Supplementary Material

A novel electrochemiluminescence immunosensing strategy fabricated by $Co(OH)_2$ Two-dimensional Nanosheets and $Ru@SiO_2$-Au NPs for highly sensitive detection of enrofloxacin

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Reagents and materials

Chloroauric acid ($HAuCl_4 \cdot 4H_2O$, 47.8%), cobalt (II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$, 99%), mono-potassium phosphate (KH_2PO_4, 99%), cyclohexane (99.5%), ammonia solution, 1-hexanol (98%) were obtained from Sinopharm Chemical Reagent Co., Ltd (shanghai China), 2-methylimidazole (2-MIM, 98%) was purchased from Tokyo Chemical Industry Co. Ltd. Tetraethyl orthosilicate (98%), (3-Aminnopropyl) triethoxysilane (APTES, 90%) and tris(2,2'-bipyridy) ruthenium(II) chloride hexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$, 98%) were purchased from Aladdin Industry Corporation. Bovine serum albumin (BSA) were acquired from Sigma-Aldrich Co., Ltd (USA). Sodium chloride (NaCl), potassium chloride (KCl, 99.5%) and sodium hydroxide (NaOH) were bought from Adamas Reagent Co., Ltd (Shanghai, China). Triton X-100 (TX-100, 98%) was purchased from Titan Scientific Co., Ltd. Acetone was bought from Linfeng Chemical Reagent Co., Ltd. Enrofloxacin (ENR), ciprofloxacin (CIP), ofloxacin (OFLX), norfloxacin (NOR), lomefloxacin (LOM) and gatifloxacin (GAT) were obtained from the National Institutes for Food and Drug Control (Beijing, China). The Ab against ENR and coating antigen of ENR were offered by our lab and evaluated by ELISA.

Buffer solution

Phosphate-buffered saline stock solution (PBS, 0.1 M) was prepared by KCl (0.1 M), NaCl (0.1 M), Na_2HPO_4 (6.4 mM) and KH_2PO_4 (1.0 mM). $K_2S_2O_8$ buffer solution (0.1 M) containing of KH_2PO_4 (0.1 M), Na_2HPO_4 (0.1 M), KCl (0.1 M) and $K_2S_2O_8$ (0.1 M) was used as ECL detection buffer solution. $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ standard solution containing

KCl (0.1 M), $Fe(CN)_6^{3-}$ (2.5 mM) and $Fe(CN)_6^{4-}$ (2.5 mM) were employed to measure electrochemical impedance spectroscopy (EIS).

Apparatus

The ECL emission was measured by an MPI- A ECL analytical system (Xi'An Remex Electronic Science & Technology Co., Ltd (Xi'An, China) in ECL buffer solution. The three-electrode system as that in the ECL detection, including working electrode, Ag/AgCl as reference electrode, platinum electrode as auxiliary electrode. The whole test was carried out under the condition that the scan potential was varied from 0 to 1.3 V and the voltage of the photomultiplier tube (PMT) was set at - 600 V. The Scanning Electronic Microscopy (SEM) was supplied by Hitachi SU8010 SEM (Hitachi Co., Japan). The Transmission Electron Microscope (TEM) images were obtained from Tecnai G2 F20 S-TWIN 200KV (FEG, FEI Co., USA). UV-vis absorption spectrum was recorded with Agilent 8453 UV-vis spectrophotometer (Agilent Co., America). EIS was undertaken using an RST electrochemical workstation (Suzhou Risetest Instrument Co., Ltd, Suzhou, China).

Preparation of $Co(OH)_2$ two-dimensional nanosheets

Briefly, 1.2 mmol of 2-methylimidazole and 0.3 mmol of $CoCl_2 \cdot 6H_2O$ were separately dissolved in 7.5 mL of H_2O. After stirring for 30 min at room temperature, the two solutions were mixed and stirred at room temperature for 12 h. The product was separated via centrifuging and washing with absolute ethanol and deionized water three times. Finally, the green product was collected, dried under vacuum and stored at room

temperature.

Preparation of Ru@SiO_2-Au NPs

In detail, it was divided into three steps. Firstly, a simple one-pot polymerization reaction was used to prepare for Ru@SiO_2, in short, 1.77 ml of TX-100, 7.5 ml of cyclohexane, 1.8 mL of 1-hexanol were added to a clean flask and the mixed solution was stirred at 25 °C water bath. After the above turbid solution was clear, 80 μL of 0.1M $Ru(bpy)_3Cl_2 \cdot 6H_2O$ were dissolved in 340 μL of ultrapure water, 100 μL of tetraethyl orthosilicate and 60 μL of NH_4OH, all were added successively in it. Subsequently, the mixed solution was stirred at 25 °C for 24 h. 10 ml of acetone was added to the obtained Ru@SiO_2, then centrifuged and washed three times with deionized water and absolute ethanol to remove excess molecules on the surface, 5ml of pure Ru@SiO_2 absolute ethanol solution was prepared. Secondly, 200 μL of aminopropyltriethoxysilane (APTES) was added to aboved Ru@SiO_2,which was stirred in a water bath at 25 °C for 40 min in dark. The obtained mixture was centrifuged and washed with absolute ethanol and deionized water four times in turn to remove redundant APTES. Finally, 1 mLof prepared luminophor was mixed with 2 mL of Au NPs in a water bath at 25 °C for 2 h, which was centrifuged and washed with deionized water to obtain the Ru@SiO_2-Au NPs.

Preparation of practical samples

Enrofloxacin was a commonly used medicine to treat livestock, poultry and aquatic diseases, two different meats (chicken and shrimp) and lake water were randomly collected from the vegetable market and lake respectively, which spiked recovery

experiments were carried out. The ethyl acetate method was used to pre-process the purchased and collected samples. First of all, the meat samples were mashed in a mortar and placed in a 50mL centrifuge tube, add 0.01, 1, 100 ng/mL ENR standard solution and 200 μL deionized water respectively, and vortex to mix well. Then add 3 g of anhydrous magnesium sulfate and 20 mL of ethyl acetate. After mixing, vortex on a shaker for 10 minutes, sonicate for 5 minutes, and centrifuge at (6000 rpm, 4min) to retain the supernatant. Aspirate the supernatant and dry it with nitrogen at 45 °C. Add 1 mL of ultrapure water to reconstitute into 0.001, 0.1 and 10 ng/mL samples and then add 3 mL of normal hexane. The alkane was vortexed and centrifuged at 10000 rpm for 5 min. Last but not least, the upper layer of n-hexane was sucked away and the lower layer of clear liquid was filtered to be tested. For water samples, we used 0.45 μm filter membrane to remove insolubles and prepared 0.001, 0.1 and 10 ng/mL with lake water respectively and kept them in a refrigerator at 4 °C for test.

Characterization of nanomaterials

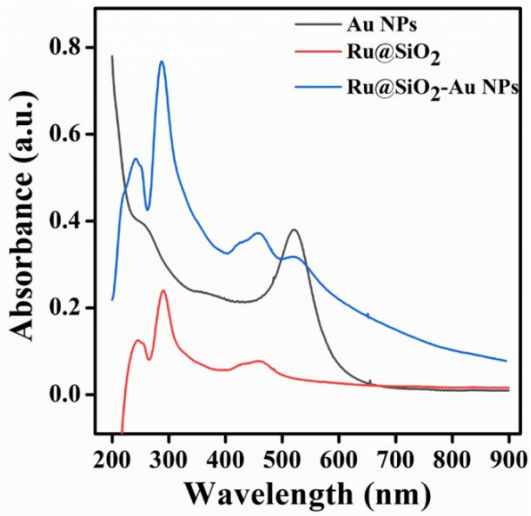


Figure S1. UV-vis spectra of Au NPs, Ru@SiO_2 and Ru@SiO_2-Au NPs

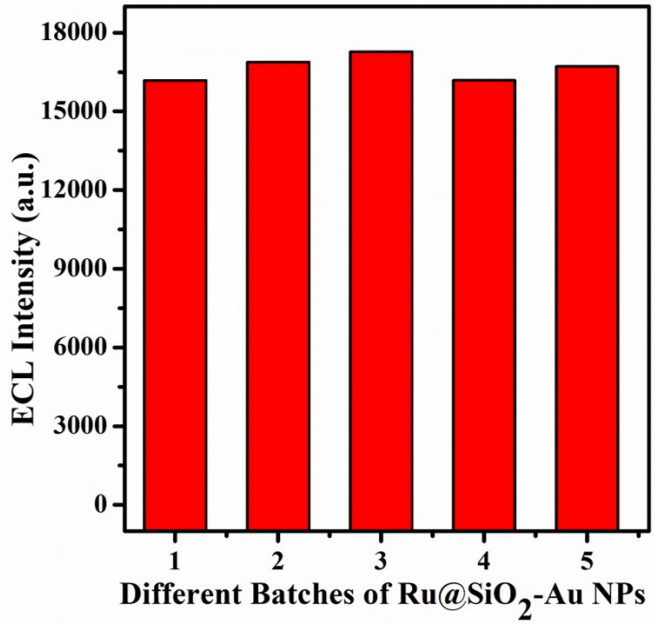


Figure S2. Five groups of $Ru@SiO_2$-Au NPs for electrochemiluminescence

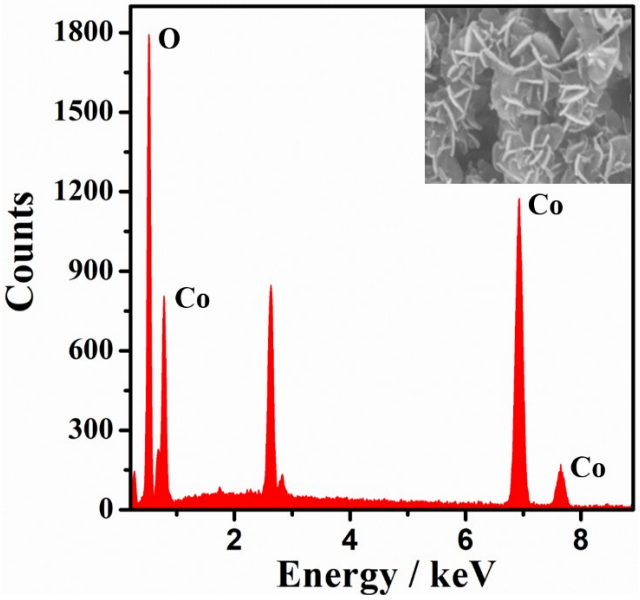


Figure S3. EDS spectrum of 2D $Co(OH)_2$ (Inset: SEM characterization of 2D $Co(OH)_2$)

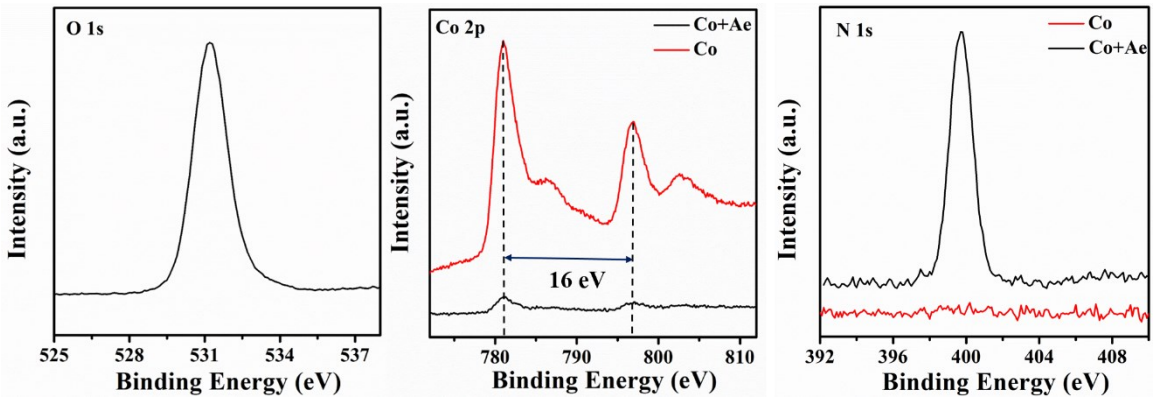


Figure S4. XPS of $Co(OH)_2$ and $Co(OH)_2$-Ae

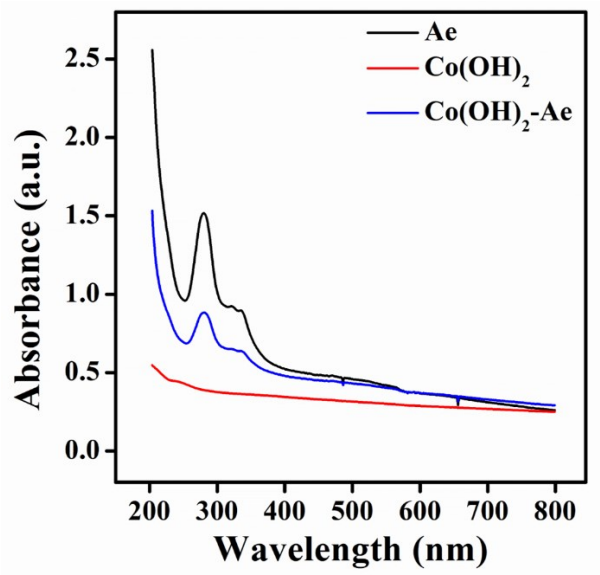


Figure S5. UV-vis spectra of the ENR coating antigen (Ae), $Co(OH)_2$ and $Co(OH)_2$-Ae

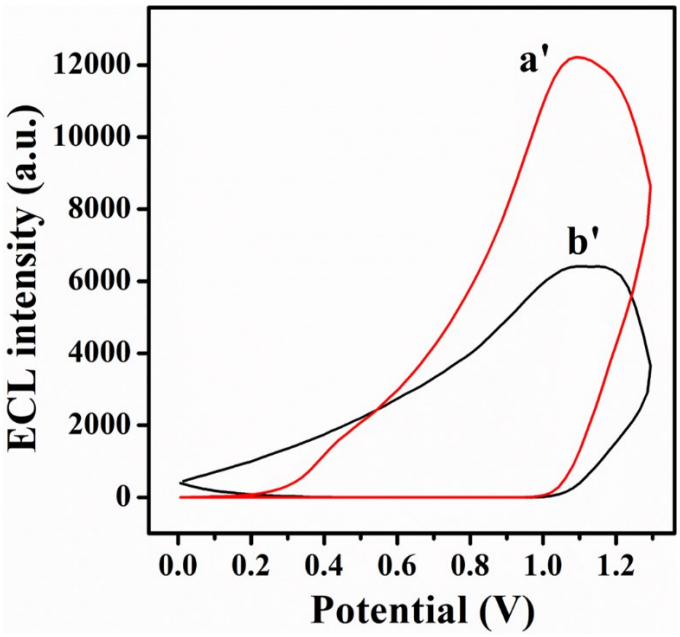


Figure S6. ECL-potential curve of immunsensor in (a') Ru@SiO_2-Au NPs and (b') Ru@SiO_2

Stability of the immunosensor

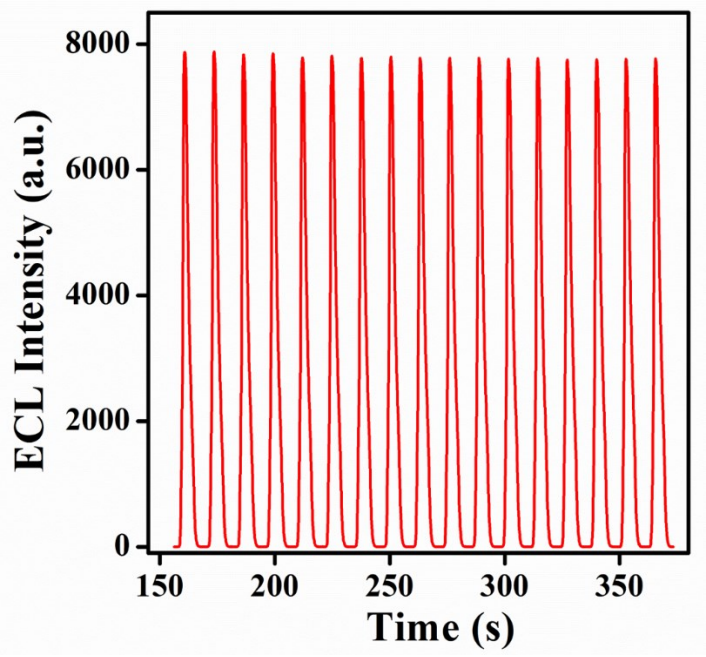
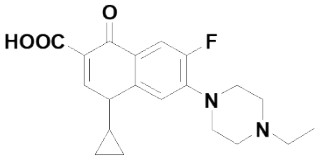
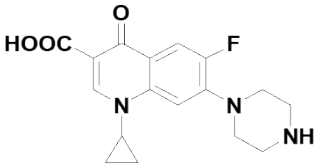
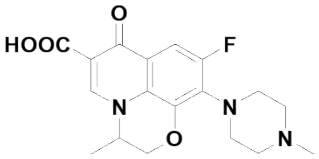
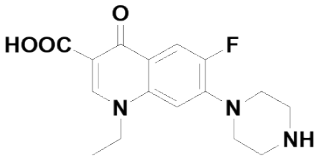
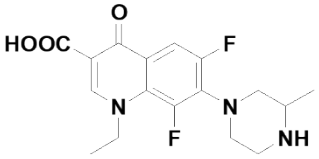
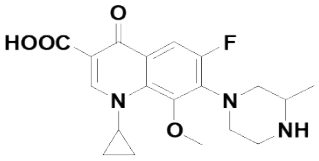


Figure S7. Seventeen continuous cyclic scans of the immunosensor formed with 0.01 ng mL^{-1} ENR standard solutions in TPA buffer solution.

Structural formula of enrofloxacin and interfering substances

Table S1 Structural formula of enrofloxacin and interfering substances

	Structural formula
Enrofloxacin	
Ciprofloxacin	
Ofloxacin	
Norfloxacin	
Lomefloxacin	
Gatifloxacin	

References

[1] L. M. Zhou, J. S. Huang, B. Yu, Y. Liu and T. Y. You, *ACS Appl. Mater. Inter.*, 2015, **7**, 24438-24445.