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Redox active molybdophosphate produced by $Cu_3(PO_4)_2$ nanospheres for enhancing enzyme-free electrochemical immunoassay of C-reactive protein

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Experimental Section

Reagents and apparatus

Dopamine Hydrochloride was purchased from Aladdin Industrial Corporation. Tris(hydroxymethyl)aminomethane (Tris) and urea was obtained from Shanghai Macklin Biochemical Technology Co., Ltd. CuSO4 and Sodium dodecyl sulfate were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). C-reactive protein (CRP) and paired antibody were acquired from Shanghai Linc-bis Science Co., Ltd. PBS (pH = 7.4) solution and Tris-HCl buffer (pH 7.4, 10 mM) were used as the buffer solution. Other reagents belong to analytical reagent and were well-preserved in the ambient temperature. Ultrapure fresh water (18.25 MΩ cm) was used throughout the experiment.

All electrochemical measurements were performed on a CHI-760E electrochemical workstation (Shanghai CH Instruments Co., Ltd. China). The transmission electron microscopy (TEM) images were acquired from a HT7700 microscope (Japan). The scanning electron microscopy (SEM) images were obtained using field emission SEM (ZEISS, Germany). X-ray diffraction (XRD) was recorded by a Rigaku D/MAX 2200 X-ray diffractometer (Tokyo, Japan). Fourier transform infrared spectroscopy (FT-IR) was collected using a FT-IR-410 infrared spectrometer (JASCO, Japan). In the electrochemical measurements: glassy carbon electrode (GCE) as the working electrode, a saturated calomel electrode as the reference electrode, and a platinum wire electrode as the counter electrode were used.

Preparation of $Cu_3(PO_4)_2$ nanospheres

$Cu_3(PO_4)_2$ nanospheres were synthesized according to a previous report.[1] Briefly, 6 g urea, 0.5 g sodium dodecyl sulfate and 80 mg $CuSO_4$ were mixed with the deionized water (100 mL) to obtain a transparent solution, and 26 µL H_3PO_4 were added to the solution, following stirred for 5 min. The solution was then transferred to the hydrothermal synthesis reactor and maintained at 80 °C for 12 h. $Cu_3(PO_4)_2$ nanospheres were recovered by centrifugation from the glaucous suspension and washed with DI water for three times to remove the residual surfactant completely, and finally dried in a vacuum drying chamber at 45 °C.

Preparation of $Cu_3(PO_4)_2$/PDA/Ab_2

Polydopamine (PDA) was used as a bridge between $Cu_3(PO_4)_2$ nanospheres and report antibody (Ab_2).[26] In order to coat a thin layer of PDA on the surface of $Cu_3(PO_4)_2$ nanospheres, $Cu_3(PO_4)_2$ nanospheres were dispersed into dopamine hydrochloride solution (Tris-HCl buffer, pH 8.5) and stirred for 1 h. After centrifugation and extensive washing with deionized water, the $Cu_3(PO_4)_2$/PDA was dispersed into PBS buffer solution (pH 7.4). 300 µL of anti-CRP (1 µg/mL) solution was then added and shaken for 6 h at 4 °C, followed by centrifuge and washed with Tris-HCl buffer solution (pH 7.4) three times. The final signal label suspension ($Cu_3(PO_4)_2$/PDA/Ab_2, 1 mg/mL) was stored at 4 °C in Tris-HCl buffer solution (pH 7.4) before use.

Preparation of PDA/rGO nanocomposites

Graphene oxide was prepared following the slight modification of the Hummers method. [2] The as-prepared purified graphene oxide (1 mg/mL, 10 mL) was dispersed in the 10 mM of Tris-HCl buffer solution (pH 8.5) containing 2 mg dopamine. The mixture was stirred for 6 h at 60 °C. Thereafter, PDA/rGO was obtained after centrifuging and washing for three times with ultrapure water, and then dried under vacuum.

Fabrication of the immunosensor

For the fabrication process of the immunosensor, a glassy carbon electrode (GCE) with the diameter of 3 mm was polished to a mirror-like surface using alumina slurry and washed extensively with pure water before use. First of all, 6 µL of 0.5 mg/mL PDA/rGO solution as substrate of sensor was dripped on the electrode and dried for 30 min. Subsequently, 6 µL of anti-CRP Ab_1 was added on the modified electrode and then incubated for 30 min to obtain Ab_1/PDA/rGO/GCE. In addition, the electrode was treated with 1 % BSA for 20 min to block the electrode from nonspecific adsorption. Consequently, 6 µL of CRP solutions of different concentrations were dripped on the electrode and incubated at 37 °C for 40 min. Then 6 µL of signal label solution was coated onto the electrode and incubated for another 40 min. Following that, the electrode was washed completely to remove unbounded labels. After the electrode incubated with 6 µL of Na_2MoO_4 solution (6 mM) for 30 min.

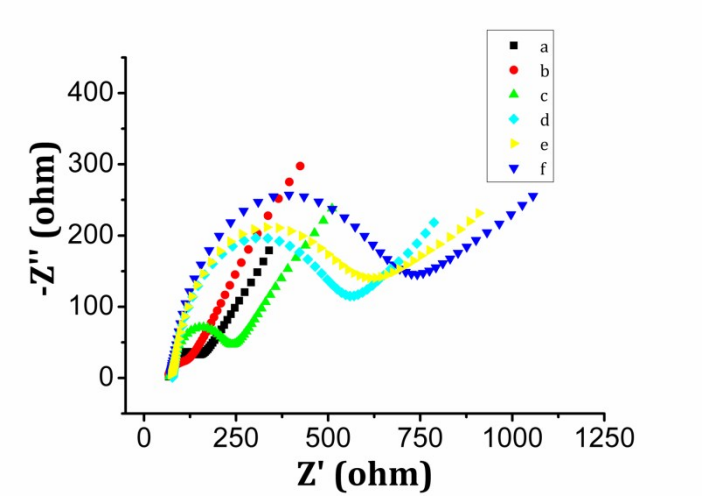

Fig. S1 Electrochemical impedance spectra of bare GCE (**a**), PDA/rGO/GCE(**b**), Ab_1/PDA/rGO/GCE (**c**), BSA/ Ab_1/PDA/rGO/GCE (**d**), antigen/BSA/Ab_1/PDA/rGO/GCE (**e**), and $Cu_3(PO_4)_2$/PDA/Ab_2/antigen/BSA/Ab_1/PDA/RG O/GCE (**f**) measured in 5.0 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.1 M KCl.

Table S1 Comparison with other methods for the detection of CRP

Method type	Linear range (ng/mL)	Detection limit (ng/mL)	Reference
Magnetic impedance-based immunosensor	1-10	1	[3]
Fiber based ELISA	0-0.1	0.013	[4]
Colorimetric immunosensor	0.03–81	0.07	[5]
Electrochemical immunosensor	5.0×10^{-4} –1	1.7×10^{-4}	This work

Table S2 Recovery test of CRP by the developed immunosensor in human serum

Sample	Amount of addition [ng/mL]	Amount of detection [ng/mL]	RSD [%, n=5]	Recovery[%]
1	0.5	0.5±0.02	3.2	99.2
2	1.5	1.5±0.05	2.7	99.6
3	2.5	2.5±0.04	1.9	99.5
4	3.5	3.5±0.06	1.3	100.2
5	4.5	4.5±0.07	1.2	99.7

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