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Supplementary Material

Construction of an Electrochemiluminescence Immunosensor Based on a Pre-Concentration Enhanced Mechanism for Ultrasensitive Detection of CEA

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37 **1. Experimental Section**

38 **1.1 Reagents and Instruments**

39 Hexadecyltrimethylammonium bromide (CTAB), methyl- β -cyclodextrin (β -CD),
40 tris(2,2'-bipyridyl)ruthenium(II) dichloride hexahydrate ($\text{Ru}(\text{bpy})_3\text{Cl}_2 \cdot 6\text{H}_2\text{O}$),
41 cyclohexane, tetraethyl orthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTES),
42 ethanol (CH_3OH), bovine serum albumin (BSA), and carcinoembryonic antigen (CEA)
43 were purchased from Shanghai Macklin Biochemical Technology Co., Ltd., China.
44 Isopropanol (IPA) and copper oxide nanoparticles (CuO NPs) were obtained from Fuyu
45 Chemical Reagent Co., Ltd., China. Zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) and
46 urea were supplied by Tianjin Sheng'ao Chemical Reagent Co., Ltd., China. Additional
47 BSA was acquired from Alfa Aesar (China) Chemical Co., Ltd., China, and additional
48 CEA was purchased from Zhengzhou Bosai Biotechnology Co., Ltd., China.

49 An electrochemiluminescence (ECL) detector (MPI-EII, Xi'an Ruimai Analytical
50 Instrument Co., Ltd., China), an electrochemical workstation (CHI760E, Shanghai
51 Chenhua Instrument Co., Ltd., China), a scanning electron microscope (SEM, Quanta
52 220 FEG, FEI Co., USA), a Fourier transform infrared (FTIR) spectrometer (Nicolet
53 Avatar 360, Thermo Fisher Scientific, USA), an X-ray diffractometer (XRD, D8
54 Advance, BRUKER, Germany), a vacuum drying oven (DZF-6030, Shanghai Yiheng
55 Scientific Instrument Co., Ltd., China), and a centrifuge (RG-160AT, Shanghai
56 Luxiangyi Centrifuge Instrument Co., Ltd., China) were used in this work.

57 **1.2 Synthesis of CDZS-1¹**

58 First, 1.50 g of $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ was uniformly dissolved in 25 mL of
59 deionized water (DI water). Subsequently, the above Zn^{2+} solution was injected into a
60 25 mL aqueous solution containing 5.60 g of 2-methylimidazole, 9 mg of CTAB, and
61 0.75 g of β -CD. The mixture was stirred magnetically for 2 min and then allowed to
62 stand at room temperature (RT) for 2 h. The resulting precipitate (CDZ-1) was
63 centrifuged, washed with DI water, and dried overnight to successfully obtain CDZ-1
64 nanoparticles.

65 CDZS-1 nanocarriers were synthesized via a selective occupation strategy. CDZ-
66 1 (0.50 g), CTAB (0.50 g), and urea (0.30 g) were uniformly dispersed in 15 mL of DI

67 water. Then, cyclohexane (15 mL), IPA (0.46 mL), and TEOS (0.50 mL) were injected
68 into the above mixture, followed by reaction at 70 °C for 16 h. The CDZS-1 nanocarriers
69 were centrifuged, washed with ethanol, and lyophilized for subsequent use.

70 **1.3 Synthesis of CDZS-Ru²**

71 Twenty-five milligrams (25 mg) of CDZS-1 composite was added to 20 mL of
72 N,N-dimethylformamide (DMF) containing 10 mg of Ru(bpy)₃Cl₂•6H₂O, and the
73 mixture was heated at 60 °C for 24 h before heating was terminated. After the composite
74 was cooled to RT, it was washed with DMF 3–5 times until the supernatant became
75 clear, and then vacuum-dried in an oven at 60 °C.

76 **1.4 Synthesis of CuO-NH₂**

77 Thirty-five point two milligrams (35.2 mg) of CuO NPs was ultrasonically
78 dispersed in 64 mL of ethanol. Then, 1.6 mL of APTES was added to the dispersion,
79 and the mixture was refluxed at 70 °C for 1.5 h. After refluxing, the product was washed
80 with ethanol three times, centrifuged, collected, and dried in an oven at 60 °C for 12 h
81 to obtain CuO-NH₂.

82 **1.5 Synthesis of Ab₂-CuO-NH₂**

83 One milligram (1 mg) of CuO-NH₂ was dissolved in 500 μL of DI water to prepare
84 a 2 mg/mL solution, which was ultrasonically dispersed uniformly. Then, 500 μL of
85 Ab₂ solution was added to the above solution, and the mixture was stirred for 12 h.
86 After stirring, 300 μL of 1 wt% BSA solution was added, and the mixture was further
87 stirred for 2 h, followed by freeze-centrifugation. The obtained solid was dissolved in
88 500 μL of DI water and stored at 4 °C in a sealed container for later use.

89 **1.6 Fabrication of the ECL Sensor**

90 Before electrode assembly, the glassy carbon electrode (GCE) was polished with
91 0.05 μm Al₂O₃ polishing powder, rinsed with DI water to remove impurities adsorbed
92 on the electrode surface, and dried under a stream of nitrogen. Ten microliters (10 μL)
93 of CDZS-Ru suspension was dropped onto the polished GCE surface and baked under
94 ultraviolet (UV) light for 30 min. After the electrode was cooled to RT, 12 μL of Ab₁
95 solution was added dropwise onto the electrode surface, and the electrode was
96 incubated in a constant-temperature incubator at 37 °C for 1 h. After incubation, 8 μL

97 of BSA solution was dropped onto the electrode surface to block non-specific binding
98 sites, followed by incubation at 37 °C for 40 min. Subsequently, 12 μL of CEA solutions
99 with different concentrations were added dropwise, and the electrode was incubated at
100 37 °C for 30 min. Finally, 12 μL of Ab₂-CuO-NH₂ secondary antibody label was
101 dropped onto the electrode surface, and the electrode was incubated at 37°C for 1 h.
102 The fabrication of the ECL biosensor was completed after the final incubation.

103 **1.7 CEA Detection Method³**

104 First, cyclic voltammetry (CV) and electrochemical impedance spectroscopy
105 (EIS) were used to characterize the assembly process of the ECL sensor (CDZS-
106 Ru/Ab₁/BSA/CEA/Ab₂-CuO-NH₂) in a mixed solution containing 0.1 M KCl and 5
107 mM [Fe(CN)₆]^{3-/4-}. The successful assembly of the ECL sensor was verified by the
108 change in interfacial electron transfer capability. After successful electrode assembly,
109 ECL measurements were performed using an MPI-EII ECL workstation to detect ECL
110 signals. A three-electrode system was adopted, with the modified GCE as the working
111 electrode, an Ag/AgCl electrode (saturated KCl) as the reference electrode, and a
112 platinum wire electrode as the counter electrode. Ten milliliters (10 mL) of PBS buffer
113 (pH 7.4) and 20 μL of tri-n-propylamine (TPrA) solution were mixed in the detection
114 cell as the co-reactant system. The photomultiplier tube (PMT) voltage was set to 420
115 V, the potential range was 0–1.3 V, and the scan rate was 0.5 V/s.

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