

Supporting Information

Carbon Nanoparticle-Protected Aptamers for Highly Sensitive and Selective Detection of Biomolecules Based On Nuclease-Assisted Signal Amplification[‡]

Xiaoyan Lin,^a Liang Cui,^a Yishun Huang,^a Ya Lin,^a Yi Xie,^a Zhi Zhu,^{*a} Bincheng Yin,^b Xi Chen^a and Chaoyong James Yang^{*a}

^a The MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, the Key Laboratory of Chemical Biology of Fujian Province, State Key Laboratory of Physical Chemistry of Solid Surfaces, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005 (China) E-mail: cyang@xmu.edu.cn, zhuzhi@xmu.edu.cn

^b State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, China

Experimental Section

Materials

ATP, UTP, CTP, GTP, kanamycin, gentamicin, streptomycin and tetracycline were purchased from Sangon Biotech Co. Ltd. (Shanghai, China). DNase I was purchased from Takara Biotechnology Co. Ltd. (Dalian, China). The DNA probes were synthesized on a PolyGen Column 12 Synthesizer and the reagents were purchased from Glen Research (Sterling, VA, USA). Carbon nanoparticles (CNPs) were purchased from Beijing DK nano technology Co. Ltd (Beijing, China).

Fluorescence measurements

Fluorescence measurements were carried out on a FluoroMax-4P Fluorescence Spectrophotometer (Horiba, Japan). The excitation and emission wavelengths were set at 490 and 520 nm, respectively, with a 3 nm bandwidth and 0.3s integration time. The emission spectra were obtained by exciting the samples at 490 nm and scanning the emission from 500 to 650 nm in steps of 1 nm/s. The reaction buffer contained 20 mM Tris-HCl (pH 8.0), 30% PEG, 5 mM MgCl₂ and 50 mM NaCl. The amplified detection of ATP was performed in 200 μL buffer with 100 nM ATP aptamer incubated with 62.5 μg/mL CNP for 30 min at room temperature. After the addition of 20 units of DNase I and different concentrations of ATP, the solution was incubated at 37°C for 90 min and the fluorescence intensities were detected afterwards. For kanamycin detection, 100 nM kanamycin aptamer was incubated with 87.5 μg/mL CNP for 30min in 200 μL buffer. After the addition of 20 units of DNase I and different concentrations of kanamycin, the solution was incubated at 37 °C for 90 min and the fluorescence intensities were detected afterwards. For biological sample analysis, DMEM cell media was used as the reaction buffer.

Gel electrophoresis

The reaction buffer contained 20 mM Tris-HCl (pH 8.0), 30% PEG, 5 mM MgCl₂ and 50 mM NaCl. The cyclic enzymatic amplification reaction was performed in 15 μ L solution with 6 μ M aptamer incubated with 3.5 mg/mL CNP for 30 min at room temperature. After the addition of DNase I and ATP, the solution was incubated at 37 $^{\circ}$ C for 120 min. A 20% non-denaturing PAGE analysis of the products from the cyclic enzymatic amplification reaction was carried out in 1 \times TBE buffer (pH 8.3) at 100V power for about 1.5 hr. After Stains-All staining, gel image was obtained using a Canon camera.

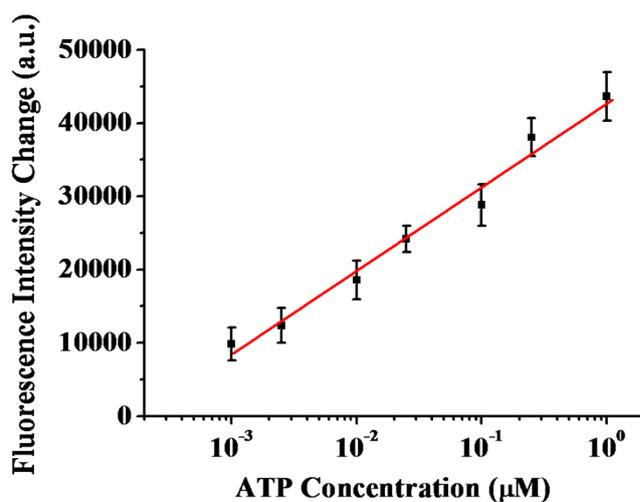


Figure S1. Change of fluorescence intensity as a function of ATP concentration.

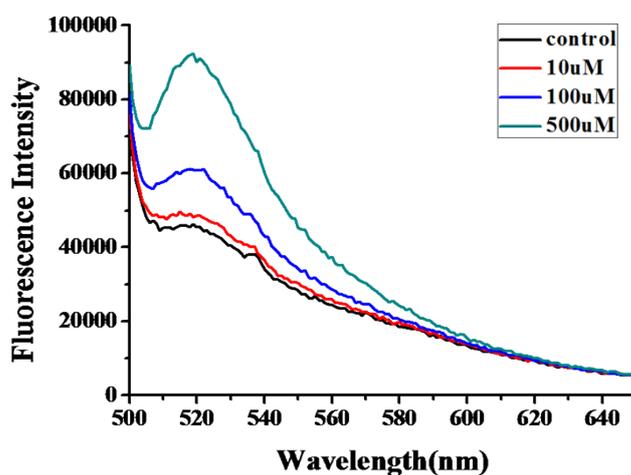


Figure S2 Detection of different concentrations of ATP in the absence of DNase I.

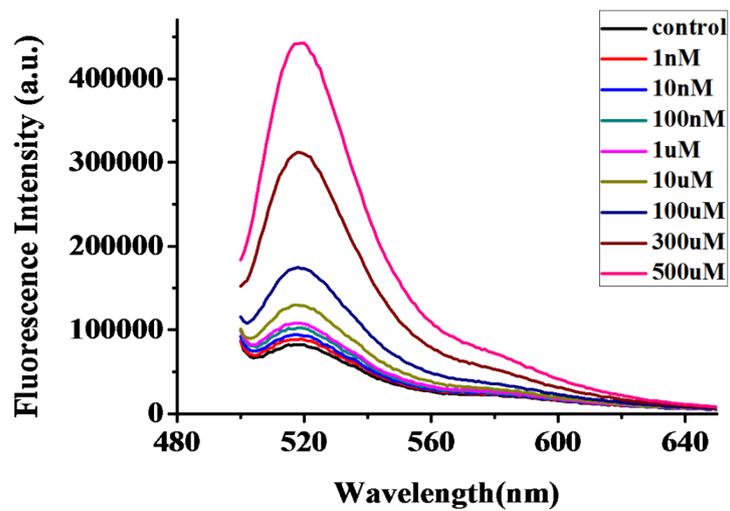


Figure S3 Fluorescence emission spectra with different concentrations of ATP in the cell media.

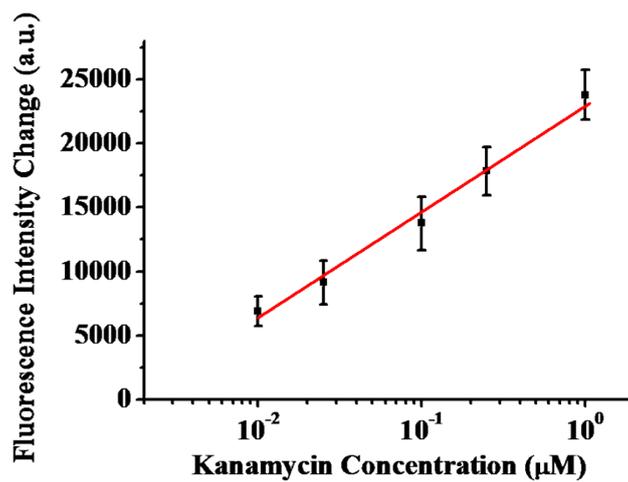


Figure S4. Change of fluorescence intensity as a function of kanamycin concentration.