Supporting Information For:

Entropy-driven signal-off DNA circuit for label-free, visual detection of small molecules with enhanced accuracy

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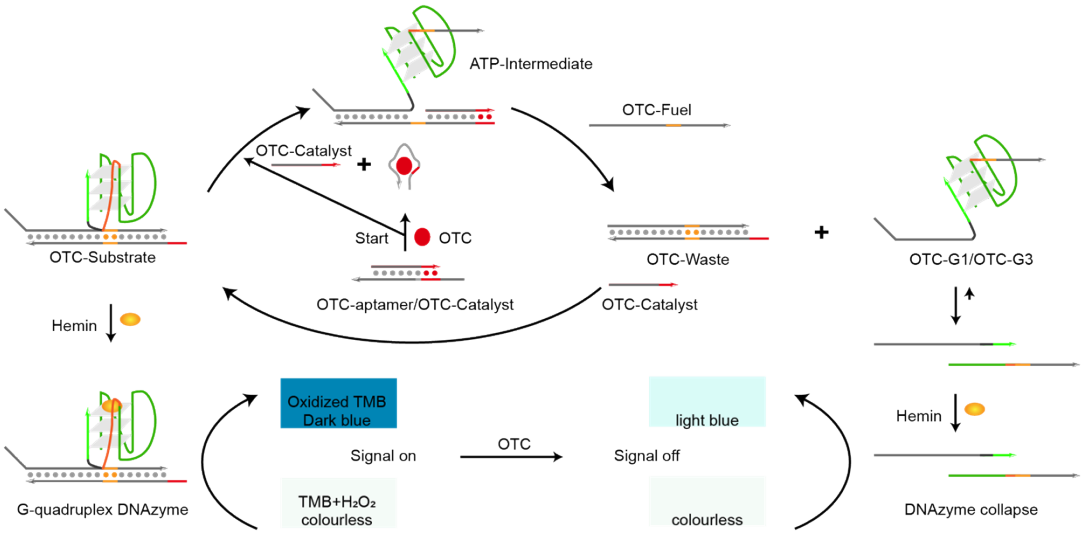
Table S1. DNA sequences used in this experiment

	DNA Sequence (5'-3')
DNA sequences used in ATP detection	
ATP-aptamer	CGGCACCTGGGGGAGTATTGCGGAGGAAGGTGCCG
ATP-catalyst	CTTCCTCCGCAATACTCCCCCA
ATP-G1	CTTTCCTACACCTACGTCTCCAACTAACTTACGGAGGG
ATP-G3	GGGTAGGGCGGGAACCCTCTTCCTCCGCAATACT
ATP-G2a	CTTTCCTACACCTACGTCTCCAACTAACTTACGGAGGGCGGGT
ATP-G2b	AGGGCGGGAACCCTCTTCCTCCGCAATACT
ATP-assistant	TGGGGGAGTATTGCGGAGGAAGAGGGCCGTAAGTTAGTTGGAGACG TAGG
ATP-fuel	CCTACGTCTCCAACTAACTTACGGCCCTCTTCCTCCGCAATACT
On-A1 (Signal strand)	CTTTCCTACACCTACGTCTCCAACTAACTTACGGAAAA
On-A2	AAAAAAAAAAAAAACCCTCTTCCTCCGCAATACT
On-G1	CCGTAAGTTAGTAGGG
On-G3	GGGTAGGGCGGGAATGGAGACGTAGG
DNA sequences used in OTC detection	
OTC-aptamer	CGACGCACAGTCGCTGGTGCGTACCTGGTTGCCGTTGTGT
OTC-catalyst	CAACCAGGTACGCACCAGCGAC
OTC-G1	TGGGGGTTTCACAGAATCTCCAACTAACTTACGGAGGG
OTC-G3	GGGTAGGGCGGGAACCCTCAACCAGGTACGCACC
OTC-assistant	GTCGCTGGTGCGTACCTGGTTGAGGGCCGTAAGTTAGTTGGAGATTC TGT
OTC-fuel	ACAGAATCTCCAACTAACTTACGGCCCTCAACCAGGTACGCACC



Scheme S1. Mechanism of the label-free, colorimetric detection of adenosine triphosphate (A) and oxytetracycline (B) based on the signal-off DNA circuit assay.

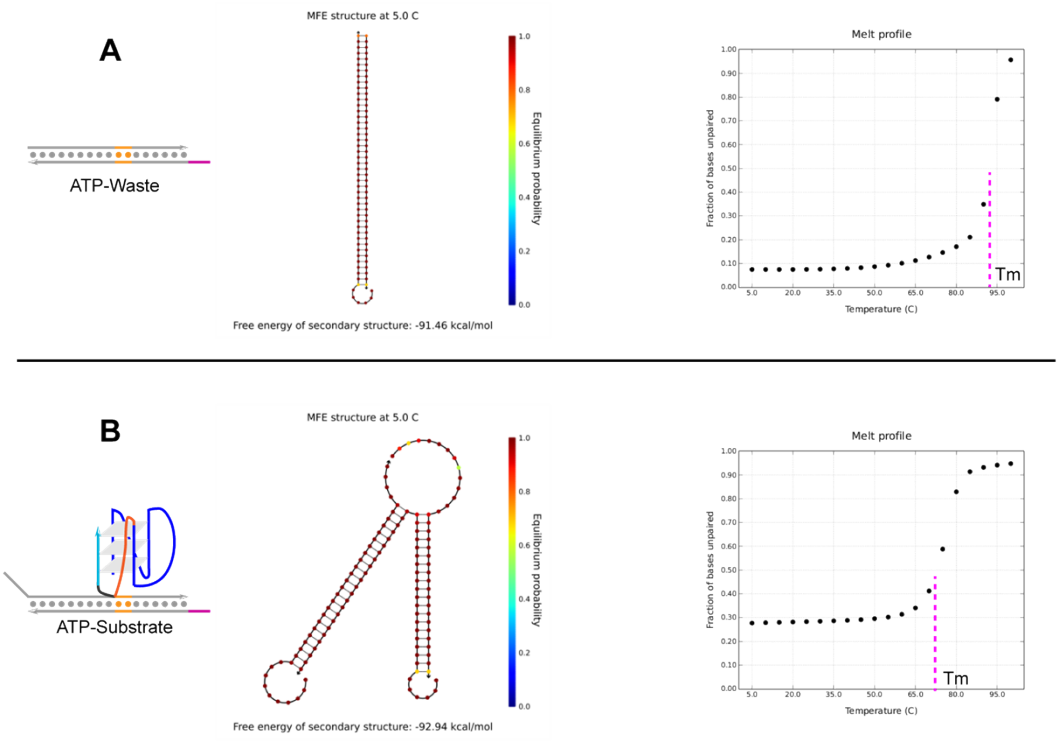
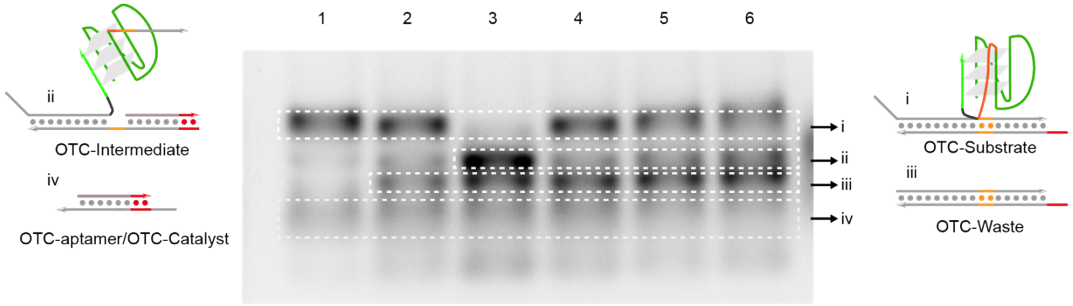


Fig. S1 The free energy and melting temperature of ATP-Waste (A) and ATP-Substrate (B) complex was assessed via NUPACK at 25 °C.



Components	Lanes					
	1	2	3	4	5	6
OTC-aptamer/OTC-Catalyst (1 μM)	+	+	+	+	+	+
OTC-Substrate (1 μM)	+	+	+	+	+	+
OTC-Fuel (1 μM)		+	+	+	+	+
OTC-Catalyst (0.5 μM)			+			
OTC (mM)				0.1	0.2	0.5

Fig. S2 Analysis by AGE (2% agarose gel) of the entropy-driven DNA circuit mechanism (OTC detection assay). lane 1, OTC-aptamer/OTC-Catalyst + OTC-Substrate; lane 2, OTC-aptamer/OTC-Catalyst + OTC-Substrate + OTC-Fuel; lane 3, OTC-aptamer/OTC-Catalyst + OTC-Substrate + OTC-Fuel + OTC-Catalyst; lane 4, OTC-aptamer/OTC-Catalyst + OTC-Substrate + OTC-Fuel + 0.1 mM OTC; lane 5, OTC-aptamer/OTC-Catalyst + OTC-Substrate + OTC-Fuel + 0.2 mM OTC; and lane 6, OTC-aptamer/OTC-Catalyst + OTC-Substrate + OTC-Fuel + 0.5 mM OTC.

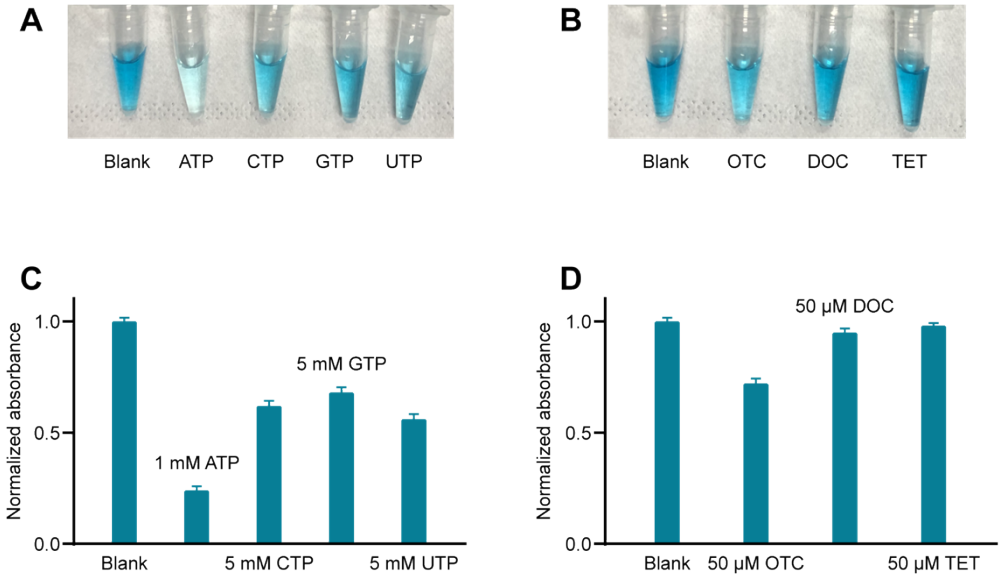


Fig. S3 (A) Photographs of samples containing various ATP (1 mM) and its analogue (5 mM). (B) Absorbance of samples in A. (C) Photographs of samples containing various OTC (50 μM) and its analogue (50 μM). (D) Absorbance of samples in C.

Table S2. Recovery of ATP in fetal calf serum samples (n = 5)

Serum Sample	Added ATP (nM)	Found (nM)	Recovery(%)	RSD
1	100	104.7	104.7	5.8
2	500	487.1	97.4	6.1
3	1000	948.9	94.9	8.8
4	5000	5016.6	100.3	3.6

Table S3. Recovery of OTC in fetal calf serum samples (n = 5)

Serum Sample	Added ATP (nM)	Found (nM)	Recovery(%)	RSD
1	500	498.3	99.7	4.3
2	1000	1034.2	103.4	5.4
3	2000	1845.2	92.3	5.6
4	5000	4785.9	95.7	9.2