Supporting Information

Synthesis of Cd^{2+}-Functionalized Titanium Phosphate Nanoparticles and Application as Labels for Electrochemical Immunoassay

Li-Na Feng, Juan Peng, Ying-Di Zhu, Li-Ping Jiang* and Jun-Jie Zhu

[*] Dr. L. P. Jiang
State Key Lab of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210093, P.R. China
Fax: (+86)-25-83597204; E-mail: jianglp@nju.edu.cn

EXPERIMENTAL SECTION

Materials and reagents

Tetrabutyl titanate (TBOT), sodium dodecyl sulfate (SDS), sodium dodecyl benzene sulfonate (SDBS) and phosphoric acid (H_3PO_4) were from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Human IgG (HIgG) (Ag), rabbit anti-human IgG (Ab_1), and goat anti-human IgG (Ab_2) were purchased from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Bovine serum albumin (BSA), poly (allylamine hydrochloride) (PAH) (M_w≈15000), and Tween-20 were from Sigma-Aldrich. Glutaraldehyde (GLU), chloroauric acid ($HAuCl_4 \cdot 3H_2O$) and trisodium citrate were obtained from Shanghai Reagent Company (Shanghai, China). Phosphate buffer saline (PBS) with different pH values were prepared by mixing the stock solution of NaH_2PO_4 and Na_2HPO_4, and then adjusting the pH with 0.1 M NaOH and H_3PO_4. HAc/NaAc solutions with different pH values were prepared by mixing the stock solutions of HAc and NaAc. All other reagents were of analytical reagent grade and used without further purification. Ultrapure fresh water obtained from a Millipore water purification system (MilliQ, specific resistivity is larger than 18 MΩcm, S.A., Molsheim, France) was used in all run.

Characterization

X-ray diffraction (XRD) patterns were performed with a Philip-X'Pert X-ray diffractometer with a Cu Kα X-ray source, λ=0.15418 nm. Scanning electron microscopic (SEM) images were obtained by using a Hitachi S-4800 scanning electron microscope. The high resolution transmission electron micrographs (HRTEM) were obtained on a JEOLJEM 200CX transmission electron microscope with an accelerating voltage of 200 kV. X-ray photoelectron spectroscopic (XPS) measurements were carried out with an ESCALAB MK II X-ray photoelectron spectrometer. Brunauer-Emmett-Teller (BET) data were collected with a Micromeritics ASAP2010 surface area and porosity analyzer at 77 K. The BET surface area was calculated from the linear part of the BET plot. Fourier transform infrared (FTIR) spectroscopic measurements were measured on a Bruker model Vector 22 Fourier transform spectrometer using KBr pressed disks. Atomic absorption spectrophotometer (AAS) was monitored by a Hitachi 180-80 spectrometer.

Electrochemical experiment

Electrochemical experiments were performed on a CHI 660D workstation (Shanghai Chenhua,

Shanghai, China) by using a conventional three-electrode system with a modified glassy carbon (GCE) as the working electrode, a saturated calomel electrode (SCE) electrode as the reference electrode, and a platinum wire as the counter electrode, respectively. SWV experiments were carried out from -1.2 to 0.2 V in 5 mL of buffer solution with different pH values at room temperature.

Synthesis of titanium phosphate (TiP) nanoparticles

Typically, 0.30 g of SDS was dissolved in ethanol (20 mL). H_3PO_4 (3 mL) was added to get a turbid solution and stilled for 4 hours to get white NaH_2PO_4 precipitate. After centrifugation, a mixture of TBOT with ethanol (1 mL/6 mL) was fast dropped into the SDS/ethanol solution to get a stable mixture solution by stirring. Subsequently, the mixture was placed in a flask equipped with a condenser and stirred at 80 °C for 6 h. The solid product was washed with DI water and ethanol three times, respectively. Finally, the white product was dispersed in 6 mL DI water to get a dispersion with the concentration of 40 mg/mL.

Typically, 0.36 g of SDBS was dissolved in ethanol (20 mL). H_3PO_4 (3 mL) was added to get a turbid solution and stilled for 4 hours to get white NaH_2PO_4 precipitate. After centrifugation, a mixture of TBOT with ethanol (1 mL/6 mL) was fast dropped into the SDBS/ethanol solution to get a stable mixture solution by stirring. Subsequently, the mixture was placed in a flask equipped with a condenser and stirred at 80 °C for 6 h. The solid product was washed with DI water and ethanol three times, respectively. Finally, the white product was obtained.

Synthesis of metal-ion functionalized titanium phosphate

First, 1 mL of TiP nanoparticles (40 mg/mL) were dispersed in 17 mL of 10 mM $Cd(NO_3)_2$ aqueous solution and stirred at 50 °C for 24 h. Then, the resulted hybrid nanoparticles were obtained by centrifugation and rinsed with water for several times. The Cd^{2+}-TiP product was dispersed in 2 mL DI water to get a dispersion TiP-Cd^{2+} with the concentration of 20 mg/mL.

Fabrication of Ab_2-TiP-Cd^{2+} bioconjugates

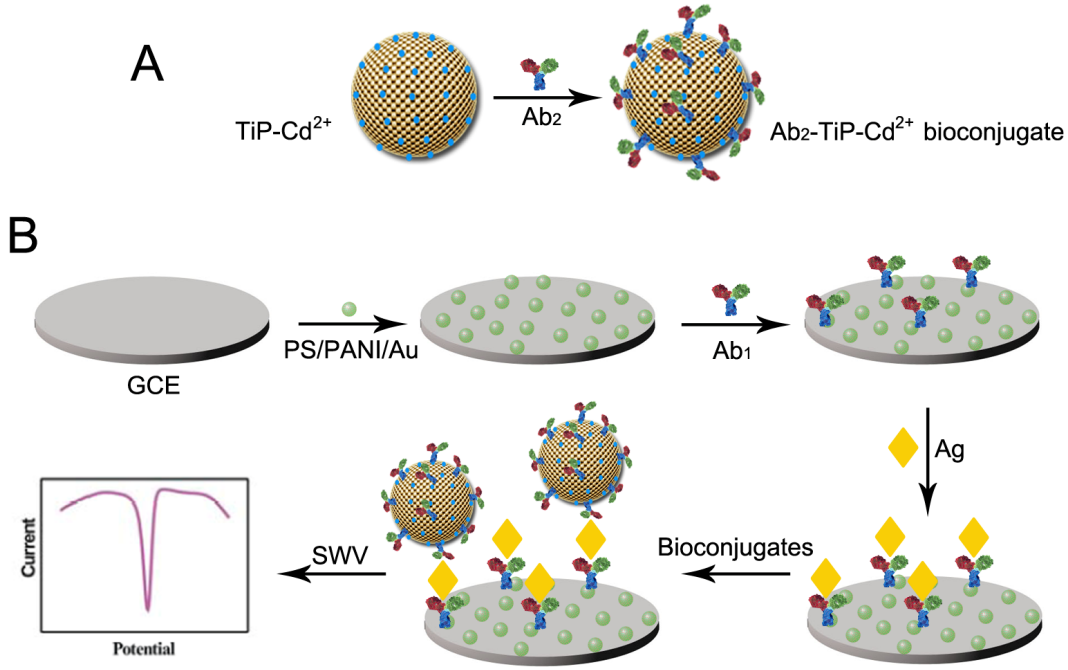
First, TiP-Cd^{2+} hybrid was dispersed in 7.5 mL PAH (2 mg/mL) aqueous solution and sonicated for 20 min. Then, the hybrid was washed with DI water and dispersed in 2 mL GLU (wt. 2.5%). Subsequently, the hybrid was added into 100 μL Ab_2 (1 mg/mL) solution and shaked for 30 min. After centrifuged and washed with PBST, the obtained biocongugate was further washed with PBS for at least three times and resuspended in 5 mL PBS as the assay solution.

Synthesis of PS/PANI/Au nanospheres

First, 10 mg of as-received PS beads was dispersed in 2 mL concentrated sulfuric acid and stirred vigorously for 24 h. The sulfonated PS beads were washed with DI water and dispersed in 3 mL deionized water. 100 μL, 0.1 M aniline solution in ethanol was added and the mixture was kept stirring in an ice bath for 1 h. Then, 1 mL of 10 mM ammonium persulfate solution in 1 M HCl was introduced and the coating reaction was allowed to proceed for 12 h in an ice bath. Subsequently, the dark-green powder was separated by centrifugation and washed with ethanol three times and then washed with water. Finally, the hybrid was dispersed in gold nanoparticle solution and stirred for 12 h. The product was obtained after centrifugation and washed with DI water.

Immunoreaction and measurement procedure

The immunoassay procedure was illustrated in Scheme S1. Prior to use, the GCE with a diameter of 3 mm was polished using 0.3 and 0.05 μm alumina slurry followed by rinsing thoroughly with water. After successive sonication in 1:1 nittric acid, acetone and water, the GCE was allowed to dry at room temperature. 5 μL of PS/PDDA/Au composite solution was dropped on the fresh pretreated GCE and allowed to dry at 4 °C overnight. Then the modified electrode was washed with PBST and immediately incubated with 50 μL of 0.1 mg/mL Ab_1 in pH 7.4 PBS for 12 h at 4 °C as the biomolecules could retain its bioactivity for a long time at the temperature. Next, the electrode was rinsed with PBST to remove physically absorbed Ab_1 and blocked with 50 μL 2 % BSA solution containing 0.05% Tween for 50 min at 37 °C to block possible remaining active sites against non-specific adsorption. After washing with PBST, the Ab_1 modified electrode was incubated with 50 μL of the target HIgG antigen for 50 min at 37 °C as the immunoreaction could obtain higher efficiency at this temperature. Finally, the electrode was incubated with 50 μL of Ab_2-TiP-Cd^{2+} bioconjugate solution for 50 min at 37 °C and then washed thoroughly with PBST to remove non-specifically bounded conjugates. Electrochemical measurement was carried out in 5 mL of pH 3.6 HAc/NaAc and SWV scan was performed from -1.2 to -0.2 V.



Scheme S1. (A) Schematic illustration of the fabrication process of Ab_2-TiP-Cd^{2+} bioconjugate. (B) Principle of sandwich immunoassay using Ab_2-TiP-Cd^{2+} bioconjugates.

RESULTS AND DISCUSSION

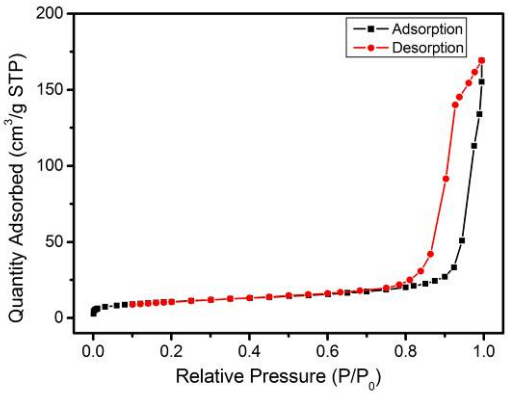


Fig. S1 N_2 adsorption-desorption isotherms of TiP nanoparticles.

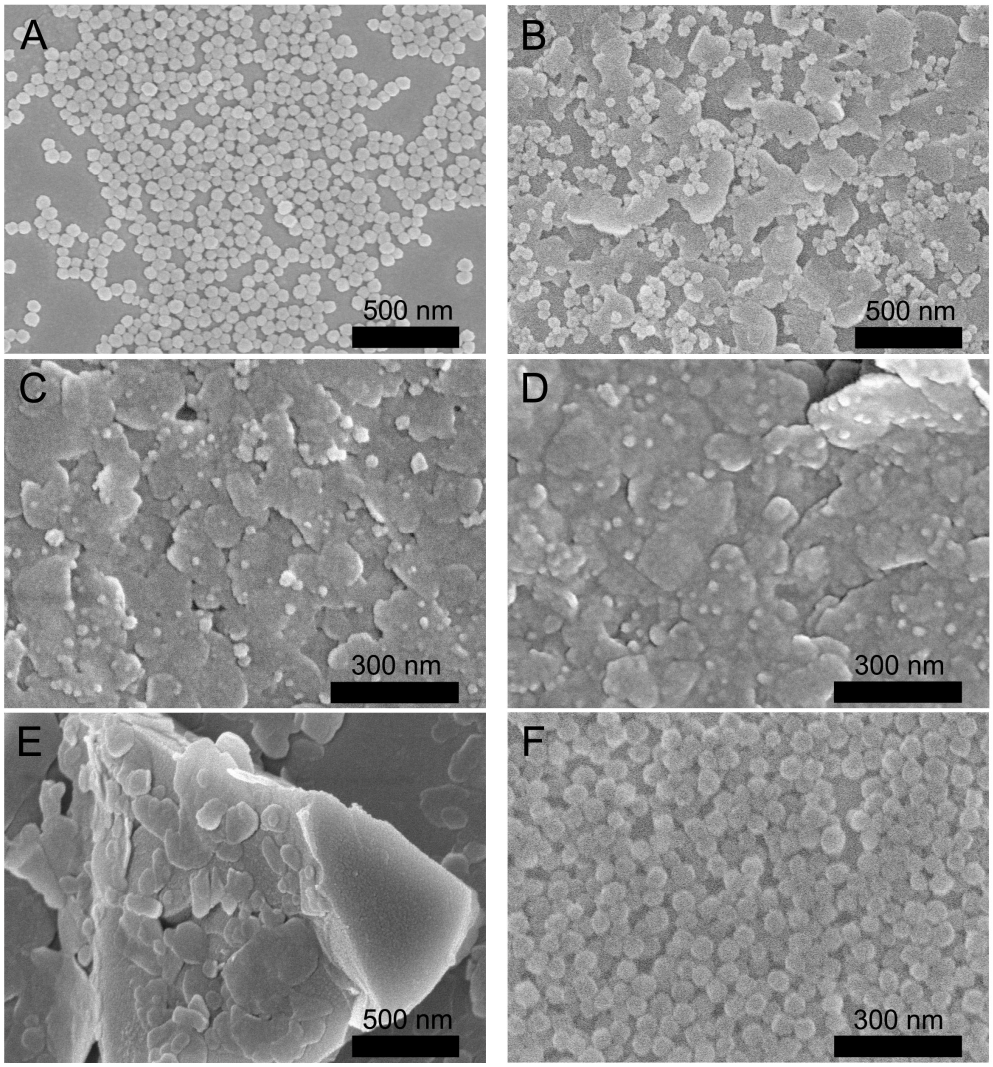


Fig. S2 SEM images of the as-synthesized TiP samples in the presence of (A) 34.7 mM SDS, (B) 23.1 mM SDS, (C) 8 mM SDS, (D) 0.8 mM SDS, (E) in the absence of SDS, and (F) 34.7 mM SDBS.

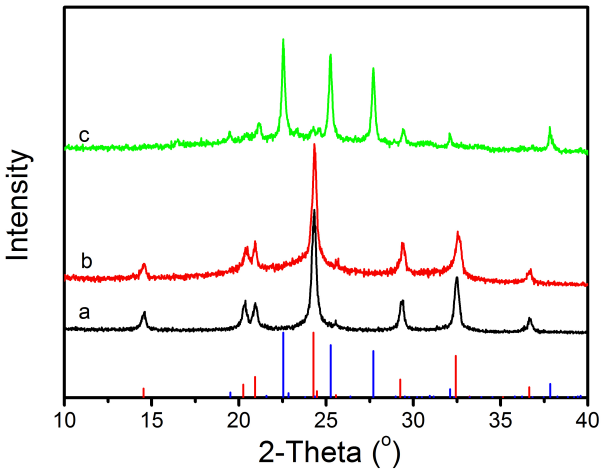


Fig. S3 XRD patterns of TiP samples with 34.7 mM of (a) SDS, (b) SDBS, (c) no surfactant. Red and blue vertical lines are relative to $NaTi_2(PO_4)_3$ (JPCDS No.33-1296) and TiP_2O_7 (JPCDS No.38-1468), respectively.

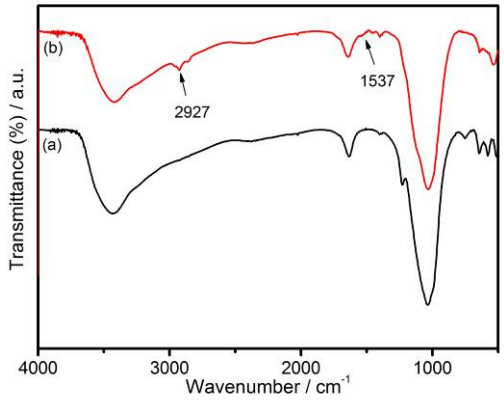


Fig. S4 FT-IR spectra of (a) TiP and (b) Ab_2-TiP-Cd^{2+}.

The TiP-Cd^{2+} hybrid can inherit the advantages of its parent materials, such as good solubility and dispersity in water. And cadmium ions could be detected as a good electrochemical signal. The TiP-Cd^{2+} hybrid offers a friendly microenvironment for immobilization of antibodies (Ab_2) to fabricate Ab_2-TiP-Cd^{2+} architecture. FT-IR spectrum was used to characterize the Ab_2-TiP-Cd^{2+} bioconjugates as shown in Fig. S4. In the spectra, the broad peak at 3430 cm^{-1} and the one at 1634 cm^{-1} correspond to the surface-adsorbed water and hydroxyl groups. The very weak peak of phosphoryl (P=O) at 1400 cm^{-1} indicates that a little amount of free PO_4^{3-} is present in the TiP nanoparticles, which is in agreement with the result of the XPS measurement. Compared with spectrum of TiP (curve a), a new band at 2927 cm^{-1} appears due to the CH_n, and the transmission around 1537 cm^{-1} matches well with that from amine group of Ab_2 in curve b.

Optimization of assay conditions

By using the Ab_2-TiP-Cd^{2+} bioconjugates as labels, a promising immunosensor for the electrochemical detection of HIgG was developed. The electrochemical signal of cadmium ions in

Ab_2-TiP-Cd^{2+} bioconjugates was directly tested by high sensitive square wave voltammetry (SWV). No stripping process was included here and the method was quite simple for detection. The SWV response was strongly influenced by the assay conditions. Therefore, the incubation time, incubation temperature and the pH value of detection solution were investigated (Fig. S5). As shown in Fig. S5A, with the incubation time increased and tended to a steady value after 50 min, indicating a tendency of thorough attachment of Ab_2 on the sensor. Longer incubation time did not enhance the peak current. Therefore, to maximize the signal and minimize the assay time, 50 min was chosen as the optimal incubation time. Fig. S5B shows the effect of incubation temperature on amperometric response. The results suggested that the maximum response occurred at incubation temperature of 37 °C. The lower responses at other temperatures were attributed to the lower reaction rate at lower temperatures and the instability of labeled antibody at higher temperatures. Therefore, 37 °C was selected as incubation temperature for the immunoassay using Ab_2-TiP-Cd^{2+} bioconjugates as a label. The pH value of the detection solution is also an important parameter. The acidity of the solution greatly affects the activity of the immobilized protein. Fig. S5C shows the effect of pH of the detection buffer solution (electrolyte) on the current of the immunoassay over a pH range from 2.0 to 8.0 at 100 ng/mL of HIgG. The results indicated that the excellent pH value was 3.6. It could be attributed to the good ion-exchange rate during acid condition.

Usually, it is important for the oxidizing signals of the cathodic polarization at a certain potential. However, herein, the response of labeled cadmium ion was not affected by electrochemical pretreatment for cathodic polarization. Particularly, the HIgG detection was not affected by the oxygen background, which could further simplify the process for the immunoassay.

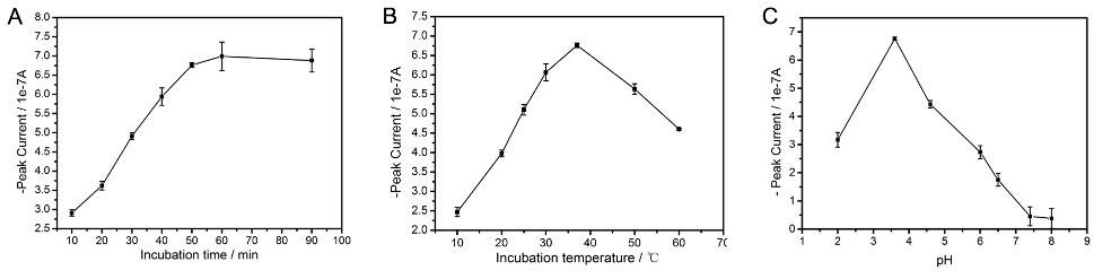


Fig. S5 Effects of (A) incubation time, (B) incubation temperature and (C) pH value of buffer solution in the immunoassay.

Specificity, reproducibility and stability of the immunoassay

Specificity is an important criterion for any analytical tool. Other proteins such as carcinoembryonic antigen (CEA), bovine serum albumin (BSA) and cardiac troponin I (cTnI) were used as the interfere to evaluate the specificity through comparing the electrochemical signals of 100 ng/mL HIgG solution with a same solution containing additional interferential substance of 500 ng/mL. The ratio of currents for HIgG alone and a mixture containing each interfering substance were 0.932, 0.895, and 0.927, respectively, that is, the specificity of the proposed immunoassay was acceptable. Immunoassay reproducibility was estimated by assaying one HIgG level for five replicate measurements with relative standard deviations (RSD) of 5.8 %. The storage stability of Ab_2-TiP-Cd^{2+} bioconjugates was investigated by comparison with the

stripping signals after a sandwich immunoreaction, which illustrated that 91.0 % of the initial response remained after 30 days' storage at 4 ^{o}C. This indicates good storage stability for the bioconjugates.

Clinical application in human serum

The feasibility of the immunoassay for clinical application was investigated by analyzing real samples, in comparison with the commercial ELISA method. The serum samples were diluted with PBS of pH 7.4. Table S1 describes the correlation between the results obtained by the proposed immunoassay and the ELISA method. No significant difference is observed between the results given by the two methods, that is, the proposed immunoassay can be satisfactorily applied to clinical determination of HIgG in human plasma.

Table S1 Assay results of clinical serum samples for the proposed and ELISA methods.

Serum	Proposed method[a] [ng/mL]	ELISA[b] [ng/mL]	Relative deviation [%]
1	140	143	-2.1
2	96	95	1.1
3	21	22	-4.5
4	823	789	4.3
5	165	162	1.8
6	269	276	-2.5

[a] The average value of three successive determinations.

[b] Given by the Affiliated Drum Tower Hospital of Nanjing University Medical School.