Phage display affibodies combined with AuNPs@Ru(bpy)$_3^{2+}$ for ultra-sensitive electrochemiluminescence detection of abrin

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2. Materials and Methods

2.1. Reagents and instruments

Abrin, antibody, M13 phage display affibody of abrin and β-BGT (β-bungarotoxin) were all prepared in our lab. Ru(bpy)$_3^{2+}$-NHS ester [Bis (bipyridine) - 4'- methyl-4-carbonylpyridine ruthenium-N-succinimide ester] and DMF (*N*,*N*-Dimethylformamide) were obtained from Sigma-Aldrich (Munich, Germany). Dynabeads ™ Protein A (a kind of magnetic microspheres coated by protein A) and M280 Dynabeads (a kind of magnetic microspheres coated by streptavidin) were supplied by Invitrogen Life Technologies (Oslo, Norway). Biotin-d(PEG)$_4$-NHS ester, Tween 20 and Polystyrene nanospheres (nano-PSs) were obtained from Aladdin Industrial Corporation (Shanghai, China). Streptavidin (SA) was obtained from Beijing Solarbio Science and Technology Co., Ltd (Beijing, China). $HAuClO_4 \cdot 4H_2O$ and Bovine serum albumin (BSA) was purchased from Shanghai Sinopharm Group Co., Ltd. (Shanghai, China) and Human IgG was purchased from Beijing Biosynthrsis Biotechnology Co., Ltd. (Beijing, China). The Procell solution (pH=6.8) containing tripropylamine (TPA) was the co-reaction reagent, and it was purchased from Beijing Biolot Diagnostics Co., Ltd. (Beijing, China). The buffers used in the experiment were PBS (0.01 mol/L, pH = 7.4) and carbonate buffer (0.05 mol/L, pH = 9.6). They were prepared with deionized water which was prepared by ultrapure water instrument.

Screen-printed gold electrode was purchased from Zensor Technology Co., Ltd (China; Fig. S1). Both working electrode with a diameter of 3mm and counter electrode are gold, and the reference electrode is Ag/AgCl. Portable ECL sensor (Fig. S1) based on the screen-printed gold electrode was jointly developed by our laboratory and Xi'an Remex Analysis Instrument Co. Ltd. (Xi'an, China). Solver Next full automatic multifunctional scanning probe microscope (NT-MDT company, Russia) was used for morphological characterization of phage display affibodies, AuNPs and gold nanocomposites. Magnetic Separation Rack (Promega Company, Madison, WI, USA) was used for magnetic separation cleaning. HS-3 vertical mixer (Scientz Biotechnology Co., Ltd., Ningbo, China) helped to incubate samples. Dialysis bags

(Beijing Solarbio Science and Technology Co., Ltd, Beijing, China) were used to purify the dual-labeled phage display affibodies and dual-labeled nano-PSs, and the MWCO (molecular weight cut off) is 8000~14000. Absorbance spectrum were determined by BioMATE 3S UV-Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Particle size and zeta potential of nano-materials and their complexes were tested by Zetasizer Nano ZS (Malvern Instruments, Britain). The pipette gun was purchased from Eppendorf company (Germany). Ultrapure water instrument was purchased from sartorius stedim company (Germany).

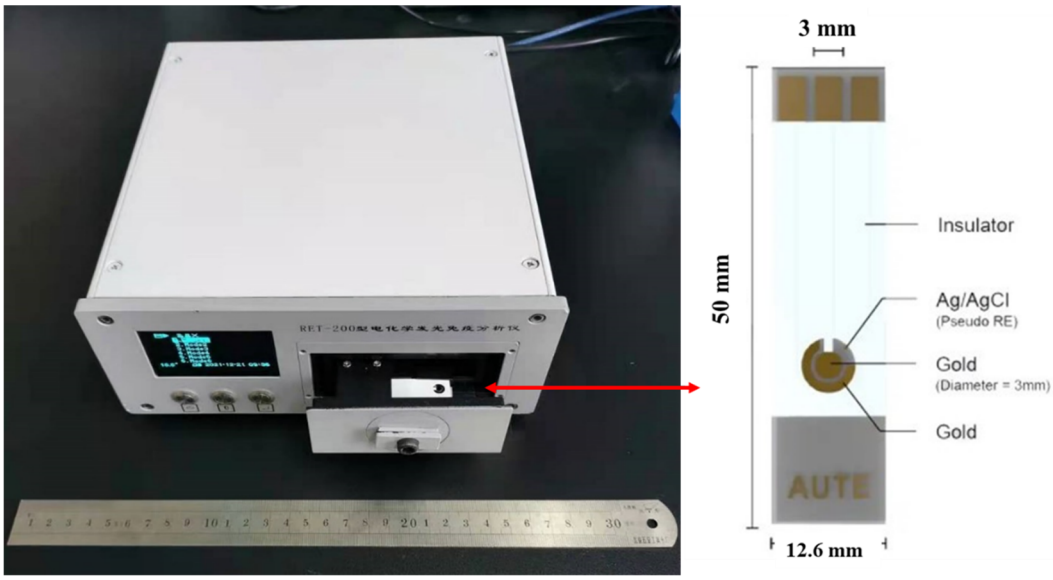

Figure S1. The portable ECL sensor and screen printed electrode (the specification parameters and picture of screen printed electrode are from the official website of Zensor Technology Co., Ltd, https://www.zensor.com.tw/PrintedElectrodes.html)

2.2 Experimental Methods

2.2.1 Preparation of magnetic capture probes

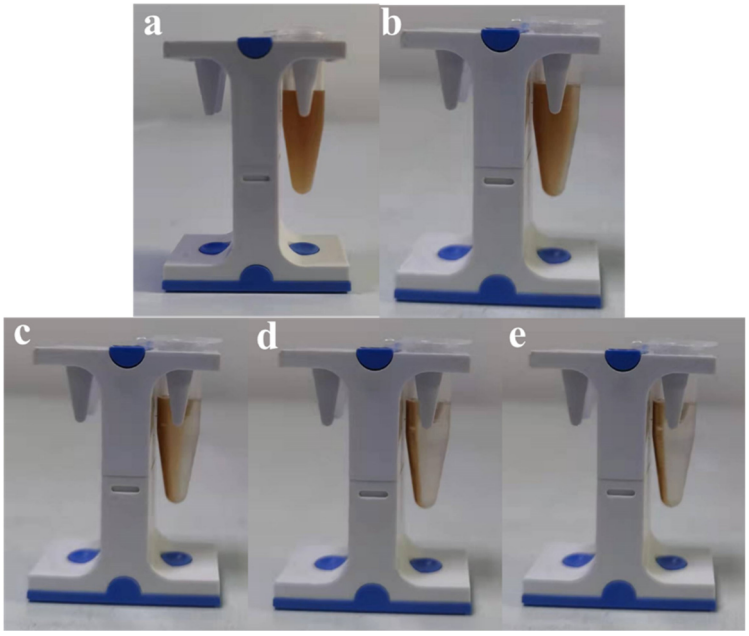

Figure S2. Magnetic separation efficiency test of magnetic microspheres (a. 0 s; b. 5 s; c. 10 s; d. 15 s; e. 20 s)

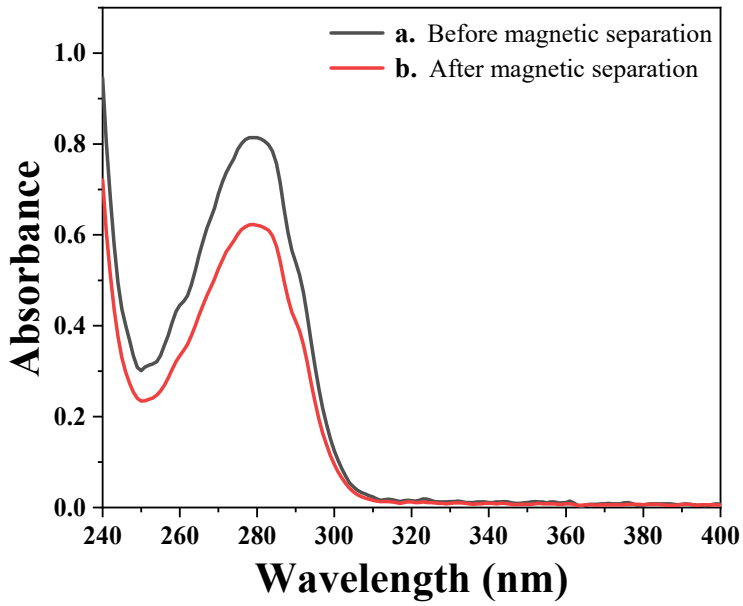

Figure S3. UV-Vis absorbance spectra of antibody solution before (a) and after (b) absorbed by protein A-coated magnetic microspheres

3. Results and Discussion

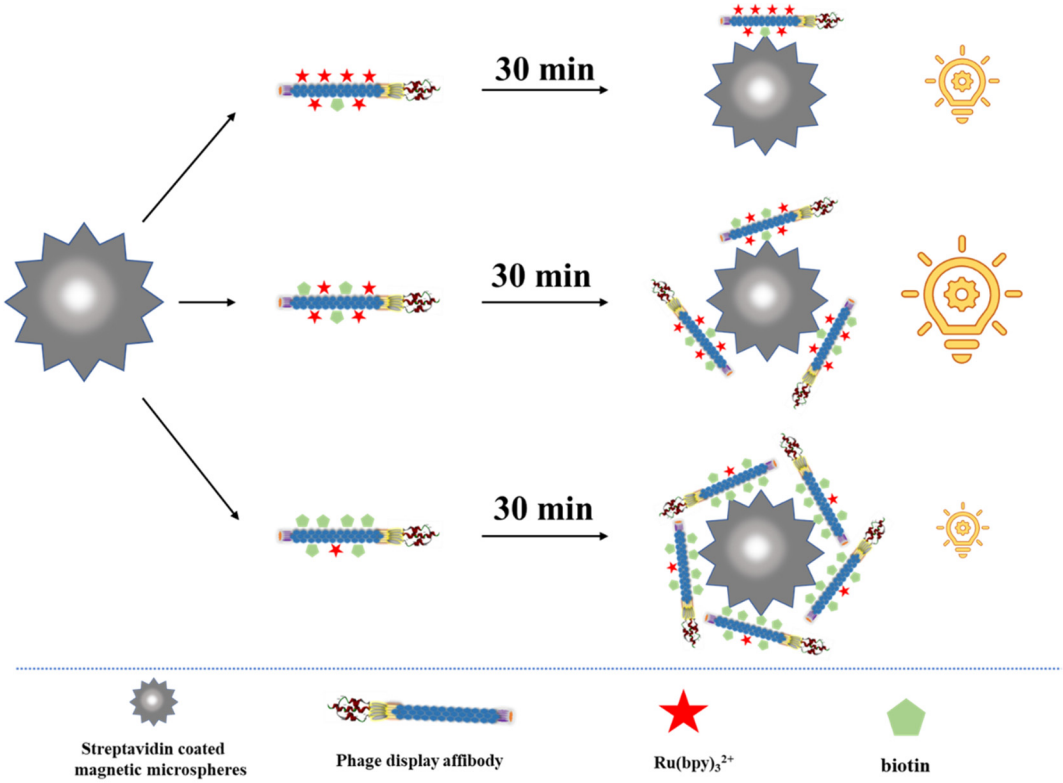

Figure S4. Schematic diagram of labeling ratio ($Ru(bpy)_3^{2+}$ and biotin) optimization principle

Table S1. Measured values and reproducibility test (n=5)

Conc. （**fg/mL**）	**Measured**（**a.u.**）					**Mean**（**a.u.**）	**RSD** （**%**）
PBS (blank)	3889	3867	3944	4003	3835	3908	1.70
5	4285	4216	4188	4334	4168	4238	1.64
10	4902	4538	4722	4744	4801	4741	2.81
50	5794	6079	6412	6733	6639	6331	6.19
100	6995	6866	6848	7043	7364	7023	2.96
500	8612	8158	7993	7940	7843	8109	3.74
1000	8581	9071	9295	8483	8746	8835	3.85
5000	9729	9691	9704	9686	9777	9717	0.38

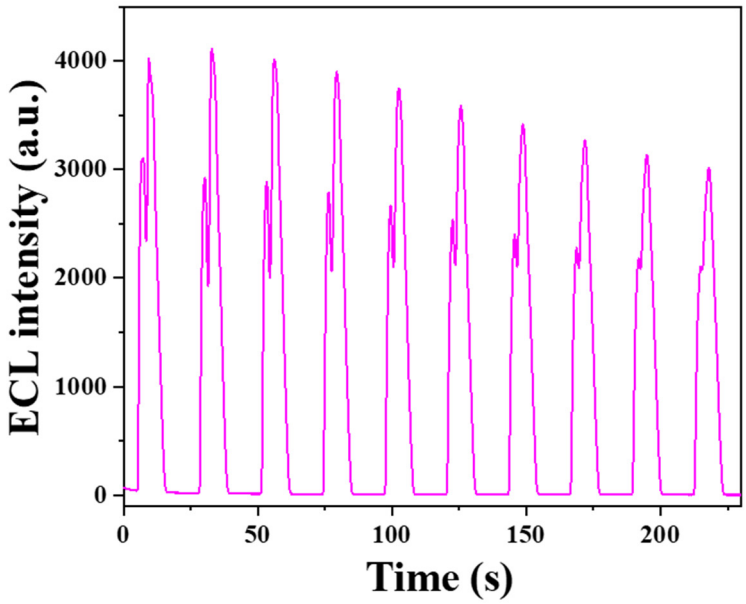

Figure S5. Stability test (ten cycles).