Electronic Supplementary Information for:

A novel L-cysteine regulated polydopamine nanoparticle-based electrochemiluminescence image application

Xin Zhang[a,b], Yixin Nie[a,b], Qian Zhang[a], Zihui Liang[a,b] and Qiang Ma[a,b]*

[a] *Department of Analytical Chemistry, College of Chemistry, Jilin University, Changchun, 130012, China*

*Corresponding author, Tel.: +86-431-85168352, E-mail address: Qma@jlu.edu.cn

Contents

(a) Chemicals and reagents

All chemical reagents in this experiment were of analytical grade. Dopamine was obtained from Xilong Chemical Co., Ltd. (Guangdong, China). L-cysteine ($C_3H_7NO_2S$), sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4) and sodium phosphate (Na_3PO_4) were purchased from Sigma Co. (Shanghai, China). PEI was provided by Aladdin Industrial Company. Reduced L-glutathione (GSH) and bovine serum albumin (BSA) were ordered from Sangon Biotech (Shanghai) Co. Ltd. Nethyl-N‘-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (NHS) were obtained from Sinopharm Chemical Reagent Co.Ltd. ATP was obtained from Beijing Dingguo Biotechnology Co. Ltd. The oligonucleotides used were synthesized and purified by Qingke Co., Ltd. (Tianjin, China). The sequences of aptamer and complementary DNA were listed in Table S1. All reagent solutions were prepared using ultrapure water (resistivity as 18 $M\Omega \cdot cm^{-1}$ at 25 ^oC). Human serum samples were obtained from the China-Japan union hospital of Jilin university.

(b) Apparatus

TEM images of the nanomaterials were obtained with a JEM-2100F field emission electron microscope operating at 200 kV acceleration voltages. FT-IR, Photoluminescence (PL), and UV-vis absorption spectra were acquired by Thermo Nicolet 360 FTIR spectrometer, Shimadzu RF-5301 PC spectrofluorophotometer and U-5100 UV/VIS spectrophotometer, respectively. The manufacturer of X-ray diffractometer is PANalytical B.V., and its model is Empyrean. The scans were run using Cu anode with a voltage of 40 KV and the intensity of 40 mA. The scan range used in the present case is 3 to 90^o-5 θ with scan step size of 0.026 θ. The nuclear magnetic resonance spectrometer is produced by Swiss BRUKER Instrument Company, and its model is Avance NEO. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a 400 MHz spectrometer in DMSO. The CV data were

acquired with a CHI 660B electrochemical workstation with the glassy carbon electrode (GCE) as the working electrode. In the all electrochemical analysis process, a platinum wire and an Ag/AgCl (saturated KCl) electrode was employed as the counter electrode and the reference electrode, respectively.

(c) Synthesis of PDAcp NPs

All synthetic steps were conduct at room temperature (23 ^{o}C). 6 mg dopamine was added into 6 mL Tris-HCl buffer (10 mM, pH = 8.5) and stirred for 2 minutes. When the color of the system varied from colorless to light brown, 3 mg L-cysteine was added into the reaction system and stirred for 1.5 h. L-cysteine contains thiol and amino groups which can undergo Michelal addition reaction and nucleophilic addition reaction with oligomers of dopamine to form internal ordered nanoparticles. 260 μL H_2O_2 (30 % aqueous) was introduced to stabilize the obtained nanoparticles. Then the obtained nanoparticles were dialyzed for 12 h to remove the unreacted L-cysteine. The obtained PDA-L-cysteine NPs were dried and stored at 4 ^{o}C. A solution with 2.5 mg/mL PDA-L-cysteine NPs was prepared in the next experiments. PEI (MW = 600) was used to regulate the surface state of PDA-L-cysteine NPs. A series of different PDAcp NPs were synthesized by adding PEI 3 mg, 6 mg, 18 mg, 30 mg, 48 mg, and 60 mg according to the mass concentration ratio of dopamine to PEI of 1/2, 1, 3, 5, 8, and 10, respectively. After stirring for 7 h, the samples were dialyzed for 12 h to remove unreacted PEI. The PDAcp NPs we final obtained was slightly darker yellow than PDA-L-cysteine NPs. The obtained PDAcp NP was dried and stored at 4 ^{o}C, and dissolved in ultra-pure water to a certain concentration before use.

Quantum yield and electrochemiluminescence (ECL) efficiency of PDAcp NPs were conducted on the basis of our previous study[1] and calculated according to the following equations:[2]

$$Y_u = Y_s\left(\frac{I_u}{I_s}\right)\left(\frac{A_s}{A_u}\right)\left(\frac{n_u^2}{n_s^2}\right) \quad (1)$$

$$\emptyset_{ECL} = \emptyset_{ECL}^{0}(\frac{I}{I^0})(\frac{Q^0}{Q}) \quad (2)$$

In eq(1) Y_u represents quantum yield, I represents the measured integrated PL intensities, A represents the ABS intensity, n is the refractive index of the solvent, u is the PDAcp NPs, and s represents the standard sample (quinine sulfate (0.54) in 0.1 mol/L H_2SO_4).[3] In eq (2) ΦECL^0 is the ECL efficiency of the standard ($Ru(bpy)^{3+}$, 5 %), I and I^0 are the integrated ECL intensities of the PDAcp NPs and the standard, and Q and Q^0 are the faradic charges (in coulombs) for the investigated and the standard species, respectively.

(d) Synthesis of Fe_3O_4 NPs

The Fe_3O_4 NPs were synthesis according to the previous study.[4] First the ferric and ferrous chlorides were mixed in water at the temperature of 35 ^oC. Under vigorous agitation, 1 mol/L NaOH solution was added to the solution at 50 ^oC then heated the system at 80 ^oC for 1 h in N_2 atmosphere. Finally, the resulting precipitate was separated by magnetic decantation and washed with ultrapure water for three times and stored in in refrigerator at 4 ^oC for use.

(e) Synthesis of cDNA@PDAcp NPs and apt @ Fe_3O_4 NPs

PDAcp NPs and cDNA were connected by the reaction of the amino group of PDAcp NPs with the carboxyl group of cDNA to form an amide bond. 4 μL 3 mmol/L EDC and 2 μL 1.5 mmol/L NHS were added with 60 μL 100 μmol/L cDNA to activate carboxyl group of cDNA. After 30 minutes, 2 mL as-prepared PDAcp NPs were added into the system with the incubated time of 16 h. Finally, cDNA@PDAcp NPs were dialyzed for 3 h to remove the unreacted cDNA. The synthesis method of Fe_3O_4NPs refers to supporting information. The thiol group of aptamer can form a coordination bond with metallic iron on Fe_3O_4 NPs surface. 1.5 mL Fe_3O_4 NPs and 20 μL 100 μmol/L aptamer were reacted at room temperature for 24 h. 0.1M NaCl aqueous solution and 2 wt% BSA were added into the solution. The obtained apt @ Fe_3O_4 NPs were separated, washed with ultrapure water for 3 times, and stored in refrigerator at 4 ^oC.

(f) Sequences of the used oligonucleotides

Table S1 Sequences of the used oligonucleotides in this work.

Name	Sequences (5’ to 3’)
Aptamer	5’-ACC TGG GGG AGT ATT GCG GAG GAA GGT-SH-3’
Complementary DNA	5’-COOH-$(CH_2)_6$-ACC TTC CTC CGC AAT ACT CCC CCA GGT-3’

(g) Results of Determination of ATP in Human Serum Samples

Table S2 Results of Determination of ATP in Human Serum Samples

Sample	Spiked (nM)	Founded (nM)	Recovery (%)	RSD (%) (n=3)
1	0.10	0.098	98.00	4.26
2	1.00	1.03	103.00	2.79
3	10.00	9.96	99.60	3.14

(h) TEM of PDAcp NPs

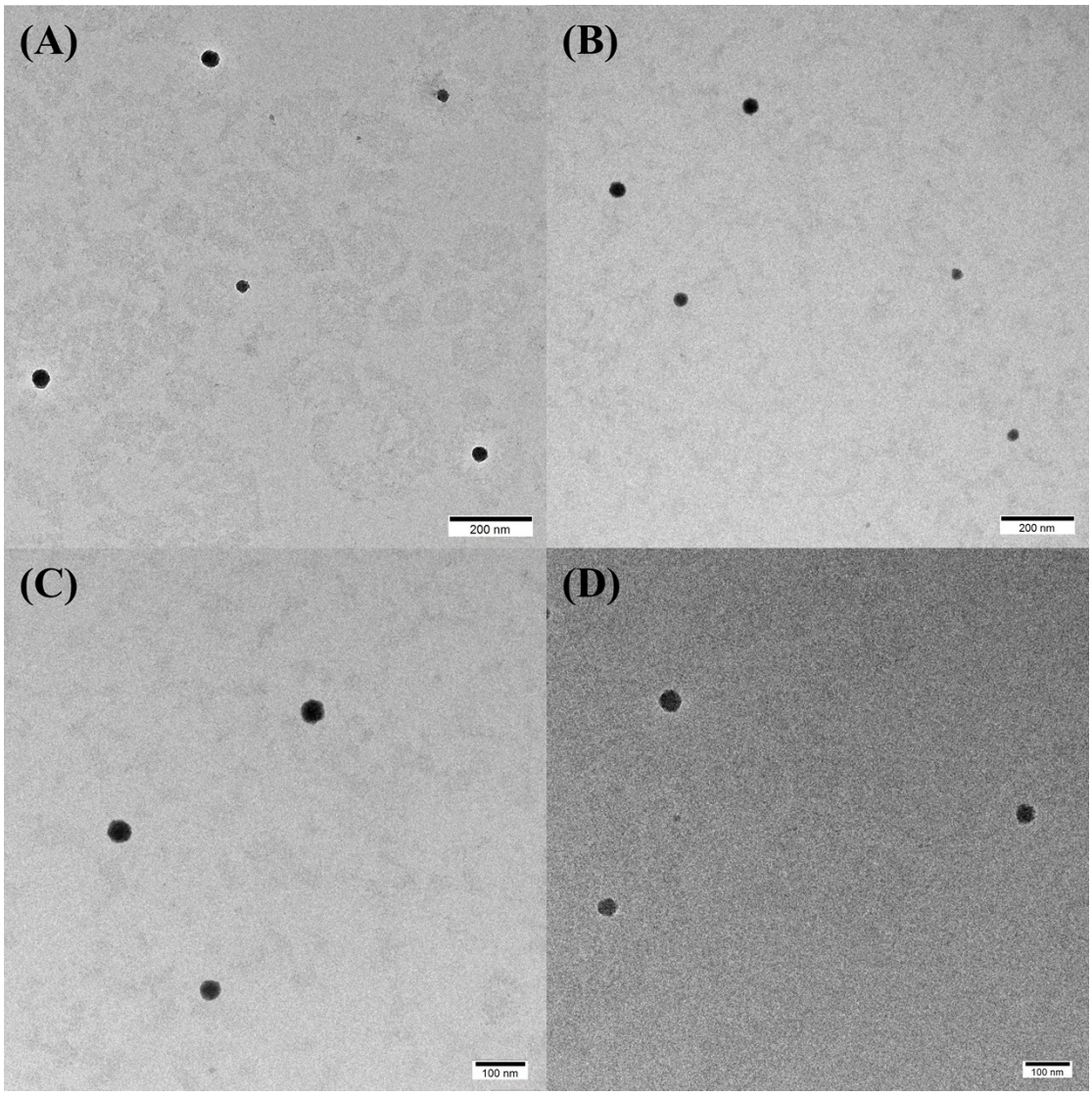

Fig S1. TEM of PDAcp NPs.

(i) XRD patterns of PDA NPs, PDA-L-cysteine NPs and PDAcp NPs

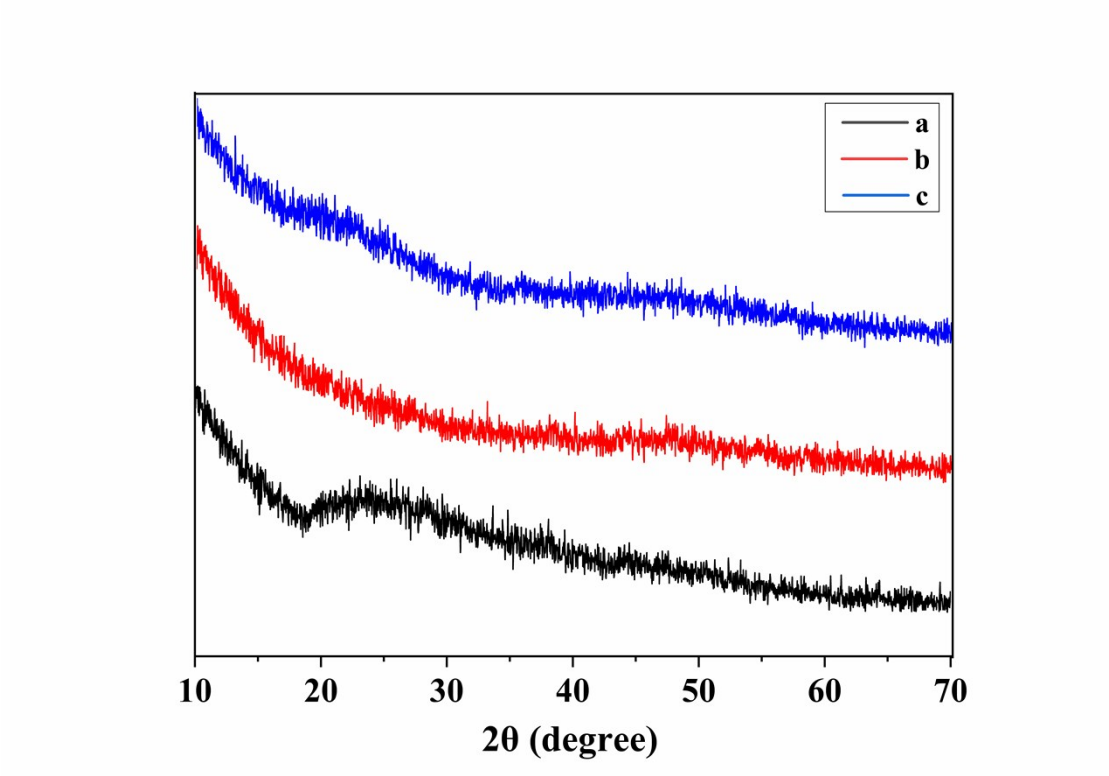

Fig S2. XRD patterns of PDA NPs (a), PDA-L-cysteine NPs (b) and PDAcp NPs (c).

(j) FT-IR of L-cysteine

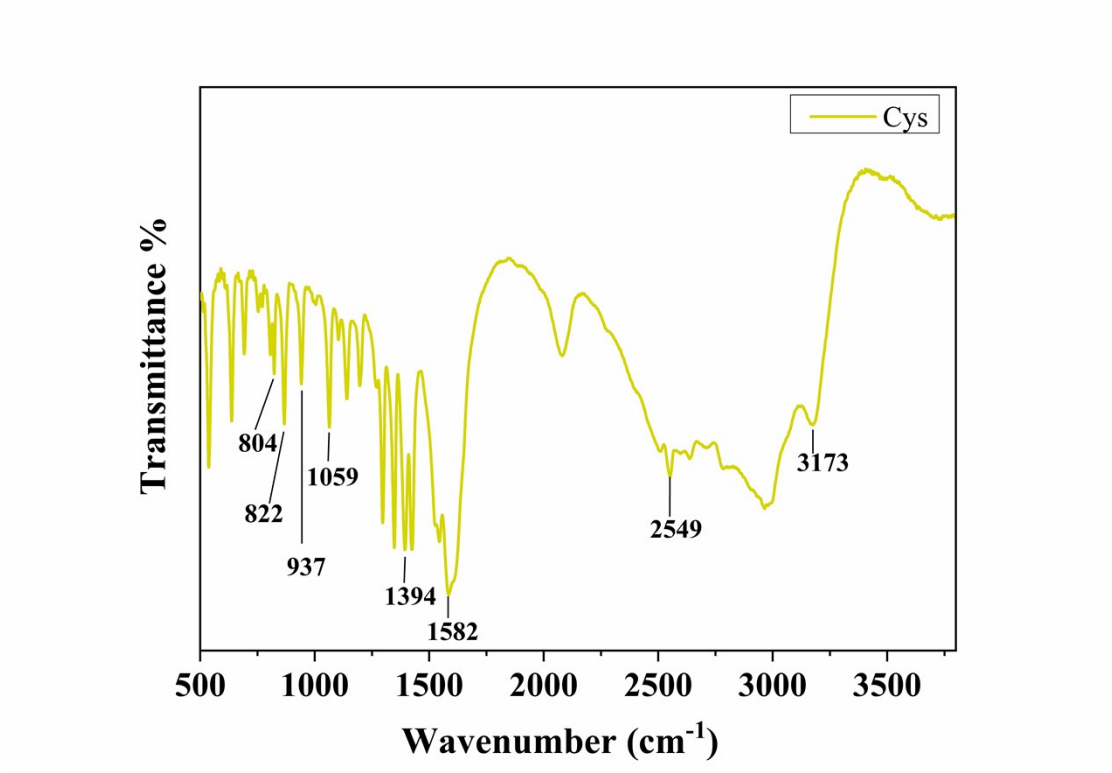

Fig S3. FT-IR of L-cysteine.

(k) ^{1}H-NMR of PDA NPs, PDA-L-cysteine NPs and PDAcp NPs in the aromatic region

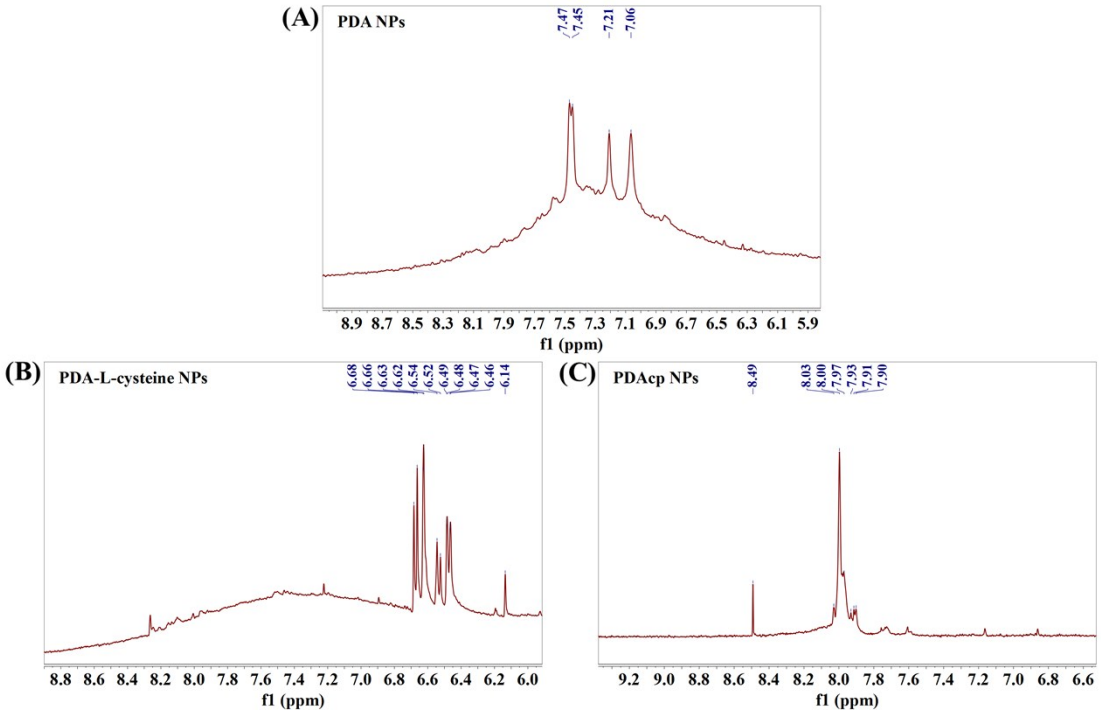

Fig S4. ^{1}H-NMR of PDA NPs (A), PDA-L-cysteine NPs (B) and PDAcp NPs (C) in the aromatic region (400 MHz, DMSO).

(l) UV-Vis absorption spectrum of PDAcp NPs

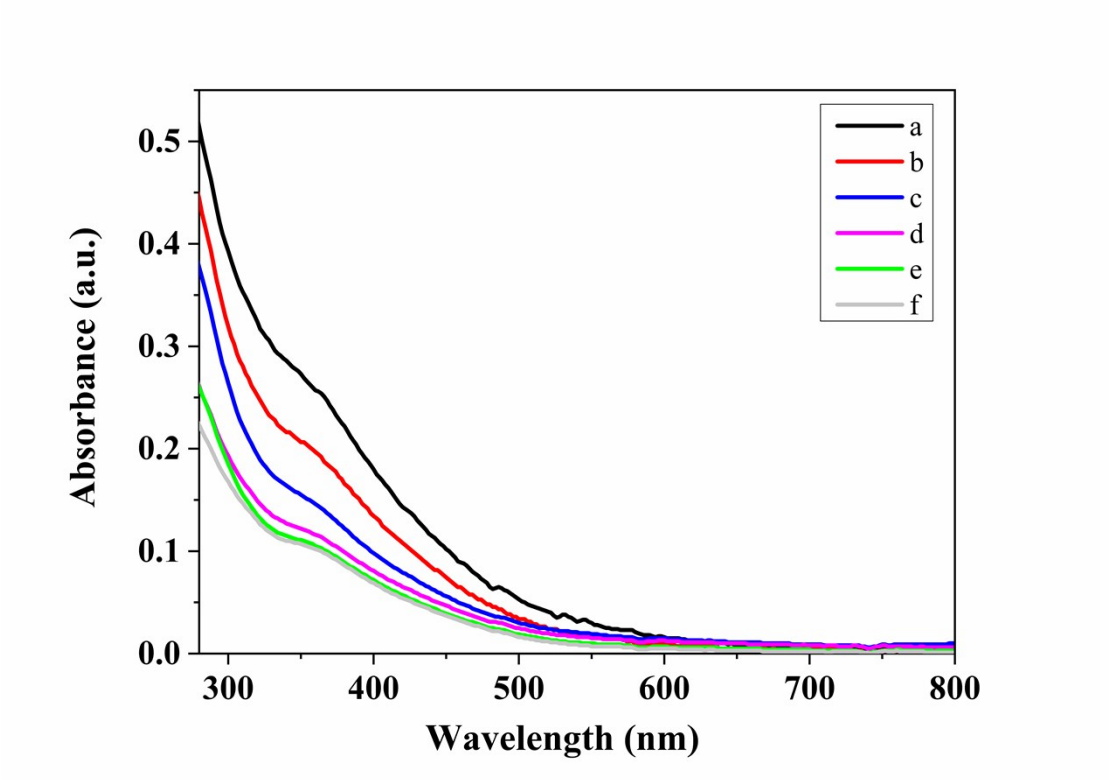

Fig S5. UV-Vis absorption spectrum of PDAcp NPs with different amount of PEI (from a to f was the mass concentration ratio of dopamine to PEI was 1/2, 1, 3, 5, 8, 10, respectively).

(m) Comparison of fluorescence spectrum of PDA-L-cysteine NPs and PDAcp NPs

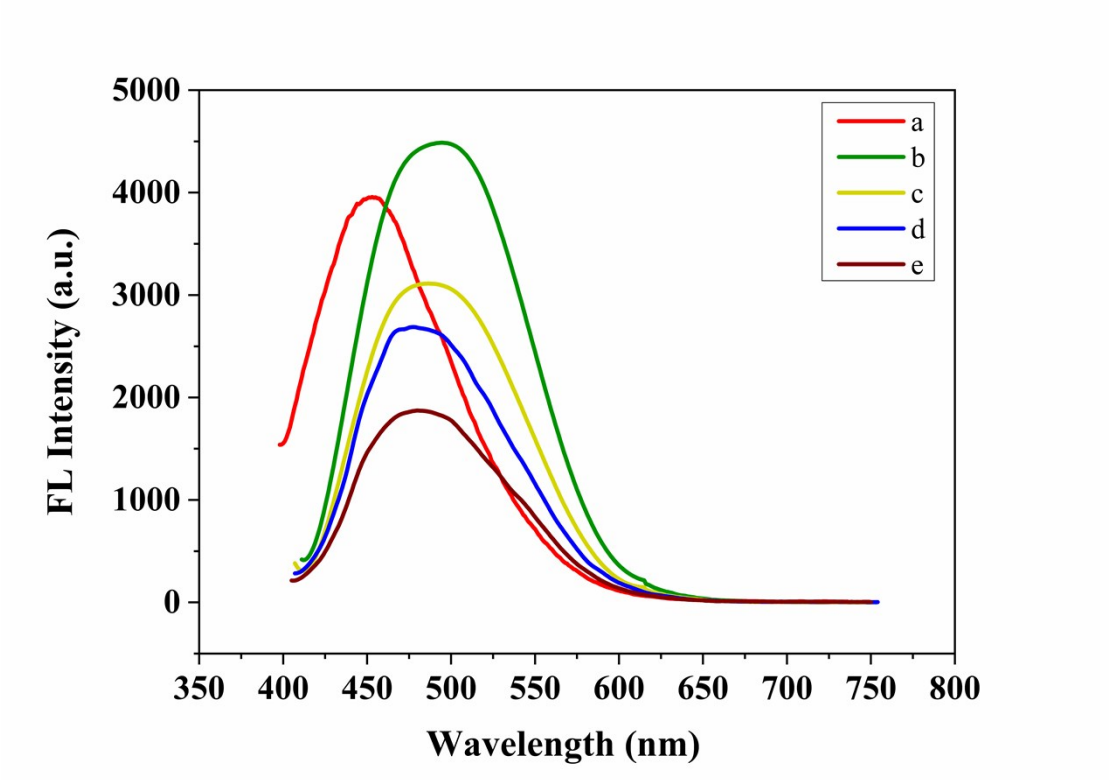

Fig S6. The fluorescence spectrum of PDA-L-cysteine NPs (a) and PDAcp NPs with different amount of PEI (from b to e was the mass concentration ratio of dopamine to PEI was 1/2, 1, 8, 10, respectively).

(n) FT-IR spectrum of glutathione-regulated PDA NPs

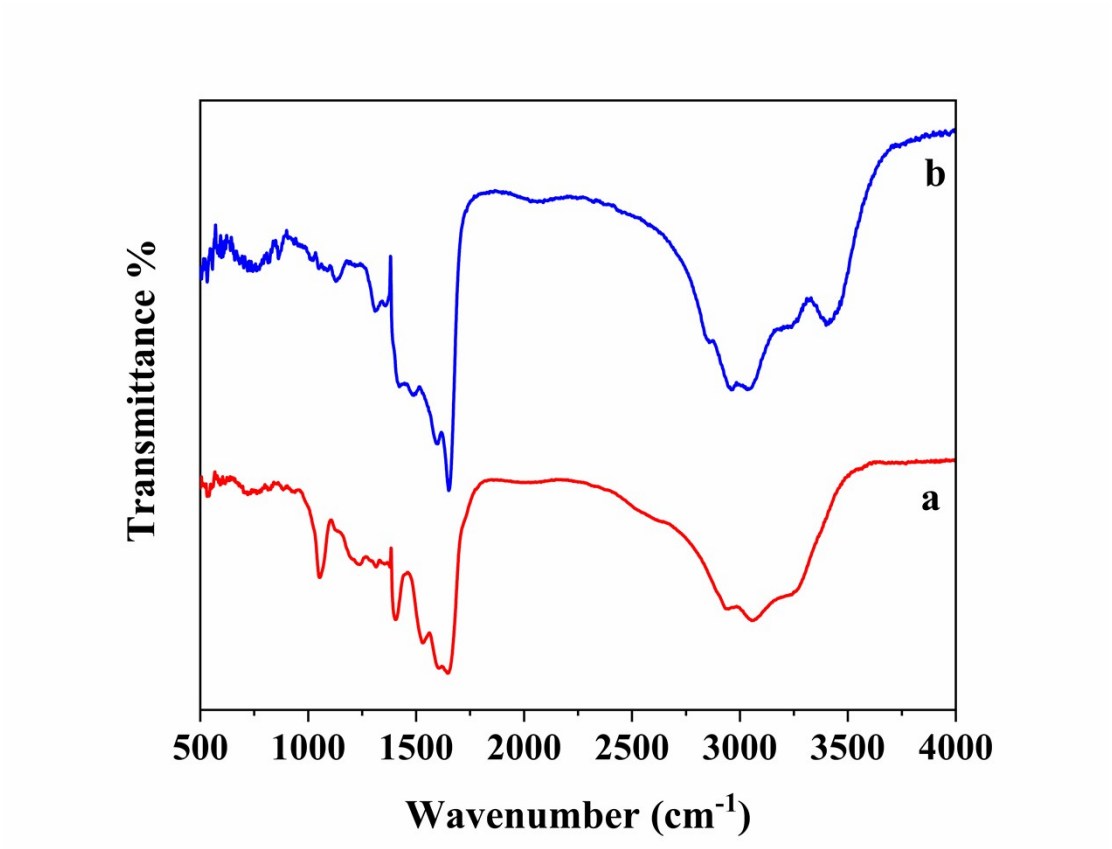

Fig S7. FT-IR spectrum of PDA-glutathione NPs (a) and PDA-glutathione/PEI NPs (b).

(o) UV-Vis absorption spectrum of glutathione-regulated PDA NPs

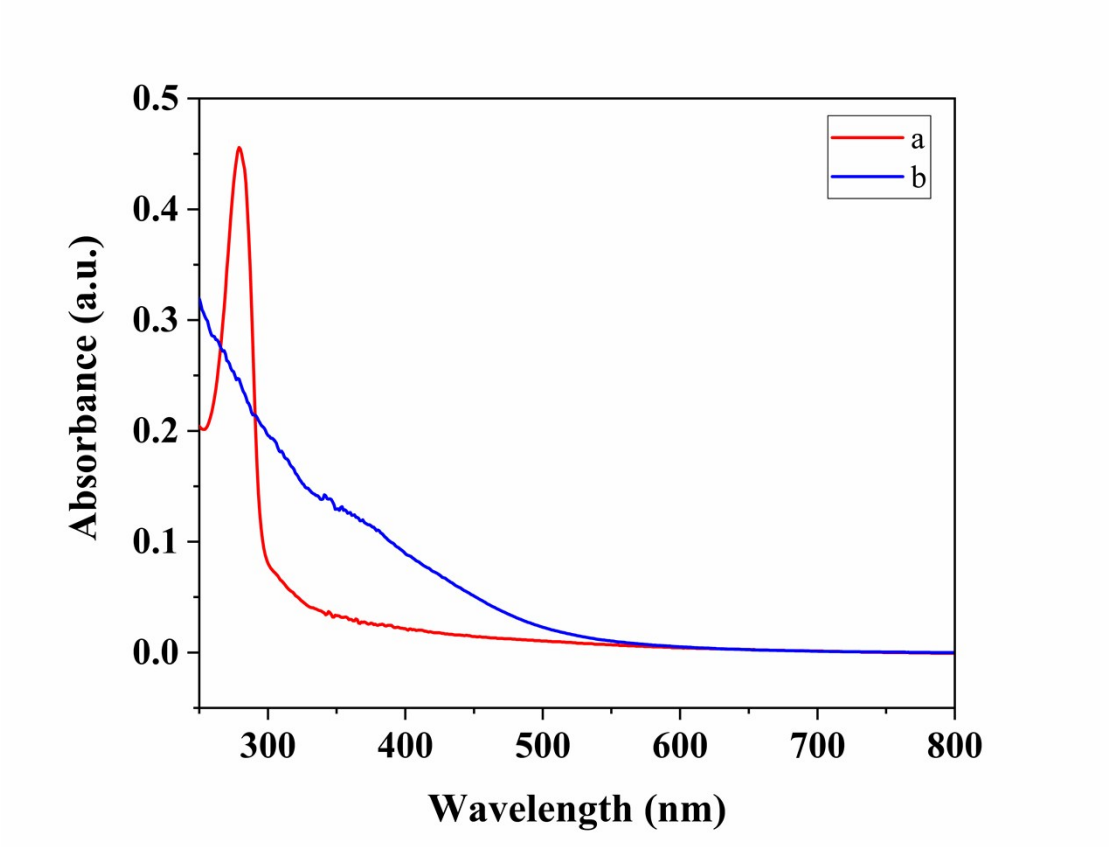

Fig S8. UV-Vis absorption spectrum of PDA-glutathione NPs (a) and PDA-glutathione/PEI NPs (b).

(p) ECL stability of PDAcp NPs

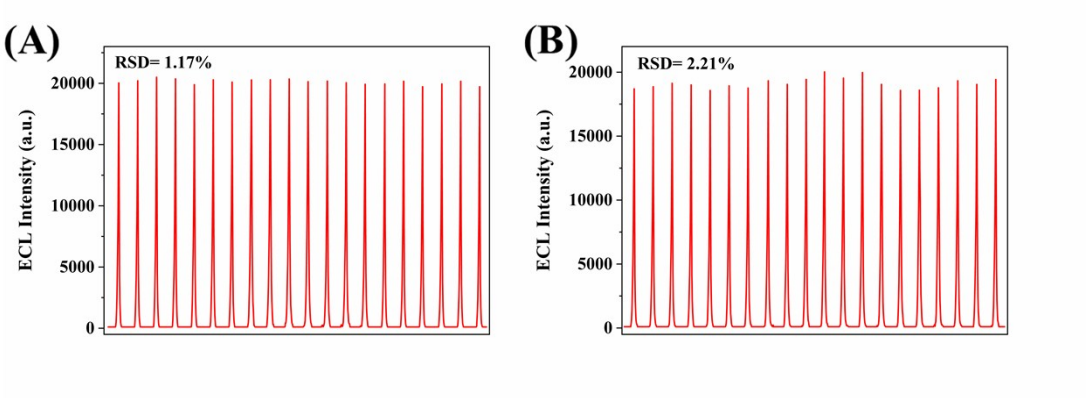

Fig S9. The ECL stability of PDAcp NPs after synthesis (A) and after stored for 1 month (B).

(q) Optimization of ATP detection conditions

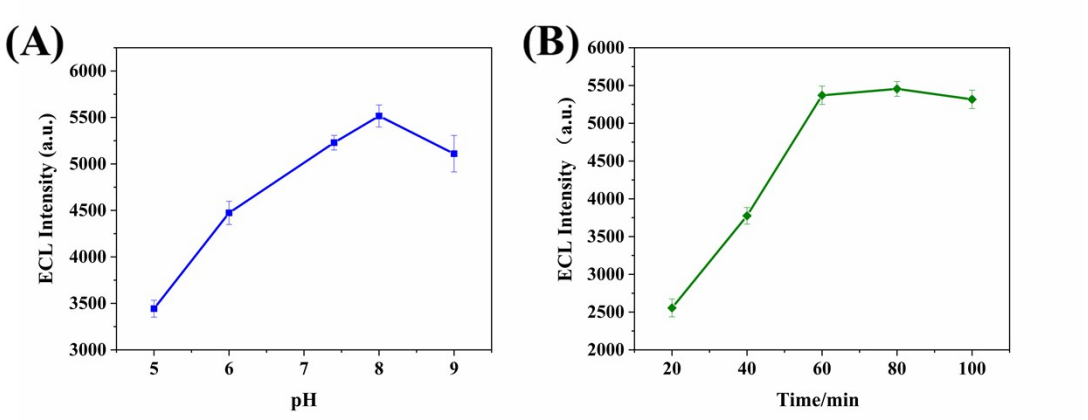

Fig S10. The effect of pH (A) and incubation time (B) on ATP detection.

(r) References

1 Y. Liu, M.K. Wang, Y.X. Nie, Q. Zhang and Q. Ma, *Anal. Chem.*, 2019, **91**, 6250-6258.

2 R.R. Hu, Z.Z. Yin, Y.B. Zeng, J. Zhang, H.Q. Liu, Y. Shao, S.B. Ren and L. Li, *Biosens. Bioelectron.*, 2016, **78**, 31-36.

3 H.P. Liu, T. Ye and C.D. Mao, *Angew. Chem. Int. Ed.*, 2007, **46**, 6473-6475.

4 J.P. Li, Q. Xu, C. Fu, Y. Zhang, *Sensor. Actuat. B-Chem.*, 2013, **185**, 146-153.