Supporting Information

Gold Nanostar Enhanced Surface Plasmon Resonance Detection of an Antibiotic at Attomolar Concentrations via an Aptamer-Antibody Sandwich Assay

Suhee Kim and Hye Jin Lee*

Department of Chemistry and Green-Nano Materials Research Center, Kyungpook National University, 80 Daehakro, Buk-gu, Daegu-city, 41566, Republic of Korea

***Corresponding author:** E-mail address: hyejinlee@knu.ac.kr; Tel. + 82 053 950 5336; Fax +82 053 950 6330; Postal address: Department of Chemistry and Green-Nano Materials Research Center, Kyungpook National University, 80 Daehakro, Buk-gu, Daegu-city, 41566, Republic of Korea*

Table of Contents

I. Experimental Details

Materials: Tetracycline (TC; Sigma-Aldrich), oxytetracycline (OTC; Sigma-Aldrich) and chlortetracycline (CTC; Sigma-Aldrich), sheep tetracycline monoclonal antibody (antiTC, MBS838726; Mybiosource), oxytetracycline-antibody (antiOTC, Orb10387; Biorbyt), hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4$; Sigma-Aldrich), sodium borohydride ($NaBH_4$; Sigma-Aldrich), sodium hydroxide (NaOH; Sigma-Aldrich), silver nitrate ($AgNO_3$; Sigma-Aldrich), hexadecyltrimethylammonium bromide (CTAB; Tokyo Chemical Industry), L-ascorbic acid (Sigma-Aldrich), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimde hydrochloride (EDC; Thermo), *N*-hydroxysulfosuccinimide (NHSS; Thermo), 11-mercaptoundecanoic acid (MUA; Sigma-Aldrich), urea (Sigma-Aldrich), glycine-HCl (GE Healthcare), 1x PBS (pH 7.4; Life Technologies) solution, acetonitrile (Sigma-Aldrich), ammonium phosphate (Sigma-Aldrich) were all used as received. A 5'-amine modified DNA aptamer[1] specific to TC, whose sequence is 5'-H_2N-CGT ACG GAA TTC GCT AGC CCC CCG GCA GGC CAC GGC TTG GGT TGG TCC CAC TGC GCG TGG ATC CGA GCT CCA GCT G -3' was purchased from IDT along with a control sequence: 5'-H_2N-CGT ACG GAA TTC GCT AGC ACG TTG ACG CTG GTG CCC GGT TGT GGT GCG AGT GTT GTG TGG ATC CGA GCT CCA CGT G -3'. All biomolecular interaction measurements were either performed in solution 1x PBS buffer pH 7.4 only, or instead in aliquots taken directly from the Sincheon river, Daegu.

Synthesis of Gold Nanostar (GNS) Colloids: GNS colloid solutions were synthesized following the procedure reported by Hafner *et al*[2]. A seed solution was first prepared by adding 250 μL of 0.01 M $HAuCl_4$ into 7.5 mL of 0.1 M CTAB followed by the addition of 600 μL of 100 mM $NaBH_4$ prepared in ice-cold water into the CTAB solution at 29 ºC. The solution was vigorously stirred for 2 min and the solution color changed from clear to pale brown. The seed solution was then stored for 1 h at 29 ºC. Separately, a growth solution was prepared by adding 150 μL of 0.01 M $HAuCl_4$, 10 μL of 0.01 M $AgNO_3$, 10 μL of seed solution and 32 μL of 0.1 M L-ascorbic acid solution into 4.75 mL of 0.1 M CTAB solution. Next, 25 μL of 0.1 M NaOH was added to the growth solution and gently mixed and stored for 3 h at 29 ºC. The growth solution color sequentially changed from clear to yellow-brown, then clear again and finally to faintly blue-purple. This solution was stored more than 3 h at 29 ºC. Centrifugation of the growth solution at 6,000 *g* for 20 min followed by resuspension of the GNS in Millipore water was performed to remove excess reagents. This process

was repeated at least two times and the stock solution (λ_{max} = 614 nm and 656 nm) was stored at 29 ºC. The size distribution of GNS was verified by transmission electron microscopy (TEM) analysis and the majority of particles were anisotropic in nature (~50%) with an average diameter of 76 ± 12 nm alongside a mixed composition of polyhedral (~39%), triangle (~10%), plus others. Even though the number of points observed varied across the nanoparticle composition, these were referred to as gold nanostars (GNS) throughout the manuscript.

Biofunctionalization of GNSs: Briefly, 10 μL of 10 mM 11-mercaptoundecanoic acid (MUA) in ethanol solution was added to 990 μL of GNS colloid solution. The solution was then sonicated for 30 min at 50 ºC and continuously sonicated for a further 2 h at 25 ºC resulting in the self-assembly of a carboxylic acid terminated monolayer on the GNS's surface. Next, 10 μL of 7.5 mM EDC and 1.5 mM NHSS prepared in Millipore water were added to the MUA coated GNSs and reacted for 30 min at 25 ºC. Next, 10 μL of 10 μM antiTC or control antibody in 1x PBS buffer solution was added to the GNS solution and left for 3 h at 29 ºC. The concentration of antiTC was 100 nM in GNS solution. This results in the covalent linking of random amine groups on the antibodies with the terminal amine-reactive NHSS ester groups on the GNS surface. The antibody linked GNS solution was washed by centrifuging (6000 *g*, 7 min) and resuspended into 1x PBS buffer solution to remove excess antibodies. The attachment of antibodies on GNS surfaces were verified using by UV-vis spectroscopy (see Figure 4a) and further utilized for SPR sensing experiments.

Creation of Aptamer Biochips for SPR sensing: A self-assembled carboxylic acid terminated monolayer was formed by soaking the bare gold chip in a 1 mM MUA in ethanol solution for 12 h at room temperature followed by thoroughly rinsing the chip with ethanol and Millipore water. The chip was then reacted with a mixed solution consisting of 7.5 mM EDC and 1.5 mM NHSS for 30 min resulting in the formation of an amine-reactive surface. Next, the chip was rinsed with Millipore water and dried under a N_2 stream. Following dropping of a 1 μL aliquot of 1 mM amine modified TC aptamer or control aptamer onto the NHSS coated chip, the chip was covered with a cover glass slip and kept in a humidity chamber for 3 h. The chip was rinsed with Millipore water and dried under a N_2 stream prior to use. A four-channel Biacore 3000 SPR system was utilized for all *in-situ* biomolecular interacting studies. A constant flow rate of 5 μL/min and 1x PBS running buffer (pH 7.4) were used throughout the measurements. Target molecules (e.g., TC) were flowed across the aptamer chip surface for at least 1 h at all target concentrations and also negative

control drugs to ensure a steady-state surface coverage was attained. All buffer and river water solution measurements were performed using a freshly prepared chip surface. Δ R.U. values were achieved by subtracting the SPR signal before the aptamer/TC surface was exposed to antiTC from the SPR signal after rinsing the surface complex of aptamer/TC/antiTC with PBS buffer. For the regeneration of the TC aptamer surface, sequential rinsing with 8 M urea, DI water, 0.01 M glycine-HCl (pH 2.5), 1x PBS (pH 12.5) and 1x PBS (pH 7.4) was performed continuously with each wash step lasting 30 min. For the measurement of TC in buffer solution, the regenerated aptamer chip was repeatedly used at least five times while for the Sincheon river water measurements the aptamer surface could not be completely regenerated after a single measurement (i.e., the R.U. signal of the used chip could not be recovered back to the levels of the freshly prepared surface). Consequently, all Sincheon river water measurements reported were performed using a freshly prepared chip surface. Normalized R.U. values were obtained by subtracting the average Δ R.U. of the non-specific control signals, NC1 to NC6 described in Table 1, from the average Δ R.U. signal obtained from TC sensing signals. For preparing the desired spiked TC concentration, various μL aliquots of the TC stock solution (10 pM) was added to river water samples.

HPLC Analysis for TC: HPLC was used to verify the results from our SPR data on river analysis. Previously established method was used to run HPLC[3]; briefly, the mobile phase composed of acetonitrile 20% and 20 mM ammonium phosphate (pH 2.5) 80% was run on a Zorbax SB C-18 reverse phase column (250 mm x 4.6 mm, d = 5 μm) in an isocratic mobile at a flow rate of 1.0 mL/min. UV-vis detector at a fixed wavelength of 280 nm was used to analyze TC. The spiked TC concentration into Sincheon river water were varied from 1 mM to 20 mM prepared in the same solution used for mobile phase. Measurements for each concentrations were repeated 3 times.

II. Supporting Figures

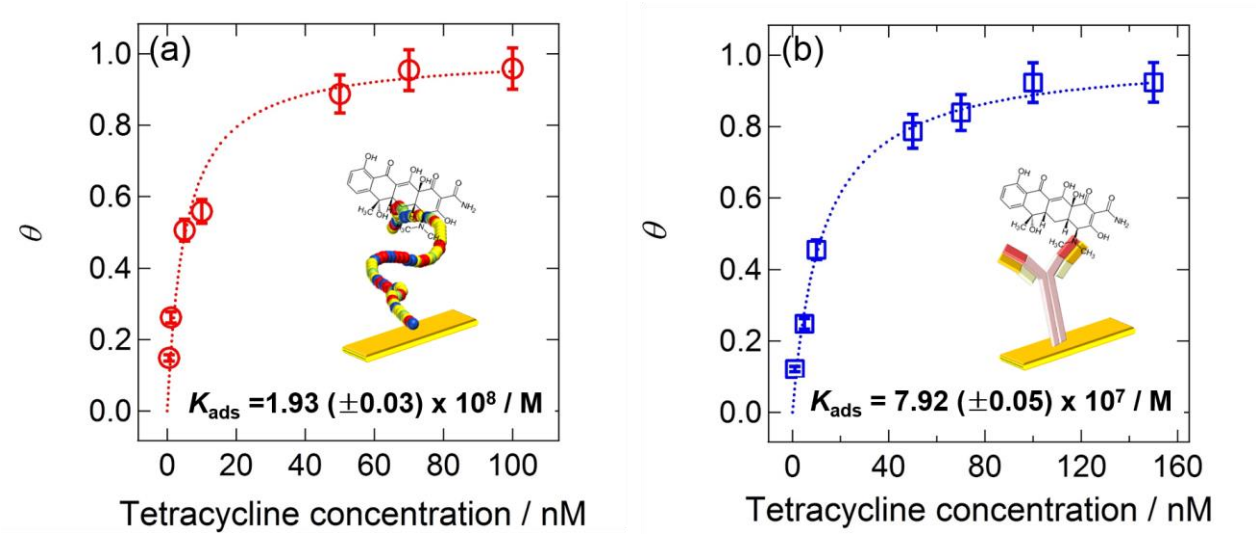

Figure S1. Langmuir adsorption isotherm plots of fractional surface coverage (θ) versus TC concentration. (a) TC specific DNA aptamer and (b) antiTC were immobilized on the SPR chip surface. The fractional surface coverage, θ, was calculated as described previously[4] by dividing the Δ R. U. response at each target concentration by the Δ R. U. for the maximum binding of TC onto the chip.

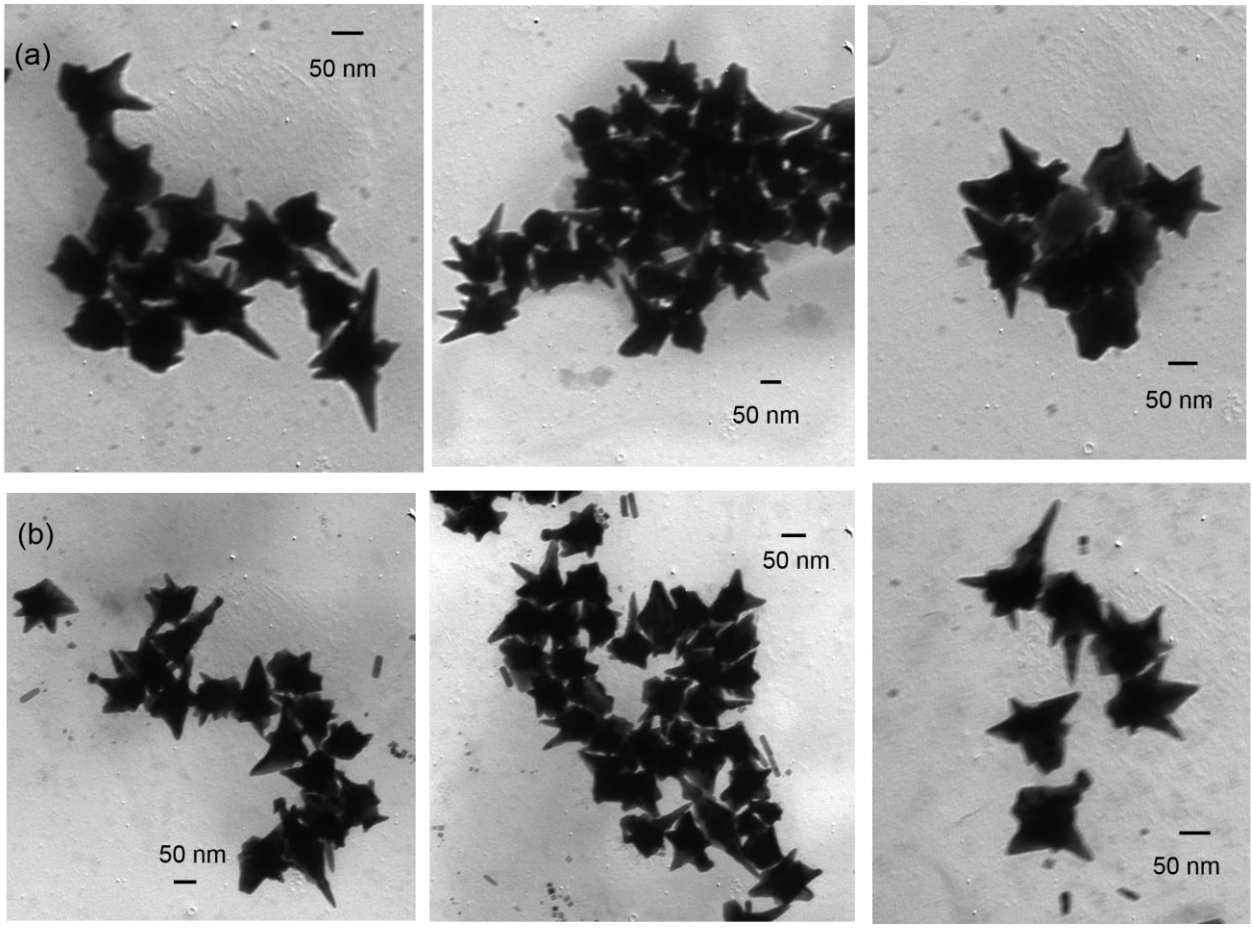

Figure S2. Representative TEM images presenting the distribution of (a) gold nanostars (GNSs) and (b) antiTC coated GNSs.

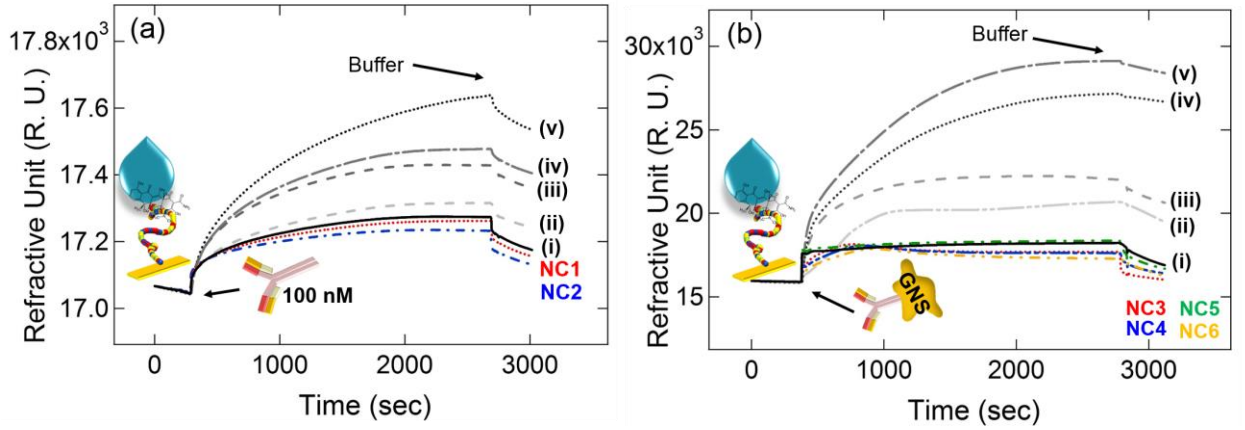

Figure S3. (a) Representative real-time SPR responses for different TC concentrations spiked in Sincheon river water aliquots. (i) Sincheon water only. (ii) 10 fM, (iii) 50 fM, (iv) 70 fM and (v) 200 fM TC spiked in Sincheon water. A fixed antiTC concentration of 100 nM was used. NC1 is a negative control where no target was used instead of TC and NC2 was the case where OTC was used instead of TC. (b) GNS enhanced SPR sensorgrams for various TC concentrations spiked in Sincheon water. A fixed OD of 1.4 (about 2.4 nM) of antiTC functionalized GNSs was used in each measurement. (i) Sincheon water itself. (ii) 50 aM, (iii) 70 aM, (iv) 100 aM and (v) 200 aM TC were spiked in Sincheon water. NC3-5 were negative controls; for NC3 no target was used instead of TC while for NC4, OTC was used instead of TC. AntiOTC was used instead of antiTC for NC5 and a control aptamer sequence modified chip was used instead of TC-specific aptamer for NC6.

References

(1) Jeong, S.; Paeng, I. R. *Sci. World J.* **2012**, *2012*, 1-10.

(2) Nehl, C. L.; Liao, H.; Hafner, J. H. *Nano Lett.* **2006**, *6*, 683-688.

(3) Oka, H.; Ito, Y.; Matsumoto, H. *J. Chromatogr. A* **2000**, *882*, 109-133.

(4) Kim, S.; Lee, H. J. *Anal. Chem.* **2015**, *87*, 7235-7240.