Electronic Supplementary Information

Photoelectrochemical aptasensor for sensitive and selective detection of kanamycin based on Au nanoparticles functionalized self-doped TiO_2 nanotube arrays

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Experimental Section

Chemicals and materials. A 0.1 mm thick titanium foil (99.6%, Jinjia Metal, China) was cut into pieces of 40 × 10 mm^2. Chloroauric acid ($HAuCl_4$), ethylene glycol (EG), ammonia fluoride (NH_4F), sodium sulfate (Na_2SO_4), phosphate buffer saline (PBS, pH = 7.4), kanamycin, chloramphenicol, ciprofloxacin, erythromycin, ofloxacin, doxycycline were purchased from Macklin Chemical and used as received. The aptamer (5'-HS-$(CH_2)_6$-TGGGG GTTGA GGCTA AGCCG A-3') was supplied by Sangon Biotech, Shanghai, China. All aqueous solutions were prepared using deionized water (DI) with a resistivity of 18.2 MΩ cm.

Preparation of TiO_2 NTs. The hierarchical TiO_2 NTs were fabricated by a two-step anodization process. Prior to anodization, the Ti foils were first degreased by sonicating in ethanol and DI water, followed by drying in pure nitrogen stream. The

anodization was carried out using a conventional two-electrode system with the Ti foil as an anode and a Pt foil as a cathode respectively. All electrolytes consisted of 0.5 wt% NH_4F in EG solution with 2 vol% water. All the anodization was carried out at room temperature. In the first-step anodization, the Ti foil was anodized at 60 V for 30 min, and then the as-grown nanotube layer was ultrasonically removed in deionized water. The same Ti foil then underwent the second anodization at 60 V for 10 min. After the two-step anodization, the prepared TiO_2 NT samples were cleaned with DI water and dried off with nitrogen gas. The as-anodized TiO_2 NTs were annealed in air at 450 °C for 1 h with a heating rate of 5 °C min^{-1}.

Fabrication of Au/SD-TiO_2 NTs. The electrochemical induced self-doping processes were conducted in a typical three-electrode system, with the TiO_2 NTs, Ag/AgCl, and Pt as working electrode, reference electrode, and counter electrode, respectively. In this step, the TiO_2 NTs underwent the electrochemical reduction under negative potentials (-1.5 V *vs* Ag/AgCl) in the supporting electrolyte of 1 M Na_2SO_4 for 30 min. Photocatalytic reduction method was used to deposit Au NPs on the SD-TiO_2 NTs. The SD-TiO_2 NTs was first soaked in 0.3 mM aqueous $HAuCl_4$ for 24 h and then was irradiated in this solution with a 300 W Xe lamp for 1 h to reduce the absorbed Au^{3+} to Au^0 by photocatalysis. For the purpose of comparison, the Au NPs were also deposited on pristine TiO_2 NTs to form Au/TiO_2 NTs.

Characterization of Au/SD-TiO_2 NTs. The morphologies of the Au/SD-TiO_2 NTs were determined by filed-emission scanning electron microscope (FESEM, FEI Quanta 600). The crystalline structure of the samples was analyzed by X-ray

diffraction (XRD, Bruker D8 Discover diffractometer, using Cu Kα radiation, λ = 1.540598 Å). The diffuse reflectance UV-vis adsorption spectra were recorded on a spectrophotometer (Shimadazu, UV 3600), with fine $BaSO_4$ powder as reference. Photoelectron Spectroscopy (XPS) data were collected by an Axis Ultra instrument (Kratos Analytical) under ultrahigh vacuum ($<10^{-8}$ torr) and using a monochromatic Al Kα X-ray source operating at 150 W. The survey and high-resolution spectra were collected at fixed analyzer pass energies of 160 and 20 eV, respectively. Binding energies were referenced to the C 1s binding energy of adventitious carbon contamination which was set at 285.0 eV.

Fabrication of PEC aptasenosr. Before incubation of aptamer with Au/SD-TiO_2 NTs, the appropriate secondary structure of aptamer was ascertained by its heat-treatment at 92 °C for 10 min, followed by snap-chilling on ice for 5 min and bringing it back to room temperature. After that, the Au/SD-TiO_2 NTs was incubated with the aptamer in immobilization buffer with optimized concentration for 100 min, followed by thoroughly rinsing with deionized water and being dried in air for further sensing applications.

Performance of the PEC aptasensor. The PEC characterization of the Au/SD-TiO_2 NTs were evaluated using a three-electrode configuration with the Au/SD-TiO_2 NTs, Ag/AgCl and Pt foil as working, reference and counter electrode, respectively. The supporting electrolyte used was PBS (1×, pH = 7.4) solution. The scan rate for the linear sweep voltammetry was 5 mV s^{-1}. The transient photoresponse was evaluated under chopped light irradiation (light on/off cycles: 60 s) at a fixed electrode potential

of 0.5 V *vs* Ag/AgCl. The photocurrent was measured under an irradiation from a 300 W Xe lamp (PLS-SXE300, PE300BF) with 420 nm cutoff filter. The electrochemical impedance spectra (EIS) were measured using a PGSTAT 302N Autolab Potentiostat/Galvanostat (Metrohm) equipped with a frequency analyzer module (FRA2) with an excitation signal of 10 mV amplitude. The impedance vs frequency spectra were acquired at the open circular potential of the system. Afterward, impedance vs potential measurement at fixed frequency of 5 kHz and 10 Hz was performed for Mott-Schottky plots and parallel capacitance plots. The PEC aptasensor was incubated kanamycin of desired concentrations at 60 °C for 20 min in PBS solution. The detection potential used for PEC sensing was 0.5 V *vs* Ag/AgCl.

Selectivity mechanism. In this PEC aptasensor, the aptamer was used as recognition unit, and the Au/SD-TiO_2 NTs was used as sensing unit, where the kanamycin was caught by the aptamer, and oxidized by the photo-generated holes from Au/SD-TiO_2 NTs. However, the interferences of ciprofloxacin, erythromycin, ofloxacin, doxycycline cannot be captured by the aptamer, and cannot reach the surface of the photoelectrode due to the steric effect as all the surface of Au NPs were occupied by the aptamer and the mercaptohexanol as blocking agent. Thus the PEC aptasensor possessed a very favorable selectivity toward kanamycin detection.

PEC aptasensor reproducibility. The detection reproducibility was also investigated by measuring the response photocurrent of more than five aptasensors, and a standard deviation of 3.75% was achieved, confirming the good reproducibility. In addition, the long-term stability of the aptasensors were also tested by studying the current

response intermittently in a period of 30 days (stored at 4 °C after the each measurements), and no obvious photocurrent differences were found, suggesting the aptasensor was quite stable for the kanamycin sensing.

Real sample analysis. The kanamycin with different concentrations (1, 10, and 100 nM) was dissolved into bovine milk. The milk solution was centrifuged at 12000 rpm (5 min) to remove fat. Finally, the supernatant was added into the PBS for analysis. Each sample was assayed three times. The PEC aptasenosr was used to detect the recoveries of different concentrations of kanamycin in commercial-available bovine milk by standard addition method to evaluate its feasibility of detection of real samples. The results were shown in Table S2, ESI†, and the recovery was in the range of 94.8-103% with low relative standard deviation in the range of 2.31-3.15%, which results were consistent with the data from enzyme-linked immunosorbent assay (ELISA) analysis, and thus revealed that the PEC aptasenosr can be utilized for determining of kanamycin in animal derived foods.

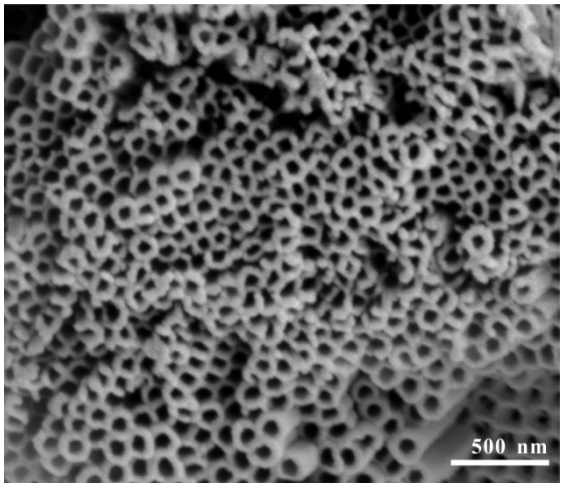

Fig. S1 SEM image of first-step anodized TiO_2 NTs.

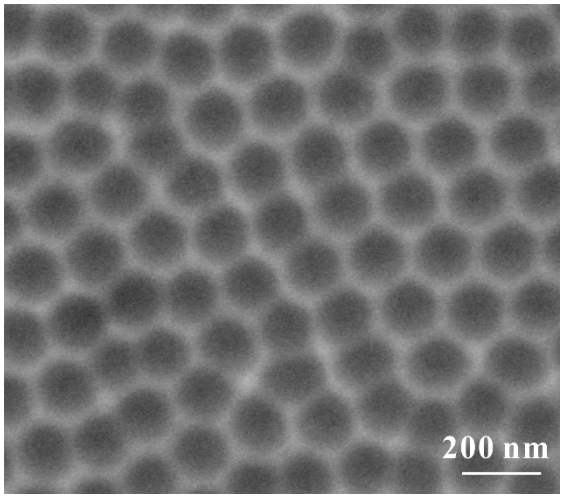

Fig. S2 SEM image of patterned Ti surface after removed the first-anodized TiO_2 NTs.

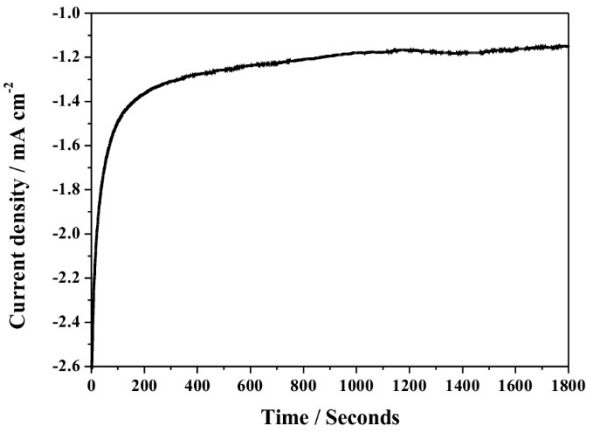

Fig. S3 I-t curve of electrochemical reduction of TiO_2 NTs at -1.5 V *vs* Ag/AgCl.

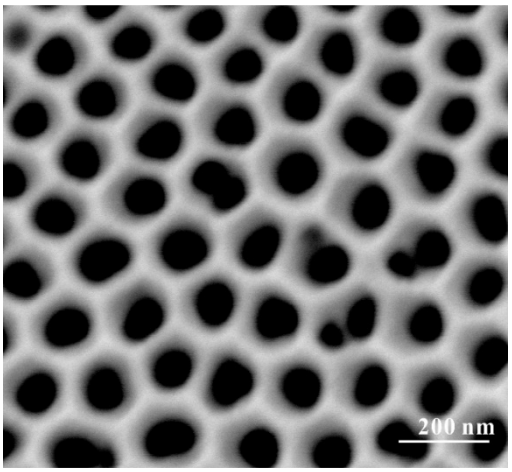

Fig. S4 SEM image of TiO_2 NTs.

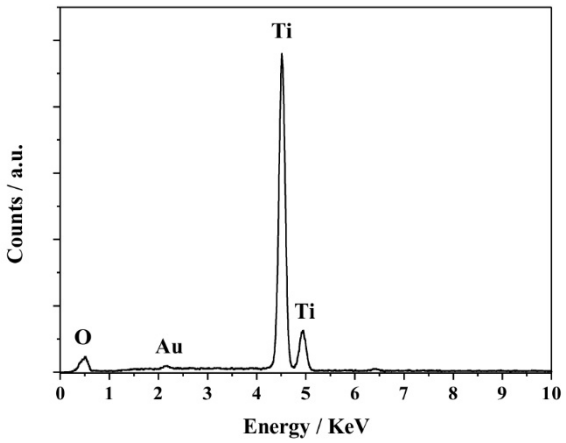

Fig. S5 EDS of Au/SD-TiO_2 NTs.

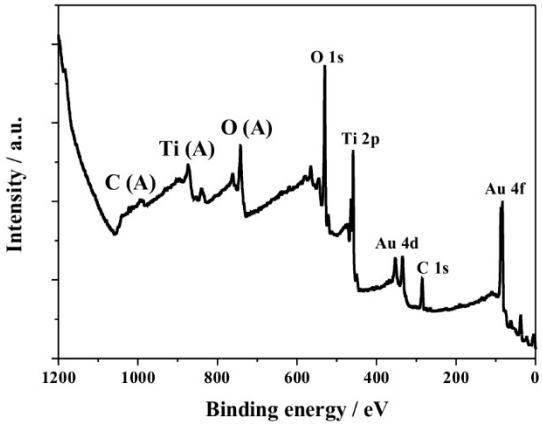

Fig. S6 XPS survey of Au/SD-TiO_2 NTs.

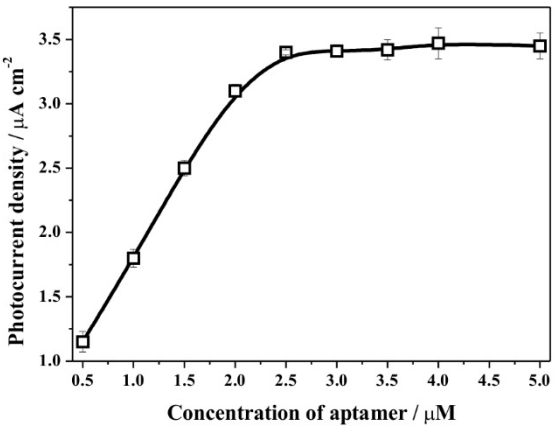

Fig. S7 Optimization of aptamer concentrations.

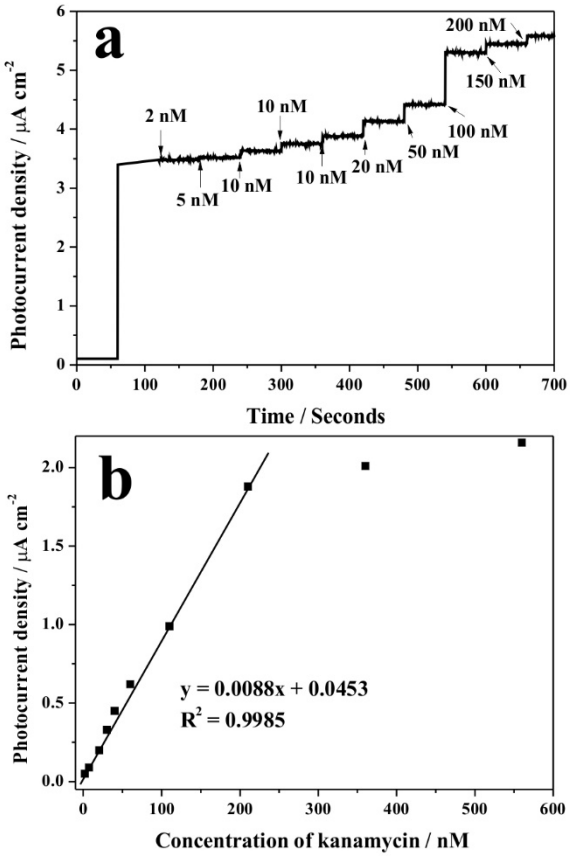

Fig. S8 (a) Photocurrent density *vs* time for successive addition of kanamycin at 0.5 V *vs* Ag/AgCl under illumination of visible light with wavelength ⩾420 nm on aptamer-Au/TiO_2 NTs; (b) photocurrent density-kanamycin concentration calibration curve on aptamer-Au/TiO_2 NTs.

Table S1 Comparison of various kanamycin sensors

Method	Material	Detection limit / nM	Linear range / nM	Reference
Electrochemistry	Graphene-Au/MWCNTs-CoPc	5.8	10-150	1
Colorimetry	Au NPs	1.49	1-100	2
Luminescence	platinum(II) complex	143	200-150000	3
Fluorescence	-	8.95	27-67000	4
PEC	C_3N_4/Graphene oxide	0.2	1-230	5
PEC	Au/SD-TiO_2 NTs	0.1	02-200	This work

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Table S2 Results from analysis of kanamycin in milk by PEC aptasensor and ELISA (n=3)

Kanamycin / nM	PEC Aptasensor		ELISA	
	Recovery / %	RSD / %	Recovery / %	RSD / %
1.0	94.8	3.15	93.6	5.32
10	99.6	2.96	105	4.63
100	103	2.31	102	3.25