Supporting Information

A potential-resolved electrochemiluminescent aptasensor for simultaneously detecting MUC1 and HER2 on breast cancer exosomes

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S1. Reagents and materials

CD63 aptamer (5'-NH_2-TTT CAC CCC ACC TCG CTC CCG TGA CAC TAA TGC TA-3'), MUC1 aptamer (5'-NH_2-TTT TTG CAG TTG ATC CTT TGG ATA CCC TGG-3') and HER2 aptamer (5'-SH-TTT GGG CCG TCG AAC ACG AGC ATG GTG CGT GGA CCT AGG ATG ACC TGA GTA CTG TCC-3') were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Cyclohexane, 1-hexanol, triton X-100, tetraethyl orthosilicate (TEOS), hydrochloric acid, acetic acid, ethanol, acetone, ammonia aqueous, glutaraldehyde, hydrogen peroxide 30% aqueous solution, tetrachloroauric(III) acid tetrahydrate, potassium persulfate and 4-aminobenzoic acid (4-ABA) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Tris(2,2′-bipyridyl) dichlororuthenium (II) hexahydrate, luminol, bovine serum albumin (BSA), N-[3-(trimethoxysilyl)propyl]ethylenediamine (DETA), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich Co. (St. Louis, MO). Dulbecco's modified eagle medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, fetal bovine serum (FBS), mucin 1 (MUC1), human epidermal growth factor receptor 2 (HER2), immunoglobulin G (IgG), bovine serum albumin (BSA), trypsin/EDTA solution, penicillin/streptomycin (P/S) and phosphate buffered saline (PBS) were purchased from Thermo Fisher Scientific Inc. (Waltham, MA). Exosome-depleted fetal bovine serum (dFBS) was purchased from System Biosciences Inc. (USA). The human breast cancer cell lines, including MCF-7, SK-BR-3, MDA-MB-231 and BT474 were obtained from Chinese Academy of Sciences (Shanghai, China). The 0.22 μm filters were purchased from Millipore Corp. (Bedford, MA). All solutions were prepared using deionized water (DI, ≥18.2 MΩ·cm) from Milli-Q water purification system (Millipore Corp., Bedford, MA).

S2. Instrumentation

MCF-7, SK-BR-3, MDA-MB-231 and BT474 cells were cultured in a humidified incubator (NU-5500E, NuAire, America). The transmission electron microscopy

(TEM) characterizations of exosomes and of $Ru@SiO_2$ and lum@Au were performed on Hitachi-7700 instrument (Tokyo, Japan) and JEOL JEM-2100 (Hitachi, Japan), respectively. The solid-state UV-vis spectra of $Ru@SiO_2$ and lum@Au was determined on a UV/vis/NIR spectrophotometer (Lambda 950, PerkinElmer, USA). Zeta potential characterization of $Ru@SiO_2$ was performed on a ZetasizerNano (Malvern, England). Nanoparticle tracking analysis (NTA) was performed on NanoSight NS300 (Malvern, US). Electrochemical measurements were carried out on a CHI1030C electrochemical workstation (Chenhua, Shanghai, China). Electrochemiluminescence (ECL) was measured with LK5100 electrochemiluminescence analyzer (Lanlike Chemical & Electronic High-Tech Co., Ltd., Tianjin, China). The indium tin oxide electrode (ITO electrode, 3.0 cm×4.0 cm) was used as working electrode. An Ag/AgCl (saturated KCl) and a platinum wire were used as reference electrode and auxiliary electrode, respectively.

S3. Simultaneous detection of MUC1 and HER2 proteins on exosomes

Table S1 Comparison of the analytical performances with other reported methods for simultaneous detection of multiple target proteins.

Method	Exosome-derived cell types	Protein types	Linear range (particles/μL)	LOD (particles/μL)	Reference
Fluorescence	SGC7901	CD63	1.0×10^2 -6.4×10^5	67	1
		MUC1		37	
SERS	MCF-7	MUC1	1.0×10^4 - 1.0×10^9	1.0-3.0×10^4	2
	SKBR-3	HER2			
	MDA-MB-231	CEA			
	BT474				
Electrochemical	MCF-7	MUC1	1.2×10^3 - 1.2×10^7	9.46×10^2	3
	SK-BR-3	HER2			
	MDA-MB-231	EpCAM			
	BT474	CEA			
Electrochemical	SK-BR-3	HER2	3.4 - 3.4 × 10^5	3.4	4
		EpCAM			
ECL	MDA-MB-231	PD-L1	2.36 - 2.36×10^4	1.620	5
		MUC1		1.586	
ECL	SK-BR-3	MUC1	3×10^2 - 1×10^7	117	This work
		HER2		92	
	MCF-7	MUC1	1×10^2 - 1×10^7	21	
		HER2		36	
	BT474	MUC1	1×10^3 - 1×10^7	105	
		HER2		231	
	MDA-MB-231	MUC1	1×10^3 - 1×10^7	307	
		HER2		128	

S4. Specificity, stability and reproducibility

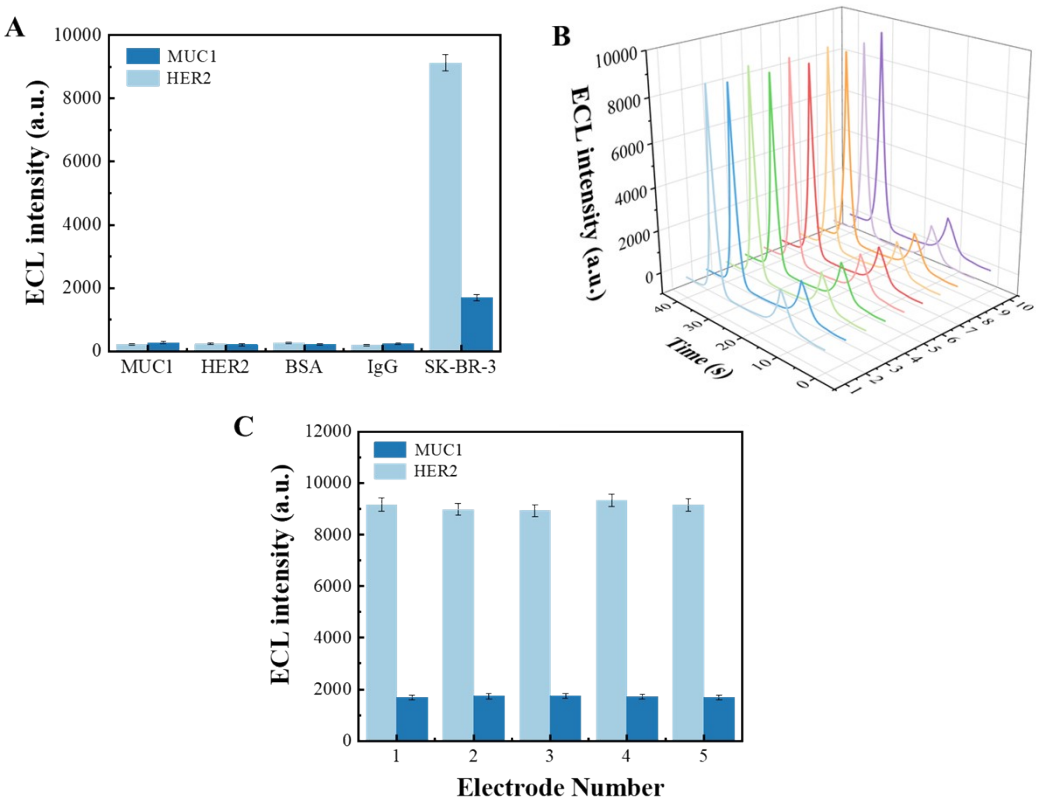

Fig. S1 (A) Specificity investigation by determining 1.0 μg/mL MUC1, HER2, lgG and BSA and 1×10^6 particles/μL SK-BR-3 exosomes; (B) Stability of the ECL aptasensor in 10 successive scans for 1×10^6 particles/μL SK-BR-3 exosomes; (C) ECL responses of 1.0×10^6 particles/μL SK-BR-3 exosomes detected by five aptasensors.

S5. Detection of exosomes in serum samples

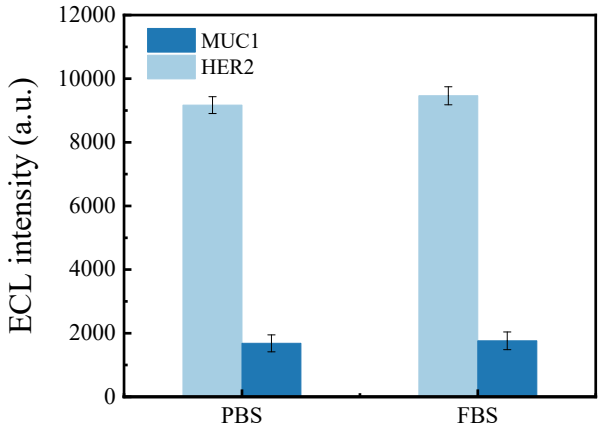

Fig. S2 Detection of 1×10^6 particles/μL SK-BR-3 exosomes in PBS and FBS.

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