Supporting Information

Real-time Investigating Two Conformational Changes of Adenosine Aptamer with Single Molecule Fingerprint Signals

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EXPERIMENTAL SECTION

Materials and reagents

Lipid 1, 2-diphytanoyl-snglycero-3-phosphocholine (DPhPC) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, U.S.A.). Wild-type α-HL nanopore, Potassium chloride, $MgCl_2 \cdot 5H_2O$, $CaCl_2$, and Trizma @hydrochloric acid were obtained from Sigma-Aldrich. Decane, goat serum, Adenosine, adenosine monophosphate (AMP), adenosine diphosphate (ADP) and Adenosine triphosphate (ATP) were purchased from Aladdin (Shanghai, China). A Teflon chamber with a 150 μm orifice was purchased from Warner Instruments (Hamden, CT, USA). All the solutions used in the experiments were prepared using ultra-pure water (18.25 MΩ). The DNA aptamers were synthesized and purified by Shanghai Sangon Biological Engineering Technology & Service Co. Ltd. (Shanghai, China). DNA sequences utilized in this work are as follows:

Ade1301:5'-ACGACGTTTGCGATGAGAAACGTATGGTTTCGAAGGTCGTT$_{15}$-3'.

AFB1 aptamer: 5'-CACGTGTTGTCTCTCTGTGTCTCGTGT$_{15}$-3'

Cd^{2+} aptamer: 5'-GACGACGGGTTCACAGTCCGTTGTC-3'

OTA aptamer: 5'-GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA-3'

Random DNA: 5'- GCTGCGAGGTAAAGG-3'

The experiments were performed using buffer with 1.0 M KCl, 50 mM $MgCl_2$, 10 mM Tris-HCl and 1mM EDTA (pH 7.5) at 25 °C. The final concentration of DNA was 200 nM. Before the combination experiment, Aps and DNA were incubated in the experimental buffer at 25℃ for 60 minutes in advance, and then added to the cis chamber.

Nanopore Electrical Recording

All experiments were conducted at 25 ± 2 ℃. 1 mL buffer were respectively added in cis chamber and trans chamber. 30 mg/mL Lipid 1, 2-diphytanoyl-snglycero-3-phosphocholine (DPhPC) was coated on both sides of orifice to form a phospholipid bilayer. 5 μg/mL α-HL protein was added into the cis chamber while the voltage was increased to 200-300 mV in order to guide the α-HL to self-assemble into heptameric transmembrane channels on the phospholipid bilayer. The DNA and molecule/aptamer complex moved from cis chamber to trans chamber under the guidance of voltage of +160 mV with Ag/AgCl electrodes. Event currents were recorded by nanopore recording system (EPC-10, HEKA Elektronik GmbH, Germany).

Data Analysis

MATBLAB (R2011b, MathWorks) was employed to data processing for gaining dwell time τ, the dwell time of different level t and current blockage I/I_0, where I was blocking current and I_0 was opening current. All the data were imported into origin 2021 and plotted as statistical histograms. Gaussian fitting was performed on the histogram to obtain the mean value of τ, t and I/I_0. Each experiment will be independently repeated three times, and all the data are presented as mean ± standard deviation (n=3) to ensure the reproducibility of the data.

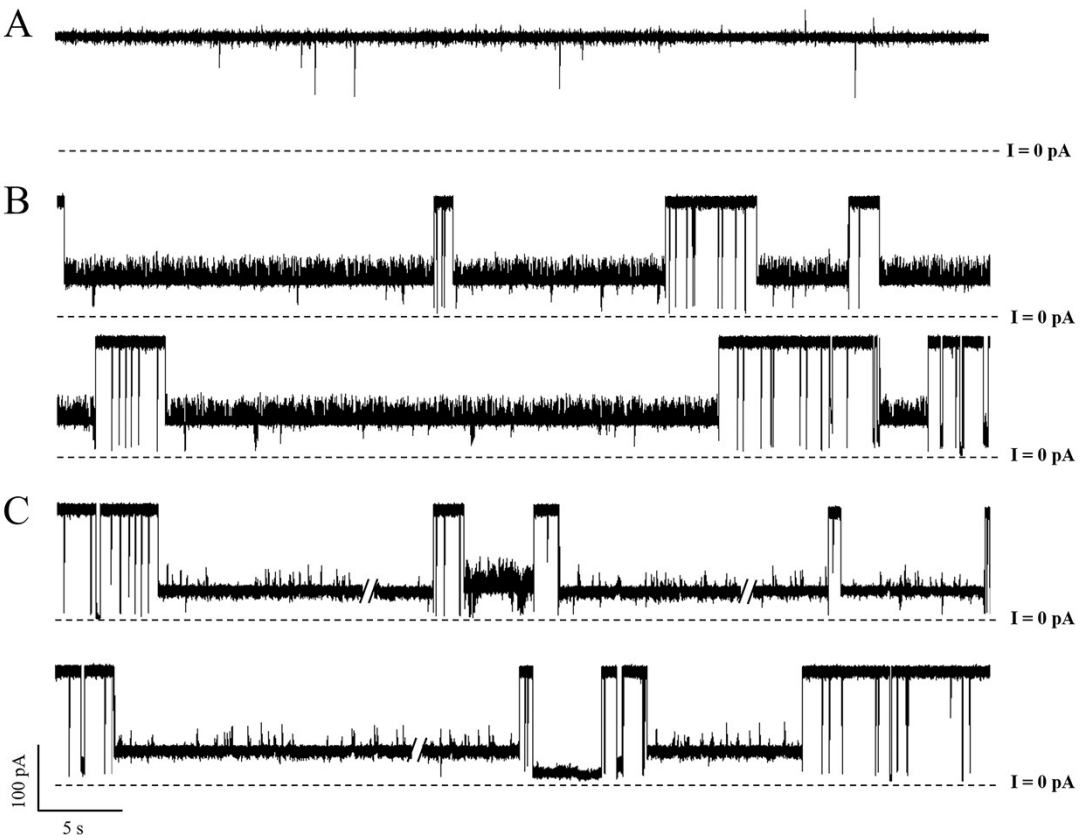

Figure S1. The current trace of Ade (A), Ade1301 (B) and Ade/Ade1301 complex (C). The concentrations of Ade and Ade1301 was 100 μM and 200 nM, respectively. (n = 3)

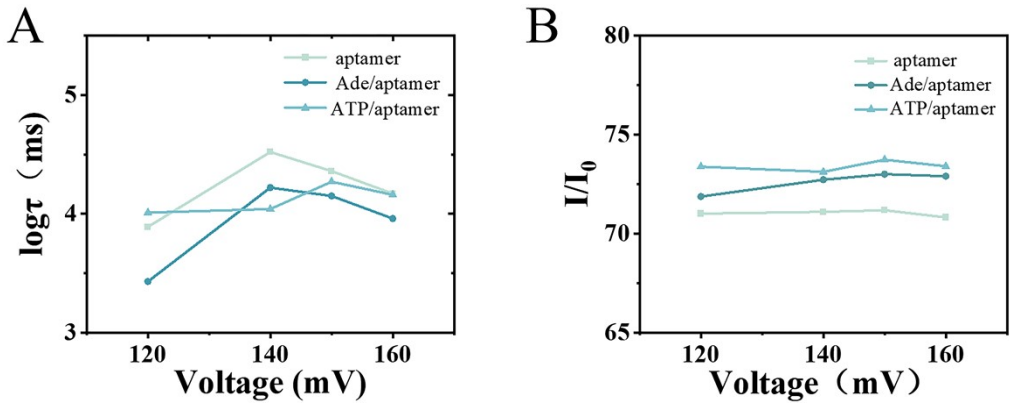

Figure S2. The logτ (A) and I/I_0 (B) of Ade1301, Ade/Ade1301 complex and ATP/Ade1301 complex at different voltages. The concentrations of molecule and aptamer was 100 μM and 200 nM, respectively. (n =3)

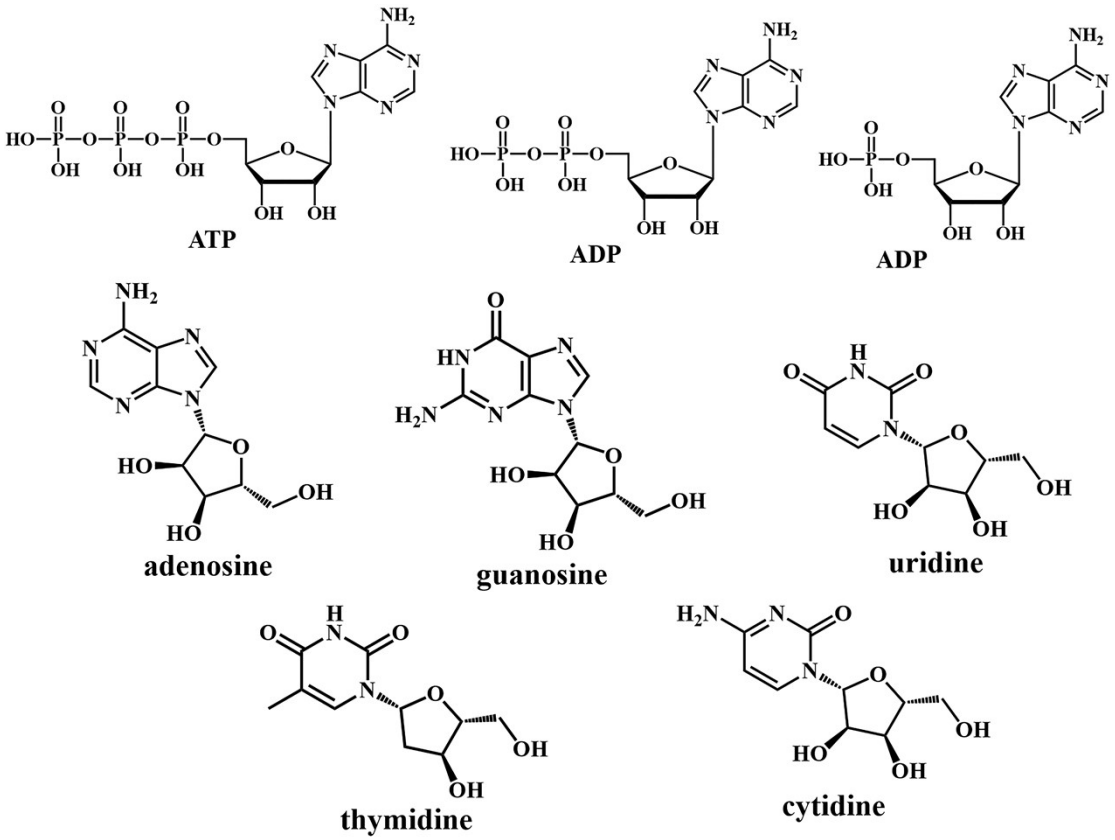

Figure S3. Chemical structural formulas of ATP, ADP, AMP, adenosine, guanosine, uridine, thymidine, cytidine.

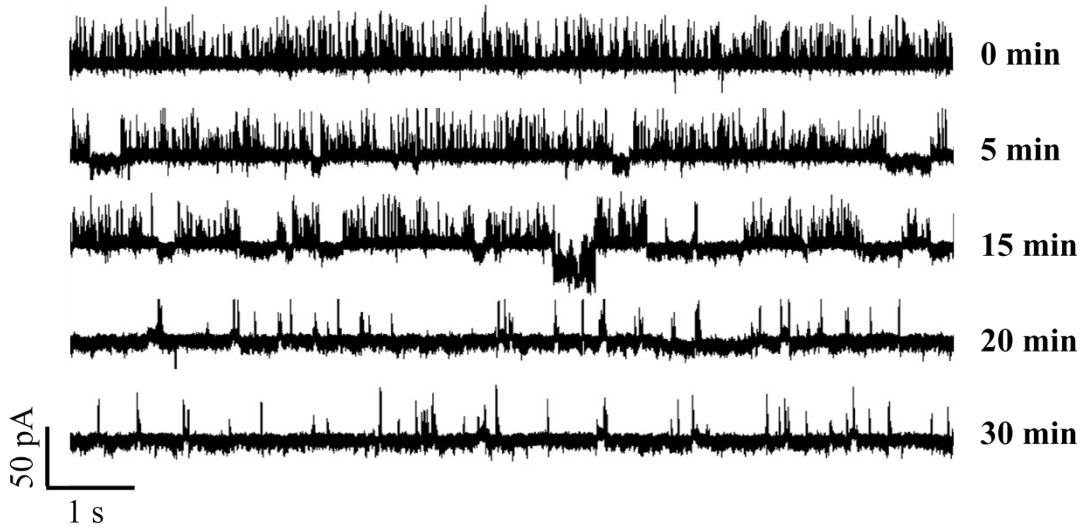

Figure S4. The current trace measured at 5, 10, 15, 20 and 30 min after the addition of adenosine.

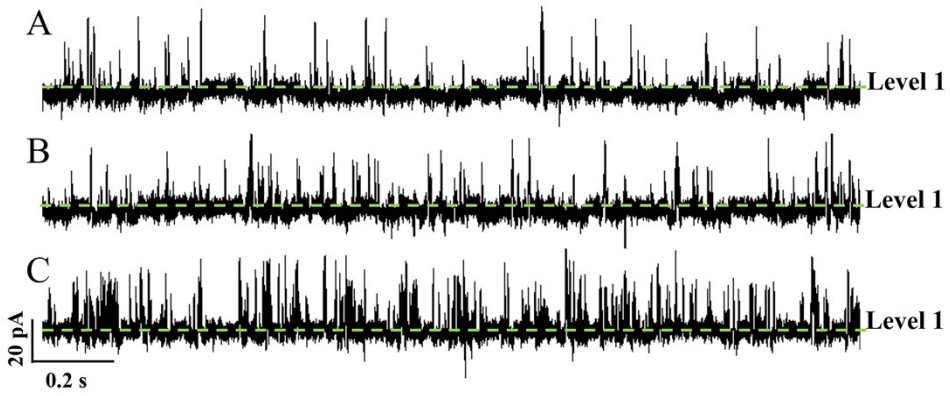

Figure S5. The current trace of (A) adenine/Ade1301 complex, (B) theophylline/Ade1301 complex and (C) theobromine/Ade1301 complex. The concentrations of molecules and Ade1301 were 100 μM and 200 nM.

Table S1. $\Delta I_2/I_0$, t2 and t1 of molecule/Ade1301 complexes with different affinities and concentrations. The final concentration of Ade1301 was 200 nM. Each experiment was repeated three times. (n =3)

	100 μM AMP	**100 μM ATP**	**100 μM Ade**	**10 μM Ade**	**1 μM Ade**
$\Delta I_2/I_0$ (%)	6.56 ± 0.16	11.34 ± 0.07	6.48 ± 0.06	6.36 ± 0.07	6.48 ± 0.08
t2(ms)	74 ± 11	534 ± 97	280 ± 60	196 ± 40	198 ± 28
t1(ms)	117 ± 27	278 ± 26	15 ± 4	121 ± 18	1323 ± 246

Table S2. T2 and t1 of Ade/Ade1301 complexes under different annealing conditions. The concentrations of molecule and Ade1301 was 10 μM and 200 nM, respectively. Each experiment was repeated three times. (n =3)

	Aptamer annealing	**Aptamer and Ade annealing**
t2(ms)	196 ± 40	270 ± 21
t1(ms)	121 ± 18	142 ± 29

Table S3. $\Delta I_2/I_0$, t2 and t1 of Ade/Ade1301 complexes at different pH. The concentrations of Ade and Ade1301 was 100 μM and 200 nM, respectively. Each experiment was repeated three times. (n

=3)

	pH	6.0	7.5	9.0
Ade	$\Delta I_2/I_0$ (%)	6.22 ± 0.05	6.48 ± 0.06	6.47 ± 0.02
	t2 (ms)	587 ± 65	280 ± 60	274 ± 28
	t1 (ms)	8 ± 2	15 ± 4	18 ± 5

Table S4. $\Delta I_2/I_0$, logt2 and logt1 of Ade/aptamer, AMP/aptamer, ADP/aptamer and ATP/aptamer. The final concentrations of molecule and Ade1301 were 100 μM and 200 nM. Each experiment was repeated three times. (n =3)

	Ade/aptamer	AMP/aptamer	ADP/aptamer	ATP/aptamer
$\Delta I_2/I_0$ (%)	6.48 ± 0.06	6.56 ± 0.16	8.04 ± 0.12	11.35 ± 0.04
logt2(ms)	2.44 ± 0.10	1.87 ± 0.07	2.03 ± 0.04	2.72 ± 0.08
logt1(ms)	1.15 ± 0.12	2.06 ± 0.10	2.05 ± 0.05	2.44 ± 0.04

Table S5. Adenosine detection in mixtures sample of Adenosine Phosphate. The molecule concentration of adenosine phosphate was 10 nM. Each experiment was repeated three times. (n =3)

	Recovery	RSD
100.00 nM Ade	102.97%	12.40%
1.00 μM Ade	98.13%	6.93%

Table S6. Comparison of sensors sensitivity and linear range for the detection of adenosine.

Method of detection	LOD	Linear range	Reference
Bragg diffraction	13.50 μM	20.00 mM - 2.00 mM	[1]
Fluorescence	84.00 nM	0.50 μM - 20.00 μM	[2]
LC-UV	1.25 μM	1.25 μM - 50.00 μM	[3]
electrochemistry	1.25 nM	10.00 nM - 10.00 μM	[4]
Colorimetry	18.00 nM	100.00 nM – 100.00 μM	[5]
α-HL nanopore	10.50 nM	20.00 nM – 500.00 nM	[6]
α-HL nanopore	1.75 nM	10.00 nM – 10.00 μM	this work

Table S7. Detection results of Ade in 10% serum. The final concentration of Ade1301 was 200 nM. Each experiment was repeated three times. (n =3)

Sample	Add	Found	Recovery (%)	RSD (%)
Serum	20.00 nM	18.45 nM	92.25	10.48
	100.00 nM	102.78 nM	102.78	12.22
	2000.00 nM	2003.03 nM	100.15	1.58

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