 mL capacity, and then 2 mL of ultra-pure water, 10 mL of glucose (7.5 mM), and 3 mL of HTAB (50 mM) solutions were sequentially added quickly to the solution under vigorous stirring. The autoclave was sealed and heated at 130 °C in a furnace for 12 h.[1] It was then cooled to room temperature, filtered and centrifuged at 4000 rpm for 10 min to obtain a precipitate which was collected and re-dispersed in ultra-pure water (2 ml) for characterization.

Characterization

The resulting solution of colloidal particles was filtered and characterized by an absorption maximum at 439 nm using a Shimadzu UV-vis-near-infrared spectrometer. The absorption spectra was obtained at room temperature. The samples for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were prepared by drying a small drop of the aqueous suspension of particles on ITO glass substrates or copper grids under ambient conditions and dried at room temperature. SEM images were taken using a Phillips microscope operated at an acceleration voltage of 5 kV. TEM images were taken using a HT770-SS electron microscope operated at an acceleration voltage of 200 kV.

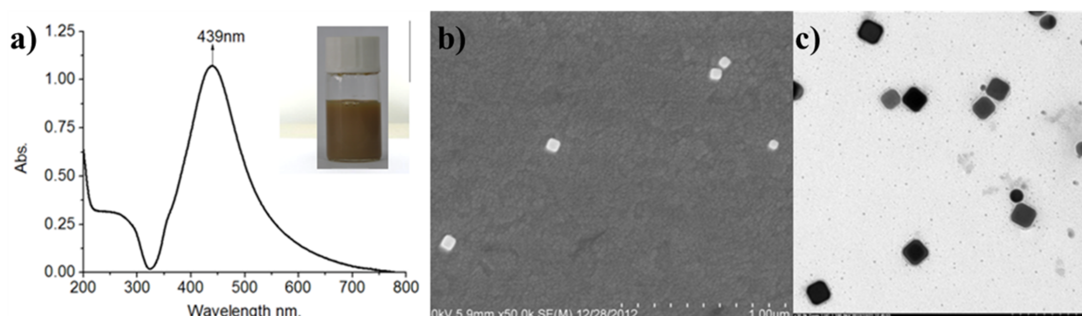

Figure S1. a) UV-Vis spectra of 55 nm AgNCs .Inset is the photograph of the suspension; b) SEM image of 55 nm AgNCs; c) TEM image of 55 nm AgNCs.

Modification procedure

The DNA plasmonic nano-probes on single AgNC were fabricated through the following steps: 1) synthesis of uniform AgNCs with an edge length of ~55 nm, which was characterized by UV-Vis spectra, SEM, and TEM (Fig. S1); then immobilization of AgNCs on a cleaned ITO (indium tin oxide) glass slide (Fig 1a-I); 2) assembly of ssDNA on the AgNCs surface (Fig 1a-II).

Substrate preparation

ITO glasses were cleaned in soapy water, ethanol, actone, ultra-pure water with resistance >18 MΩ•cm, respectively. They are dried in a weak stream of nitrogen. Then ITO glasses were pretreated with a UV/Ozone ProCleaner (Bioforce Nanosciences) for 30 min and washed them with ultra-pure water (deionized with resistance >18 MΩ•cm). After that, the cleaned ITO glasses were immersed in a dilution 100 times of aqueous AgNCs (55 nm) solution for 1 min.

Immobilization of ssDNA on the AgNC surface

Thiolated single-stranded DNA (ssDNA) molecules (concentration, 1 pM) were immobilized on the freshly prepared AgNCs surface with high-salt buffer (1 M NaCl, 50 mM phosphate buffer, pH 7.2). Immobilization was carried out at room temperature (25 °C) under constant mixing in a shaker for 12 h. Then the AgNCs modified ITO glass were rinsed with ultra-pure water and subsequent incubation with mercaptohexanol (1 mM in ultra-pure water) for 2 h. The ssDNA molecules were immobilized on the AgNCs surface.[2] ITO glass

platform, covered the surface by sample solution (about 100 ul), and then the scattering spectra of a typical single AgNC@DNA was recorded.

DNA–RNA hybridization

Before hybridization, immobilized ssDNA ITO glasses were thoroughly washed with ultra-pure water and dried in a weak stream of nitrogen. ssDNA–miRNA hybridization reactions were done at room temperature (25 °C) for 2 h in hybridization buffer (3×3 SSPE with Tween 20, pH 7.4;). Then, ITO glasses were washed with DNase- and RNase-free water and gently dried in a weak stream of nitrogen.

DNA–RNA melting process

After addition to hot ultra-pure water (90 °C, 200 ul) to the suface of immobilized AgNC@DNA-miRNA ITO glasses with 5min, the ITO glasses were washed with ultra-pure water and dried in a weak stream of nitrogen.

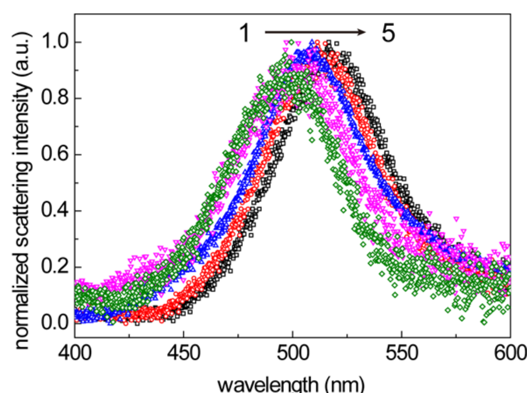

Figure S2. Representative scattering spectra of AgNC@ssDNA probe at different times upon treating with miR-21(1 PM), showing that the λ_{max} of the LSPR spectra is red-shifted, spectra 1–5: 0, 30, 60, 90, 120 min. (The scattering intensities of the all the spectra have been normalized.)

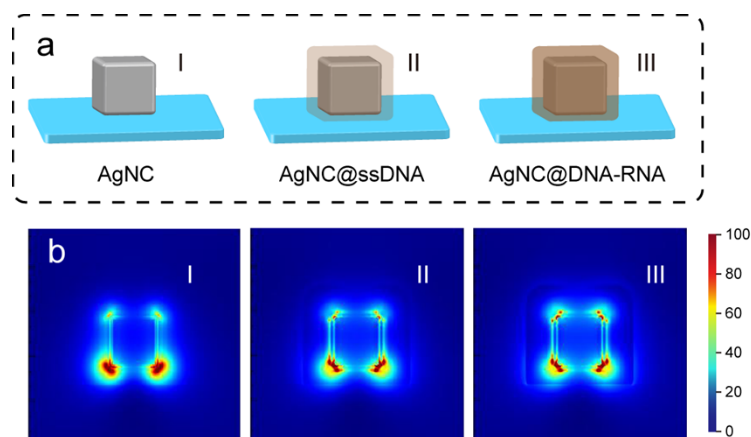

Figure S3. a) 3D model for FDTD simulation (57 nm AgNC with 10 nm nucleic acids molecules shell).

b) The calculated electric field distributions ($|E|^2/|E_0|^2$) for AgNC (b-I), AgNC@ssDNA (b-II) and AgNC@DNA-miR-21 (b-III).

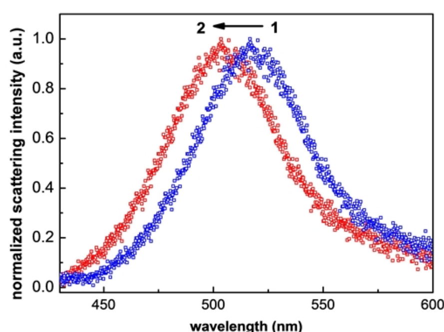

Figure S4. Typical scattering spectra of AgNC@DNA-miRNA before (1) and after (2) removal of miR-21 from the ssDNA-AgNCs surface by adding 90 ^{o}C water, showing that the λ_{max} of the LSPR spectra is blue-shifted. (The scattering intensities of the all the spectra have been normalized.)

Single nanoparticle DFM imaging and scattering spectroscopy

The dark-field spectrum measurements were carried out on an inverted microscope (eclipse Ti-U, Nikon, Japan) equipped with a dark field condenser (0.8 < NA < 0.95) and a 60× objective lens (NA = 0.8)，a 100 W halogen lamp, a true-color digital camera (Nikon DS4fi), and a monochromator (Acton SP2358) equipped with a spectrograph CCD (PIXIS 400BR:excelon, Princeton Instruments) and a grating (grating density: 300 L/mm; blazed wavelength: 500 nm). The true-color scattering images of AgNCs were taken using a 60× objective lens (NA = 0.8). The AgNC-functionalized slides were immobilized on a platform. At first, the scattered light by single AgNC modified with ssDNA as the original AgNC peak (λmax) in the scattering spectrum. Then, occuring hybridization between ssDNA and miRNA on AgNCs surface after treated with miR-21, the scattering peak red-shifted to longer wavelength continuously. The color change in DFM and time-dependent PRRS spectroscopy of the individual particle were obtained to detect the marker of early lung cancer. The scattering spectra from the single nanoparticle were corrected by subtracting the background spectra taken from the adjacent regions without the ssDNA modified AgNCs and dividing with the calibrated response curve of the entire optical system. The spectra were integrated as 20 second.

FDTD simulations

FDTD simulations were performed using a commercial simulation program (FDTD solutions 8.6, Lumerical Solutions, Inc., Vancouver, Canada). In all calculations, a total-field scattered-field plane wave source with the wavelength ranging from 300 nm to 1000 nm is selected to estimate the interaction between propagating plane wave and nanocube. To get accurate results, an override mesh region with mesh size of 0.5 nm is used for the nanocube. According to experiments, the AgNC is placed onto a glass substrate with the surrounding environment to be solution with a RI of 1.33. The dielectric functions of silver and glass substrate are both obtained from Palik.[3] According to the in-situ SEM images (Fig. 1c), edge length of the nanocube is set to be 57 nm. In addition, all corners and edges of the cube are rounded with a radius of 14 nm. To simulate the influence of ssDNA attachment on the LSPR of AgNC, a dielectric layer covering the nanocube with thickness of 10 nm is used.[4] The thickness of the dielectric layer is chosen based on the ssDNA strands used in experiment. Since subsequent attachment of miRNA has little influence on the layer thickness, additional miRNA attachment is simulated by increasing the RI of the dielectric layer with fixed thickness of 10 nm. By setting the RI of dielectric layer to be 1.57 and 1.72, respectively, the calculated

scattering spectra of AgNC@ssDNA and AgNC@DNA-miRNA were in extraordinary consistence with the scattering spectra obtained in experiment after ssDNA and miRNA attachment (Fig. 1f). The values of 1.57 and 1.72 are reasonable since they both lie in the range from 1.33 to 1.75, where 1.75 is the consensus DNA refractive index.[5]

Reference

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