Supporting Information

# A multicolor immunosensor for sensitive visual detection of breast cancer biomarker based on sensitive NADH-ascorbic acid-mediated growth of gold nanobipyramids

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**1. Separation and detection of AAP and AA with HPLC**

An Agilent 1100 series high performance liquid chromatography (HPLC) together with a ZORBAX SB-C18 column (4.6 × 250 mm, 5-μm particle size) was employed to achieve the separation and detection of AAP and AA at a flow rate of 1 mL/min. The mobile phase consisted of methanol and $KH_2PO_4$ (20 mM, pH=3) with a volume ratio of 1:9. The sample injection volume was 10 μL. The column temperature was set at 30 ℃. The wavelength of the UV-Vis detector was set at 250 nm. The ALP hydrolysis solution was diluted 50-fold with the mobile phase before HPLC-MS/MS detection.

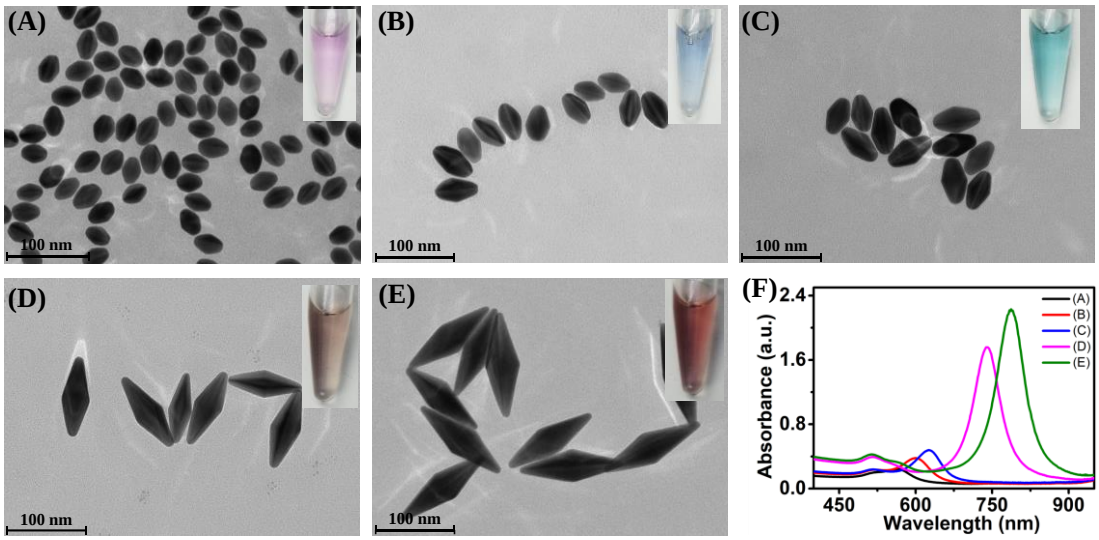


**Figure S1:** TEM images and photographs of the AuNBPs solution (A-E) prepared with only AA reduction (without NADH assistance) and their corresponding UV-visible spectra (F). AA concentration: 560 μM (A), 580 μM (B), 600 μM (C), 670 μM (D) and 750 μM (E).

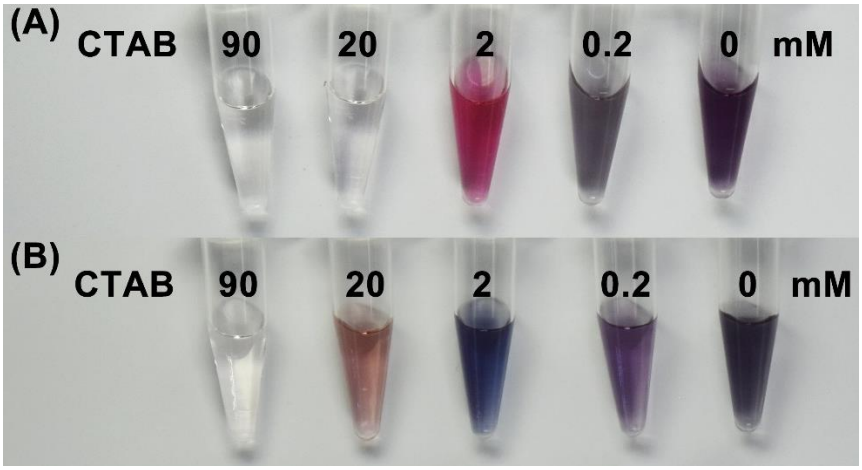


Figure S2: The effect of CTAB on the AuNBPs growth mediated with NADH reduction in the presence (A) and absence (B) of 25 mM HCl. (A): NADH-mediated AuNBPs growth was carried out in 130 µL of growth solution containing 0.48 mM HAuCl4, 0.96 mM $AgNO_3$, 1.25 µL seed solution, 25 mM HCl and different concentrations of CTAB; (B): NADH-mediated AuNBPs growth was carried out in 130 µL of growth solution containing 0.48 mM HAuCl4, 0.96 mM $AgNO_3$, 1.25 µL seed solution and different concentrations of CTAB.

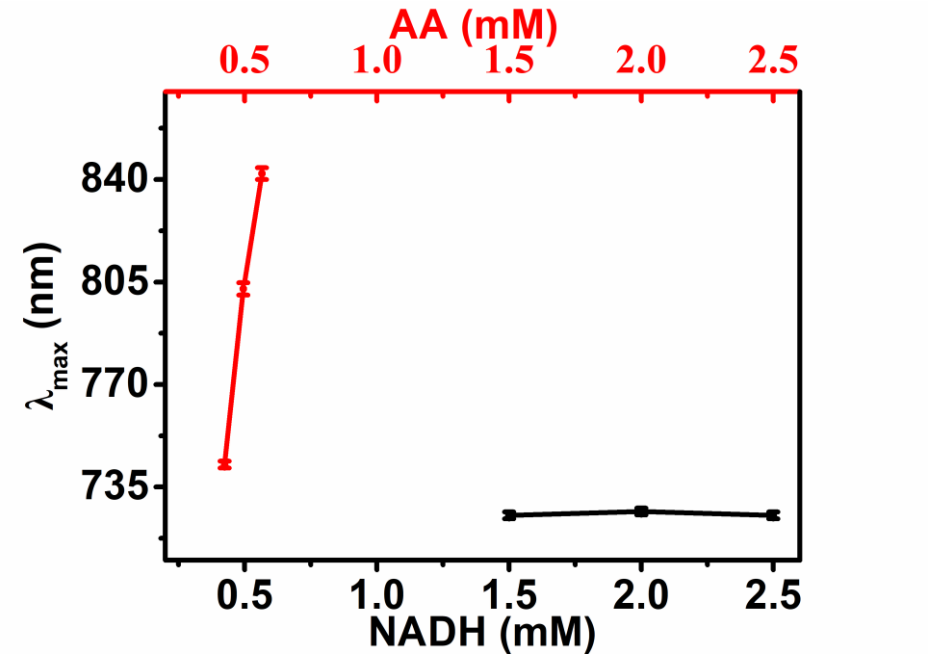


Figure S3: Effect of AA concentration and NADH concentration on the maximum absorption wavelength of longitudinal LSPR of NADH-assisted AA-mediated AuNBPs.

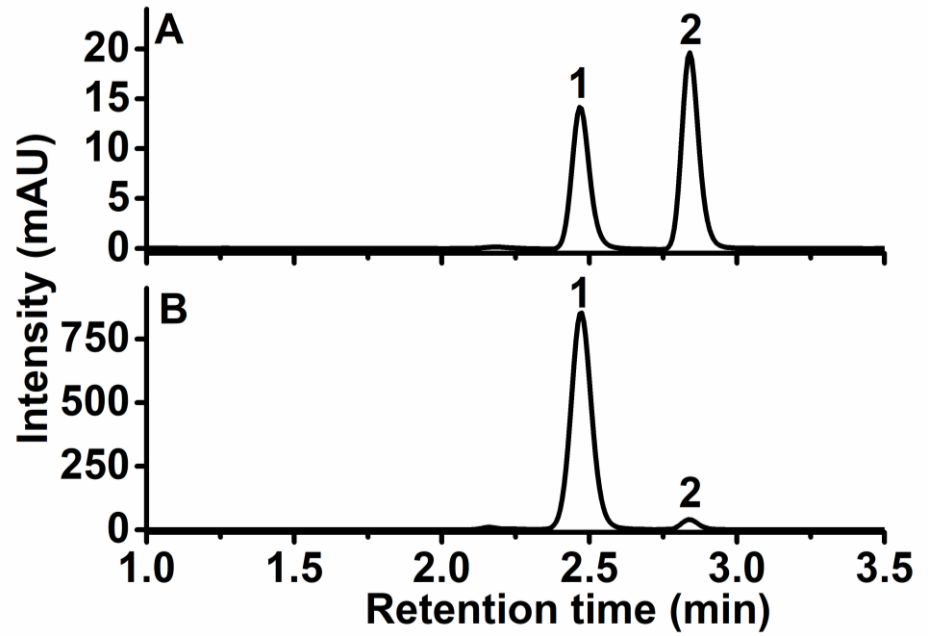


Figure S4: (A): HPLC chromatogram of AAP and AA standard; (B): HPLC chromatogram of AAP solution catalyzed for 1 hour by ALP. Peak 1 and Peak 2 represent AAP and AA, respectively. The concentration of AAP and AA standard was all 20 μM.

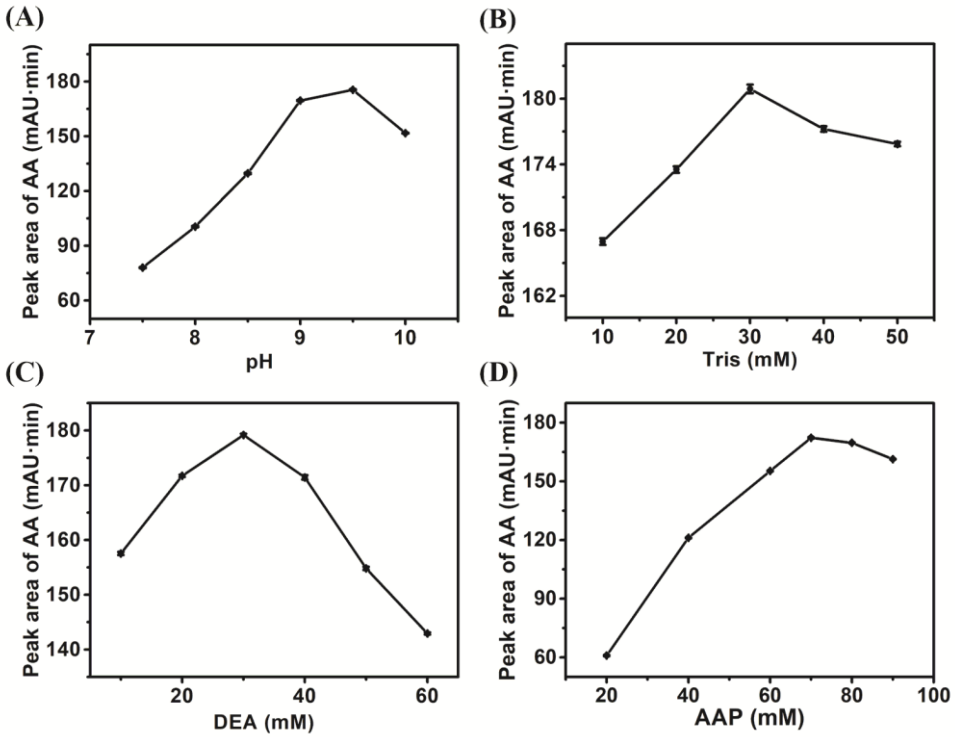


Figure S5: Effect of pH (A) and concentration (B) of Tris-HCl buffer, DEA concentration (C) and AAP concentration (D) on the peak area of AA generated from AAP, which was hydrolyzed by ALP (8 mU/mL) for 1 hour.

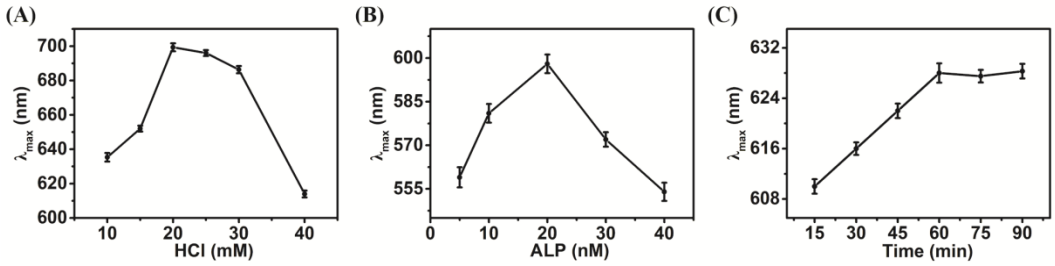


Figure S6: Effects of HCl concentration in Au NBPs growth solution (A), streptavidin-linked ALP concentration (B) and incubation time of streptavidin-linked ALP (C) on the maximum absorption wavelength of longitudinal LSPR of NADH-assisted AA-mediated AuNBPs.

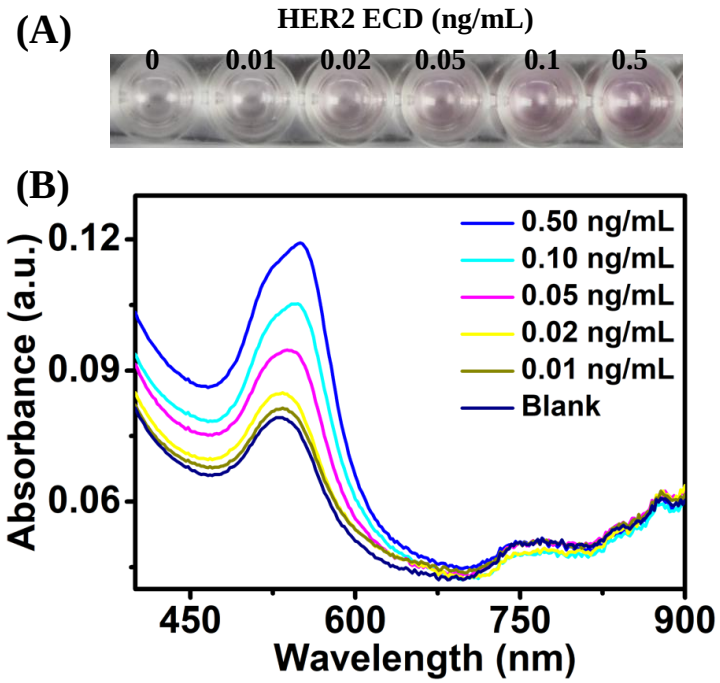


Figure S7: The photographs (A) and the UV-visible adsorption spectra (B) of the multicolor immunosensor for detecting low concentrations of HER2 ECD (0 - 0.5 ng/mL).

Table S1: Comparison of analytical performance among the previous methods and our multicolor immunosensor for HER2 ECD detection

| Analytical methods | Recognition element | Liner range (ng/mL) | Detection limit (ng/mL) | Reference |
|---|---|---|---|---|
| Fluorescence | Antibody | 0.025-5 | 0.025 | 1 |
| Fluorescence | Aptamer | 6250-31250 | 4750 | 2 |
| Electrochemistry | Aptamer | 0.2-2 | 0.2 | 3 |
| Electrochemistry | Antibody | 15-100 | 4.4 | 4 |
| Electrochemistry | Affibody | 0-40 | 6 | 5 |
| Electrochemistry | Imprinted polymer | 10-70 | 1.6 | 6 |
| Colorimetry | Antibody | 2.5-100 | 1.5 | 7 |
| Multicolor immunosensor | Antibody | 1.0-7.0 | 0.05 (UV-visible spectrophotometry) 0.5 (bare eye observation) | This work |


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