Supporting Information

Ionic liquid-assisted chemiluminescent immunoassay of prostate specific antigen using nanoceria as alkaline phosphatase-like nanozyme label

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Experimental Section

Reagents and materials

4-(2-Hydroxyethyl)-1-piperazine-1-ethanesulfonic acid (HEPES) was purchased from Biofroxx. Cerium(III) nitrate hexahydrate, polyacrylic acid (PAA, 5000 MW) and ammonium hydroxide were obtained from Sinopharm Chemical Reagent Co., Ltd. 1-Butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), p-nitrophenyl phosphate (*p*-NPP), 4-methylumbelliferyl phosphate disodium salt (4-MUP), CDP-star, prostate specific antigen (PSA) and hemoglobin (Hb) were purchased from Sigma-Aldrich. Mouse monoclonal antibody to anti-prostate specific antigen (mAb), and rabbit polyclonal antibody to anti-prostate specific antigen (pAb) were obtained from Abcam plc. Bovine serum albumin (BSA), human serum albumin (HSA), lysozyme, trypsin and cellulase were obtained from Beijing Soleibao Science & Technology Co., Ltd. Superoxide dismutase (SOD) was purchased from Shandong Xiya Reagent Co., Ltd. Xanthione oxidase (XOD) was purchased from Shanghai Yuanye Biological Technology Co., Ltd. Glucose oxidase (GOx) was obtained from Shanghai Sangon Biotechnology Co., Ltd. All other reagents were of analytical grade. The 96-well polystyrene microplates were purchased from Greiner bio-one GmbH.

Instrumentation

Transmission electron microscopic (TEM) images were acquired on a Tecnai G2 20 S-TWIN transmission electron microscope (Thermo Fisher Scientific, USA). Zeta potential (ζ) measurements were performed using a NanoBrook Omni (Brookhaven Instruments, USA). X-ray photoelectron spectroscopy (XPS) measurements were performed on an X-ray photoelectron spectrometer with monochromatized Al Kα radiation (Thermo Fisher Scientific, USA). Chemiluminescent (CL) intensity was collected from a CL analyzer (Xi'an Remex Analyse Instrument Co., Ltd, China). UV-vis absorption measurements were carried out on a UV2550 UV-Vis Spectrophotometer (Shimadzu, Japan). Fluorescence measurements were performed on a PerkinElmer Model LS-55 Luminescence Spectrometer (PerkinElmer, USA). CL measurements of 96-well microplates were carried out on a SpectraMax i3x reader (Molecular Devices, USA). CL images were obtained on an IVIS Lumina III imaging system (PerkinElmer, USA).

Synthesis of PAA-CeO_2

PAA-CeO_2 were synthesized through a precipitation method by using ammonium hydroxide as the precipitating agent in the presence of PAA.[1] Briefly, 0.54 g of cerium (III) nitrate and 0.05 g of PAA was completely dissolved in 1.25 mL of deionized water. Then the resulted mixture was introduced into 7.5 mL of ammonium hydroxide. After continuous stirring for 1 day at room temperature, the suspension was centrifuged at 5000 rpm for 5 min and washed with deionized water until the supernatant was neutral. Finally, the precipitation PAA-CeO_2 was redispersed in 10 mL deionized water for further use.

Effect of [BMIM][BF_4] on the hydrolysis of different phosphatase substrates

In brief, [BMIM][BF_4] with different concentrations (i.e. 0%, 4%, 8%, 16% and 20%) were mixed with chromogenic substrate *p*-NPP (2 mM), fluorescent substrate 4-MUP (20 μM) or chemiluminescent substrate CDP-star (25 μM) in 20 mM of HEPES buffer (pH 8.5). Then, the time-dependent response of the mixture in the presence of PAA-CeO_2 as phosphatase mimic was monitored.

Preparation of PAA-CeO_2-pAb

The detection antibody (pAb) and PAA-CeO_2 were conjugated with reference to previous literature with slight modifications.[2] Firstly, 2 mL PAA-CeO_2 (1 mg/mL) was adjusted to pH 8.5 with 50 mM of K_2CO_3 and then 20 μL pAb (1 mg/mL) was added. After incubation at room temperature for 6 h, the product was centrifuged and washed three times with 10 mM of Tris-HCl buffer (pH 7.4). Finally, the precipitation PAA-CeO_2-pAb was dispersed in 40 μL of Tris-HCl buffer (10 mM, pH 7.4) containing 1% BSA for future use.

CL immunoassay of PSA

A typical sandwich-type CL immunoassay by using PAA-CeO_2 as a label was performed as follows. Firstly, 50 μL of the capture antibody (mAb) solution (4 μg/mL) in Na_2CO_3-$NaHCO_3$ coating buffer (50 mM, pH 9.6) was added into each well of the 96-well polystyrene microplates and kept at 4 ^oC overnight. Secondly, after removing the uncoated mAb solution, the wells were washed for three times with 200 μL of PBST (10 mM PBS containing 0.05% v/v Tween 20, pH 7.4) and then sealed with 200 μL 1% BSA in PBST buffer for 1 h at 37 ^oC. Thirdly, after washing the wells three times with PBST to remove excess BSA, 50 μL PSA standards with different concentrations from 0 pg/mL to 10 ng/mL diluted in PBST buffer was

added. After incubation for 2 h at 37 ^{o}C, the wells were washed three times with PBST. Fourthly, 50 μL of 100-fold diluted PAA-CeO_2-pAb was injected into each well. After incubation at 37 ^{o}C for 1 h, the unbound PAA-CeO_2-pAb was washed with Tris-HCl buffer (10 mM, pH 7.4) for three times. Finally, 100 μL of 25 μM CDP-star solution including 0% or 16% [BMIM][BF_4] in 20 mM HEPES (pH 8.5) was added into each well. After 14 min of incubation in the dark at room temperature, the microplates were immediately placed in a SpectraMax i3x plate reader to record the CL intensities.

For the detection of PSA in serum samples, serum were diluted 50-fold with PBST and then different concentrations of PSA (i.e. 5 pg/mL, 50 pg/mL and 500 pg/mL) were spiked. The spiked samples were analyzed through the same procedure as described above for PSA standards.

Characterization of PAA-CeO_2.

TEM characterization shows the well-dispersed PAA-CeO_2 with an average particle size of *ca.* 6 nm (Fig. S1, ESI†). For Ce 3d XPS spectrum of PAA-CeO_2, the two doublets including v_0 (879.9 eV), v' (884.4 eV), u_0 (897.9 eV), and u' (902.6 eV) were corresponded to Ce^{3+} and the three doublets denoted as v (882.0 eV), v'' (888.4 eV), v''' (896.0 eV), u (900.4 eV), u'' (907.0 eV) and u''' (916.2 eV) were attributed to Ce^{4+} (Fig. S2A, ESI†).[3] This indicates that Ce^{3+} and Ce^{4+} oxidation states coexist in PAA-CeO_2. According to the semi-quantitative formula $\%Ce^{4+} = \frac{A_{Ce^{4+}}}{A_{Ce^{3+}} + A_{Ce^{4+}}} \times 100$ (where $A_{Ce^{3+}}$ and $A_{Ce^{4+}}$ represents the total integrated area of the peaks relevant to Ce^{3+} and Ce^{4+}, respectively)[4], the ratio of Ce^{4+}/Ce^{3+} was estimated to be 1.56:1, suggesting the dominant valence of cerium in the sample is tetravalent. For the O 1s XPS spectrum, the peak centered at 530.7 eV was associated to the surface oxygen while the peak located at 528.9 eV was attributed to the lattice oxygen of Ce-O (Fig. S2B, ESI†), which is consistent with previous reports.[5, 6] The apparent zeta potential of nanoparticles was measured to be -8.9 mV, suggesting the successful coating of negatively charged PAA on nanoceria.[7] The results indicate the successful preparation of PAA-CeO_2.

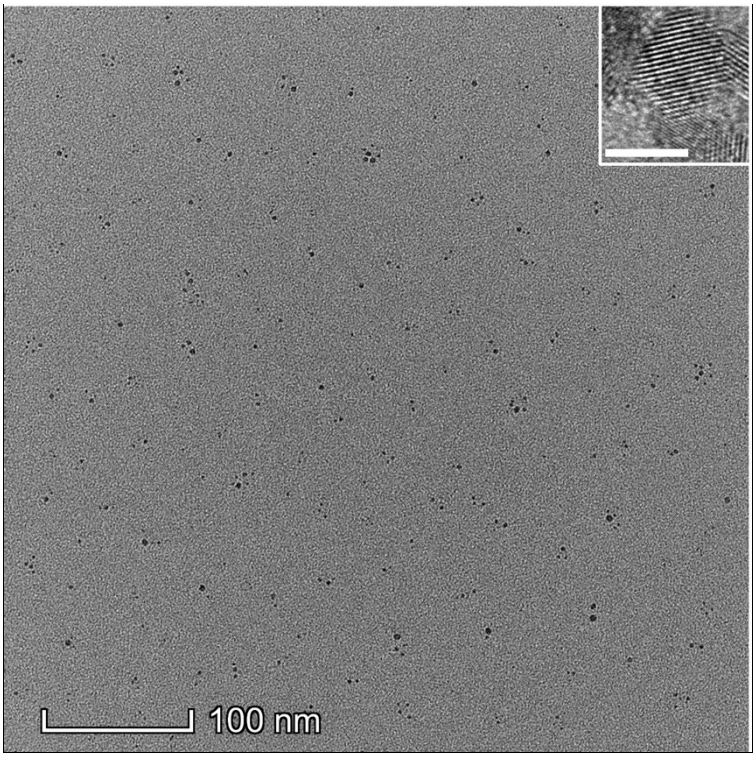

Fig. S1 TEM image of PAA-CeO_2. Inset: the high-resolution TEM image of PAA-CeO_2, the scale bar is 5 nm.

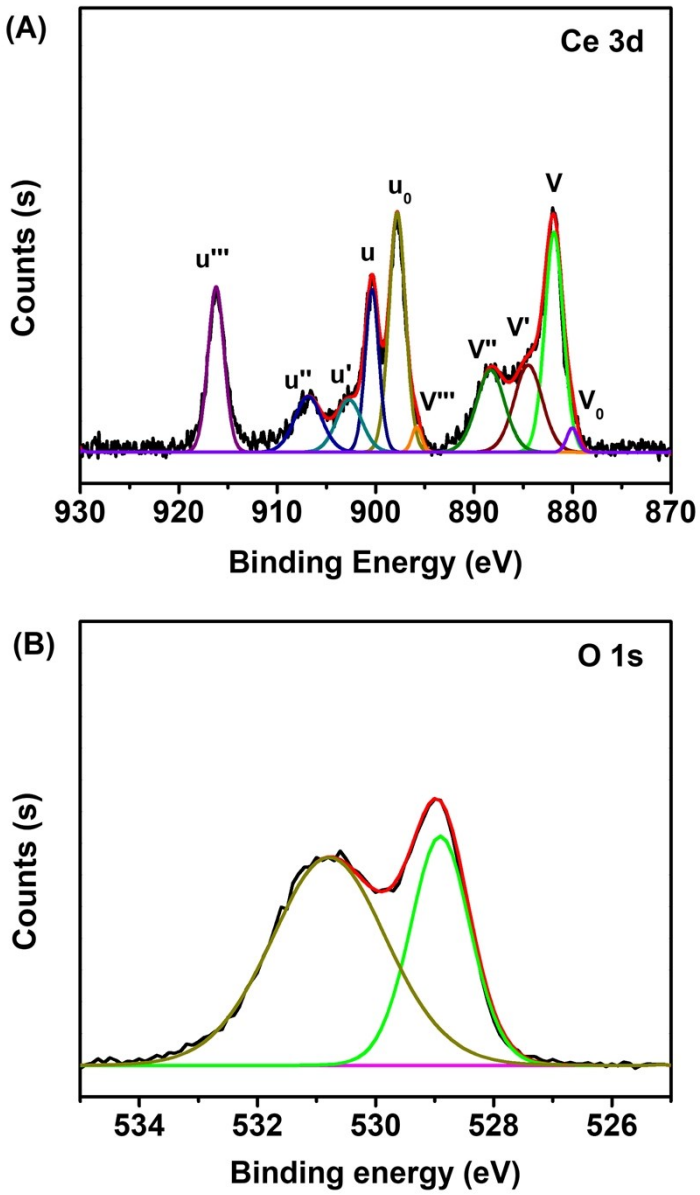

Fig. S2 (A) Ce 3d and (B) O 1s XPS spectra of PAA-CeO_2.

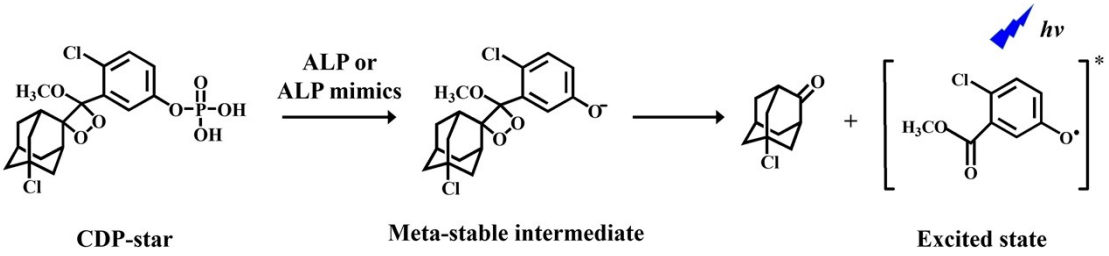

Fig. S3 CL reaction scheme of CDP-star under the catalysis of ALP or ALP mimics. In the presence of the same concentration of CDP-star, higher activity of ALP or ALP mimics leads to higher CL intensity.

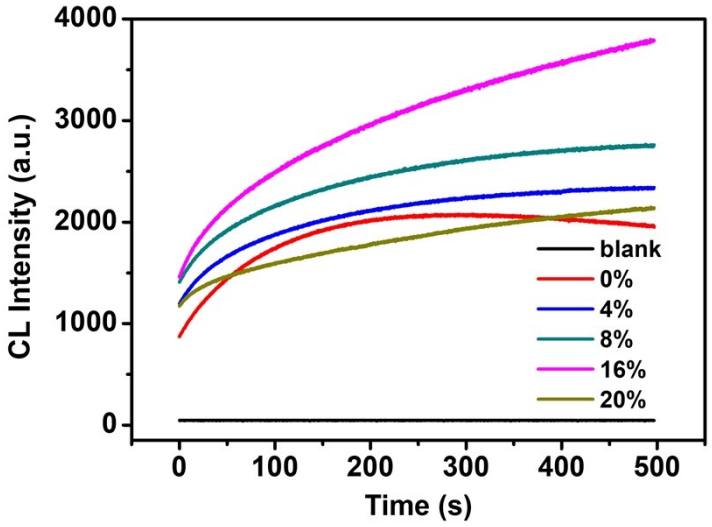

Fig. S4 The time-dependent CL intensity of 0.1 mg/mL PAA-CeO_2 and 25 μM CDP-star in the presence of different concentrations of [BMIM][BF_4]. The blank curve represents the CL intensity of 25 μM CDP-star. The CL experiments were performed in 20 mM HEPES buffer (pH 8.5).

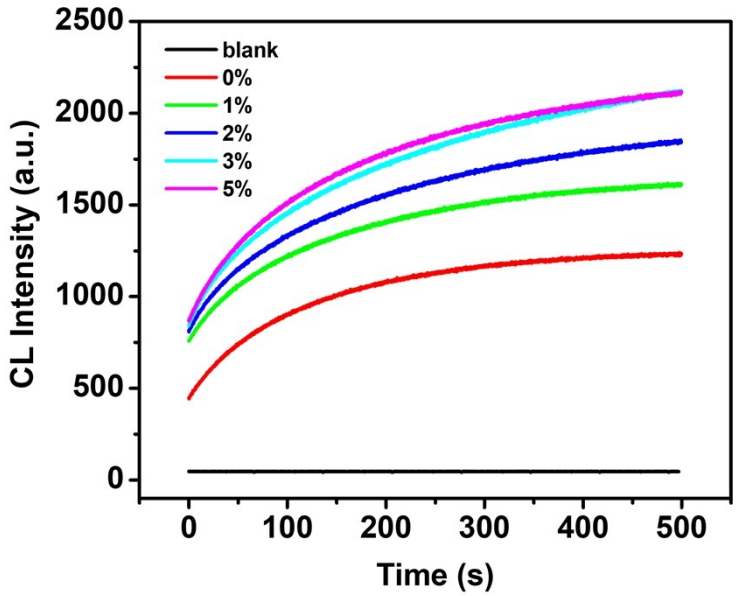

Fig. S5 The time-dependent CL intensity of 0.1 mg/mL PAA-CeO_2 and 25 μM CDP-star in the presence of different concentrations of [BMIM][BF_4]. The blank curve represents the CL intensity of 25 μM CDP-star. The CL experiments were performed in 20 mM HEPES buffer (pH 7.0).

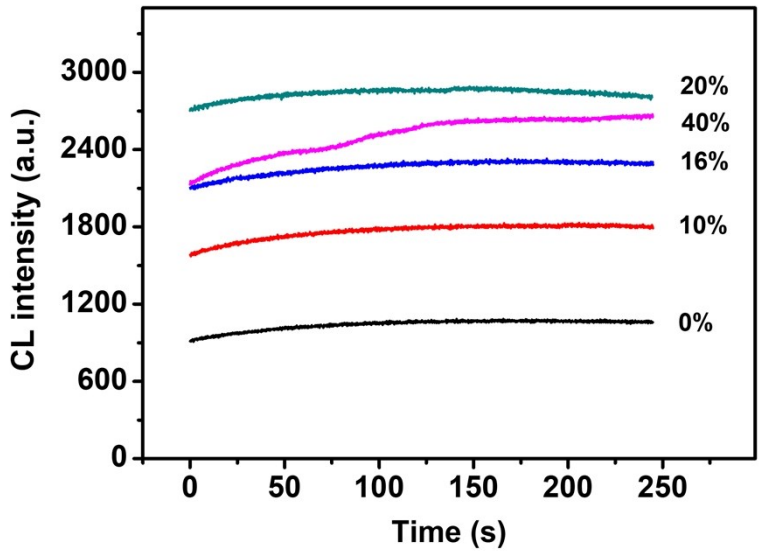

Fig. S6 The time-dependent CL intensity of 0.05 mg/mL PAA-CeO_2 and 25 μM CDP-star in the presence of different concentrations of ethanol. The CL experiments were performed in 20 mM HEPES buffer (pH 8.5).

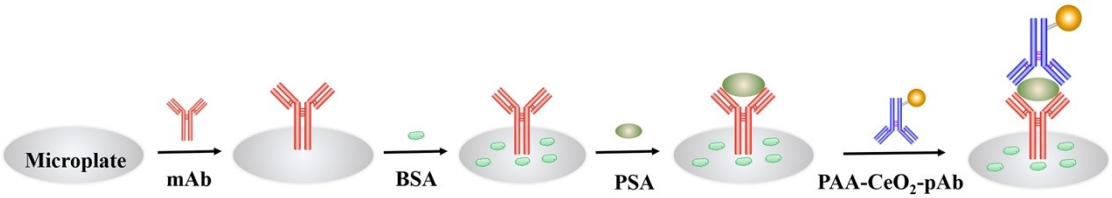

Fig. S7 Schematic illustration of the stepwise formation of PAA-CeO_2-labelled CL immunosensor in 96-well microplates.

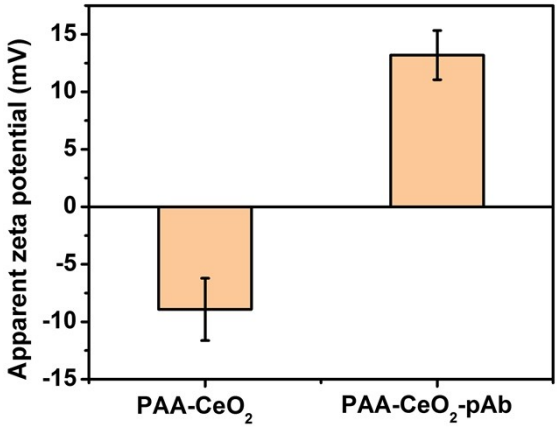

Fig. S8 Apparent zeta potential of PAA-CeO_2 before and after conjugation with pAb.

Table S1 The analytical performance comparison of the proposed method with previous PSA detection methods.

Method	Label probe	Linear range (ng/mL)	Detection limit (pg/mL)	Reference
Electrochemiluminescence	GO@AuNRs	0.0005-5	0.17	8
Surface plasmon resonance	Ab-SA-biotin-FNP NPs	0.001-0.2	1	9
Electrochemiluminescence	SA-PEG UCNPs	0.001-10	0.41	10
Chemiluminescence	Au NPs	0.0001-100	0.05	11
Electrochemistry	Cu^{2+}-ppi	0.01-100	5.2	12
Fluorescence	Fe-MOFs	1-20	180	13
Electrochemistry	Ag/CdO	0.05-50	28	14
Colorimetric/photoacoustic /fluorescence triple read-out detection	Au@Ag@SiO_2	0-10	100	15
Chemiluminescence with ionic liquid assistance	PAA-CeO_2	0.0001-10	0.053	This work

Table S2 Determination of PSA in spiked serum samples (n=3)

Sample	Spiked (pg/mL)	Found (pg/mL)	Recovery (%)	RSD (%)
1	5	4.87	97.4	4.72
2	50	53.72	107.4	5.96
3	500	498.92	99.8	4.20

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