Electronic Supplementary Information (ESI) for Analyst

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Hybridization chain reaction-enhanced enzyme-biomineralization for ultrasensitive colorimetric biosensing of a protein biomarker

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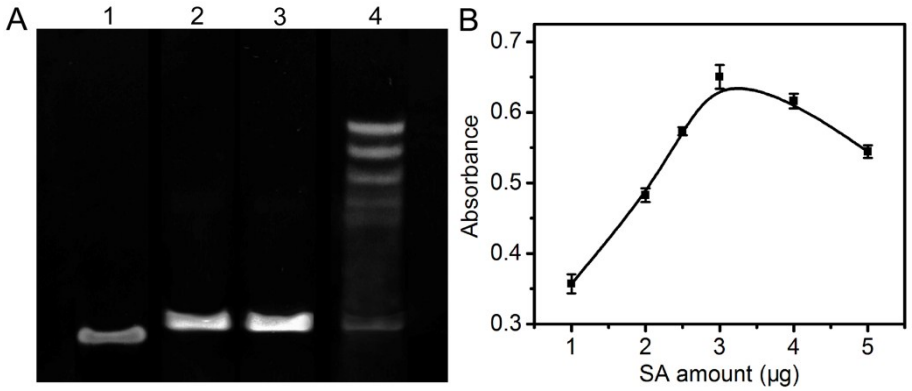

Fig. S1 (A) PAGE images of different samples: CEA-aptamer (lane 1), H1 (lane 2), H2 (lane 3), and the reaction products of the mixture of CEA-aptamer, H1 and H2; (B) Effect of the SA amount used for the Au NP nanotag preparation on the absorbance response of 50 ng mL^{-1} CEA.

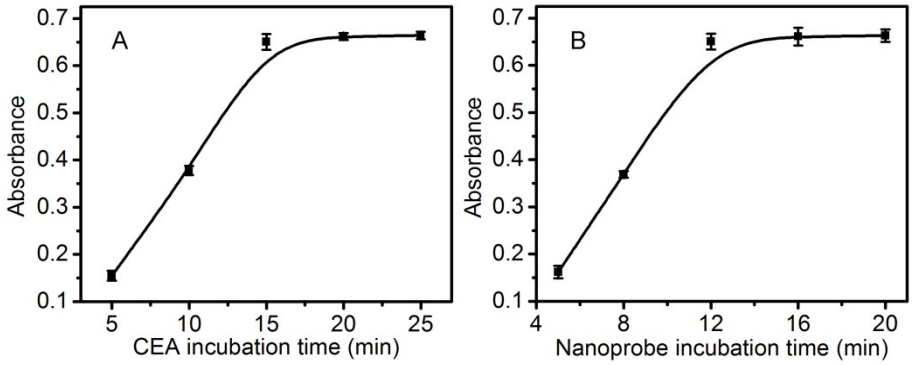

Fig. S2 Effects of the incubation time of CEA (A) and nanoprobe (B) on the absorbance responses of 50 ng mL^{-1} CEA.

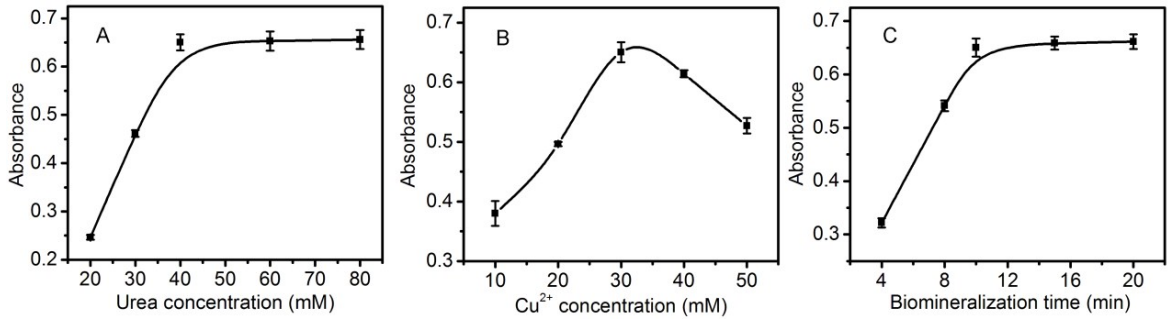

Fig. S3 Effects of the concentrations of urea (A) and Cu^{2+} (B), and the biomineralization time (C) on the absorbance responses of 50 ng mL^{-1} CEA.

Table S1 An overview on the specific features of the proposed method and their comparison with several biosensing methods previously reported for CEA analysis.

Signal transduction strategy	Detection method	Linear range	LOD	Ref.
Enzymatic chromogenic reaction by HRP-labeled antibody-functionalized Au NPs	Colorimetry	2–40 ng mL^{-1}	0.02 ng mL^{-1}	9
Enzyme-induced biomineralization of copper ions by urease-functionalized Au NPs	Colorimetry	1 pg mL^{-1}– 100 ng mL^{-1}	0.45 pg mL^{-1}	16
DNAzyme chromogenic reaction based on biobarcode and enzyme-assisted DNA recycling amplification	Colorimetry	0.025–40 ng mL^{-1}	0.025 ng mL^{-1}	35
Catalytic chromogenic reaction by $ZnFe_2O_4$@MWCNTs nanocomposite	Colorimetry	5 pg mL^{-1}– 30 ng mL^{-1}	2.6 pg mL^{-1}	36
Enzymatic chromogenic reaction by HRP at a paper-based microfluidic immunodevice	Colorimetry	0.1–20 ng mL^{-1}	0.03 ng mL^{-1}	37
Catalytic chromogenic reaction by Pd/Fe_3O_4@C peroxidase mimetics	Colorimetry	5 pg mL^{-1}– 30 ng mL^{-1}	1.7 pg mL^{-1}	38
Label-free bioassay at a graphene/Au NPs based immunosensor	EIS	0.1–1000 ng mL^{-1}	0.06 ng mL^{-1}	39
Electrocatalytic reaction of HRP at a nitrogen-doped graphene based immunosensor	DPV	0.02–12 ng mL^{-1}	0.01 ng mL^{-1}	40
Electrocatalytic reaction of Au@Pt DNs/NG/Cu^{2+} nanocomposite	Amperometry	0.5 pg mL^{-1}– 50 ng mL^{-1}	0.167 pg mL^{-1}	41
HCR-enhanced biomineralization of copper ions	Colorimetry	0.1 pg mL^{-1}– 50 ng mL^{-1}	0.071 pg mL^{-1}	This work

HRP: horseradish peroxidase; MWCNT: multi-walled carbon nanotube; EIS: electrochemical impedance spectroscopy; DPV: differential pulse voltammetry; Au@Pt DNs/NG/Cu^{2+}: Cubic Au@Pt dendritic nanomaterials-functionalized nitrogen-doped graphene loaded with Cu^{2+}

Table S2 Comparison on the CEA analyzed results of human serum samples by using the proposed and reference methods (ng mL^{-1}).

Sample no.	1	2	3
Reference method	13.05	0.50	0.050
Proposed method (RSD, %)	12.93 (2.4)	0.47 (3.3)	0.052 (2.5)
Relative error (%)	-0.92	-6.0	4.0