

Supporting Information

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Multicolor biosensor for alkaline phosphatase activity detection based on peroxidase activity of copper nanoclusters and etching of gold nanorods

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1 **1. Reagents and instruments**

2 Bovine serum albumin (BSA) was purchased from Shanghai Shenggong
3 Biological Engineering Co., Ltd. Alkaline phosphatase (ALP) was purchased from
4 Shanghai Yuanye Biotechnology Co., Ltd. 3,3',5,5'-Tetramethylbenzidine (TMB),
5 cetyltrimethylammonium bromide (CTAB), chloroauric acid (HAuCl_4), copper sulfate
6 (CuSO_4), ascorbic acid (AA) were purchased from Tianjin Fuchen Chemical
7 Reagent Co., Ltd. Silver nitrate (AgNO_3) and sodium borohydride (NaBH_4) and were
8 purchased from Sinopharm Chemical Reagent Co., Ltd. All reagents are of analytical
9 grade. All related solutions were prepared with deionized water obtained by DirectQ3
10 UV system (equipment purchased from Merck Millipore, Germany, conductivity 18.2
11 $\text{M}\Omega/\text{cm}$). The UV-Vis absorption spectrum was obtained by Multiskan GO full-
12 wavelength microplate reader from Thermo Fisher Scientific. A Nicolet iS50 FT-IR
13 system (Thermo Fisher, USA) was used to scan Fourier Transform Infrared
14 Spectroscopy (FT-IR).

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16 **2. Preparation of AuNRs**^{1,2}

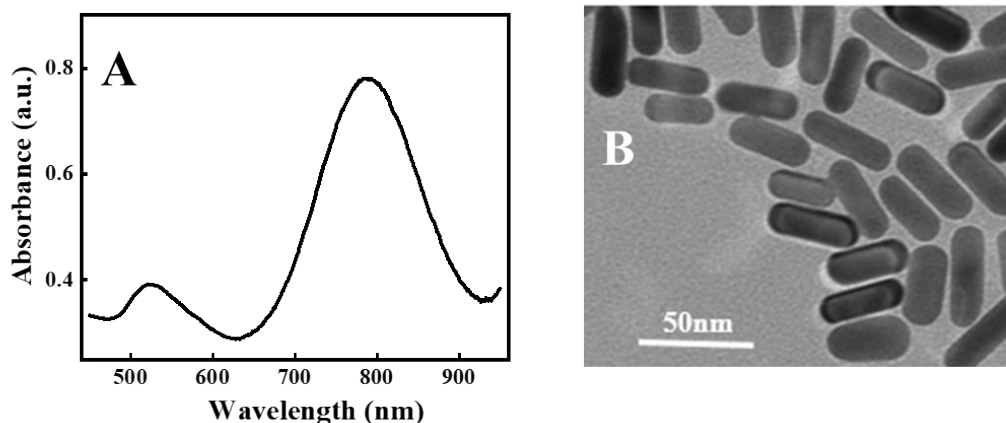
17 AuNRs are synthesized briefly by seed growth methods, including the synthesis
18 of gold seeds and the growth of AuNRs. The preparation method of gold seed solution
19 is as follows: NaBH_4 was dissolved in ice water to reduce HAuCl_4 to prepare gold
20 seeds. HAuCl_4 solution (0.25 mL, 0.01 mol/L), CTAB solution (1 mL, 0.2 mol/L) and
21 4.75 mL water were mixed in small glass bottles, and stirred at 1200 r/min for 20 s.
22 The solution changed from yellow to dark brown, and the solution was left for 30 min
23 to age the prepared gold seeds.

24 Preparation of growth solution: CTAB solution (12.5 mL, 0.2 mol/L), 9.675 mL
25 deionized water, AgNO_3 solution (0.15 mL, 0.01 mol/L) and HAuCl_4 (1 mL, 0.01
26 mol/L) were put in a round-bottom flask, stirred and mixed. Then, ascorbic acid (1.6
27 mL, 0.01 mol/L) was added to the mixed solution, and the solution quickly became
28 colorless. Finally, 0.05 mL of the newly prepared seed solution was added to the
29 growth solution, which was stirred vigorously for 20 s and then stood for 24 h to wait
30 for the growth of the AuNRs.

1 The prepared solution was centrifuged at 8000 r/min for 10 min and washed
2 three times with water, and then centrifuged AuNRs were dissolved in an appropriate
3 volume of CTAB solution (0.06 mol/L) for later use.

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5 3. Characterization of AuNRs



6 Fig. S1 (A) UV-visible absorption spectrum of AuNRs. (B) TEM image of
7 AuNRs.

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9 4. Preparation of CuNCs³

10 CuNCs were synthesized by reported methods³. Briefly, CuSO₄ aqueous solution
11 (1 mL, 20 mmol/L) was added to the BSA solution (5 mL, 15 mg/mL) and stirred
12 vigorously at room temperature for 5min, then NaOH solution (0.6 mL, 100 mmol/L)
13 was added to the above solution. The resulting mixture was stirred at 55 °C for 8h,
14 and then the solution was dialyzed in deionized water with a 3 KDa dialysis bag for
15 48h to remove the unreacted Cu²⁺. The synthesized copper nanoclusters were stored
16 in a 4 °C refrigerator.

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2 **References**

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