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Electronic Supplementary Information (ESI)

Regenerable electrochemical immunological sensing at DNA nanostructure-decorated gold surfaces

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Experiment section

Materials: tris-(hydroxymethyle)aminomethane was from Cxbio Biotechnology Ltd. Ethylenediaminetetraacetic acid (EDTA), 11-MU (11-mercapto-1-undecanol), 11 MUA (11-mercaptoundecanoic acid), and tris(2-carboxyehtyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (St. Louis, MO). TMB substrate (TMB = 3,3',5,5'-tetramethylbenzidine; Neogen K-blue low activity substrate) was from Neogen (U.S.A.). Avidin-HRP(horseradish peroxidase) was from Roche Diagnostics (Mannheim, Germany). Affinity purified anti-mouse TNF-α, biotin anti-mouse TNF-α cocktail and recombinant mouse TNF-α were purchased from eBioscience. The buffer solutions involved in this study are as follows: the hybridization buffer was 1M NaCl and 10mM TE buffer (pH 7.4). The buffer for iTSP assembly was 20mM Tris(pH8.0), 50mM $MgCl_2$. The DNA immobilization buffer was 10mM Tris-HCl, 1mM EDTA, 10mM TECP(pH 7.4), and 1M NaCl. The washing buffer was 0.1M NaCl and 10mM PB buffer (pH 7.4). Enzyme diluent was 0.1M PBS buffer with 0.5% casein (pH 7.2). All solutions were prepared with Milli-Q water (18MΩ·cm resistivity) from a Millipore system.
All oligonucleotides were synthesized and purified by TaKaRa Inc.(Dalian, china), , which are shown in table S1.

Table S1: DNA sequence

DNA Name	***sequence (5'-3')***
iTSP-A	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA TTTTTTTTTTGTATCCAGTG GCTCA
iTSP-B	HS-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
iTSP-C	HS-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
iTSP-D	HS-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
T1	Biotin-TGAGCCACTGGATAC
T2	Biotin-TGAGCCGCTGGATAC
Linker1	HOOC-TGAGCCACTGGATAC
Linker2	HOOC-TGAGCCGCTGGATAC

Formation of DNA Tetrahedra probe

Mixed the four oligonucleotides (iTSP-A, iTSP-B, iTSP-C and iTSP-D) in equimolar quantities in TM buffer (20 mM Tris, 50 mM $MgCl_2$, pH 8.0), heated the mixture to 95℃ 2min and then cooled to 4℃ in 30 s.

Gel electrophoresis

The iTSP were analyzed using polyacryamide gel electrophoresis (PAGE, 10%) in TBE buffer at a constant current of 5 mA at 4℃.

Gold Electrode treatment.

The gold electrodes are 2-mm-diameter Gold working electrode (CHI101 CH Instruments Inc.)

The procedure of the electrode treatment before DNA self-assembly is as followed:

First, Polish gold electrodes on microcloth with three micropolish deagglomeated alumina suspensions (1.0, 0.3 and 0.05 μm in diameter) in sequence for 5 min each. Rinse the electrodes extensively with water after each polishing step. Second, sonicate polished electrodes sequentially in ethanol and Milli-Q water for 5 min each, to remove residual alumina powder. Remove any residual impurities form the gold electrodes through electrochemical oxidation and reduction of the metal. Third, apply a positive potential of 2 V to the electrodes for 5 s, followed by a negative potential of -0.35 V for 10 s. Then run 5-10 cycles of cyclic voltammetry (CV) in 3 mL of 0.5 M H2SO4 solution with potential range (-0.3 to 1.55V) in a 4 V s-1 scan rate. Fourth, check the cleanness of the gold electrodes by running a CV cycle in a fresh 0.5 M H2SO4 solution with potential range (-0.3 to 1.55V) in a 0.1 V s-1 scan rate.

The roughness factor of the gold surface was determined electrochemically from the charge required for the formation and reduction of gold oxide and was estimated about 1.2.

Electrochemical measurements.

Electrochemical measurements were performed with a CHI 660 electrochemical workstation (CH Instruments Inc., Ausin, TX). A conventional three-electrode configuration was employed all through the experiment, involving a gold working electrode, a Ag/AgCl reference electrode, and a platinum counter electrode. A glass cell with 3 mL of Neogen K-blue low activity substrate was placed on a cell stand. All potentials were referred to the Ag/AgCl (3 M KCl) electrode, and all measurements were carried out at a scan rate of 100mV/s. Amperometric detection was performed at a fixed potential of 100 mV, and the steady state was usually reached and recorded within 100s.

TSP-based immunoassays

iTSP (1 μM) was first incubated with the cleaned electrodes for 3 h at room temperature, and then hybridized with a protein linker (100 nM). Capture antibodies (Ab_1) were attached by first depositing 3 μL of freshly prepared 400 mM EDC and 100 mM NHS in 1 × PBS onto the TSP-modified electrode and washing with 1 × PBS after 15min. This was followed by treatment with 3 μL of 100 μg/mL anti-mouse TNF-α in pH 7.0 PBS buffer for 2 h. After washed with 1 × PBS, the electrodes was incubated with 3 μL of standard solution containing TNF-α (0 - 2.5 ng/mL) in 1 % BSA, 1 × PBS buffer for 1 h. The electrodes were incubated with 50 μg/mL biotinylated anti-mouse TNF-α cocktail for 1 h, rinsed thoroughly with 1 × PBS, incubated with strepavidin-HRP conjugates for 0.5 h, consecutively. The sensor was then extensively rinsed and subjected to electrochemical measurements. For regenerate experiments, the electrodes were rinsed with 1 × PBS at 45 ℃ for 30 s.

Control experiment:

We carried out a control experiment to confirm that the observed current change was only specific to the binding of TNF-α. As shown in Figure 2b, 1% BSA did not lead to signal increase (~35 nA), which was markedly lower than that for 5 ng/mL of TNF-α (~1276 nA). This study confirms that the antibody retains high activity after attaching onto iTSP surface and iTSP platform possesses excellent specificity for protein.

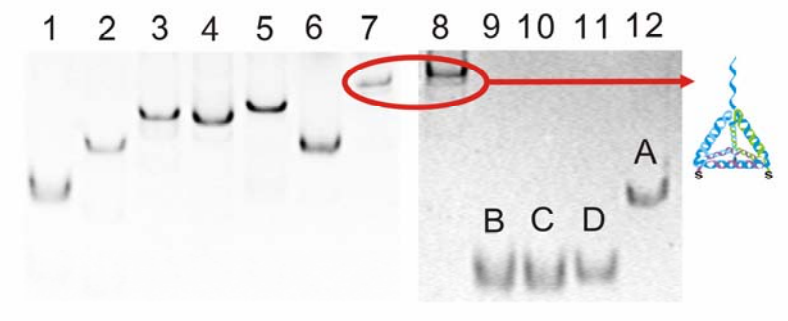

Fig. S1 Gel electrophoretic analysis of the formation of iTSP. Lane 7 and Lane 8 stand for lane 8 for iTSP. Control experiment for single-stranded (ss-) DNA (lane 9 stands for iTSP-B, lane 10 stands for iTSP-C, lane 11 stands for iTSP-D, lane 12 stands for iTSP-A) or any other combinations lacking one (lane 3 stands for iTSP-A + iTSP-B+ iTSP-C, lane 4 stands for iTSP-A + iTSP-B+ iTSP-D, lane 5 stands for iTSP-A + iTSP-C+ iTSP-D, lane 6 stands for iTSP-B + iTSP-C+ iTSP-D) or two strands(lane 1 stands for iTSP-C + iTSP-D , lane 2 stands for iTSP-A + iTSP-B).

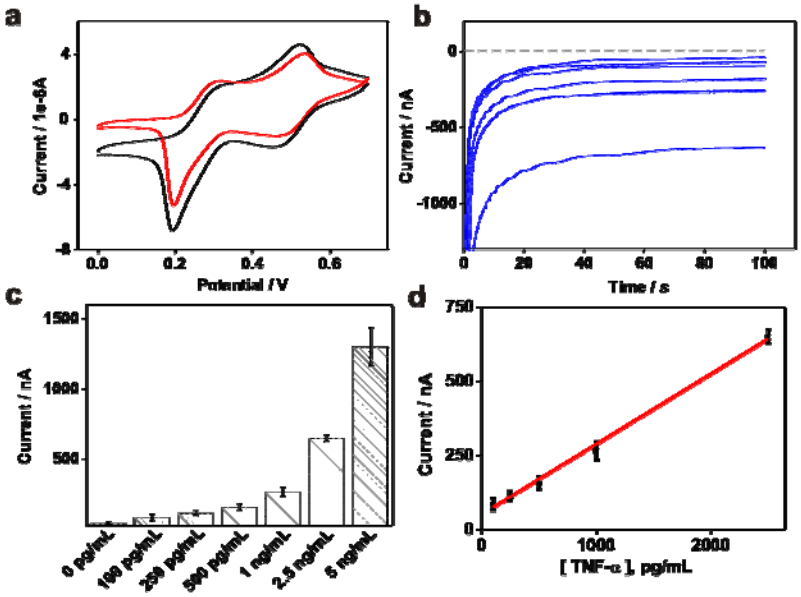

Fig. S2 (a) CVs for iTSP in the absence (red line) and presence of 5 ng/mL TNF-α (black line). Scan rate: 100 mV / s. (b) Amperometric curves (i-t) for iTSP tested in solution with a series of TNF-α concentrations. From top to bottom: 0, 100 pg/mL, 250 pg/mL, 500 pg/mL, 1 ng/mL, 2.5 ng/mL. (c) Concentration profiles for the detection of TNF-α. (d) Plot for concentation of TNF-α vs. amperometric current.

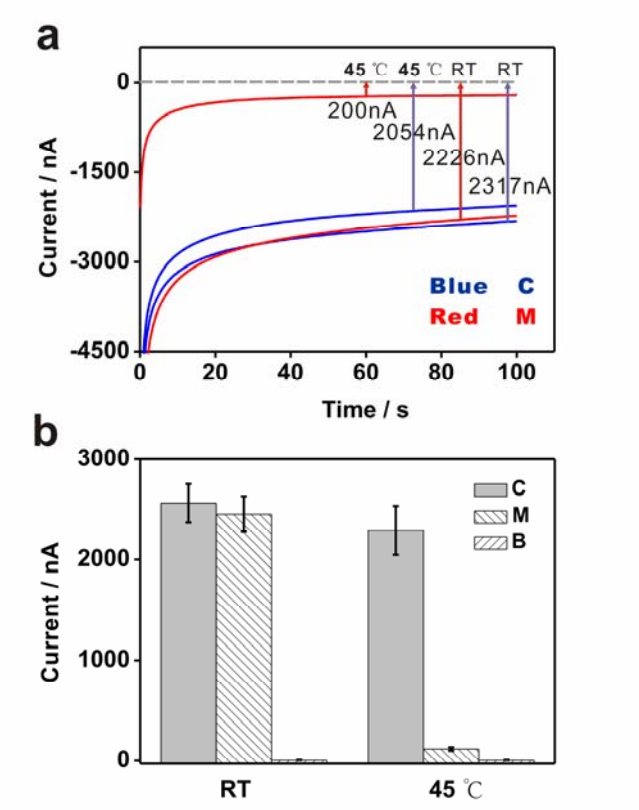

Fig. S3 (a) Amperometric curves (i-t) for the iTSP sensor at different temperature. The bridge DNA contains either C**:** fully complementary (A:T), or M: one-base mismatch (G:T). B stands for blank. **(b)** Comparison of hybridization efficiency at different temperature for fully complementary bridge DNA and single-base mismatched (G:T) bridge DNA.