Supporting Information

Experimental Sections

Reagents

The following labeled oligonucleotides were synthesized by Sangon Inc. (Shanghai, China) and used as received, their sequences are following below:

ssDNA1:

3'-HS$(CH2)_6$-TCTTGGACCCCCTCATAACGCCTCCTTCCA-5' (1)

ssDNA2:

5'-AGAACC **TGGGGGAGTATTGCGGAGGAAGGT**-NH_2-3' (2)

ssDNA1 contains complementary sequences to ssDNA2 which contains adenosine aptamer (in bold).[1]

Adenosine, cytidine, uridine, guanosine, 2-(Dibutylamino) ethanol (DBAE), 1-ethyl-3-[(3-dimethylamino)propyl]carbodiimide (EDC), ferrocene acetic acid, n-hydroxysuccinimide(NHS), and 6-mercaptohexano (MCH) were purchased from Sigma (St. Louis, MO) and used as received. Tris (2, 2'-bipyridyl) ruthenium (II)-doped silica nanoparticles (Ru-SiNPs) were synthesized and functioned as reported.[2]

Other chemicals employed were of analytical grade and used without further purification. DNA buffer solution was prepared by dissolving DNA into 0.05mol/L pH 7.4 phosphate buffer solutions (PBS). All solutions were prepared with deionized water.

Apparatus

ECL detection system contains a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) and a CHI 660a electrochemical system (Shanghai Chenhua Equipments, China). The three-electrode system was used. A gold electrode (3 mm in diameter) was used as the working electrode, an Ag/AgCl (saturated with KCl) was employed as the reference electrode and a platinum wire was served as the auxiliary electrode. A glassic cell with optically flat bottom was used as the electrochemical cell which was directly placed on the photomultiplier (PMT operated at -800 V).

Probe synthesis

The ECL probe was synthesized according to ref.[3] Briefly, 50μL 1.0×10^{-5} mol/L ssDNA1 solution was added into 50μL functionalized Ru-SiNPs solution and stirred for 120 min in 37 ℃ water bath, the functionalized Ru-SiNPs surface finally covalently bound with ssDNA1 and labeled on the terminal of the oligonucleotides. After this, the production was washed, centrifuged and resuspended in PBS, and then stored at 4 ℃ for the following experiments.

The Fc-labeled-ssDNA2 was synthesized according to the previously reported procedure. [4] Briefly, 1 mg ferrocene acetic acid are added to 2 mL 0.30 mol/L PBS containing EDC/NHS (0.1 mol/L each) and then stirred 2 h to get the activated ferrocene acetic acid solution. Then, 50 μL activated ferrocene acetic acid solution was mixed with 50 μL 1.0×10^{-5} mol/L ssDNA2 solution and reacted at room temperature overnight, the mixture was stored in refrigerator at 4 □ for the following

experiments.

Sensor preparation

The gold electrodes were polished with alumina slurry, then ultrasonic washed with water and ethanol for 3 min, respectively. Finally, they were further electrochemically activated in 0.50 mol/L H_2SO_4 until an ideal and stable voltammogram observed. The synthesized ECL probe solution was dropped on the cleaned electrode surface and kept for 4h at 37℃, and the ECL probe could be modified on the electrode surface through Au-S binding. In order to cover the nonspecific sites, the above modified electrode was immersed in MCH solution for 1h. Then Fc-labeled aptamer probe solution was dropped onto the electrode surface to react for 2h, by this means, the Fc-aptamer can hybridize with the ECL probe and the aptamer based ECL biosensors could be developed. The biosensors were incubated with different concentrations of adenosine at room temperature for 120 min for following assay.

Serum samples detection

Human serum samples were kindly provided by the Hospital of Fuzhou University (Fujian, China). Before detection, the samples were centrifuged for 10 min at 4 ℃, and the above solution were diluted 1000 times with the buffer solution, then vary concentrations of adenosine were added into the diluted samples and then tested by the standard procedures.

References

1 B. Chakraborty, Z. Jiang, Y. Li and H.Yu, *J.Electroanal. Chem.* 2009, **635**, 75-82.

2 S.Santra, P.Zhang, K. M. Wang, R. Tapec and W.Tan, *Anal.Chem.* 2001, **73**, 4988-4993.

3 N. N. Zhu, H. Cai, P. G. He and Y. Z. Fang, *Anal. Chim. Acta* 2003, **481**,181-189.

4 S. Takenaka, Y. Uto, H. Kondo, T. Ihara and M. Takagi, *Anal. Biochem.*1994, **218**, 436-443.

Table S1 Recovery of adenosine detection in human serum samples

Sample No.	Added	Found	Recovery	RSD (%)
1	2.0×10^{-8}	1.8×10−8	90%	4.3%
2	2.0×10^{-9}	2.1×10^{-9}	105%	6.2%
3	5.0×10^{-9}	4.9×10^{-9}	98%	5.8%

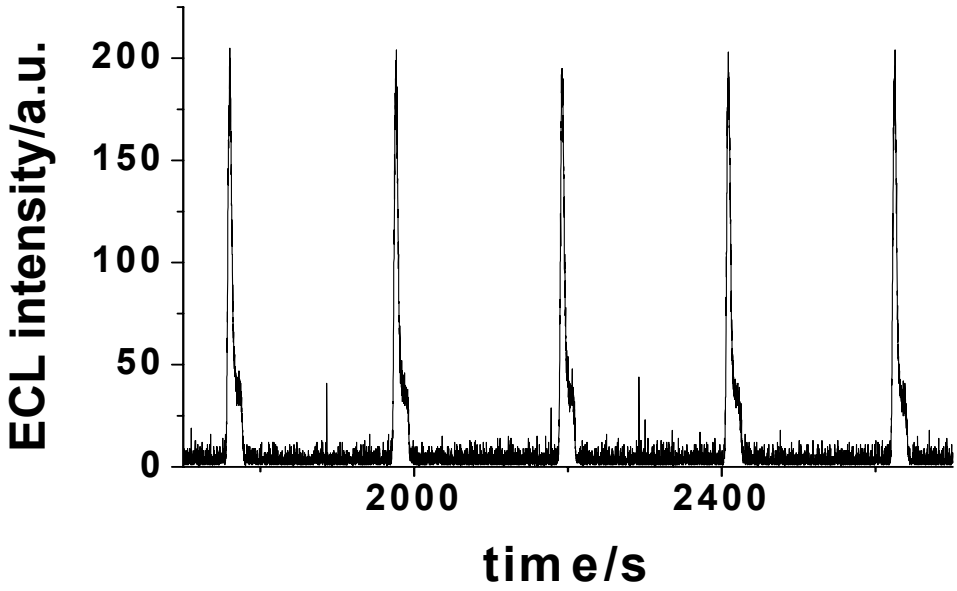

Fig. S1 Stability of the biosensor after reaction with 1.0×10^{-8} mol/L adenosine

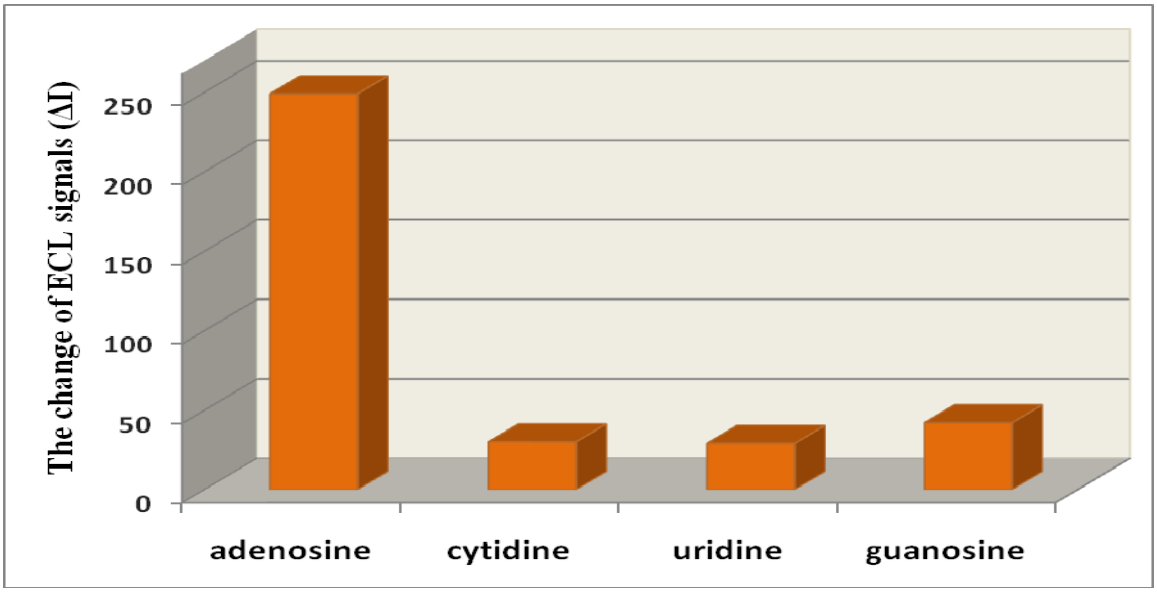

Fig. S2 Selectivity of biosensor toward to adenosine, cytidine, uridine and guanosine. The concentration of adenosine is 5.0×10^{-10} mol/L, the others are 5.0×10^{-8} mol/L.