## **Supporting Information**

## Dual Amplification Fluorescence Assay for Alpha Fetal Protein Utilizing Immuno-Hybridization Chain Reaction and Metal-Enhanced Fluorescence of Carbon Nanodots

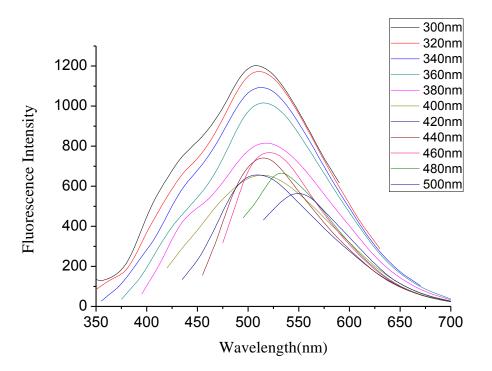
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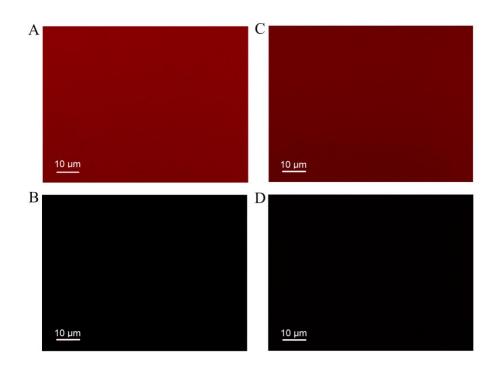
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**Figure S1.** Excitation dependent fluorescence emission of CDs under irradiation in the range of 300-500 nm. When excited at 360 nm, the CDs exhibit a strong and broad fluorescence spectrum centered at ~515 nm.



**Figure S2.** Fluorescence images of the Ab<sub>c</sub>-plasmonic slide (A), plasmonic slide (B), Ab<sub>c</sub>-normal slide (C), and normal slide (D) after reacted with Cy3-labeled sheep anti-mouse IgG.

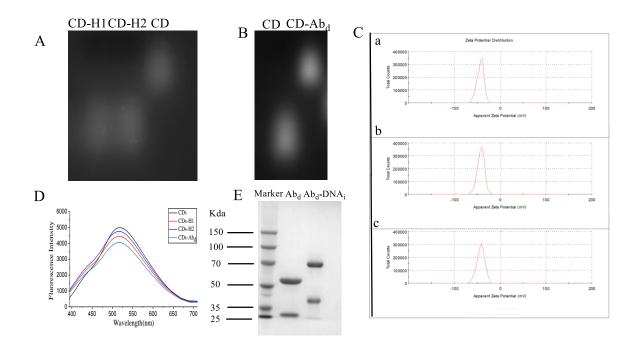
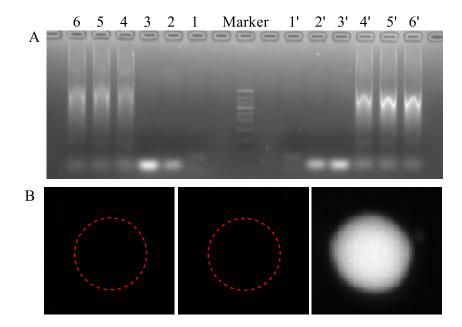
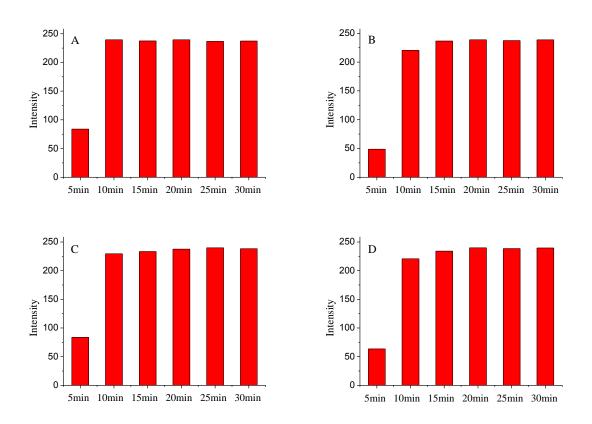


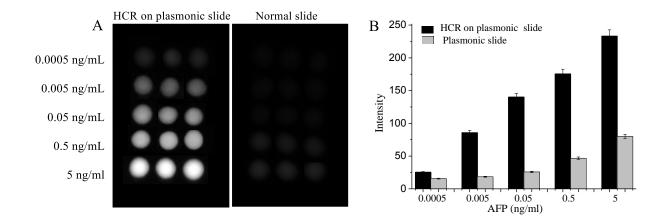
Figure S3. (A)Analysis of CD-DNA conjugation and (B) CD-antibody conjugation by garose gel electrophoresis. (C) The the zeta potentials of CDs-Ab<sub>d</sub> (-43.1mV) (a),CDs-H1(-42.1mV) (b), CDs-H2(-42.3mV) (c). (D) The fluorescence emission spectra of CDs, CDs-H1, CDs-H2, CDs-Ab<sub>d</sub>. (E) Analysis of initiator-antibody conjugation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).



**Figure S4.** (A)Agarose gel electrophoresis image for HCR-based assay with or without coupling CD and annealing steps. Lane marker: DNA size marker; Lane 1: Initiators; Lane 2: H1; Lane 3: H2 ; Lane 4: Initiators/H1/H2:1:5:5; Lane 5: Initiators/H1/H2:1:10:10; Lane 6: Initiators/H1/H2:1:100:100; Lane 1': Initiators; Lane 2': CD-H1; Lane 3': CD-H2 ; Lane 4': Ab<sub>d</sub>-DNA<sub>i</sub>/CD-H1/CD-H2:1:5:5; Lane 5': Ab<sub>d</sub>-DNA<sub>i</sub>/CD-H1/CD-H2:1:10:10; Lane 6': Ab<sub>d</sub>-DNA<sub>i</sub>/CD-H1/CD-H2:1:100:100. (B) Characterization of the as-proposed detection system in different condition(Ab<sub>d</sub> without conjugating oligonucleotide initiators reacted with CD-H1 and CD-H2; Ab<sub>d</sub>-DNA<sub>i</sub> reacted with CD without conjugating H1 and H2; Ab<sub>d</sub>-DNA<sub>i</sub> reacted with CD-H1 and CD-H2).



**Figure. S5** (A) Fluorescence intensities of the detection system in different reaction between capture antibody and AFP (20 min for initiator-detection antibody and AFP, 25 min for initiator-detection antibody and DNA solution (H1 and H2)). (B) Fluorescence intensities of the detection system in different reaction between initiator-detection antibody and AFP (15 min for capture antibody and AFP, 25 min for initiator-detection antibody and DNA solution (H1 and H2)). (C) Fluorescence intensities of the detection system in different reaction between CDs-detection antibody and AFP(20 min for capture antibody and AFP). (D) Fluorescence intensities of the detection system in different reaction between initiator-detection between initiator-detection antibody and AFP). (D) Fluorescence intensities of the detection system in different reaction between initiator-detection antibody and AFP). (D) Fluorescence intensities of the detection system in different reaction between initiator-detection antibody and AFP). (D) Fluorescence intensities of the detection system in different reaction between initiator-detection antibody and AFP). (D) Fluorescence intensities of the detection system in different reaction between initiator-detection antibody and AFP).



**Figure. S6** (A) Fluorescence diagram of the system upon the addition of increasing amount of AFP on HCR-plasmonic slide and normal slide. (B) The histogram of contrastive fluorescence intensity between HCR-plasmonic slide and normal slide.