Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Highly Sensitive and Selective Fluorescent Biosensor of Breast Cancer Derived Exosomes Using Click Reaction of Azide-CD63 Aptamer and Alkyne-Polymer Dots

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1. Characterization of isolated exosomes by TEM

Transmission electron microscopy was performed to visualize exosomes. Initially, exosomes suspended in PBS were carefully placed onto a 200-mesh formvar carbon-coated copper grid and allowed to stand for 10 minutes. The excess solution was gently absorbed from the edge of the copper grid using filter paper. Subsequently, a 2% phosphotungstic acid solution was applied to the grid, staining the exosomes, and incubated for 3 minutes. Afterward, the grid was dried under an incandescent lamp. Finally, the exosomes were observed using a transmission electron microscope operating at 120 kV (JEM-1400plus).

2. Western Blot

The western blot experiment was carried out to confirm the presence of the CD63 and TSG101 proteins in exosomes. Here, the exosomes from the serum of breast cancer patient were lysed in a SDS-PAGE sample buffer and boiled for 10 min for protein denaturation. The protein sample was separated by 12% SDS-polyacrylamide gel electrophoresis and transferred into a polyvinylidene difluoride (PVDF) microporous membrane. Then the membrane was cut into two pieces and treated with blocking reagent for 1 h. Subsequently, after washing with TBST containing 0.1% Tween 20, the upper part of the membrane (containing proteins with a molecular weight larger than 33 kDa) was incubated with the anti-TSG101 antibody overnight at 4°C, while the lower part of the membrane (containing proteins with a molecular weight below 33 kDa) were incubated with the anti-CD63 antibody overnight at 4°C. After washing with TBST, the membranes were incubated with a secondary antibody (anti-rabbit antibody

labeled with horseradish peroxidase) for 2 hours. Finally, blots were presented by ChemiDoc™ Touch Imaging System (Bio-Rad Laboratories).

3. Measurement of the fluorescence quantum yield of Pdots

Fluorescence quantum yield represents the luminescence ability of a fluorescent substance and has a value between 0 and 1. We use quinine sulphate as the standard fluorescent substance, and the specific formula for calculating the fluorescence quantum yield of Pdots is as follows:

$$\varphi_f = \frac{number\ of\ light-emitting\ photons}{number\ of\ photons\ absorbed}$$

$$\varphi_f = \varphi_s \times \frac{F_f}{F_s} \times \frac{A_s}{A_f}$$

Note: s stands for Standard; f stands for the substance to be measured. F_f and F_s are the fluorescence intensities measured at the same excitation wavelength for quinine sulphate and Pdots respectively.

The actual detected values are obtained by bringing the actual detected values into Eq:

$$\varphi = 0.54 \times \frac{2153704}{30459887} \times \frac{0.15}{0.027} \approx 0.212$$

4. Measurement of fluorescence lifetimes of Pdots

When a substance is excited by a beam of laser light, the molecules of the substance absorb energy and jump from the ground state to some excited state, and then emit fluorescence back to the ground state in the form of radiative leaps. When the excitation light is removed, the fluorescence intensity of the molecule drops to 1/e of the maximum fluorescence intensity $I(t)$ at the time of excitation, the time required is called the fluorescence lifetime, often expressed as τ.

First set the x-axis to time in ns or μs, while setting the y-axis to fluorescence intensity

and taking a logarithmic function. The data on the y-axis was normalized and then re-plotted. After that a nonlinear fit was performed and ExpDec2 function was chosen and fitted to convergence.

The equations involved are:

$$\tau_{ave} = \frac{A_1{\tau_1}^2 + A_2{\tau_2}^2}{A_1\tau_{1+}A_2\tau_2}$$

$$I(t) = I_0 + A_1 exp(-\frac{t}{\tau_1}) + A_2 exp(-\frac{t}{\tau_2})$$

The fitted units of τ_1 and τ_2 are still ns, which need to be divided by 1000, i.e., $\tau_1{}' = \frac{\tau_1}{1000}$, $\tau_2{}' = \frac{\tau_2}{1000}$.

Finally, the fitting results are:

$A_1 = 0.28134, A_2 = 655.0532$

$\tau_1 = 11.081, \tau_2 = 1.521$

$$\tau_{ave} = \frac{0.28134 \times 11.081^2 + 655.0532 \times 1.521^2}{0.28134 \times 11.081 + 655.0532 \times 1.521} = 1.55\ \mu s$$

5. Calculation of the limit of detection

The fluorescence intensity corresponding to the lowest concentration in the linear curve is added to the standard error of the blank sample and the resulting value is brought into the linear equation.

The fluorescence intensity measured at an exosome concentration of $6.5*10^7$ particles/μL was 490.00766666666667.

The standard error of the measured fluorescence intensity at an exosome concentration of 0 is 5.71.

The relevant equation was F = 149.92 Log C - 671.41 (R^2 = 0.989), where F was the fluorescence intensity of the corresponding exosome.

Bring the values in:

$$Log\ C = \frac{(490.00766666666667 + 5.71) + 671.41}{149.92} = 7.785003113$$

$$C = 10^{7.785003113} = 60953689.72$$

6. **The fluorescence of the PBS buffer eluents was tested to confirm the removal free alkyne-Pdots.**

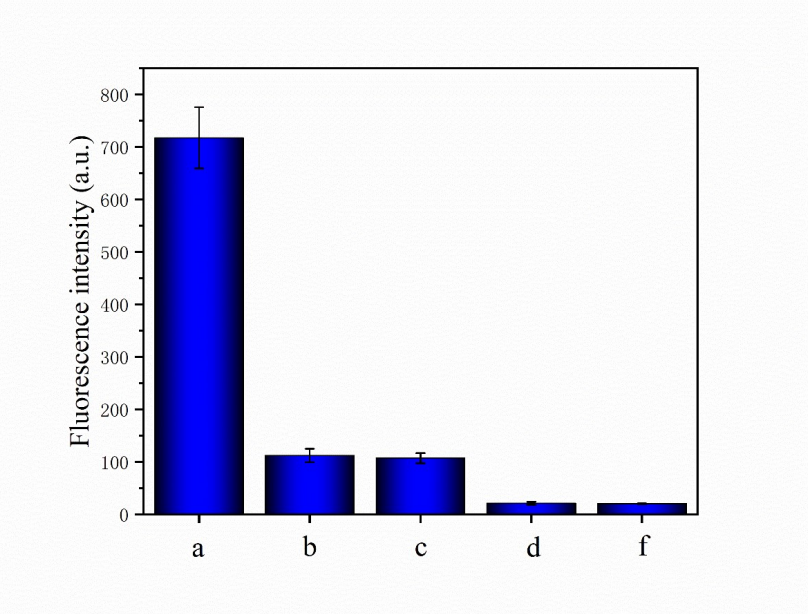

Fig. S1 Fluorescence intensities of (a) click reaction supernatant; (b) first eluent; (c) second eluent; (d) third eluent; (e) control

7. **The FT-IR analysis was also detected to confirm the introducing of alkyne groups of Pdots.**

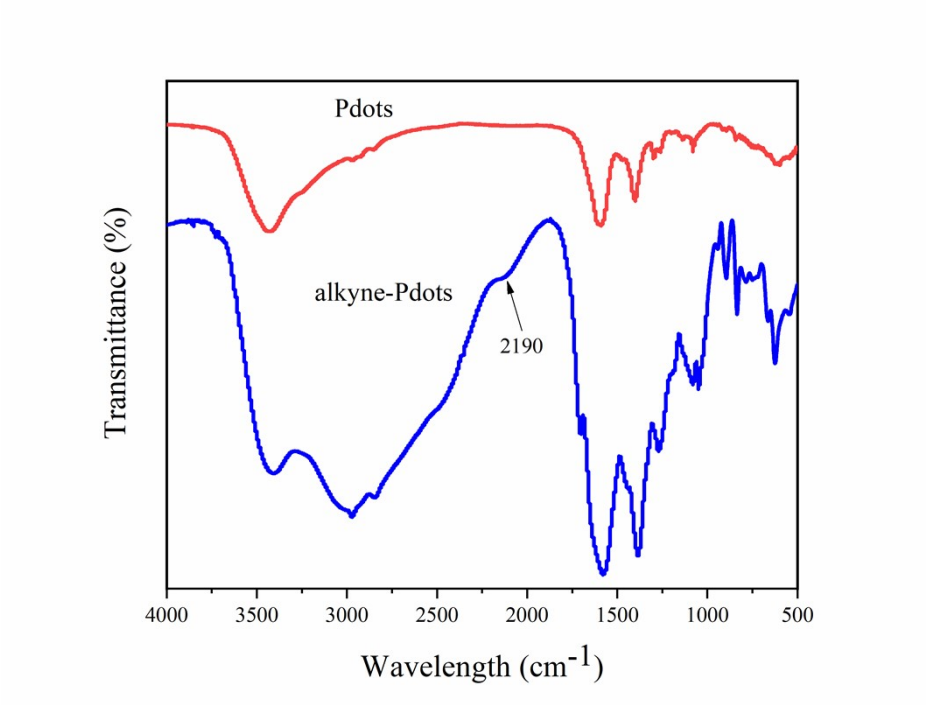

Fig. S2 FT-IR spectra of Pdots and alkyne-Pdots.

8. **In the blank control, the fluorescence intensity is very weak in the absence of exosomes.**

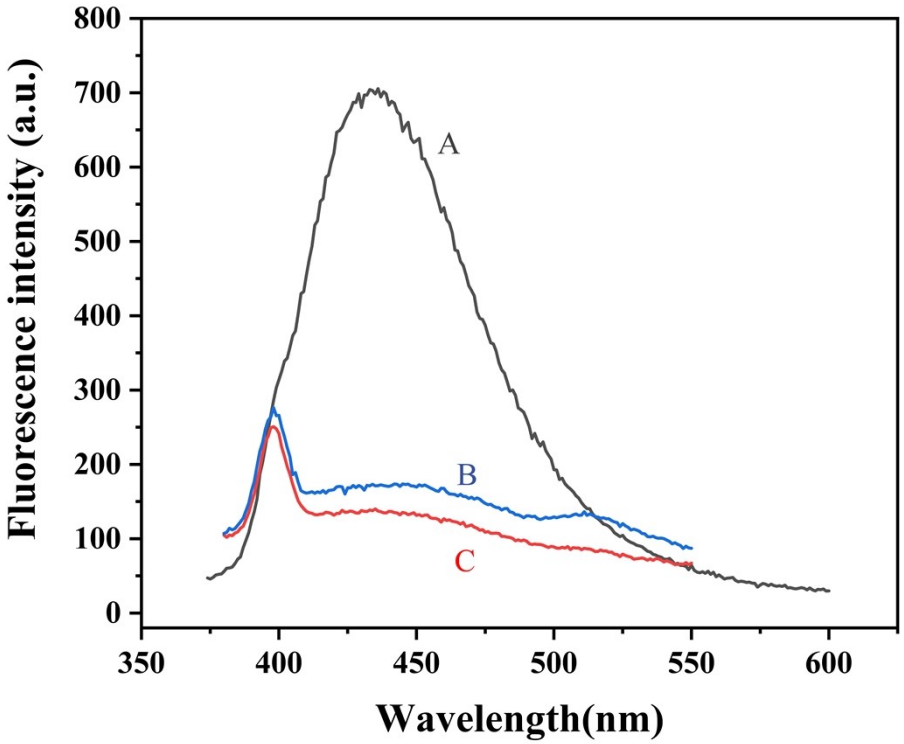

Fig. S3 Fluorescence intensity of click-reaction supernatant under different conditions. (A) Exosomes are present and alkyne-Pdots are used as fluorescent probes. Exosomes

are absent and (B) alkyne-Pdots are used as fluorescent probes; (C) Pdots are used as fluorescent probes.

9. **Table S1.** Average fluorescence intensity values of the probes in various concentrations of exosomes (6.50×10^7, 9.75×10^7, 3.9×10^8, 6.50×10^8, 1.17×10^9, 1.30×10^9 particles/μL).

Concentrations of exosomes (particles/μL)	**Average ± S. D.**	**RSD (%)**
6.50×10^7	490.0 ± 3.9	0.80
9.75×10^7	532.9 ± 6.8	1.28
3.9×10^8	627.3 ± 7.6	1.20
6.50×10^8	650.4 ± 8.3	1.28
1.17×10^9	677.4 ± 2.5	0.37
1.30×10^9	697.3 ±9.9	1.42

n = 3

10. Table S2. Comparison of different detection strategies for exosomes

Strategies	Materials	Detection target	LODs	Detection capability	Ref.
Integrated concentration and determination system of exosomes (ICDSE)	quadruplet supramolecular dendrimer and zirconium metal-organic framework. A plasmonic biosensor	Exosomes and exosomal miRNA from the plasma of patients with non-small cell lung cancer	LOD of the biosensor for miR-155 approached 2.03 fM	/	1
A light-controlled recruitable HCR (LCR-HCR) strategy	Three cholesterol-modified hairpins (H1, H2, and H3)	miR-21	3.3 pM	/	2
Electrochemiluminescence (ECL)	Ru@SiO2 NPs	The MUC1 protein expression on breast cancer cells and their	0.83 μg/mL	Linear range: 7.53 - 753 μg/mL	3

		derived exosomes			
Integrating mass spectrometry imaging and gold nanoparticle (AuNP)-based signal amplification	Organic oligomers as mass tags and specific antibodies are modified on AuNPs to form biomarker probes	Protein biomarkers on the surface of exosomes	50 exosome particles	/	4
Fluorescence detection	Fe_3O_4@TiO_2 and Pdots	Exosomes from human serum	6.09×10^7 particles/μL	Linear range: 6.50×10^7 - 1.30×10^9 particles/μL	This work

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