

Electronic Supporting Information

A novel aptameric nanobiosensor based on self-assembled DNA-MoS₂ nanosheet architecture for biomolecule detection

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EXPERIMENTAL SECTION

Reagents and Apparatus. The DNA oligonucleotides used in this work were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China). The sequences of the DNA oligonucleotides were as follows:

P1 (ATP aptamer): 5'-FAM-CCTGGGGGAGTATTGCGGAGGAAGGTT-3'

P2 (Thrombin aptamer): 5'-FAM-AGTCCGTGGTAGGGCAGGTTGGGGTGACT -3'

P3 (control DNA sequence for ATP): 5'-FAM-TGTCCGTGCTAGAAGGAAACAGTTACCA-3'

Adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine triphosphate (GTP), and uridine triphosphate (UTP) were all purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Thrombin and lysozyme (Lys) were bought from Sigma-Aldrich (U.S.A.). Bovine serum albumin (BSA) and human IgG antibody were from Dingguo Biotech (Beijing, China). All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd. All reagents were used as received without further purification. All solutions were prepared using ultrapure water, which was obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA) and had an electric resistance $>18.3 \text{ M}\Omega$.

The fluorescence measurements were carried out on an FL-7000 spectrometer (Hitachi, Japan). The fluorescence emission spectra were collected from 500 nm to 600 nm at room temperature with a 488 nm excitation wavelength.

Material Synthesis:

MoS₂ nanosheets were synthesized by natural MoS₂ crystals (Sigma-Aldrich) according to the Eda method.²⁶ MoS₂ nanosheets were dissolved in Milli-Q ultrapure water

and then sonicated for 2 h to give a homogeneous solution and stored for use.

Sensor Preparation for ATP Detection.

Under optimized sensing conditions 20 mM Tris-HCl buffer (pH 7.4, containing 100 mM NaCl, 5 mM KCl and 5 mM MgCl₂), the probe solutions containing P1(50 nM) was mixed with MoS₂ (40 µg/mL) to form P1–MoS₂ solution in buffer. Then the complex solution was incubated for 30 min at 30°C. An aliquot of the targets (appropriate concentrations of ATP solution) was added to the P1–MoS₂ solution. After allowing the mixture to incubate for 30 min at room temperature the fluorescence emission spectra of the mixture were recorded.

Sensor Preparation for Thrombin Detection.

Under optimized sensing conditions 20 mM Tris-HCl buffer (pH 7.4, containing 20 mM KCl and 5 mM MgCl₂), the probe solutions containing P2 (20 nM) was mixed with MoS₂ (40 µg/mL) to form P2–MoS₂ solution in buffer. Then the complex solution was incubated for 30 min at room temperature. An aliquot of the targets (appropriate concentrations of thrombin solution) was added to the P2–MoS₂ solution. After allowing the mixture to incubate for 30 min at room temperature the fluorescence emission spectra of the mixture were recorded.

Cellular ATP Assay

Human lung adenocarcinoma A549 cells were chosen for the experiment. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 µg mL⁻¹ streptomycin, and 100 units mL⁻¹ penicillin. Cells were all cultured at 37°C in a humidified incubator containing 5% CO₂ and passaged every 2–3 days. Vigorous growth

cells were collected after trypsin digestion. The collected cells were centrifuged at 2000 rpm for 3 min at 4°C and washed twice by PBS buffer (20 mM phosphate, 137 mM NaCl, and 2.7 mM KCl, pH 7.4) and then suspended in Tris–HCl buffer. Cell lysis (approximately 4×10^6 cells/mL, 1 mL) was performed with cell lysis buffer for 10 min at 4°C, and then the lysate was centrifuged at 2000 rpm for 3 min at 4°C. The supernatant was ready for ATP assays. The ATP detection protocol was the same as above.

Fig. S1. Values of the ζ potential for MoS₂ sheets.

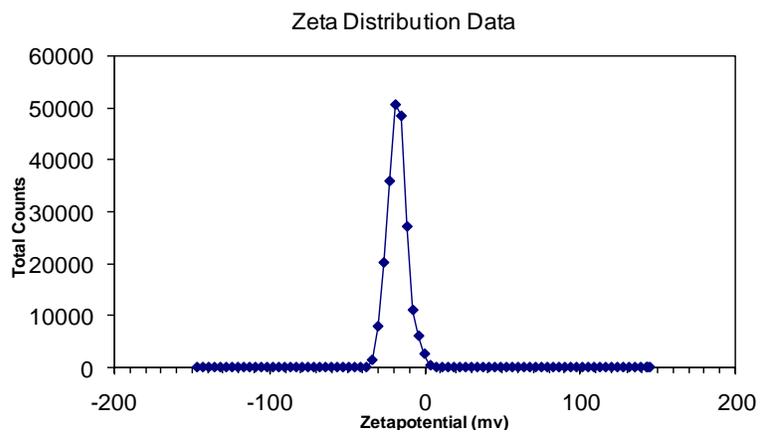


Fig. S2. (a) Fluorescence quenching of P1 in Tris-HCl buffer by MoS₂ nanosheets as a function of time, and (b) Fluorescence restoration of P1 in MoS₂ nanosheets solution by ATP as a function of time. (P1 50 nM, ATP 1 mM)

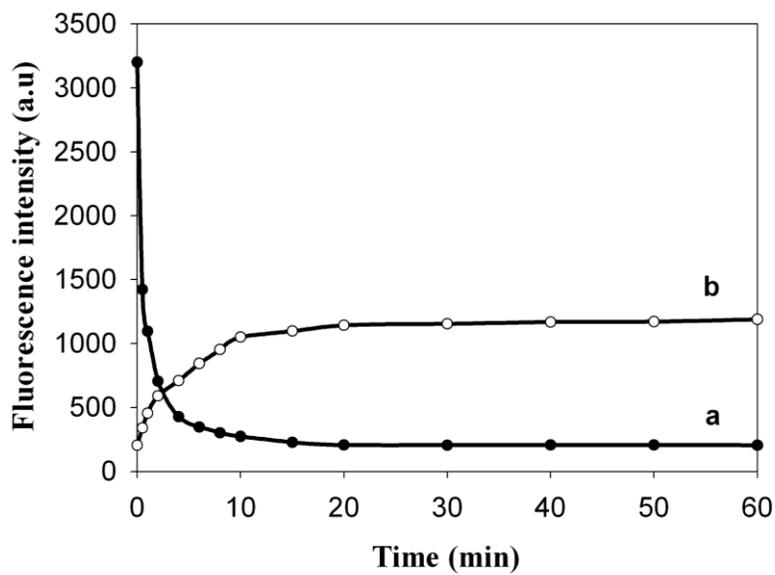


Fig. S3. Fluorescence intensity histogram of P1 + MoS₂ (black histogram) and P1 + MoS₂ + ATP (gray histogram) in the presence of 0, 10, 20, 40, 60, and 80 μg mL⁻¹ MoS₂ (P1 50 nM, ATP 1 mM).

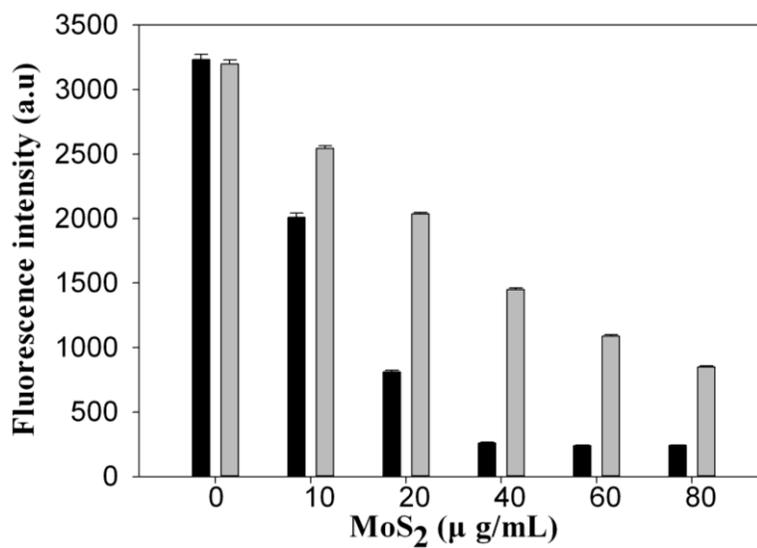


Fig. S4. Calibration curve of DNA-MoS₂ nanosheet biosensor in the presence of increasing amount of ATP. (MoS₂ 40 μg mL⁻¹, P1 50 nM).

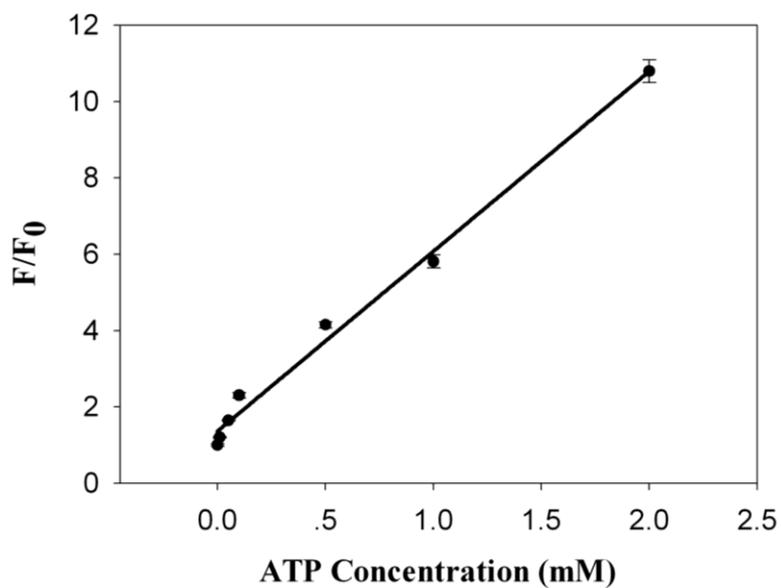


Fig. S5. Fluorescent spectra of DNA-MoS₂ nanosheet biosensor with various concentrations of ATP in 1% human serum (0, 10, 50, 100, 1000, 2000 μM).

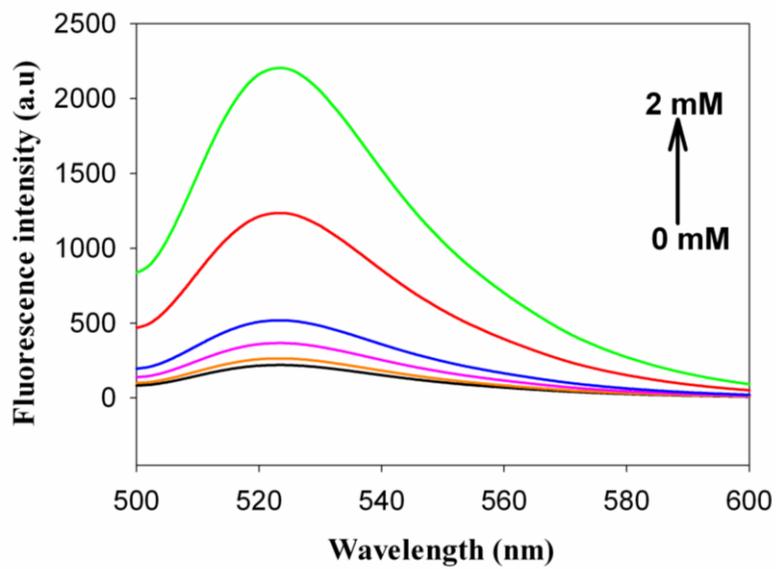


Fig. S6. Fluorescence spectral responses of DNA-MoS₂ nanosheet biosensor to (a) freshly prepared cell extraction (ATP present) and (b) cell extractions after 24 h aging (ATP absent), (c) blank.

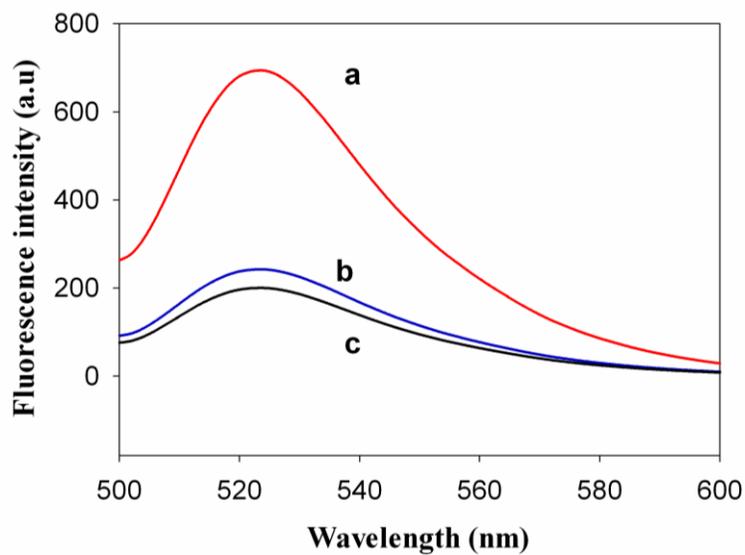


Fig. S7. Fluorescence emission spectra of P2 under different conditions: (a) P2; (b) P2 + thrombin; (c) P2 + MoS₂ + thrombin; (d) P2 + MoS₂. (MoS₂ 40 μg mL⁻¹, P2 20 nM, thrombin 100 nM).

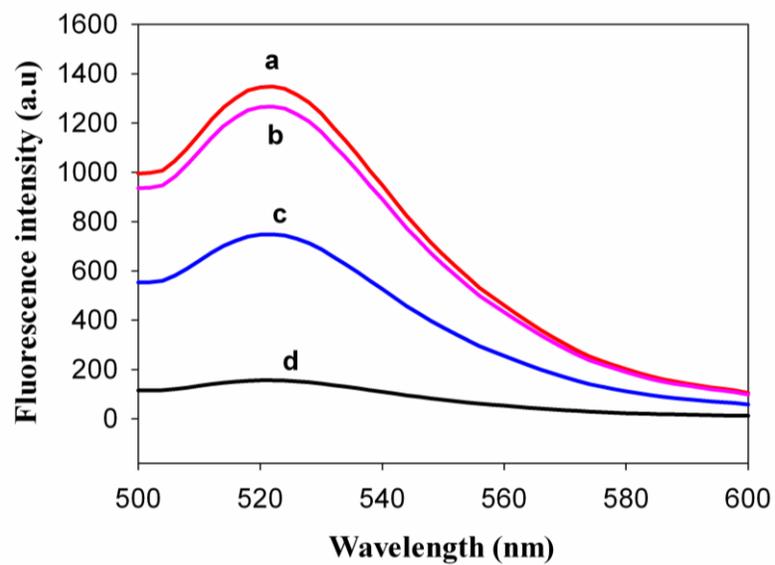


Table S1. The concentration of ATP in freshly lysed cells and lysed cells after 24 h aging. ^a

Sample ^b	aptamer–MoS ₂ nanoassembly biosensor for ATP detection (mM) ^c	ATP assay kit for ATP detection (mM) ^c
1-1	1.19	1.25
1-2	1.03	1.13
2-1	0.13	0.14
2-2	0.11	0.12

^a Samples 1-1 and 1-2 were from freshly lysed cells, and 2-1 and 2-2 were from lysed cells after 24 h aging. ^b Each sample was analyzed in triplicate, and the results were the average values. ^c Average of three measurements.