

# **Immunoassay- aptasensor for the determination of tumor- derived exosomes based on the combination of magnetic nanoparticles and hybridization chain reaction**

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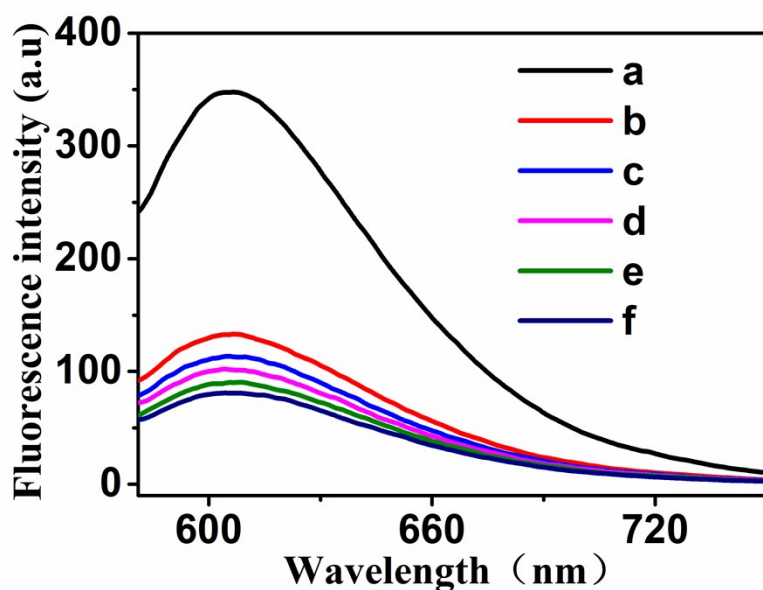
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**# Hua Zhang and Yajuan Zhou are the Co-first authors.**

## **S1 Feasibility analysis of the developed method for exosomes detection**

To further verify the feasibility of this sensing strategy, fluorescence measurements were performed to record the fluorescence emission spectra of different

mixtures. As shown in Figure. S1, the fluorescence intensity of curve a ( curve a: MNPs + CD63 + exosome + probe 1 + probe 2 + probe 3) is relatively strong. Under the conditions of without probe3 (curve b) or probe 2 + probe 3 (curve c), the fluorescence intensity was relatively weak. It demonstrates the HCR mediated signal amplification was based on probe 2 and probe 3. And the fluorescence response experiment without probe 1 (curve d) or CD63 antibody (curve e) were both weaker than curve a, it demonstrates that the determination of exosomes was based on the binding specificity (PDL-1 and aptamer of PDL-1, CD63 antibody and exosomes). Finally, the fluorescence intensity of the group without exosomes (curve f) can be negligible. These results confirm the feasibility of this proposed fluorescence platform for exosomes determination.

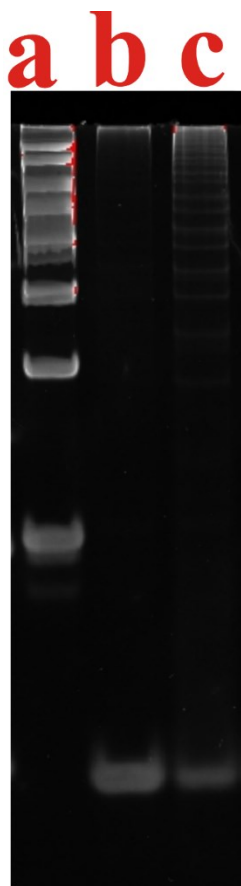


**Figure.S1** Representative fluorescent emission spectra of different mixtures. From curve a to f: (a) MNPs + CD63 + exosome + probe 1 + probe 2 + probe 3; (b) MNPs + CD63 + exosome + probe 1 + probe 2; (c) MNPs + CD63 + exosome + probe 1; (d) MNPs + CD63 + exosome + probe 2 + probe 3; (e) MNPs + exosome + probe 1 + probe 2 + probe 3; (f) MNPs + CD63 + probe 1 + probe 2 + probe 3.

## **S2 HCR characterization of the determination platform**

In figure.S2, each band represents: lane a: marker; lane b: 5  $\mu$ M probe 2+5  $\mu$ M probe 3(reacted for 30 minutes before electrophoresis); lane c: 5  $\mu$ M probe 2+5  $\mu$ M

probe 3 +100 nM trigger sequence (reacted for 30 minutes before electrophoresis). And the condition of electrophoresis is: 10% PAGE; electrophoresis buffer: 0.5×TBE buffer; voltage : 80V; electrophoresis time: 100min; volume of sample: 10  $\mu$ L. The loading samples were prepared by mixing 10  $\mu$ L of the product with 2  $\mu$ L 6×loading buffer. DNA gel electrophoresis was carried out in 0.5×TBE buffer (45 mM Tris, 45 mM boric acid, 1.3 mM EDTA, pH 8.1) on a horizontal electrophoresis system (DYY-6C, Beijing Liuyi Instrument Factory, China) at 80V for 100 min. The size of the extended product was estimated using a 0.1-1 Kb DNA ladder from Takara Biotechnology Co. Ltd. (Dalian, China).



**Figure.S2** The electrophoresis results of different mixtures. lane a: marker; lane b: probe 2+ probe 3; lane c: probe 2+ probe 3 + trigger sequence.