Supporting Information

Polydopamine nanosphere based highly sensitive and selective aptamer cytosensor with Enzyme amplification

Daoqing Fan[a], Kun Wang[ac], Changtong Wu[ac], Xiaoxiao Gu[ac], Yaqing Liu[ab*] and Erkang Wang[a*]

[a] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin,130022, PR China, University of Chinese Academy of Sciences, Beijing, 100039, China

[b] Key Laboratory of Food Nutrition and Safety (Tianjin University of Science and Technology), Ministry of Education, Tianjin, 300457, P. R. China.

[c] Department of Chemistry and Environmental Engineering, Changchun University of Science and Technology, Changchun, China.

Corresponding authors: yaqingliu@tust.edu.cn; ekwang@ciac.ac.cn, Tel: +86-22-60912484; +86-431-85262378; Fax: +86-431-85689711.

Experimental Section:

Chemicals: The oligonucleotide (Sangon, Shanghai)was dissolved in water as stock solution and quantified by UV-vis absorption spectroscopy with the following extinction coefficients (ε260 nm, M-1cm-1): A = 15400, G = 11500, C = 7400, T = 8700. The water used in the experiments was purified through a Millipore system. Exonuclease III was obtained from NEB Biolabs (New England). Dopamine hydrochloride (99%) was obtained from Alfa Aesar (USA). Standard PTK 7 protein was obtained from Abnova (Taiwan, China).Other chemicals were reagent grade and were used without further purification.

Apparatus: The fluorescence emission spectra of different samples were collected on Fluoromax-4 Spectrofluorometer (HORIBA Jobin Yvon, Inc., U.S.A.) under room temperature from 505 to

650 nm with the excitation wavelength of 494 nm. Slit widths for both the excitation and emission were 5 nm. FTIR analysis was performed on a Bruker Vertex 70 spectrometer, Germany (Transmission mode, RES=2 cm^{-1}, Scan times=32, Scan range=400~4000 cm^{-1}).

Polydopamine nanospheres (PDs) synthesization:

Polydopamine nanospheres were synthesized according to previous report (*Chem. Sci*., 2014, 5, 3018) with minor modification. Briefly, 100 mg dopamine hydrochloride (99%) was added to the mixture of 100 mL Tris-buffer and 40 mL isopropyl alcohol, after stirring for 72h in dark, the final solution was centrifuged at 10000 rpm for 5min and washed with distilled water for five times until the supernatant become the same color as water. Then PDs were re-suspended with suitable volume of distilled water for future experiment.

Cell culture:

For the cell lines, HeLa (cervix adenocarcinoma), MCF-7 (human breast cancer cells) and A549 cells were cultured in DMEM medium (GIBCO, Invitrogen, U.S.A.); CCRF-CEM (human leukemia) cells were cultured in RPMI-1640 medium (Hyclone, U.S.A.); All of the cell lines were supplemented with 10% fetal bovine serum (TBD, Tianjing, China.), 100 U/mL penicillin, and 100μg/mL streptomycin and incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

Experimental procedure:

The cells were collected and centrifuged at 1500 rpm for 3 min in culture medium and then washed twice with PBS buffer. The cell density was determined by a Petroff Hausser cell counter (USA). Then 1.0×10^6 cells were mixed with 100uL excess aptamer stock solution (10 μM) and incubated at 37°C for 30min. After that, the mixture was centrifuged at 1500 rpm for 3 min and washed twice with PBS buffer to separate unbonded aptamer. After being diluted by PBS buffer, cell suspensions with different concentrations were obtained. The cell suspension with different

concentrations were added into the mixture of 40μL PDs and 5uL FAM-DNA (10μM) and incubated at 37°C for 30 min. Appropriate units of Exonuclease III were added and then incubated at 37°C for 1.5h. Finally, the fluorescence intensity was measured.

Specificity testification experiment:

For the specificity of aptamer probe towards PTK 7 protein, same amount of BSA and PTK 7 protein (5ug) was added into the solution containing 100nM Label-ap DNA, the reaction buffer used is Tris-HCl (pH=8.0, 10mM Reduced glutathione).The mixture was incubated at 37°C for 30 min, then the fluorescence spectra were collected.

For the specificity of PTK 7 protein to the aptamer probe, same amount of PTK 7 protein was added into the solution containing 200nM random dual-labeled probe, 100nM Label-ap DNA, respectively. (Reaction buffer used is Tris-HCl (pH=8.0, 10mM Reduced glutathione)). Then the mixture was incubated at 37°C for 30 min, then the fluorescence spectra were collected.

Fluorescence estimation of PTK7 protein's amount on different cell's membrane:

To estimate the different amount of PTK 7 protein on different cell lines' membrane, we performed the fluorescence verification experiment using single fluorophore (FAM) labeled aptamer probe. For control cells and target CEM cells, 200nM Single-Label (same with that used in our experiment) was used. The experiment was performed as following: 200nM Single-Label was added into 1.0×10^5 cells/mL CEM cells and MCF-7, A549 and Hela cancer cells, respectively. After 30 mins' incubation at 37°C, the solution was centrifuged at 2600 rpm for 3min, the supernatant was removed and the precipitation was re-suspended with 500uL 1×PBS buffer. Then the fluorescence spectra were collected.

Table S1. Sequences of DNA used in this work, and hybridization region and poly-T region are colored in red and green, respectively.

Strand Name	DNA sequences
Aptamer probe	5’ -ATCTAACTGC TGCGCCGCCG GGAAAATACT GTACGGTTAGA TTTTTTTTTTTT ACCTCAGCAGTTAGGCCA TTTT -3′
Label-ap	5’ -6-FAM-ATCTAACTGCTGCGCCGCCGGGAAAATACT GTACGGTTAGA TTTTTTTTTTTT ACCTCAGCAGTTAG-6-BHQ1-3′
FAM-DNA	5′-6-FAM- TGGCCTA ACTGCTGAGGT-3'
Random probe	5’ -6-FAM-TACCTGCAATCCGCCGTGCGTACTAAAAGG GTACGGTTAGA CGCACGGCGG ATTGCAGGTA-6-BHQ1-3′
Single-Label	5’ -6-FAM-ATCTAACTGCTGCGCCGCCGGGAAAATACT GTACGGTTAGA TTTTTTTTTTTT ACCTCAGCAGTTAG-3′

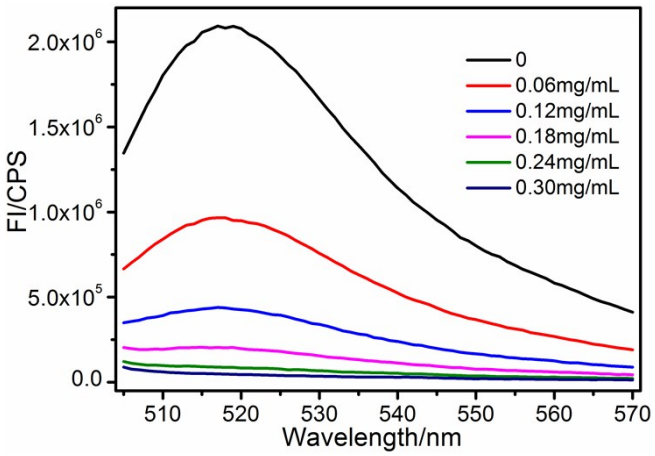

Figure S1. The fluorescence spectrum of 100nM FAM-DNA with different concentrations of PDs.

Figure S1 shows the fluorescence response of 100nM FAM-DNA with different concentrations of PDs. As the increasement of PDs, fluorescence intensity decreases gradually and reaches the lowest value in the presence of PDs at concentration of 0.30mg/ml.

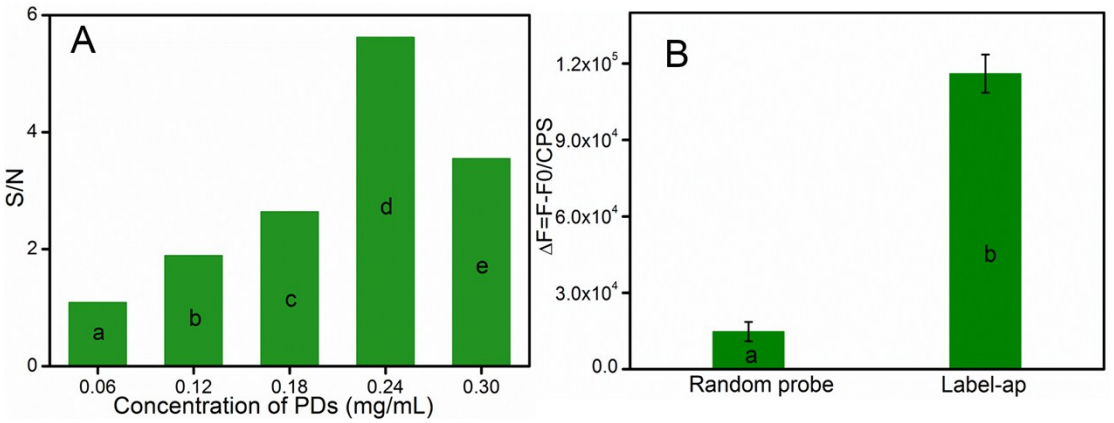

Figure S2.(A) The relationship between S/N ratio and different concentrations of PDs and (B) Comparison of the fluorescence intensity (△F) produced by same amount of PTK7 using the aptamer probe, where F and F0 are fluorescence intensities of 200nM random probe or 100nM Label-ap in the presence and absence of PTK 7 protein, respectively. Error bars were estimated from three replicate measurements.

The S/N ratio is calculated according to the equation $S/N = F/F_0$, where F_0 represents the fluorescence intensity of FAM in the presence of different concentrations of PDs; F represents fluorescence intensity of FAM in the presence of different concentrations of PDs and 1.0×10^6 cells/Ap complex. As can be seen in Figure S2(A), the S/N ratio shows the maximum in the presence of 0.24mg/ml PDs, to perform the detection at the optimized condition, we choose 0.24mg/ml of PDs in our detection platform.

To testify the specificity of standard PTK 7 protein towards the used aptamer probe, a random hairpin probe with 5'-FAM and 3'-BHQ1 labeled (same labeled position with Label-ap) was used. As can be seen in Figure S2(B), after adding 200nM random probe, 100nM Label-ap into 5ug PTK 7 protein, respectively, the changed values of fluorescence intensity (△F) produced by Label-ap is about 10-fold of that caused by 2-fold concentration of random probe. The result indicated the high specificity of standard PTK 7 protein towards our used aptamer probe.

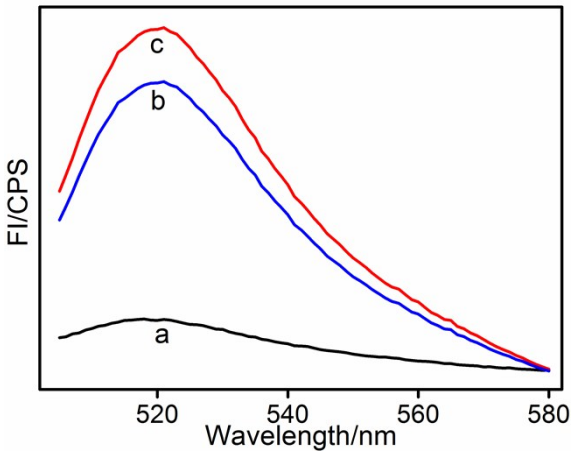

Figure S3. Fluorescence spectra of 10uM, 5uL 5'-FAM and 3'-BHQ1 Label-ap DNA (a); after incubated with 1.0x10^5 CEM cells (b), 1.0x10^6 CEM cells at 37° for 1.5 h (c).

As shown in **Figure S3**, the fluorescence intensity of Label-ap DNA is very low as a result of FRET between FAM and BHQ1. While, after incubating with 1.0x10^5 CEM cells at 37℃ for 1.5h, we could see obvious fluorescence response after the Label-ap DNA incubated with 1.0x10^5 cells and it showed just a little difference with that in the presence of 1.0x10^6 cells. This result reveals that the aptamer used in our work could efficiently bind with PTK7 protein and could be used for cell detection.

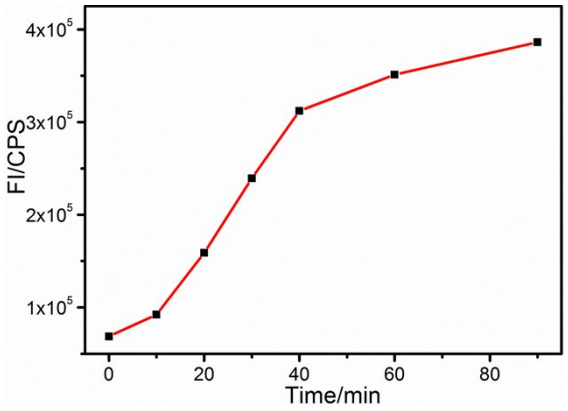

Figure S4. The fluorescence intensity of 1.0x10^5 cell with other reaction mixture under different incubation time.

As shown in **Figure S4**, the fluorescence intensity increases slowly after incubating 40min. To keep cell bioactivity during detection, 90min is selected as the incubation time in our work.

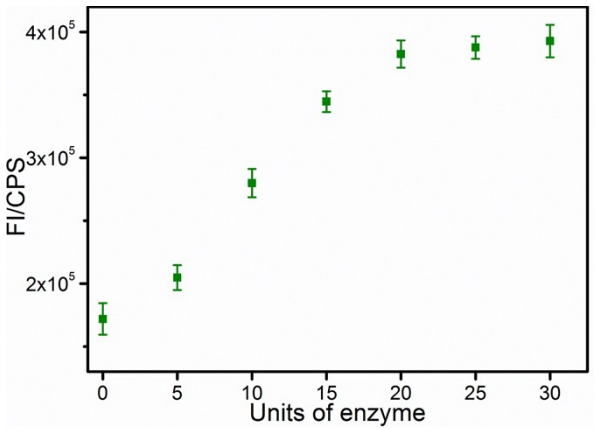

Figure S5. The relationship between fluorescence intensity of $1.0x10^5$ cell with other reaction mixture and different units of Exonuclease III.

As shown in **Figure S5**，the intensity reaches a plateau when the units of enzyme is 25 U, so we chose 25 U as the optimal enzyme units in our work.

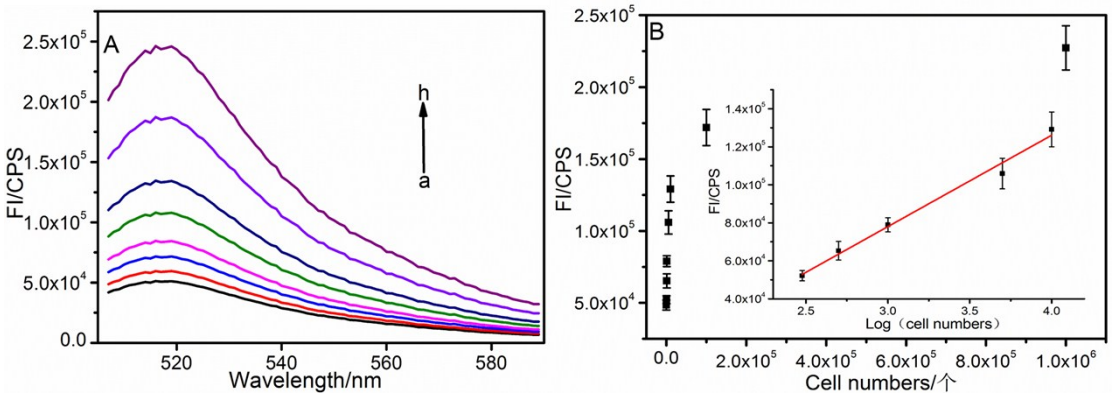

Figure S6.（A）Fluorescence emission spectra of analyzing different concentrations of CEM cells (from a to h): 0, 500, 700, 10^3, 0.5×10^4, $1.0x10^4$, $1.0x10^5$ and $1.0x10^6$ cells/mL. (B) The relationship between the fluorescence intensity at 520nm and the concentration of CEM cells. The inset shows a linear relationship ($R^2 = 0.985$) over logarithmic value of cells in the range from 500 to 1×10^4 cells. (it is better to fit the data from 500 to 10^6 cells/mL)

As can be seen from **Figure S6 A**, fluorescence signal gradually increases with the increase of concentration of cells. **Figure S6 B** shows the relationship between fluorescence intensity at 520 nm and the concentration of cells. The intensity would not have obvious increasement when the

concentration of target cells reaches 10^6 cells/mL (From Fig. S6A, fluorescence in the presence of 10^6cells/mL is quite different from the others). It might be attributed to reaction of FAM-DNA and red region of Ap reached a balance. The inset shows calibration curve between fluorescence intensity at 520 nm and logarithm of cell concentration. The intensity linearly dependents on the cell concentration in the range from 500 to 10^4 cells (R=0.985), and the LOD is about 218 cells (three times the standard deviation of background signal).

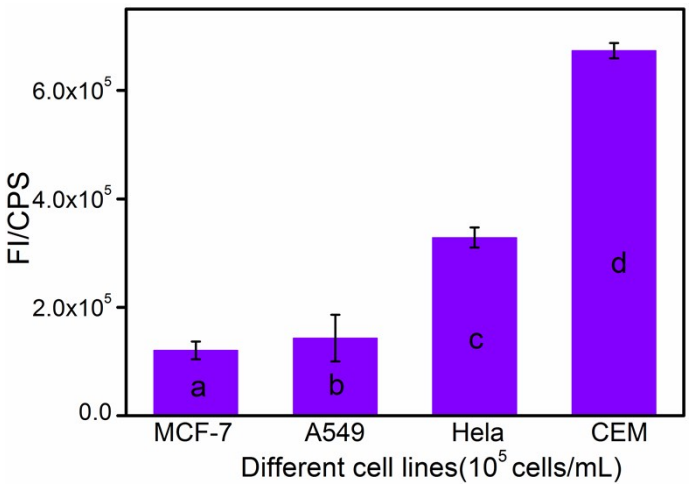

Figure S7.Column bars of fluorescence responses produced by $1.0x10^5$ different cell lines after the reaction with 200nM FAM-labeled Single-ap strand.

To estimate the different amount of PTK 7 protein on different cell lines' membrane, we performed the fluorescence verification experiment using single fluorophore (FAM) labeled aptamer probe (Single-Label strand). 200nM (Single-Label strand) was added into 1.0×10^5 different cell lines, then the mixture was incubated at 37°C for 30min, then the solution was centrifuged at 2600 rpm for 3 min and washed twice with PBS buffer to separate unbonded single-ap strand. After that, the fluorescence spectra were collected. As can be seen in Figure S7, MCF-7 (a) and A549 (b) showed low fluorescence responses as a result of the low expression of PTK 7 protein on MCF-7 and A549 cell lines. And Hela cell line produced a much lower fluorescence intensity than CEM cells, which could be ascribed to the less than a half of PTK 7 protein density of Hela cells (550 ±90 receptors/um^2) than target CCRF-CEM cells (1300 ±190 receptors/um^2). The results further confirmed the reliability of our selectivity phenomenon and consistent with the report (*Chem. Eur. J.* **2009**, 15, 5327).