Electronic Supporting Information

Catalytic assembly of DNA topological nanostructure on nanoporous gold arrays as 3D architectures for label-free telomerase activity sensing

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Experimental section

Chemicals and instruments

Human telomerase ELISA kit was obtained from MyBioSource, Inc. (USA). Zidovudine (3'-azido-3'-deoxythymidine, AZT), *tris*(2-carboxyethyl)phosphine (TCEP), Tris-HCl buffer solution (50 mM, pH 7.6), deoxynucleotide triphosphates (dNTPs), malachite green (MG), magnesium chloride ($MgCl_2$), mercapto-1-undecanol (MCU) and polystyrene (PS) beads were purchased from Sigma Aldrich. Polystyrene beads were further purified by centrifugation with a mixture of ethanol and DI water (1:1, volume ratio), and then dried in an oven at 50 ^oC for 24 h, and stored in 4 ^oC before use. Without special indication, all reagents and solvents were of analytical grade or better and used directly. The thiolated sequence (TS) primer was purchased from Integrated DNA Technologies (IDT, Coralville, IA) and listed as below:

5'- HS-$(CH_2)_6$-TTT TTA ATC CGT CGA GCA GAG TT -3'

Surface enhanced Raman spectroscopy (SERS) measurements were carried out using a home-built line-scan Raman microscopy system with 785 nm excitation.[1] All the SERS spectra were presented after baseline correction.[2] Extinction spectra were measured by a Cary 50 Scan UV-

visible spectrometer at room temperature. Scanning electron microscopy (SEM) images were performed using a FEI XL-30 FEG SEM.

Fabrication of nanoporous gold array (NPGA) substrate

The fabrication process is described in detail in Ref [3] and will be briefly outlined here. A gold film with a preselected thickness was first evaporated onto a silicon wafer substrate. A 100 nm thick Au-Ag alloy film was then sputter deposited using an alloy target ($Au_{30at.\%}Ag_{70at.\%}$, ACI Alloy). A monolayer of polystyrene (PS) beads with a 460 nm diameter (Sigma Aldrich) was then formed on top of the alloy film, followed by a timed oxygen plasma treatment to shrink the PS beads and guarantee the separation of neighboring beads. The sample was then sputter etched in Argon plasma to transfer the bead pattern into the alloy film. Once the pattern transfer was completed, the PS beads were removed by sonication in chloroform. The samples were then dipped in 70% nitric acid for 1 minute for dealloying, followed by rinsing in deionized (DI) water for 2 minutes. The process thus far produced the patterned NPGA structures.

Preparation of human telomerase standard solution and inactivated telomerase solution

The human telomerase standard solution was prepared by adding diluent solution from ELISA kit into human telomerase lyophilized standard sample, and kept it for 30 min to make sure the lyophilized standard completely dissolved and well mixed. The inactivated telomerase solution was achieved by heating the telomerase standard solution to 90 ^{o}C for 15 min. The activity of telomerase at 1.0 mg/mL was estimated to 5.0 IU.

Preparation of TS immobilized NPGA substrate

Firstly, 5 μL TCEP (20 mM) and 2 μL TS primer (10 μM) were added into Tris-HCl buffer solution for 1 h at room temperature, obtained the activated TS primer solution. The as-prepared

ANPG was cut into chips with 3 mm × 3 mm and washed with DI water. Subsequently, the ANPG chips were immersed into the above mixture for overnight to form TS modified ANPG.

Telomerase activity assay

The TS modified NPGA chips were immersed into the Tris-HCl buffer (20 mM, pH 7.6) containing dNTPs (10 μL, 10 mM each), KCl (10 μL, 1.0 M), $MgCl_2$ (8 μL, 30 mM) and telomerase (1×10^{-4} IU), incubated for 15 min at 37 ^{o}C. The two negative control experiments were carried out in here. The first one used the diluent solution to instead of telomerase; second one was the inactivated telomerase. After that, the above telomerase processed ANPG chips were taken out and washed with DI water, and immersed into the MG solution (2 μM) for 35 min at 37 ^{o}C, formed MG/G4 decorated NPGA. Finally, the well prepared MG/G4 modified ANPG chips were washed with deionized water and transferred into the MCU solution (0.3 mM) for 3 h to remove the nonspecific binding of MG and physical absorption of TS primer.

SERS measurement

The excitation laser was shaped into a 1×133 μm^2 line at the sample plane with ~22.1 mW power. The SERS spectra over the laser spot were recorded by a spectrograph charge-coupled device system (LS-785, Princeton Instruments, Acton, Massachusetts). The spectra were readout by WinSpec software (PI Acton), followed by curvature correction and background removal in MATLAB using automated polynomial-based techniques.[4] Five random spots from each sample were measured and the spectrum for each sample were taken as the average of these measurements.

Calculation of the telomeric repeats' number on the end of TS primer

Due to the nitrogen's number in the TS primer sequence is constant, and the telomeric repeats is periodic oligonucleotide bases (ATTGGG). The number of telomeric elongated repeats could be calculated according to the increase factor of the N 1s/Au 4f ratio of the TS primer before and after incubating with telomerase. The calculated equation is listed bellow:

$$N_{telomeric\ repeats} = \frac{(I-1) \times N_{TS\ primer}}{N_{ATTGGG}} \quad \text{(Eq. 1)}$$

Where $N_{telomeric\ repeats}$ is the number of telomeric elongated repeats on the end of TS primer. *I* is the increase factor of the N 1s/Au 4f ratio of the TS primer modified ANPG before and after incubating with telomerase by XPS measurement. N_{ATTGGG} and $N_{TS\ primer}$ are the number of nitrogen in AATGGG sequence and TS primer, respectively. The number of nitrogen in each base, AATGGG sequence and TS primer are listed in Table S1.

Table S1 The number of nitrogen in each base, ATTGGG and TS primer*

	N_A	N_T	N_C	N_G	N_{ATTGGG}	$N_{TS\ primer}$	I	$N_{telomeric\ repeats}$
Value	5	2	3	5	24	80	5.545	15.5

* N_A, N_T, N_C, N_G, N_{ATTGGG} and $N_{TS\ primer}$ are the number of nitrogen in A, T, C, G, AATGGG and TS primer, respectively.

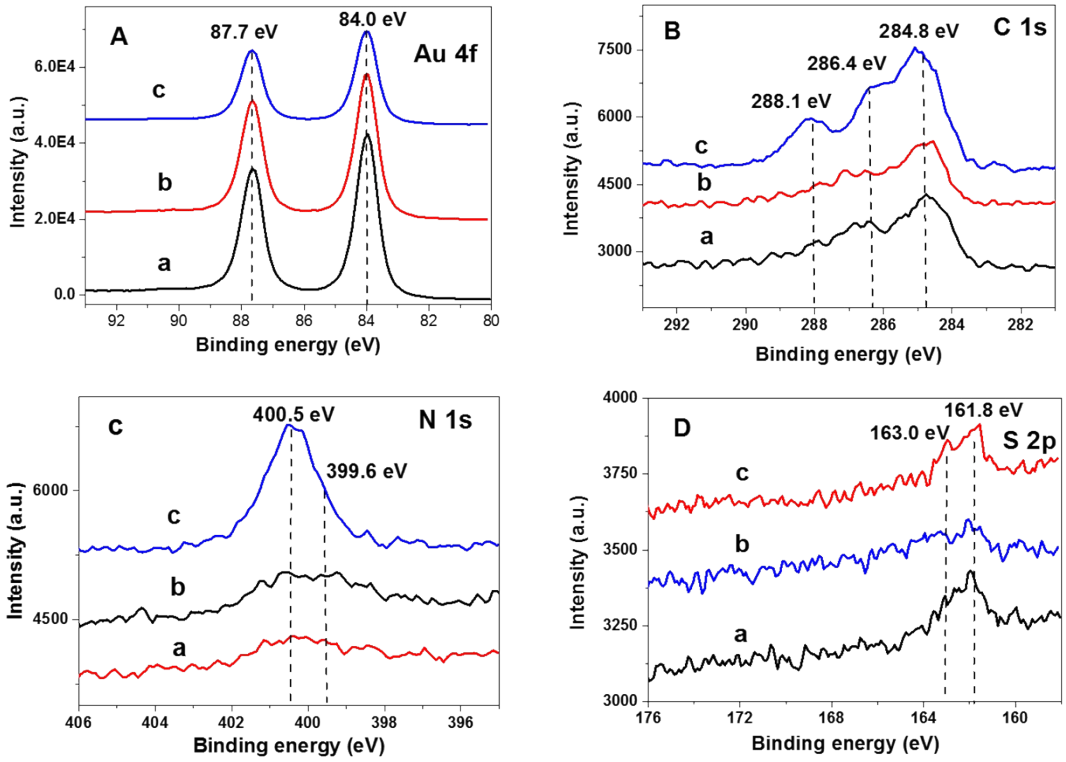
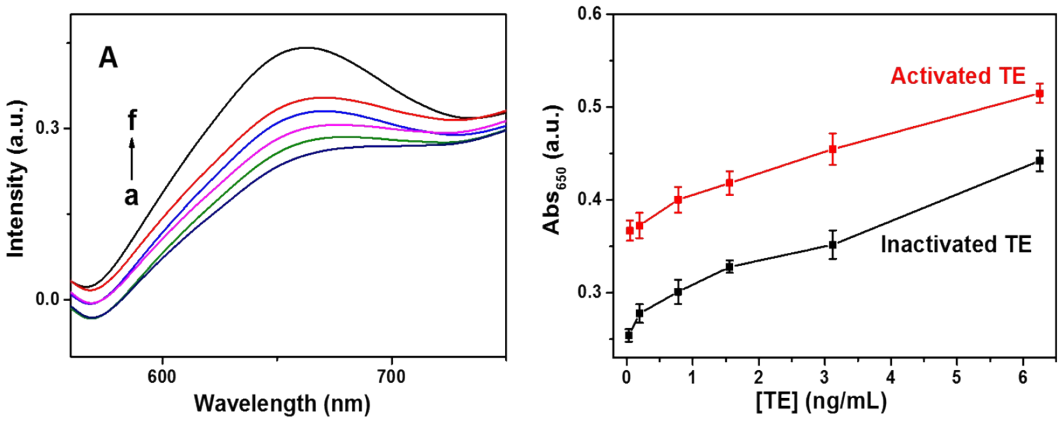


Fig. S1. Au 4f (A), C 1s (B), N 1s (C) and S 2p (D) XPS spectra of different modified NPGA. (a) Bare NPGA+TS primer; (b) Bare NPGA+TS primer+dNTPs; (c) Bare NPGA+TS primer+dNTPs+ telomerase. Concentrations of TS primer, dNTPs, telomerase and MG were 1.0 μM, 30 μM, 6.45×10^{-7} IU and 2.0 μM, respectively.



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101 **Fig. S2.** UV/Vis spectra of inactivated telomerase using ELISA-based colorimetric assay, from a
102 to f were 0.034 ng/mL, 0.195 ng/mL, 0.78 ng/mL, 1.56 ng/mL, 3.12 ng/mL and 6.25 ng/mL,
103 respectively; (D) Dependence of UV/Vis absorption intensity at 650 nm on telomerase
104 concentration with activated telomerase (a) and inactivated telomerase (b).

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