Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Support information

Total Membrane Lipid Assay (MLA): Simple and Practical Quantification of Exosomes Based on Efficient Membrane-Specific Dye Unaffected by Protein

Ning Li, Zhenlong Huang, Zhiwei Ye, Xinfu Zhang, Lingcheng Chen and Yi Xiao*

State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116024, China. Email: xiaoyi@dlut.edu.cn.

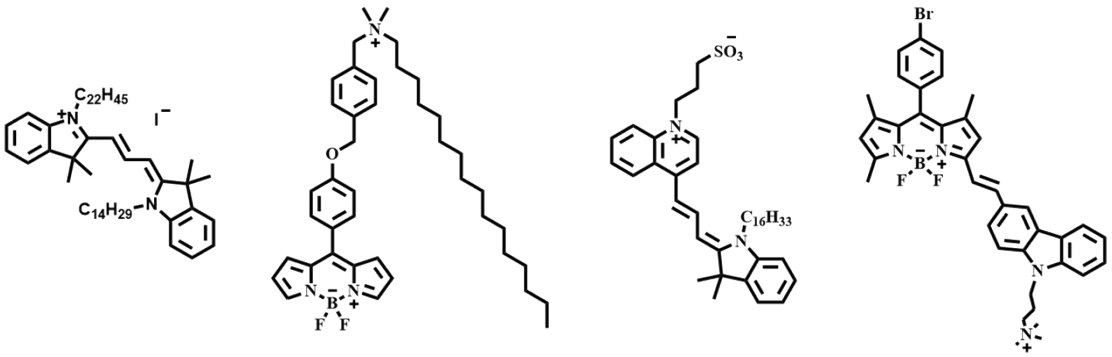


Figure S1. Structures of **PKH26**, **Mem-BDP**, **Mem-CA** and **Mem-SQAC**.

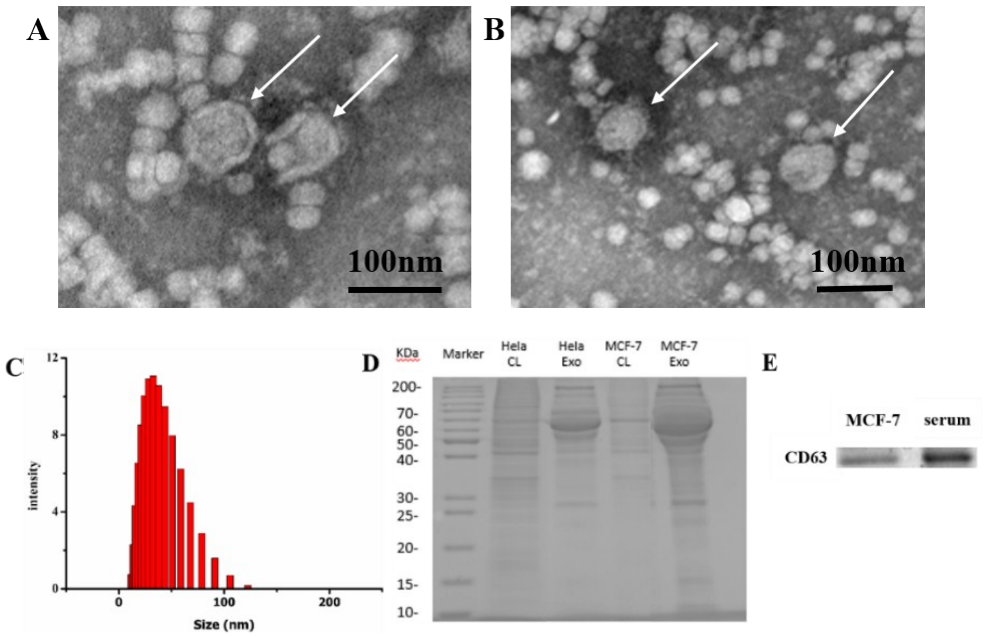


Figure S2. Characterization of exosomes. (A) Transmission electron microscopy (TEM) image of exosomes from MCF-7 cells culture supernatant. (B) Transmission electron microscopy (TEM) image of exosomes from serum. (C) Dynamic Light Scattering (DLS) of exosomes from MCF-7 cell culture supernatant. (D) Coomassie brilliant blue stained 12% SDS-PAGE analysis of Hela cell lysates (CL), exosomes from Hela cell culture supernatant, MCF-7 cell lysates (CL) and exosomes from MCF-7 cell culture supernatant (20 μg). (E)The CD63 proteins of exosomes form MCF-7 cells culture supernatant and serum are treated by western blotting.

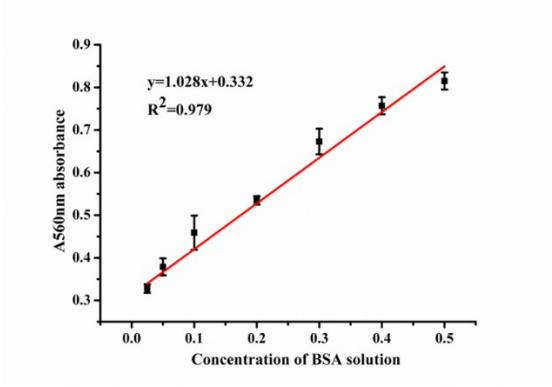
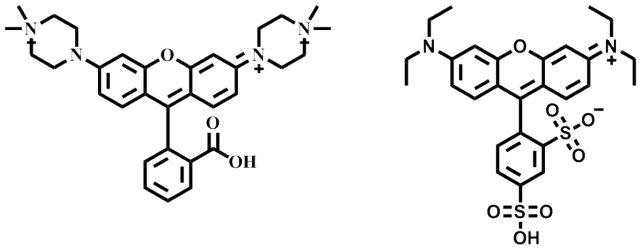
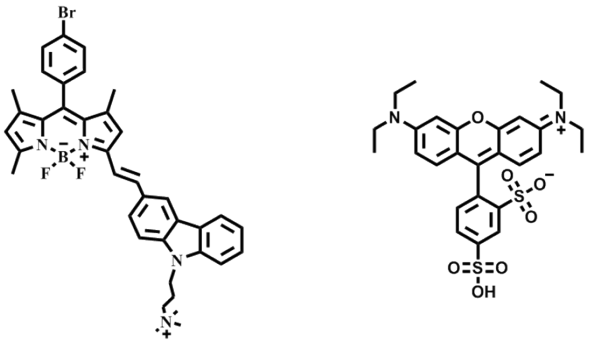


Figure S3. Standard curve for BCA protein assay.



Rho 1 (Piperazine rhodamine) **Rho 2** (Sulfonyl rhodamine)

Figure S4. Structures of **Rho 1** and **Rho 2**.



Dye A (**Mem-SQAC**) **Dye B (Rho 2**)

Figure S5. Structures of **Dye A** and **Dye B**.

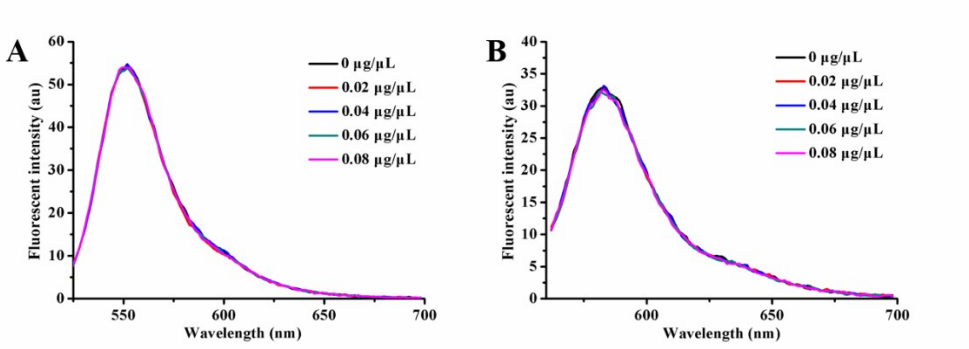


Figure S6. Fluorescence spectra changes of two **Dye B** candidates with different concentrations of BSA protein. (A) Fluorescence spectra change of **Rho 1** with different concentration of BSA protein. (B) Fluorescence spectra change of **Rho 2** with different concentration of BSA protein.

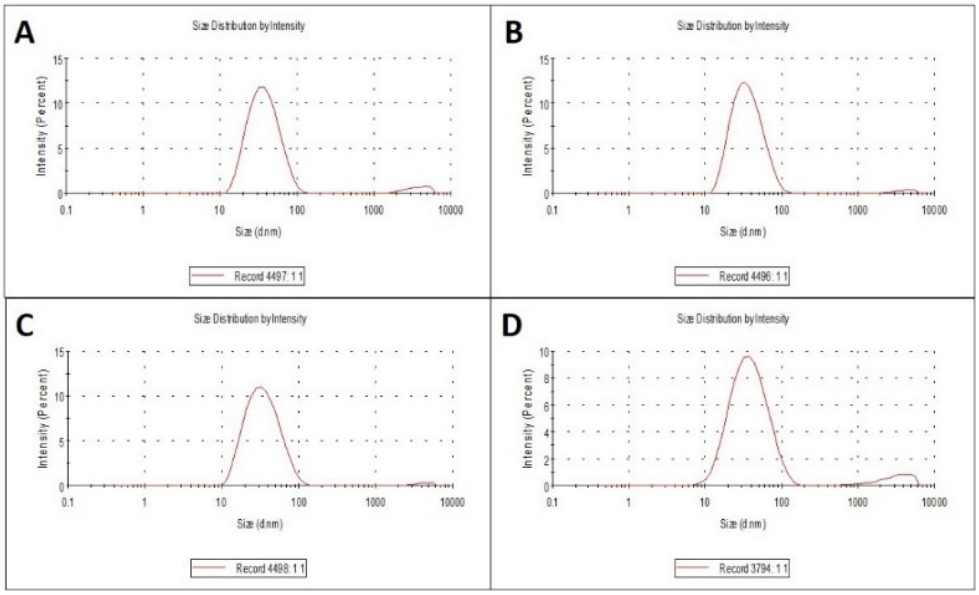


Figure S7. Dynamic light scattering (DLS) size distribution of exosomes. (A) Dynamic light scattering (DLS) of exosomes from Hela cell culture supernatant. (B) Dynamic light scattering (DLS) of exosomes from MCF-7 cell culture supernatant. (C) Dynamic light scattering (DLS) of exosomes from MCF-10A cell culture supernatant. (D) Dynamic light scattering (DLS) of exosomes from breast cancer patient serum.

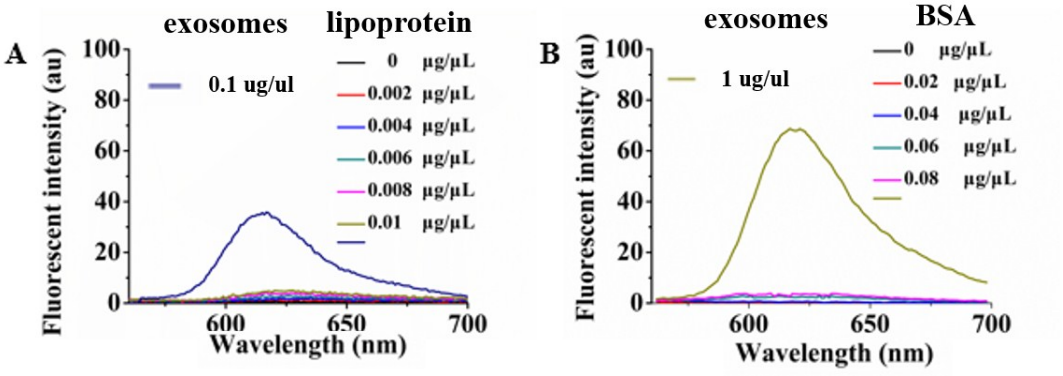


Figure S8. Mem-SQAC was insensitive to residue proteins mixed with exosomes. (A)Fluorescence spectral changes of Mem-SQAC induced by different concentrations of lipoprotein (0-0.01 μg/μL) and exosomes (0.1 μg/μL). (B)Fluorescence spectral changes of Mem-SQAC induced by different concentrations of BSA (0-0.08 μg/μL) and exosomes (1 μg/μL). Exosome quantification measured by BCA assay kit (total protein concentrations).

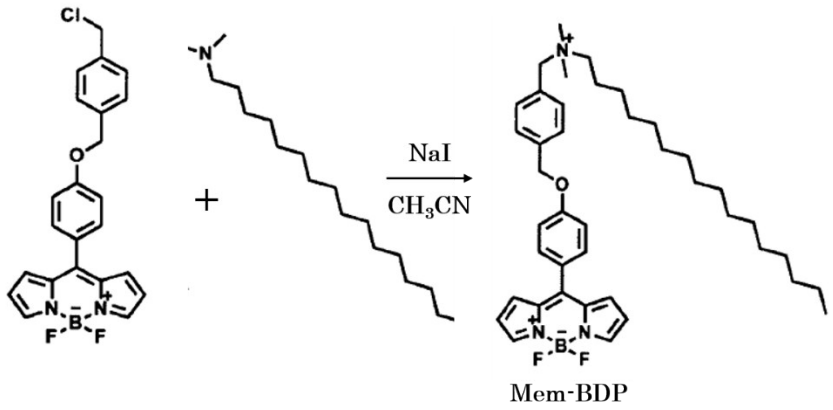


Figure S9. Synthetic route of **Mem-BDP**.

Under the protection of argon, compound 1 (25 mg, 0.059 mmol), Nal (35 mg, 0.24 mmol) and dimethylamino hexadecylamine (19 mg, 0.07 mmol) were dissolved in 5 mL of tetrahydrofuran. The reaction solution was heated to reflux for 4 hours, and the reaction mixture was cooled. The organic solvent was evaporated under reduced pressure and dissolved in dichloromethane to remove solid impurities, followed by column chromatography (slip: CH2CI2/CH3OH 15:1). The product **CM-BDP** (36 mg, 0.046 mmol, 77%) was obtained.

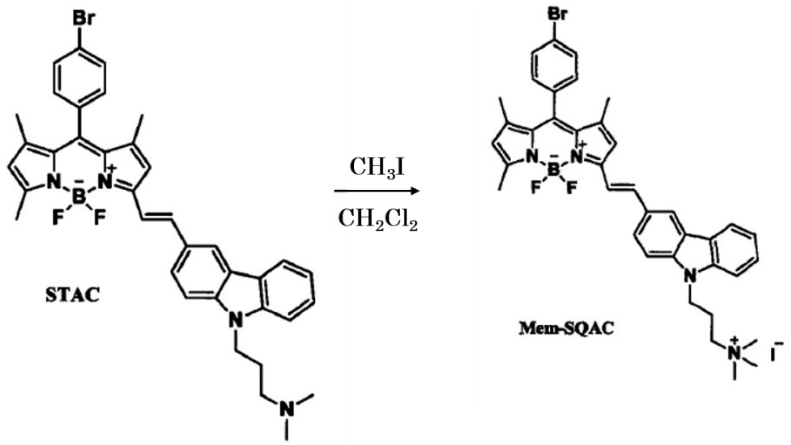


Figure S10. Synthetic route of **Mem-SQAC**.

The intermediate STAC (100 mg, 0.15 mmol) was dissolved in 5 mL of dry dichloromethane. Add 0.2 mL of methyl iodide and stir at room temperature overnight. Solid black particles were formed, filtered, and washed three times with dry diethyl ether to obtain the standard molecule.

references：

1.Song Xinbo, PhD Dissertation, Dalian University of Technology, 2017.

2.Hu Mingyu, Master Dissertation, Dalian University of Technology, 2017.

3.X. Zhang, C. Wang, L. Jin, Z. Han and Y. Xiao, ACS Appl. Mater. Interfaces, 2014, 6, 12372-12379.