

Supporting Information

Plasmonic Nucleotide Hybridization Chip for Attomolar Detection: Localized Gold and Tagged Core/Shell Nanomaterials

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EXPERIMENTAL SECTION

Reagent and Materials. Thiol-modified custom-designed oligonucleotides (see the sequences presented in Table 1), gold(III)chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), sodium borohydride, sodium citrate, and 1,4-benzenedithiol were purchased from Sigma-Aldrich (Woodlands, TX, USA). The immobilized AuNPs (50 nm diameter) on SPR chips were purchased from BBI Solutions (Cardiff, UK). SpotReady-16 gold spotted glass microarray chips (spot size 1 mm diameter, SPR-1000-016) were purchased from GWC Technologies (Madison, WI, USA). All solutions were prepared in ultrapure distilled water (DNase and RNase free) that was purchased from Life Technologies (Carlsbad, CA, USA). Citrate-stabilized magnetic nanoparticles (Fe_3O_4 NPs, 50 nm hydrodynamic diameter) were purchased from Chemicell GmbH Inc. (Berlin, Germany). PBS buffer, pH= 7.4, was prepared using ultrapure distilled water (DNase and RNase free), and used for preparing all solutions as well as run the SPRi experiments.

Instrumentation. All experiments were performed using a surface plasmon resonance imager (GWC, SPRImager-II, Horizon SPR imager model, Madison, WI, USA) operating at a SPR source wavelength of 800 nm at room temperature. A dual channel set-up connected to two syringe pumps (flow rate $50 \mu\text{L min}^{-1}$ and $100 \mu\text{L}$ sample loop, New Era Pump System, Inc., Farmingdale, NY, USA) was used to evaluate the hybridization of the specific capture and detection nucleotides with miRNA-155 versus control (no miRNA-155 present). The difference images were collected using Digital Optics V++ software built with the instrument. The 3D images were represented using ImageJ 1.49 v software (National Institutes of Health, USA).

Coating Au NPs on an SPRi Au array surface. To form a self-assembled monolayer of 1,4-benzenedithiol on gold array chips, we followed a similar procedure reported in literature with slight modifications.¹ Briefly, an SPRi Au gold array chip was immersed in a freshly prepared solution of 1 mM 1,4-benzenedithiol in n-hexane for about 1.5 h at

50 °C to form the self-assembled monolayer by thiol chemisorption on the Au surface. During this process, the solution was degassed under constant nitrogen purging. After that, the chip was washed with n-hexane and dried under nitrogen. The SPRi Au array chip then was placed with 50 nm Au NPs solution for overnight in a moisturized chamber at 4 °C to allow the immobilization of Au NPs on the surface via the benzene dithiol SAM linker. Finally, the chip was rinsed with deionized water (Figure S1) and dried with nitrogen gas for further immobilization of the miRNA-155 capture nucleotide probe.

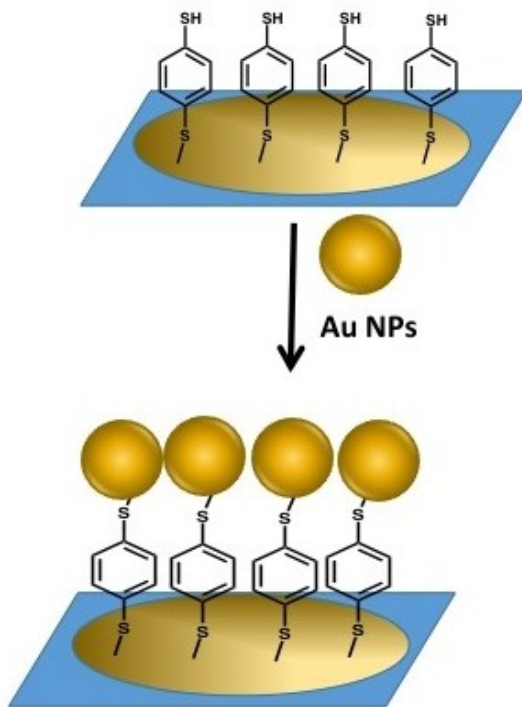


Figure S1. Schematic representation of decorating an SPRi Au array surface with Au NPs to generate a localized surface plasmon using 1,4-benzenedithiol SAM linker.

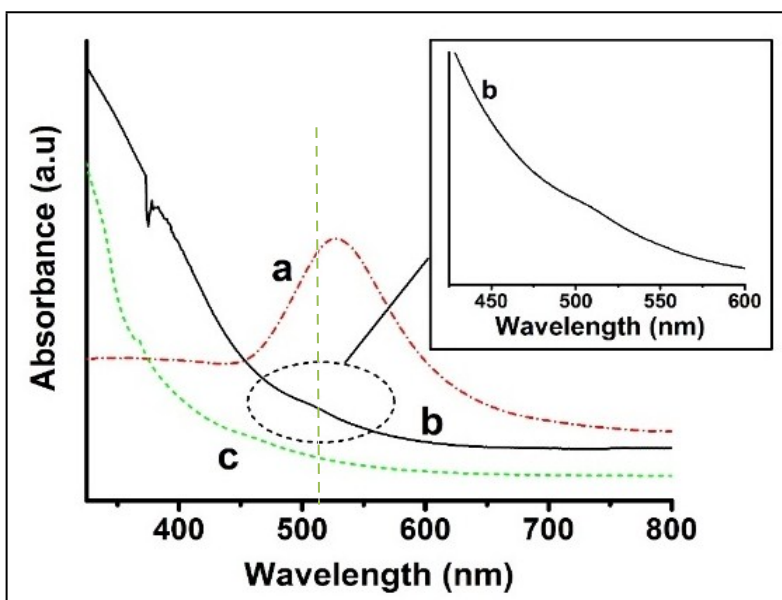


Figure S2. UV-Visible absorption spectra of **a.** Au NPs, **b.** Fe₃O₄@Au NPs, and **c.** Fe₃O₄ NPs. Inset represents the enlarged spectrum of Fe₃O₄@Au NPs.

Immobilization of hairpin capture oligonucleotide: The Au NPs-Au SPRi array chip was covered with 150 μ L of 1 μ M hairpin capture nucleotide probe and incubated for two hours to allow thiol chemisorption between the end thiol group of the capture probe and Au NPs. The chip then was washed with ultrapure water and dried with nitrogen.

Preparation of detection nucleotide-Fe₃O₄@Au NPs conjugates. To prepare the signal amplification nucleotide conjugate, we followed our previously described method with slight modifications.² Briefly, 250 μ L of 1 μ M detection nucleotide solution prepared in PBS buffer was mixed with 150 μ L of Fe₃O₄@Au NPs suspension and allowed to conjugate for two hours at room temperature with a gentle mixing. Upon completion of the incubation, the solution was centrifuged at 10,000 rpm for 15 min and the supernatant was removed and the nanoparticles were washed in buffer twice and suspended again in 400 μ L of fresh PBS solution.

SPRi measurements. After assembling the SPRi array chip in the dual microfluidic channel setup, a reference image was obtained in PBS. Then, different concentrations of miRNA-155 were injected including the zero control (PBS containing no spiked miRNA-155) and allowed to incubate for 10 min. Following a wash in PBS, the detection nucleotide-Fe₃O₄@Au NPs conjugate was injected and incubated for 10 min as to accomplish the double hybridization and additionally amplify the signals. Again, the chip was washed with buffer and the difference image was acquired to measure SPRi pixel intensity differences. The SPRi pixel intensities were measured for different concentrations of miRNA-155 ranging between 0.08-80 fM. The lengths of 1,4-benzenedithiol, miRNA-155, hairpin capture nucleotide of miRNA-155, and detection nucleotide probe were estimated by Avogadro software.³

Finite-difference time-domain (FDTD) simulations. We utilized the Lumerical FDTD package to perform FDTD simulations.⁴ The simulations were used to calculate the scattering cross sections for the nanostructures as a function of the incident light wavelength. The optical properties for Au were taken from the literature.^{5,6} The perfectly

matched layer (PML) boundary conditions were used in the simulations. The scattering cross sections as a function of wavelengths were calculated using the total field/scattered-field (TFSF) formalism. The incident light source used for the simulation was a Gaussian source in the simulated wavelength region.

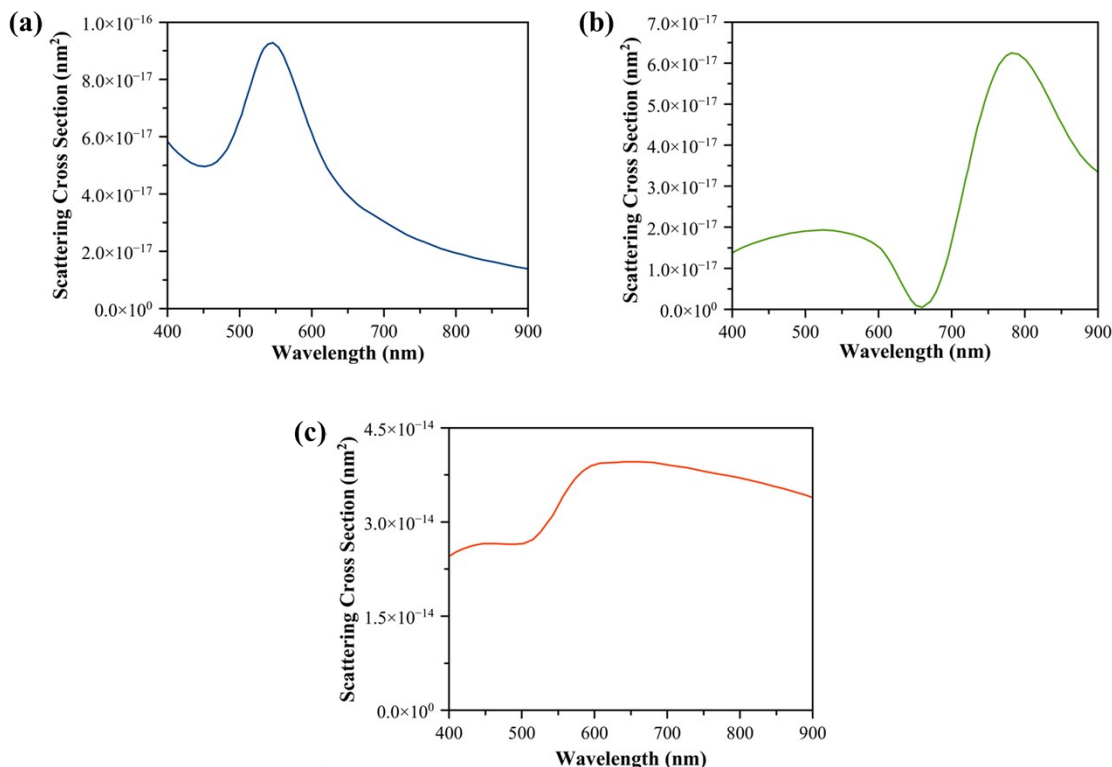


Figure S3. Scattering cross-section as a function of incident light wavelength for **a.** single Au nanosphere of 50 nm diameter, **b.** nine core@shell Fe₃O₄@Au nanoparticles of 10 nm core diameter and 2 nm shell thickness, and **c.** a system consists of Au nanosphere of 50 nm diameter and nine core@shell Fe₃O₄@Au nanoparticles of 10 nm core diameter and 2 nm shell thickness. The distance between the core-shell nanoparticles is 1 nm. In the simulation, we used the following distances: (i) distance between the Au spherical nanoparticle and the core-shell nanoparticle is 21 nm, and (ii) distance among core-shell nanoparticles from each other is 1 nm.

1 Y. J. Lee, I. C. Jeon, W.-k. Paik, K. Kim, *Langmuir* 1996, 12, 5830-5837.

2 G. Premaratne, Z. Al Mubarak, L. Senavirathna, L. Liu, S. Krishnan, *Sens. Actuator B Chem.* 2017, **253**, 368–375.

3 M. D. Hanwell, D. E. Curtis, D. C. Lonie, T. Vandermeersch, E. Zurek and G. R. Hutchison, *Journal of Cheminformatics*, 2012, 4, 17.

4 Lumerical Inc., <http://www.lumerical.com/tcad-products/fdtd/>, (accessed September

28, 2016).

5 E. D. Palik, Handbook of Optical Constants of Solids, Academic Press, San Diego, Calif., 1 edition., 1997.

6 OSA | Optical properties of fourteen metals in the infrared and far infrared: Al, Co, Cu, Au, Fe, Pb, Mo, Ni, Pd, Pt, Ag, Ti, V, and W.,
<https://www.osapublishing.org/ao/abstract.cfm?uri=ao-24-24-4493>.