Electronic Supplementary Material (ESI) for Biomaterials Science.
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Supplementary information for

Two-point immobilization of conformation-specific beta$_2$-adrenoceptor for recognizing the receptor agonists or antagonists inspired by binding-induced DNA assembly

Juan Gao, Xinyi Yuan, Xinxin Zheng, Xue Zhao, Taotao Wang, Chaoni Xiao, Qi Liang, Jing Wang, Qian Li, and Xinfeng Zhao*

College of Life Sciences, Northwest University, Xi'an 710069, China

*Corresponding author: Xinfeng Zhao

Tel: (+86) 029 88302686

E-mail: zhaoxf@nwu.edu.cn

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1 Supplementary method

1.1 Cryo-field emission scanning electron microscopy (cryo-FESEM)

We characterized the morphology of the microspheres by a Hitachi SU8000 field-emission scanning electron microscope, which consists of a field emission gun, lower and upper secondary electron detectors, and a Genesis software system. The microspheres were dispersed on double-sided tape and the tape was immobilized on a sample holder. The sample holder was placed on a cold stage and the temperature was set as -150 °C. The accelerating voltage and the working distance were set as 5 kV and 9.5 mm, respectively. Observations were conducted at 1.0 k × and 10.0 k × magnification.

1.2 Fluorescence analysis

Fluorescence microscope Olympus BX61 (Olympus Corporation, Tokyo, Japan) was used to characterize the DNA and RNA strands on the microspheres. The excitation wavelengths were set as 575 nm and 495 nm to detect ROX and FAM, respectively. The samples were placed over glass slides, covered with glass coverslips, and exposed to the microscope.

1.3 X-ray photoelectron spectroscopy (XPS)

XPS was carried out on a Thermo Fisher Esca Lab 250Xi analyser using a monochromatic Al Kα source (1486.6 eV) with the filament voltage and electric current of 14.7 keV and 10 mA. The measurements were carried out with 0.5 mm entrance slit of the spectrometer and a pass energy of 30 eV. A charge neutralizer was used to compensate for the charging of the sample. The binding energy was calibrated via the

C1s peak at 284.8 eV.

1.4 Immuno-TEM analysis

We immersed the bare, aptamer modified and β_2-AR coated microspheres into tris-buffered saline containing 1% Tween 20 (TBST) and blocked the microspheres with 5% skimmed milk for 1 h. The microspheres were washed with TBST three times and then incubated with 0.68 μg β_2-AR antibody (Abcam, Cambridge, UK) at 4 °C overnight. After washing with TBST, we incubated the microspheres with 10.0 μg goat anti-rabbit IgG/Gold (Abcam, Cambridge, UK) for 1 h. The microspheres were washed by TBST and dried at 55 °C for immuno-TEM analysis.

The immuno-TEM analysis was performed by FEI Talos-S (FEI company, USA) with Super X energy spectrum at an accelerating voltage 200 kV. The dried microspheres were dispersed in ethanol for about 10 minutes and dropped onto copper mesh grids. The grids were fixed using a molybdenum ring and clamp. The magnifications of images were set at 2.0 k ×, 5.5k ×, 28.5k ×, and 150.0k ×, respectively.

2 Supplementary Figures

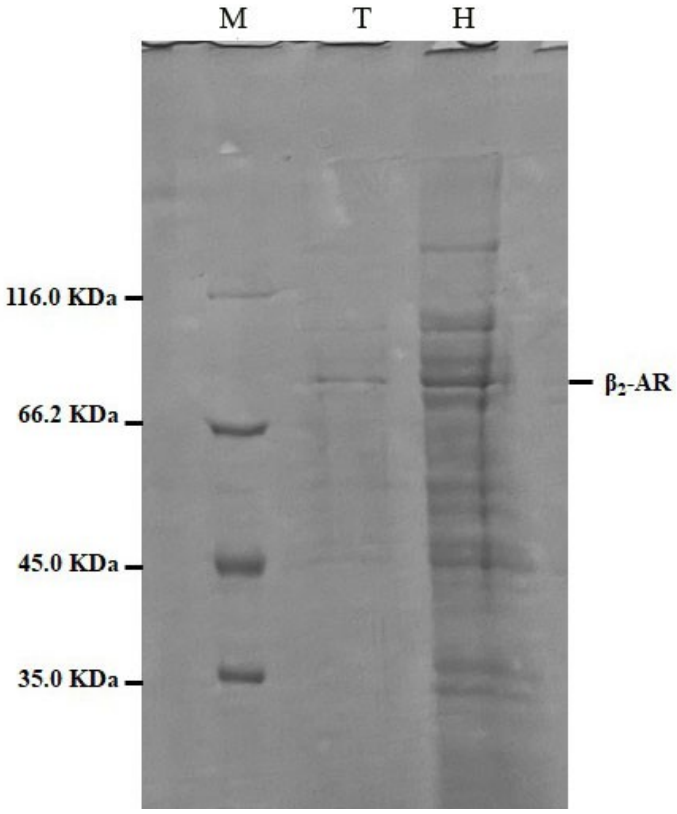
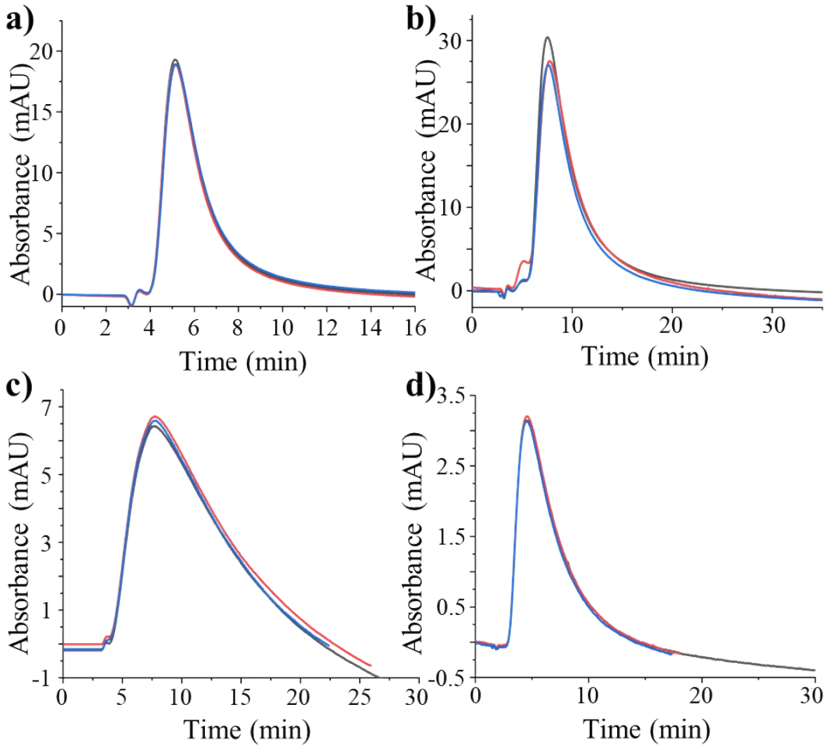


Figure S1 Purity analysis of the immobilized β_2-AR by SDS-PAGE. The proteins were dissociated from the microspheres by heating the receptor conjugated microspheres for five minutes at 95 °C and washing the microspheres with phosphate buffer (pH 7.4) in the presence of 250 mM imidazole. Lane M: marker; Lane T: the receptor was immobilized by the current two-point method; Lane H: the receptor was immobilized by Ni^{2+}-NTA system.



e)

Ligands	Retention time (min)			RSD(%)
	10 days	20 days	30 days	
Salbutamol	5.99	6.01	6.04	0.34
Terbutaline	7.47	7.74	7.53	1.53
Methoxyphenamine	7.59	7.71	7.72	0.77
Ephedrine	4.47	4.65	4.58	1.62

Figure S2 Stability evaluation of the immobilized β_2-AR by chromatographic methods. A, B, C and D were salbutamol, terbutaline, methoxyphenamine, and ephedrine hydrochloride respectively.

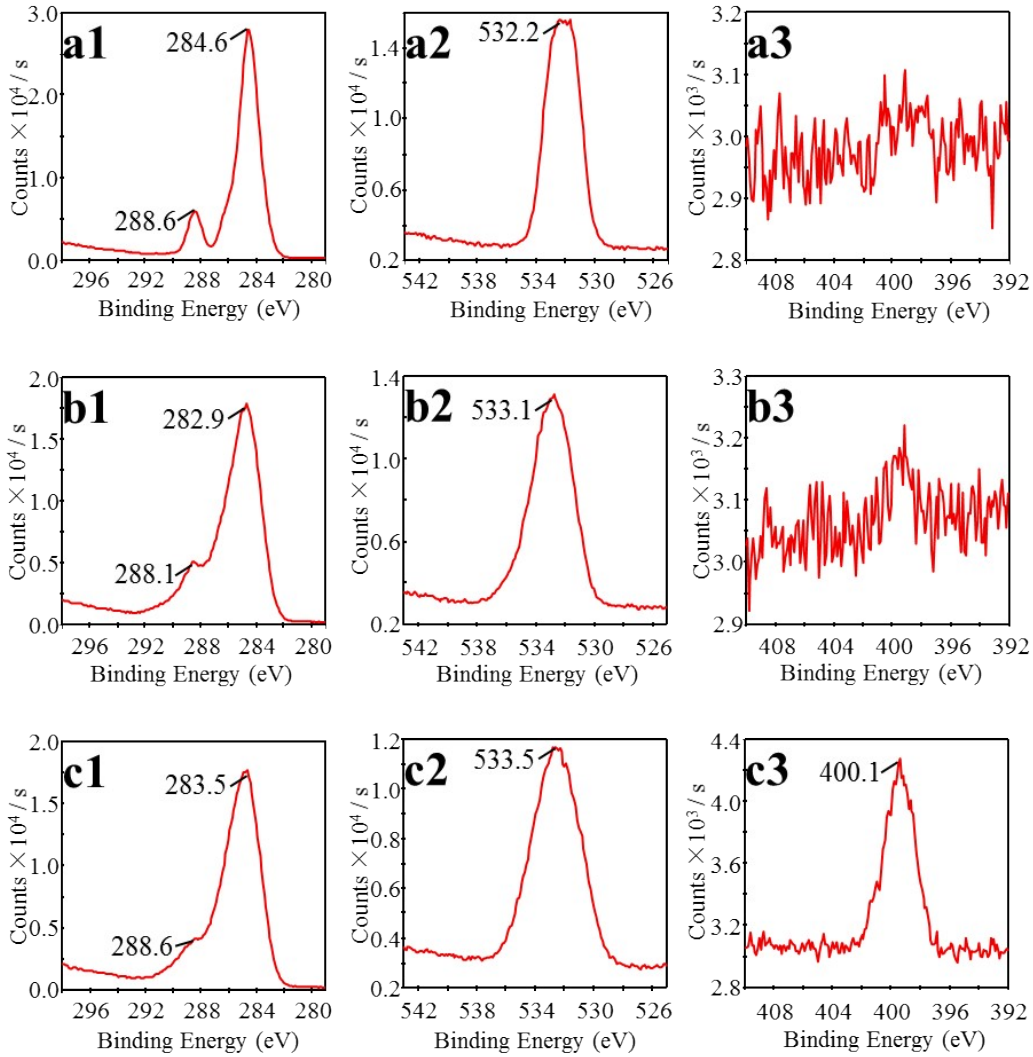


Figure S3 Detailed elemental analysis of the microspheres by X-ray photoelectron spectroscopy. a1-a3) Amine microsphere; b1-b3) Strand B modified microsphere; c1-c3) β_2-AR coated microsphere.

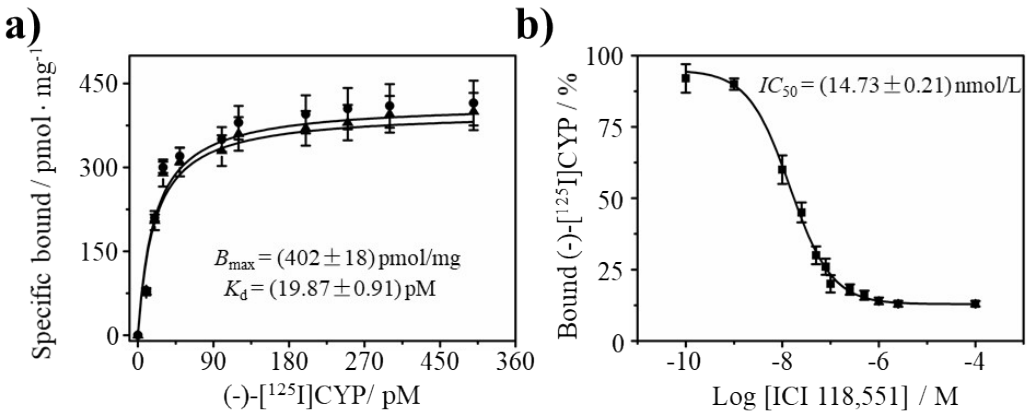
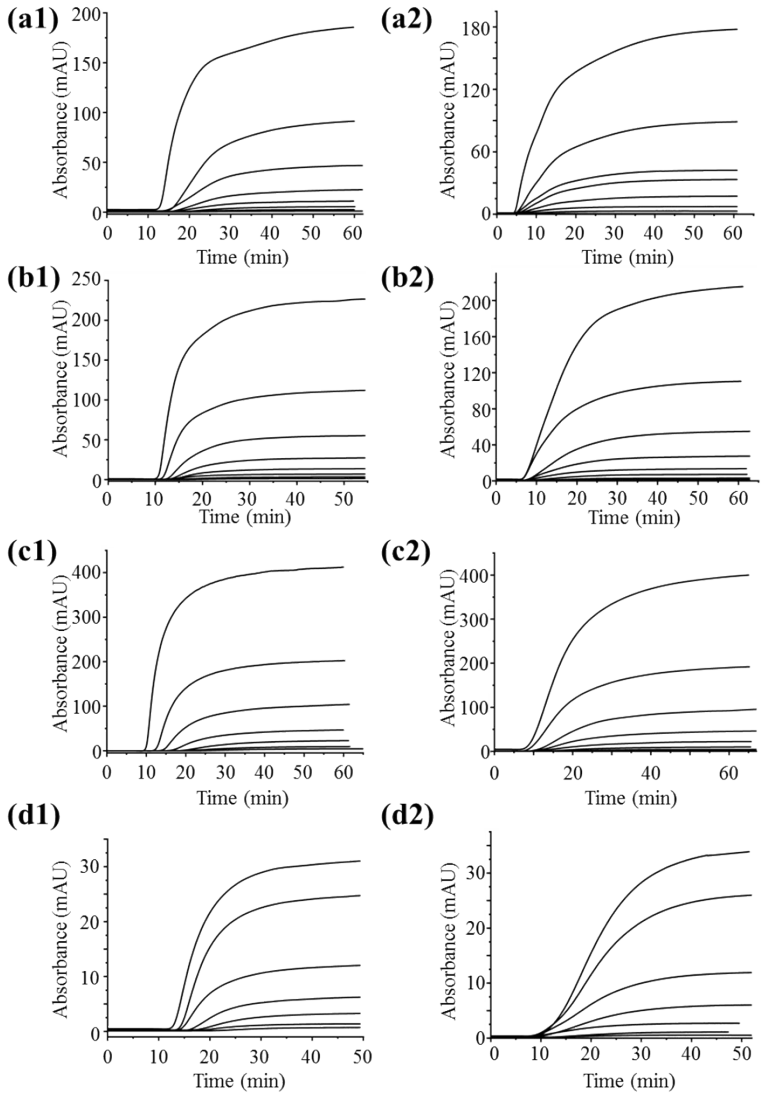


Figure S4 Saturation (a) and competition (b) binding for (-)-[^{125}I]CYP to β_2-AR. β_2-AR in cell lysate (●); immobilized β_2-AR (▲); ICI 118,551 (■).



(e) Ligand	Single-point immobilization method		Double-point immobilization method	
	K_A ($\times 10^5$ M^{-1})	m_L ($\times 10^{-9}$ M)	K_A ($\times 10^5$ M^{-1})	m_L ($\times 10^{-9}$ M)
Salbutamol	1.71	43.70	3.62	14.30
Terbutaline	0.35	18.40	1.34	16.70
Ephedrine	0.16	712.00	0.22	333.00
Methoxyphenamine	0.59	61.40	0.84	50.00

Figure S5 Frontal analysis of four drugs on single-point and two-point immobilization β_2-AR column. a) Salbutamol, b) Terbutaline, c) Methoxyphenamine, d) Ephedrine. From bottom to top, the mobile phase concentrations of the drugs were 0.5, 1.0, 2.0, 4.0, 8.0, 10.0, 20.0 and 40.0 mM for salbutamol; 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 64.0 mM for terbutaline; 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 64.0 mM for methoxyphenamine; and 5.0, 10.0, 20.0, 40.0, 80.0, 160.0 and 200.0 mM for ephedrine. e) Binding parameters of β_2-AR and the four drugs.