Supporting Information

Materials and chemicals

Levonorgestrel was purchased from Hubei Goto Biopharm Co., Ltd. $H_2PtCl_6 \cdot 6H_2O$ and $[Cu(CH_3CN)_4]PF_6$, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and N-Hydroxysuccinimide (NHS) were purchased from Energy Chemical. Poly (styrene-*co*-maleic anhydride) (PSMA) was purchased from Macklin® Co., Ltd. 4-morpholineethanesulfonic acid (MES) was purchased from Aladdin® Co., Ltd. Monoclonal mouse antibodies for IL-6 (Ab1 and Ab2 recognize the different position of IL-6), goat anti-mouse IgG, IL-6 standard were purchased from Suzhou Hongxin Biotechnology Co., Ltd. sodium dodecyl sulfate (SDS) was purchased from Beijing Solarbio Science & Technology Co., Ltd.

Instrumentation

TEM images were acquired with a Tecnai G2 F20 S-TWIN transmission electron microscope and JOEL JEM-2100 (operated at an acceleration voltage of 200 kV). DLS and Zeta potential measurements were performed on a Horiba SZ-100 Nanoparticle Size Analyzer. UV-Vis spectroscopy was acquired using a TU-1901 double-beam UV-Vis spectrophotometer. Steady-state emission spectra of the compound were analyzed using a Horiba FluoroLog-3 spectrofluorometer. ESI-TOF-MS spectroscopy was conducted on an AB SciexX500R Q-TOF spectrometer. Fourier transform infrared (FT-IR) spectroscopy was conducted using a Bruker TENSOR 27 FT-IR spectrometer in the 1000–4000 cm^{-1} region with KBr pellet method. Elemental analyses (EA) were carried out with a Perkin-Elmer 240 elemental analyser.

Synthesis of Pt_2Cu_4 cluster

Pt_2Cu_4 cluster was prepared according to a previously reported method. 1 mL $H_2PtCl_6 \cdot 6H_2O$ (6.25 μmol) dissolved in methanol was added to 0.5 mL levonorgestrel solution in CH_2Cl_2, followed by addition of 8 μL Et_3N. 1 mL $[Cu(CH_3CN)_4]PF_6$ (4.6 mg, 12.5 μmol) in DCM was added, and then stirred for 2 h. The resultant suspension was centrifuged at 10000 rpm for 3 min, then allowed to evaporate slowly at room temperature to yield yellow strip crystals.

Preparation of carboxyl Pt_2Cu_4 nanobeads (NBs)

15 mg PSMA and 5 mg Pt_2Cu_4 cluster were dissolved in 5 mL DCM, then slowly added to 45 mL water containing 20 mg SDS for crushing with ultrasound at a power of 500W for 30 minutes. The obtained solution was stirred at room temperature for 12 h and centrifuged at 14,000 rpm for 10 min, then suspended in water. Carboxyl Pt_2Cu_4 NBs was acquired by hydrolysis of the anhydride groups with addition of ammonia.

Antibody conjugation to Pt_2Cu_4NBs (Pt_2Cu_4NBs-Ab1)

100 μL carboxyl Pt_2Cu_4NBs in MES buffer (pH 5.0, 50 mM) was activated with addition of 10 μL NHS (2 mg/mL) and 10 μL EDC (2 mg/mL) for 30 min. The above solution was centrifuged and suspended in CBS buffer (pH 9.6, 50 mM), followed by addition of 5 μL IL-6 Ab1 for 2 h. Then, 20 μL 4% BSA solution was added and stirred for 2 h to block the surface of nanospheres, and centrifuged at 14,000 rpm for 15 min at 4 °C.

Fabrication of Pt_2Cu_4-based immunochromatography test strips

The test strip consists of nitrocellulose membrane, sample pad and absorbent pad. The IL-6 antibody Ab2 and goat anti-mouse IgG was diluted to proper concentrations, filtered, and dispensed on test line (T line) and control line (C line) at a jetting rate of 1 μL/cm respectively. The distance between T line and C line was approximately 10 mm, and the width of strip was allowed cutting to 4 mm.

Lateral flow immunoassay for IL-6

20 μL sensing probe and 80 μL sample with different concentrations of IL-6 were mixed in micro-well for 5 min reaction. Then, the strips were taken out for fluorescence imaging under UV light. Images were analyzed by image-J software to calculate the ratio of fluorescence intensity of test and control zones.

To evaluate specificity of the as-fabricated sensor, normal cytokines and proteins such as IL-12，IL-10，TNF-α，IFN-γ，HSA and BSA were tested.

To verify the applicability of this method in serum samples, 100, 1000 and 5000 pg/mL IL-6 in bovine serum were tested.

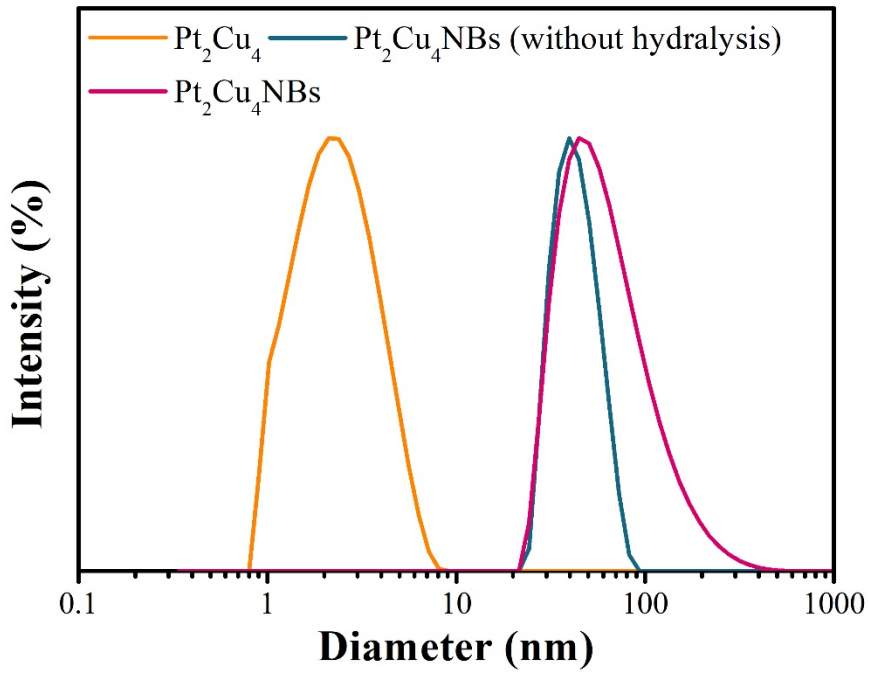

Fig. S1. Hydrodynamic diameter of Pt_2Cu_4, Pt_2Cu_4NBs without hydrolysis and Pt_2Cu_4NBs.

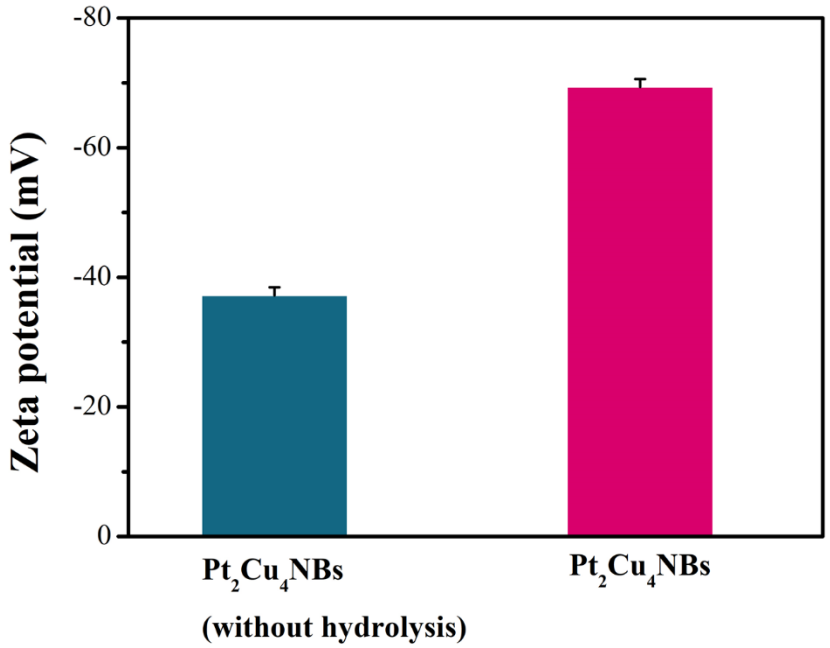

Fig. S2. Zeta potential of Pt_2Cu_4NBs without hydrolysis and Pt_2Cu_4NBs.

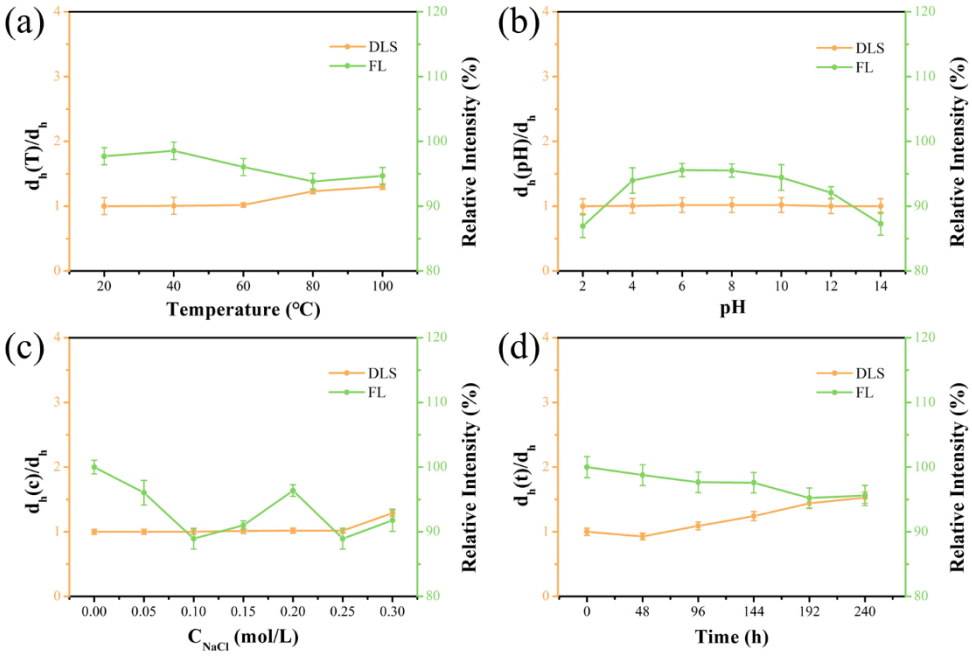

Fig. S3. Stability of Pt_2Cu_4NBs under (a) different temperatures, (b) different pH values, (c) different concentrations of NaCl solution, and (d) PBS for different time. $d_h(C)$, $d_h(pH)$, $d_h(T)$, and $d_h(t)$ are the size of Pt_2Cu_4NBs in tested physiological environments, which was normalized to the DLS diameter (d_h) of Pt_2Cu_4NBs in Milli-Q water at 25 ℃, respectively.

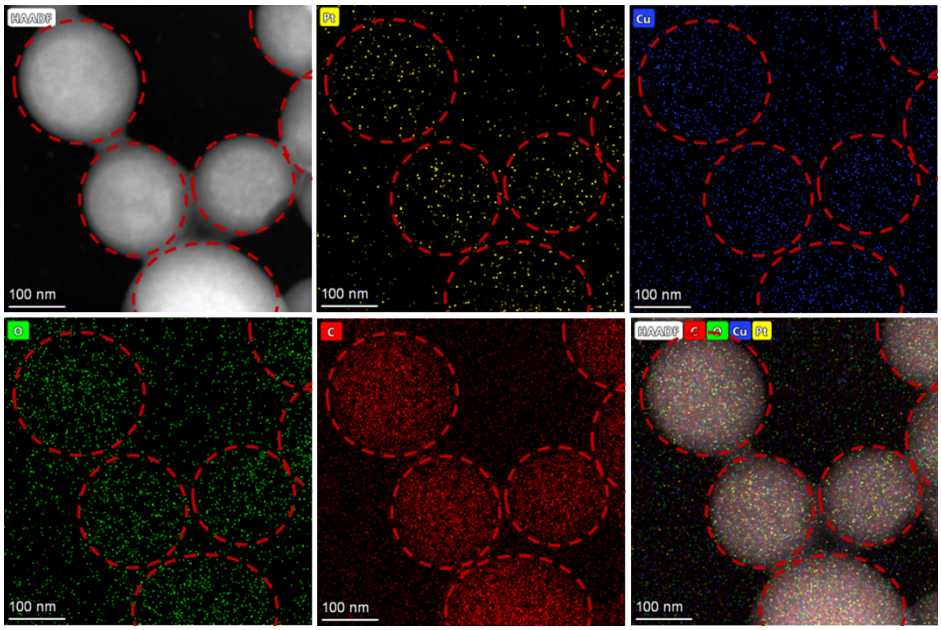

Fig. S4. TEM-EDXS mapping for Pt_2Cu_4 NBs.

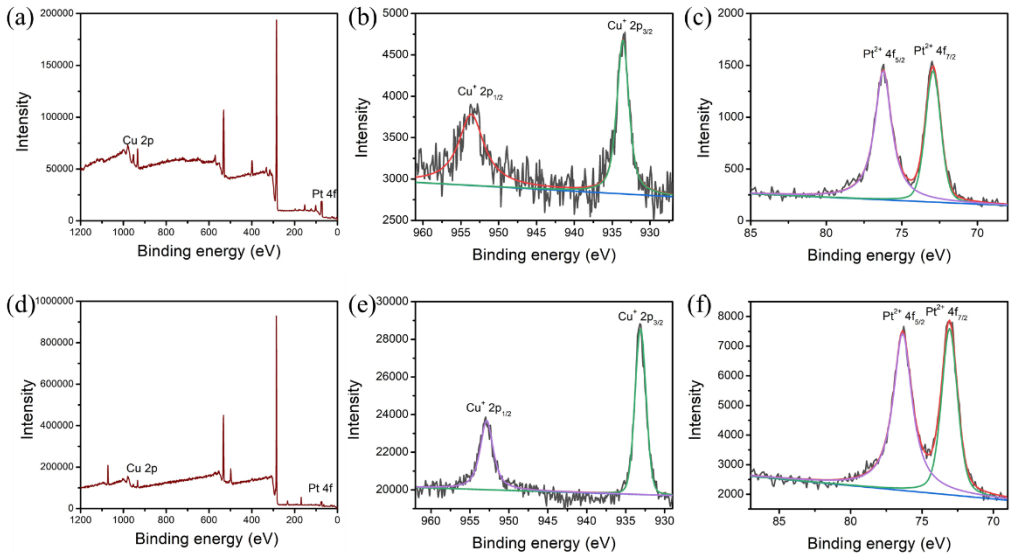

Fig. S5. XPS analysis for Pt_2Cu_4 (a-c) and Pt_2Cu_4 NBs (d-f).

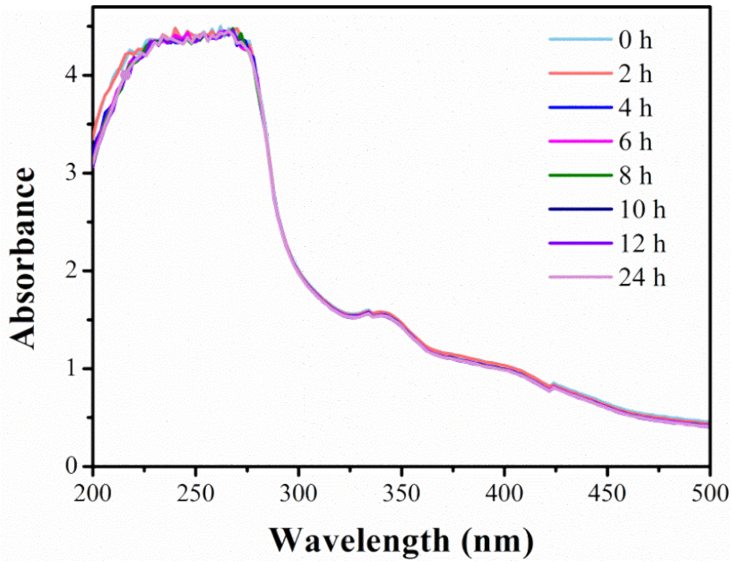

Fig. S6. The stability of UV-Vis absorption of Pt_2Cu_4NBs-Ab1.

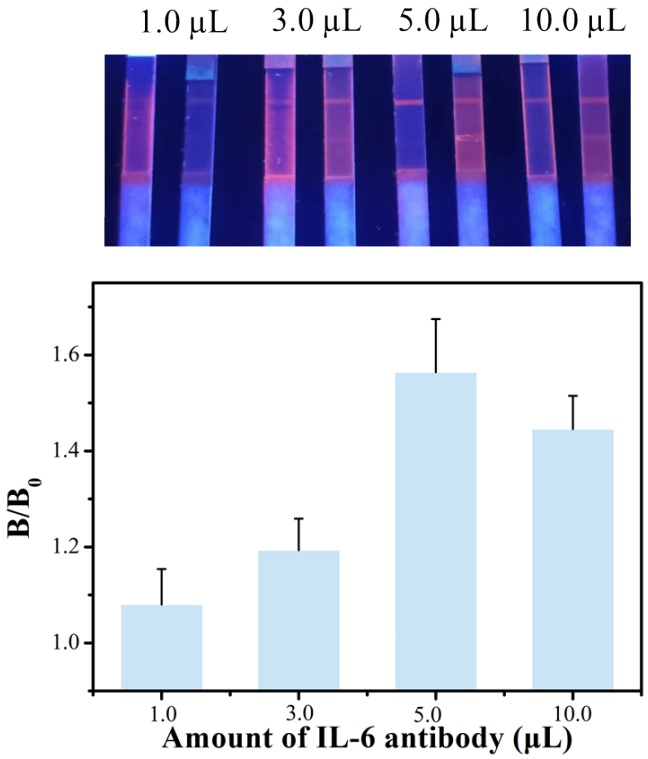

Fig. S7. Optimization of amount of IL-6 Ab for conjugation.

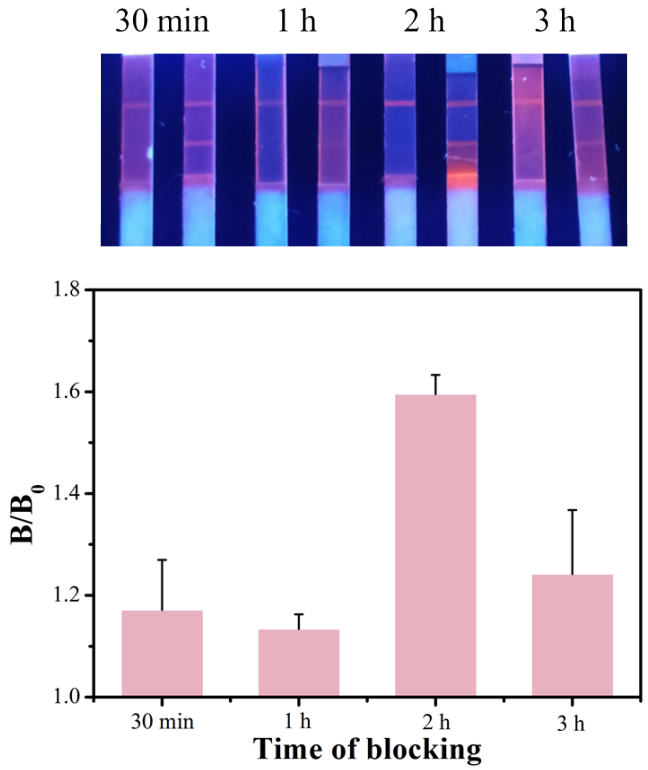

Fig. S8. Optimization of different time of blocking.

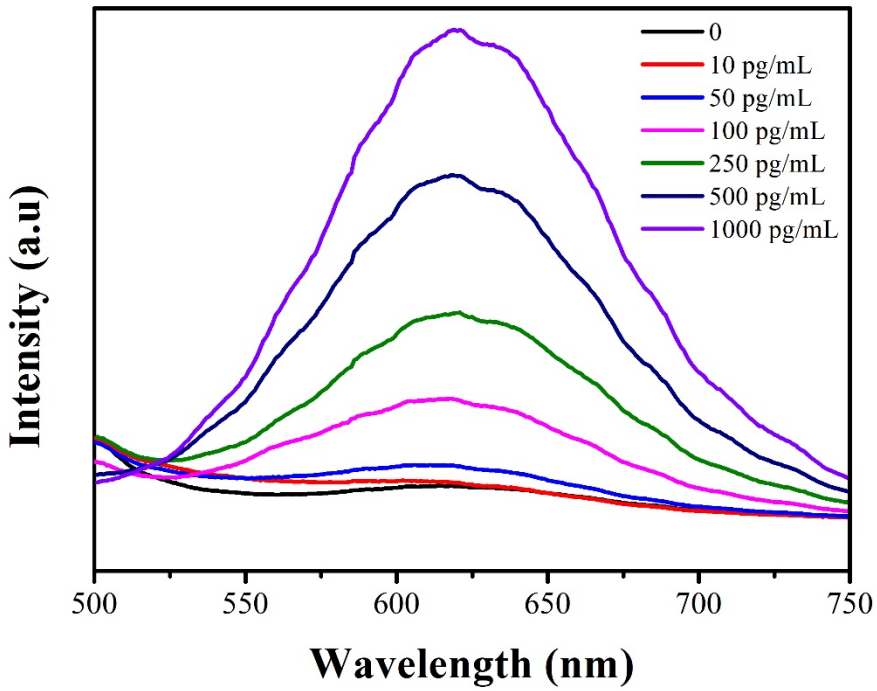

Fig. S9. Fluorescence spectrum of T lines with addition of 0–1000 pg/mL IL-6 under 365 nm laser excitation.

Table S1. EA (%) data of Pt_2Cu_4 clusters.

Sample	Element	Calculated (%)	Experimental (%)
Pt_2Cu_4	Carbon	64.35	63.41
	Hydrogen	6.94	6.97

Table S2. Comparison of different sensor platforms for the detection of IL-6.

Signal probe type	Detection or amplification strategy	Limit of detection	Incubation time (min)	Real sample analysis	Reference
AuNP	LFICS	65.2 ng/mL	10	Serum	[1]
Label free	Voltammetric immunosensors	4.8 pg/mL	30	Serum	[2]
TiO_2/CdS/CdSe	Photoelectro-chemical	0.38 pg/mL	60	/	[3]
Thermal	Functionalized screen-printed electrodes	3.4 pg/mL	45	Plasma	[4]
Au@Fe_3O_4	SERS	0.028 pg/mL	60	Serum	[5]
AuNPs	Electrochemical immunosensor	0.654 pg/mL	About 13 h	Serum	[6]
Fluorescence cluster	LFICS	42.66 pg/mL	15	Serum	This work

Table S3. Recovery of IL-6 in serum samples.

Sample	Spiked concentration (pg/mL)	Detect concentration (pg/mL)	Recovery (%)	CV(%)
Serum	10	9.84±1.05	98.1	8.53
	100	96.79±6.16	95.8	6.33
	500	491.01±56.84	97.7	8.79

Table S4. Standard addition experiment of IL-6 in serum.

Sample	Spiked concentration (pg/mL)	Detected concentration (pg/mL)	Calculation of unknown concentration	Calculated value of addition
Serum	20	30.93±0.76	10.93±0.76	9.37±1.87
	50	58.79±1.39	8.79±1.39	
	100	108.39±3.48	8.39±3.48	

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