Supporting information

Magnetic-enzymatic synergy driven photoelectrochemical aptasensor on a microfluidic chip for Sub-pM Kanamycin detection

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Materials and Reagents

Macklin Reagent Inc. (Shanghai, China) provided the kanamycin. Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), hydrochloric acid (HCl) and sodium chloride (NaCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. Shanghai China. Melamine and roxithromycin were purchased from Adamas-Beta Reagent Co., Ltd. (Shanghai, China). L-Ascorbic Acid 2-Phosphate sesquimagnesium (AAP) Salt Hydrate from Leyan, Shanghai, China. Chloramphenicol was obtained from Aladdin Reagent Inc. (Shanghai, China). Dexter Biotechnology Co., Ltd. (Chengdu, China) provided erythromycin and ofloxacin; Yuanye Biotechnology Co., Ltd. (Shanghai, China) provide oxytetracycline. Sangon Biotechnology Co., Ltd (Shanghai, China) also provided streptavidin-alkaline phosphatase (SA-ALP), complementary DNA (cDNA), and kanamycin aptamer as well. The cDNA probe and kanamycin aptamer were synthesized in the form of, as follows: 3′-CCG-ATT-CGG-CTA-TTT-TTT-TTT-(CH_2)6-NH2-5′and3′-AGC-CGA-ATC-GGA-GTT-GGG-GGT-biotin-5′, respectively.

Experimental Equipment

The morphology and structure of the synthesized samples were characterized employing a Scanning Electron Microscope (SEM, JSM-7800F, Japan). A JEM-2100F microscope (Japan) with an accelerating voltage of 200 kV was used to observe transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HR-TEM). The X-ray diffraction (XRD) patterns were recorded with a Bruker D8 Advance diffractometer (Germany) using Cu Kα radiation. Raman spectroscopy was carried out on a Horiba LabRAM HR Raman spectrometer (Japan) with an excitation wavelength of 532 nm. X-ray photoelectron spectroscopy (XPS) was performed on a Thermo Fisher Scientific K-Alpha+ spectrometer (with all high-resolution XPS spectra calibrated to the C1s peak). The UV-visible absorption spectra were recorded on a UV-2550 spectrophotometer (Shimadzu, Japan) from 200 to 800 nm. Electrochemical performance of portable system was evaluated by using a Chenhua CHI 660e electrochemistry workstation (China).

Fabrication of TiO_2 Nanorod Arrays

The genel method to synthesize the TiO_2 nanorod arrays consists of blending 2.27 mmol citrate, 2.27 mmol tetrabutyl titanate and 24 mL hydrochlorid acid (36−38% w/w) with also be in 20-25mL

deionized water. The mixed solution is poured into a Teflon-lined stainless steel autoclave of 100 mL, after stirring the mixture for 15 minutes. Immerse the cleaned FTO substrate with the conductive side facing down, and the two are immersed. The autoclave is then sealed and heated at a temperature of 150 °C for 6 hours. The substrate is peeled off from the bottom after cooled to room temperature, rinsed with deionizing water and ethanol, dried at 80 °C for 2 hours. The prepared TiO_2 nanorod arrays are then annealed at 500 °C for 1 hour, with a heating rate of 5 °C per minute.

Preparation of g-C_3N_4 sheets and TiO_2/Nb_2C/CN

Due to the prominent optical activity and photocatalytic mobility of g-C_3N_4, melamine was used as precursor for the pyrolysis method in fabrication of g-C_3N_4 sheets. In brief, a total of 6g of melamine was heated in a crucible at 520 °C for 2 h with ramp rate of up to 5 °C/min under atmosphere, leading to the bulk of light-yellow g-C_3N_4. The bulk material was then pulverized in a mortar and pestle to obtain a fine powder. The powder was then heated at 10 °C/min in an open crucible to 550 °C for 3 h to yield the g-C_3N_4 powder. Lastly, 0.1 g of the powder was sonicated in 100 mL of isopropanol for 10 h and centrifuged at 4000 rpm for 1 h to obtain a dispersion of g-C_3N_4.

Adding MXene: 1 mL of the g-C_3N_4 dispersion was then diluted in 1 mL of ultrapure water and then mixed up with 10 μL from a solution of MXene (2 mg/mL) and sonicated for another hour. A 100 μL portion of this mixture was deposited on the TiO_2/FTO substrate and dried in air at 60 °C.

cDNA Conjugation to CMBs

200 μL of washed carboxylated magnetic beads (CMBs, 50 mg/mL) are resuspended in cationic MES buffer (0.05 M, pH 5.0) and washed three times to ensure proper cleaning. Beads were resuspended in 500 μL of clean MES buffer prior to the next step. In order to activate carboxyl groups on CMBs, 500 μL of a solution of EDC (0.1 M) and NHS (0.01 M) was added to the CMBs, followed by incubation at room temperature for 30 minutes. After activation, the CMBs were washed three times with MES buffer and redispersed in 1 mL PBS buffer (0.01 M, pH 7.4). Conjugation efficiency was carried out by mixing 100 μL of amino-modified cDNA with activated CMBs (100 μL, 10 mg/mL) and incubating at 37 °C for 12 hours. After incubation, the reaction

mixture was also incubated at 37 °C for 1 h to allow removal of excess cDNA by magnetic separation.

Application in real samples.

To validate real-world applicability, commercially sourced milk samples were analyzed. Sample pretreatment followed established protocols: Acetic acid (20% v/v) was incrementally added to adjust the pH to 4.6, inducing isoelectric precipitation of denatured proteins (primarily tyrosine). The mixtures were incubated at 45°C for 10 min in a water bath to maximize protein aggregation, followed by centrifugation (10,000 rpm, 30 min) to pelletize precipitated proteins and lipids. The supernatant was filtered through a 0.22 μm membrane to eliminate residual lipids and neutralized prior to analysis. Kanamycin concentrations were spiked into the pretreated milk, and detection was conducted.

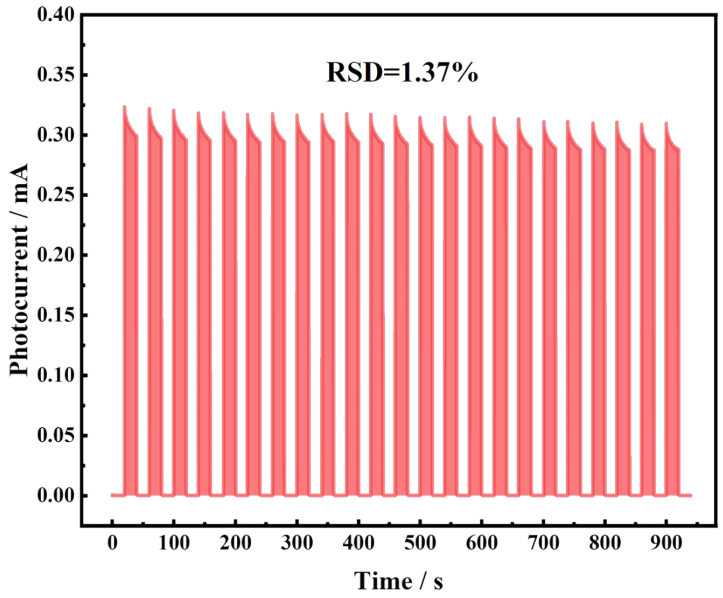

Figure S1. Stability of the sensor processed by 20 consecutive photoelectrochemical (PEC) measurements of 1 nM Kana.