Supporting Information

Target-induced charge reversal of aptamers for visual detection of lysozyme based on positively charged gold nanoparticles

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Experimental Section

Materials and reagents: Lysozyme, thrombin, cytochrome *c*, bovine serum albumin, $HAuCl_4 \cdot 4H_2O$, 6-mercaptohexanol (MCH) and Tris-HCl were purchased from Sigma (St. Louis, MO, USA). Sodium borohydride ($NaBH_4$) was obtained from Kelong Chemical Inc. (Chengdu, China). Cysteamine was purchased from Aladdin (Shanghai, China). The lysozyme binding aptamer (LBA) with and without thiol modified at the 5'-terminus was ordered from Shanghai Sangon Engineering Technology and Services Co., Ltd. (Shanghai, China) with the following sequence: 5'-ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3'.

All other reagents were of analytical grade and used without further purification. Ultrapure water (18 MΩ·cm) was used in all experiments. The experiments were conducted at room temperature (20 °C).

Apparatus: The UV-vis absorption spectra were recorded with a UV-2450 spectrophotometer (Shimadzu, Japan) at room temperature using a 300 μL black-body quartz curette with 1 cm path length. Transmission electron microscopy (TEM) image was recorded on a JEM-2100 transmission electron microscope (Jeol Co. Ltd, Japan). The photographs were taken with a canon EOS 550D digital camera. Cyclic voltammograms were recorded on a CHI 852C electrochemistry work station (CH Instruments Inc., Shanghai, China). A conventional three-electrode configuration was used, with a modified gold working electrode (3 mm in diameter, CH Instruments Inc., Shanghai, China), a Ag/AgCl (3 M KCl) reference electrode, and a platinum wire counter electrode.

Preparation of the (+)AuNPs: All glasswares used in the following procedure were cleaned in a bath of freshly prepared 3:1 HNO_3-HCl, rinsed thoroughly in water and dried with N_2 prior to use. (+)AuNPs were prepared by $NaBH_4$ reduction of $HAuCl_4$ in the presence of cysteamine according to the published protocol with slight modifications.[1] Briefly, a cysteamine solution (400 μL, 213 mM) was added to a 40 mL of 1.42 mM $HAuCl_4$ solution. After stirring for 20 min at room temperature, 10 μL of 10 mM $NaBH_4$ solution was added and the mixture was vigorously stirred for 10 min at room temperature in the dark. After further mild stirring, the resulting wine-red solution was stored in refrigerator (4 °C) and ready for use. The average diameter of the as-prepared (+)AuNPs is ~30 nm according to the typical TEM image (Fig. S2).

Electrochemical Characterization of the modified electrodes: The gold electrode was first immersed in a fresh warm piranha solution (3:1 mixture of concentrated H_2SO_4 and 30% H_2O_2) for 30 min, followed by rinsing with water. The electrode was then polished

with 0.3 and 0.05 μm aluminum slurry respectively and sonicated sequentially in water, ethanol and water for 5 min each. Next, the electrode was electrochemically cleaned in 0.5 M H_2SO_4 until a remarkable voltammetric peak was obtained. Finally, the electrode was rinsed thoroughly with water and dried with nitrogen. The LBA strands were immobilized on the cleaned gold electrode by spreading a droplet of 10 μL thiolated LBA (1 μM) in incubation buffer (IB, 20mM Tris-HCl, 0.1M NaCl, 5mM $MgCl_2$, pH 7.5) for overnight at room temperature in humid atmosphere. Prior to use, the thiolated LBA was first heated to 80°C in the IB for 4 min, and then allowed to slowly cool down to room temperature. The LBA-modified electrode was then rinsed with IB and blocked with 1 mM MCH (in IB) for 1 h. After that, the gold electrode was rinsed again with IB and deionized water, followed by drying with nitrogen before electrochemical characterization. To explore the change of the surface charge of aptamer after incubation with lysozyme, a 10 μL droplet of lysozyme of 1.05 μM in IB was deposited onto the LBA-modified gold electrode and incubated for 1 h. This was followed by washing with buffer (20 mM Tris-HCl, 0.1 M NaCl, 5 mM $MgCl_2$, 0.01% Tween 20, pH 7.5) to remove non-specifically bound lysozyme. Finally, cyclic voltammetry was performed on the modified electrode in 0.1 M KCl solution containing 1 mM $[Fe(CN)_6]^{3-/4-}$ by scanning the potential from -0.1 V to 0.6 V at a scan rate of 50 mV s^{-1}.

Visual detection protocol for lysozyme: The LBA was first treated with the pre-heating and cooling steps as mentioned above. For protein visual assay, lysozyme at appropriate concentration was incubated with the annealed LBA solution at room temperature for 30 minutes and 10 μL of the mixture was transferred into 200 μL (+)AuNP solution to reach a final concentration of LBA at 100 nM. The photographs of the (+)AuNP solutions were

taken after 1 min of incubation. The UV-vis spectra were recorded by diluting the resulting colored solution five times and the absorption spectra were collected in the range of 400-800 nm at room temperature.

Supplementary Figures:

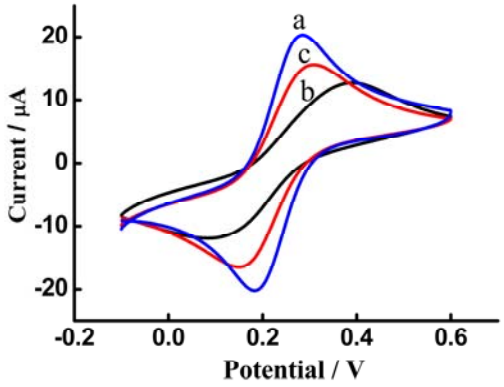

Fig. S1 Cyclic voltammograms of 1 mM $[Fe(CN)_6]^{3-/4-}$ on a bare gold electrode (a) and on a LBA-modified gold electrode before (b) and after (c) incubation with 1.05 μM lysozyme in 0.1 M KCl solution by scanning the potential from -0.1 V to 0.6 V at a scan rate of 50 mV s^{-1}.

As illustrated in Fig. S1, a couple of quasi-reversible, well-defined redox peak of $[Fe(CN)_6]^{3-/4-}$ is observed on the pretreated bare gold electrode (curve a). After self-assembly of the thiolated LBA and MCH on the gold electrode, the current response of $[Fe(CN)_6]^{3-/4-}$ on the modified electrode (curve b) is much smaller than that of the bare electrode (curve a). This is due to the fact that the negatively charged LBA probes and MCH act as electrostatic barriers to repel the negatively charged $[Fe(CN)_6]^{3-/4-}$ electrochemical markers from the electrode surface and hinder the interfacial electron transfer reaction, leading to the reduced current responses.[2] The binding of lysozyme with the LBA switches the surface charge of the LBA-modified electrode and an apparent increase in current response is observed (curve c).

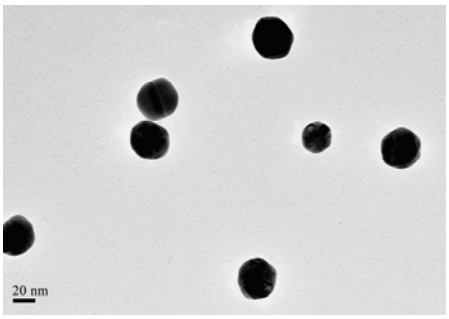

Fig. S2 Typical TEM image of the as-prepared (+)AuNPs.

References:

1 T. Niidome, K. Nakashima, H. Takahashi and Y. Niidome, *Chem. Commun.*, 2004, 1978.

2 R. Levicky, T. M. Herne, M. J. Tarlov and S. K. Satija, *J. Am. Chem. Soc.*, 1998, **120**, 9787.