SUPPORTING INFORMATION

Palindromic Molecular Beacon-Based Z-Scheme BiOCl-Au-CdS Photoelectrochemical Biodetection

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TABLE OF CONTENTS

PARTIAL EXPERIMENTAL SECTION

Material and Reagent. Kanamycin (Kana), erythromycin (ERY), streptomycin (STR), cefradine (CED) and tetracycline (TCY) were purchased from Aladdin (Shanghai, China). Klenow fragment polymerase (KF polymerase), nicking endonuclease (Nt.BbvCI), bovine serum albumin (BSA) and deoxyribonucleoside triphosphates (dNTPs) were obtained from New England Biolabs Inc. (Beijing, China). Ultrapure water used in this work was prepared by a Millipore water purification system (18.2 MΩ cm, Milli-Q, Merck KGaA, Germany). 3-Mercaptopropionic acid (MPA), thiourea, cadmium chloride ($CdCl_2$), bismuth nitrate pentahydrate [$Bi(NO_3)_3 \cdot 5H_2O$], potassium chloride (KCl, 99.5%), gold(III) chloride hydrate ($HAuCl_4 \cdot 3H_2O$), ethanol (C_2H_5OH, 99.7%), 2-(morpholino) ethanesulfonic acid (MES) and *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide (EDC), tris(2-carboxyethyl) phosphine (TCEP), 2-hydroxy-1-ethanethiol (MCH), and ascorbic acid (AA) were obtained from Merck KGaA (Darmstadt, Germany). All buffers including phosphate-buffered saline (PBS; 10 mM, pH 7.4, 136.89 mM of NaCl, 2.67 mM of KCl, 8.24 mM of Na_2HPO_4, 1.76 mM of KH_2PO_4) solution and TNaKT buffer (20 mM Tris, 300 mM NaCl, 5 mM KCl, pH 7.5) were the products of Sigma-Aldrich. All reagents (analytical grade) were used as received without further purification. All oligonucleotides were acquired from Shanghai Sangon Biotech. Co., Ltd. (Shanghai, China). Prior to use, all the oligonucleotides were first heated to 95 °C for 5 min and then naturally cooled to room temperature (RT) for use. These sequences are listed as follows:

name	sequence (5'- 3')
Aptamer for Kana (Apt)	TGGGGGTTGAGGCTAAGCCGA
Complementary DNA (c-DNA)	TCGGCTTAGCCTCAACCCCCA
Hairpin DNA1 (HP1)	CGTACGTATTACGCGTAAGCTGAGGTGGGGGTTGAGGCTAAGCCGATTACGCGTAA
Hairpin DNA2 (HP2)	NH_2-$(CH_2)_6$-TCAGCTTACCGTACGTATTACGCGTAAGCTGA-$(CH_2)_6$-SH
Nick	TCAGCTTACGCGTAATACGTACG

Preparation of BiOCl and BiOCl-Au Nanostructures. BiOCl nanostructures were synthesized by a modified solvothermal method similar to previous report.[1] Typically, $Bi(NO_3)_3 \cdot 5H_2O$ (1.0 mM) and KCl (1.0 mM) were initially added into 15-mL ultrapure water, and then stirred for 60 min to obtain a uniform suspension. Following that, the mixture was transferred into a 50-mL

Teflon-lined steel autoclave and kept in a drying oven at 160 °C for 24 h. After that, the resulting products (*i.e.*, BiOCl nanostructures) was collected by centrifugation (10 min, 10 000*g*), washed with ultrapure water and ethanol thoroughly, and dried at 60 °C in a vacuum oven.

Next, the stepwise facets-selective photo-reduction was employed to deposit Au^0 on the surface of BiOCl nanostructures. In a typical procedure, 100 mg of the as-prepared BiOCl powder was initially added into 30-mL methanol/water solution (1:5, v/v) under vigorous stirring. Following that, 1.0 mL of $HAuCl_4$ aqueous solution (80 mM) was injected into the suspension in two separate times. At every turn, the suspension was stirred for 1 h in dark to achieve the preferential adsorption and then exposed to a 500-W xenon arc lamp for 3 h. Finally, the product was centrifuged, rinsed with ultrapure water and dried at 60 °C as before to obtain BiOCl-Au power.

Synthesis of Carboxylated CdS QDs. 3-Mercaptopropionic acid (MPA)-coated CdS QDs were synthesized on the basis of the literature with minor modification.[2] Briefly, CdCl2 (0.175 mM) and thiourea (0.289 mM) were initially dispersed into ultrapure water (15 mL) under vigorous stirring in order to obtain the Cd^{2+}/thiourea precursor. Thereafter, 0.395 mM of MPA was added into the resulting precursor (note: The mixture should be fixed to 35 mL by using ultrapure water), and the pH of the mixture was adjusted to 10 by using 1.0 M NaOH. After that, the resultant mixture was deaerated for 30 min by nitrogen bubbling, followed by transferring to a 100-mL Teflon-lined steel autoclave. The heating temperature was maintained at 100 °C for 60 min. Finally, the as-prepared CdS QDs capped with MPA were dialyzed into 12 000 – 14 000-molecular weight cut off dialysis and concentrated for further use.

Synthesis of CdS QD-Labeled Hairpin DNA2 (QD-HP2). The carboxylated CdS QDs and the aminated hairpin DNA2 (HP2) were conjugated by typical carbodiimide coupling, as described in our previous work. [3] Briefly, 1.0 mL of the carboxylated CdS QDs (5.0 mg mL^{-1}) was initially dispersed into 1.0-mL 2-(morpholino)ethanesulfonic acid (MES; 0.1 M, pH 6.0) containing 3.0-mg NHS and 2.0-mg EDC. Following that, the resulting solution was gently shaken for 60 min at room temperature, followed by the centrifugation for 10 min at 11 000g and washed with ultrapure water for three times to remove excess EDC and NHS. Meantime, the thiolated HP2 was activated by 10 μM TCEP for 60 min to reduce the disulfide bonded. Afterwards, the activated CdS QDs was re-dispersed in the aminated HP2 (5.0 μM), and slightly shaken for 6 h at 4 °C. During this process, hairpin HP2 was covalently conjugated to the carboxylated CdS QDs. The excess hairpin HP2 was

removed by centrifugation as before. Finally, the obtained CdS QD-labeled hairpin HP2 (QD-HP2) was dispersed into TNaKT buffer (20 mM Tris, 300 mM NaCl, 5 mM KCl, pH 7.5) containing 1.0 wt % BSA and 10 mM TCEP at 4 °C.

Target-triggered PMB-based Target Recycling. Prior to experiment, 5.0 μL of 10 μM Kana-aptamer and 5.0 μL of 10 μM c-DNA were initially mixed into 90-μL PBS buffer (10 mM, pH 7.4) containing 20 mM KCl, 200 mM NaCl and 10 mM $MgCl_2$. Thereafter, the resulting mixture was denatured for 4 min at 95 °C and finally cooled to RT. During this process, the added aptamer hybridized with c-DNA to form an Apt-c-DNA duplex with a concentration of 0.5 μM. Next, the as-prepared Apt-c-DNA duplex was used for the reaction of target. At this step, 25 μL of PBS (10 mM, pH 7.4) containing the different-concentration Kana, 2.5 μL of 10× NEBuffer, HP1 (10 μM), KF polymerase (5.0 U), dNTPs (250 μM) and Nt.BbvCI (5.0 U) were simultaneously added into 25 μL of the above-prepared Apt-c-DNA duplex. After mixing gently, the mixture was incubated at 37 °C for 120 min. During this process, the complementary DNA strand (c-DNA) was displaced from the Apt-c-DNA duplex because of the specific Kana-aptamer reaction. Following that, the displaced c-DNA was triggered the strand-displacement reaction after hybridization with HP1 to produce palindromic fragments. In the presence of KF polymerase, dNTP and Nt.BbvCI endonuclease, the strand-displacement reaction was repeatedly carried out to release a new nick strand. All the experiments were conducted using the standard procedure unless otherwise specified.

Fabrication of PEC Biosensing Platform. BiOCl-Au-coated fluorine-doped tin oxide (FTO) electrode was prepared as follows. Prior to modification, FTO electrode was successively washed in toluene, acetone, ethanol and ultrapure water with 15 min ultrasonic, and dried in air. Thereafter, the cleaned FTO electrode was stuck by the waterproof transparent tape with a hole (r = 2.5 mm). Following that, the above-prepared BiOCl-Au aqueous suspension (25 μL, 20 mg mL^{-1}) was thrown on the active surface of FTO electrode and dried at RT. Then the resulting FTO electrode was immersed into pH 7.5 TNaKT buffer containing QD-HP2 (600 nM) and incubated for 6 h at RT. During this process, hairpin HP2 was assembled onto the BiOCl-Au through the Au-S bond. The unbound active sites on gold nanoparticles were blocked by incubation with MCH (500 nM) for 60 min at RT. Finally, HP2-conjugated BiOCl-Au/FTO (BiOCl-Au-QD-HP2/FTO) photosensitive electrode was washed with PBS (10 mM, pH 7.4) and stored at 4 °C for use.

Analysis of Real Milk Sample. Milk samples were obtained from the local Walmart

supermarket (Fuzhou, China). Initially, 2.0 mL of milk sample was placed into the centrifugal tube, and then the mixture containing 4.0 mL of trichloroacetic acid and 2.0 mL of chloroform were added into the sample to deposit the organic substances. After removing the large-sized substance, the resulting suspension was centrifuged for 10 min at 6000*g* to remove possible organic precipitates again. Following that, the resulting supernatant was passed sequentially through a 100-nm nylon mesh, no. 1 filter and a GF/B filter in sequence. Finally, Kana standards with different concentrations were spiked into the supernatant, respectively, for the recovery experiment. The commercial Kana ELISA kit was also employed as the reference for the accuracy of photoelectrochemical (PEC) biosensing method.

Statistical Analysis. All measurements were carried out in triplicate. A statistical data analysis was performed using Statistics Analysis System (SAS) ver. 9.0 and Statistical Program for Social Sciences (SPSS) ver. 9.0 software packages. Comparisons between dependent variables were determined using analysis of variance (ANOVA), Duncan multiple range test, correlation analysis and multiple regression analysis. Results are expressed as mean value ± standard deviation (SD) of three determinations and statistical significance was defined at $P \leq 0.05$.

Table S1. Comparison of Different Kana Detection Methods on Analytical Properties.

method	linear range	limit of detection	Ref.
Photoelectrochemistry	0.2 – 450 nM	50 pM	(4)
Fluorescence aptasensor	1.0 – 80 nM	0.29 nM	(5)
Surface plasmon resonance	0.1 – 10 μM	12 nM	(6)
Electrochemical aptasensor	0.05 – 200 pM	36 fM	(7)
Gas pressure-based bioassay	0. 2 – 50 pM	63 fM	(8)
Surface-enhanced Raman scattering	2.0 pg mL^{-1} – 80 ng mL^{-1}	2.0 pg mL^{-1}	(9)
Photoelectrochemistry bioassay	0.05 – 5.0 pM	29 fM	this work

REFERENCES

(1) Jiang, J.; Zhao, K.; Xiao, X; Zhang, L. Synthesis and facet-dependent photoreactivity of BiOCl single-crystalline nanostructures. *J. Am. Chem. Soc*. **2012**, *134*, 4473−4476.

(2) Aboulaich, A.; Billaud, D.; Abyan, M.; Balan, L.; Gaumet, J.; Medjadhi, G.; Ganbaja, J.; Schneider, R. One-pot noninjection route to CdS quantum dots via hydrothermal synthesis. *ACS Appl. Mater. Interfaces* **2012**, *4*, 2561-2569.

(3) Qiu, Z.; Shu, J.; Tang,D. Near-infrared-to-ultraviolet light-mediated photoelectrochemical aptasensing platform for cancer biomarker based on core–shell $NaYF_4$:Yb,Tm@TiO_2 upconversion microrods. *Anal. Chem*. **2018**, *90*, 1021-1028

(4) Liu, X.; Liu, P.; Tang, Y.; Yang, L.; Li, L.; Qi, Z.; Li, D.; Wong, D. A photoelectrochemical aptasensor based on a 3D flower-like TiO_2-MoS_2-gold nanoparticle heterostructure for detection of kanamycin. *Biosens. Bioelectron*. **2018**, *112*,193-201.

(5) Deng, J., Liu, Y., Lin, X., Lyu, Y., Qian, P., Wang, S. A ratiometric fluorescent biosensor based on cascaded amplification strategy for ultrasensitive detection of kanamycin. *Sens. Actuators B* **2018**, *273*, 1495-1500.

(6) Dehghani, S.; Danesh, N.; Ramezani, M.; Alibolandi, M.; Lavaee, P.; Nejabat, M.; Abnous, K.; Taghdisi, S. A label-free fluorescent aptasensor for detection of kanamycin based on dsDNA-capped mesoporous silica nanoparticles and Rhodamine B. *Anal. Chim. Acta* **2018**, *1030*, 142-147.

(7) Zeng, R.; Su, L.; Luo, Z.; Zhang, L.; Lu, M.; Tang, D. Ultrasensitive and label-free electrochemical aptasensor of kanamycin coupling with hybridization chain reaction and strand-displacement amplification. *Anal. Chim. Acta* **2018**, *1038*, 21-28.

(8) Zeng, R.; Luo, Z.; Zhang, L.; Tang, D. Platinum nanozyme-catalyzed gas generation for pressure-based bioassay using polyaniline nanowires-functionalized graphene oxide framework. *Anal. Chem*. **2018**, *90*, 12299-12306.

(9) Zengin, A.; Tamer, U.; Caykara, T. Extremely sensitive sandwich assay of kanamycin using surface-enhanced Raman scattering of 2-mercaptobenzothiazole labeled gold@ silver nanoparticles. *Anal. Chim. Acta* **2014**, *817*, 33-41.