Supporting information for

DNA nanolantern-based split aptamer probes for *in-situ* ATP imaging in living cells and lighting up mitochondria

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1. Reagents and instruments

Materials and reagents.

All DNA oligonucleotides (Table S1) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China) and purified by ultra-PAGE (polyacrylamide gel electrophoresis) and high-performance liquid chromatography methods. ATP was purchased from Sigma-Aldrich Chemical Co. Ltd. UTP, GTP, CTP were obtained from NEW ENGLAND BioLabs® Inc. Mito-Tracker Green was purchased from Yeasen Biotech Co., Ltd (Shanghai, China). Cell Counting Kit-8 was obtained from Beyotime Biotech Co., Ltd. (Shanghai, China). Camptothecin and oligomycin was purchased from Yuanye Biotech Co., Ltd. (Shanghai, China). Deionized and sterilized water (resistance > 18.25 MΩ·cm) was used in all experiments. All chemical reagents were of analytical grade and without any further purification before use.

Instruments

All the fluorescence measurements were conducted on a Shimadzu RF-5301 PC fluorescence spectrometer (Shimadzu Ltd., Japan) with an excitation wavelength of 540 nm. Excitation and emission slits were set at 5.0 and 3.0 nm, respectively. Dynamic light scattering (DLS) experiments were performed on the Malvern Zetasizer Nano ZS90 (Malvern Instruments, Ltd., Worcestershire, UK) at room temperature. Atomic force microcopy (AFM) characterization was carried out using Bruker Dimension Icon (USA). The cell fluorescence images were captured by confocal laser scanning microscope (Nikon A1R) equipped with 60 × objective lens

and 100 × objective lens. The cytotoxicity assay was performed via microplate reader (Synergy 4).

Table S1. Oligonucleotides used in this work

Oligonucleotide	Sequence (5'→3')
A1	ATT GTG ACC CAC CAG TAT GAC CCG TTC GGA
A2	TCC GAA CGG GTC ATA GTG TCA CTC TTG ACA TCC
A3-apt1	**Cy3-ACCTGGGGGAGTAT** GGA TGT CAA GAG TGA GTG GTC ACG ACG TCA TTA
A3-apt2	**TGCGGAGGAAGGT(Cy5)** GGA TGT CAA GAG TGA GTG GTC ACG ACG TCA TTA
A4	TAA TGA CGT CGT GAC GTG CTG GTG GGT CAC AAT
apt1	Cy3-ACCTGGGGGAGTAT
apt2	TGCGGAGGAAGGT-Cy5

NLa-1 is constructed by A1 + A2 + A3-apt1 + A4.

NLa-2 is constructed by A1 + A2 + A3-apt2 + A4.

Underline is the split ATP aptamer sequence.

2. Fluorescence spectra NLa-1, NLa-2 and their mixture

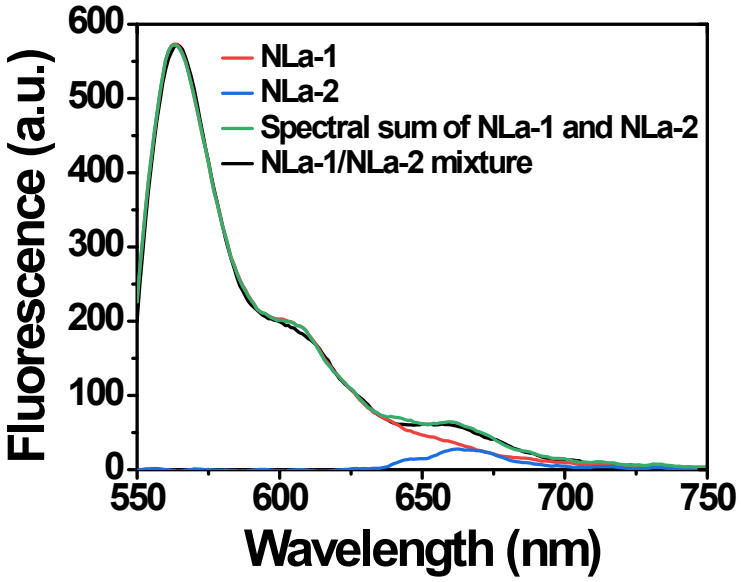


Figure S1. Fluorescence spectra of NLa-1, NLa-2, NLa-1/NLa-2 mixture and the spectral sum of NLa-1 and NLa-2.

3. ATP-sensing using single-stranded split aptamer probes (apt1/apt2)

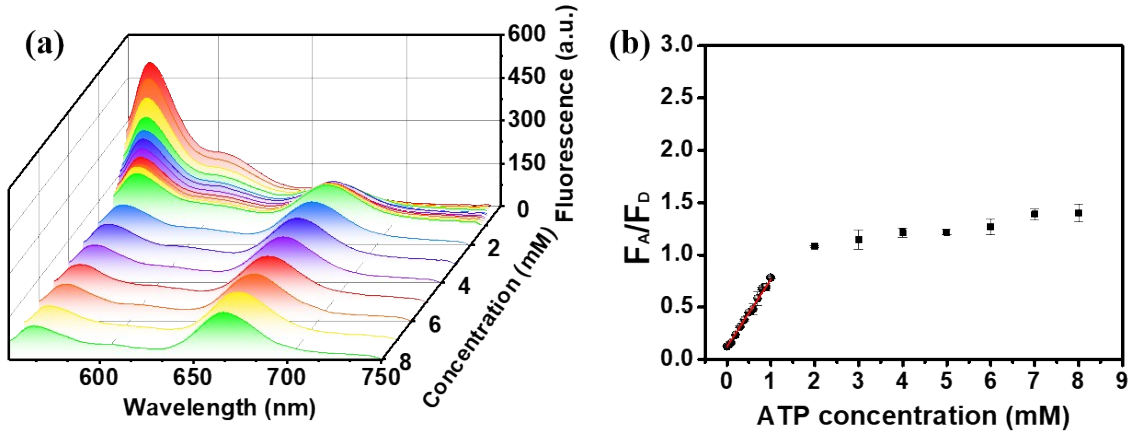


Figure S2. ATP-sensing using apt1/apt2 mixture. (a) Fluorescence spectral change of the apt1/apt2 mixture in response to different concentrations of ATP. (b) ATP concentration-dependent F_A/F_D ratio change. The red line represents the linear relationship between F_A/F_D ratio and ATP concentration in the range of 0 – 1 mM.

4. Incubation time-dependent ATP-imaging

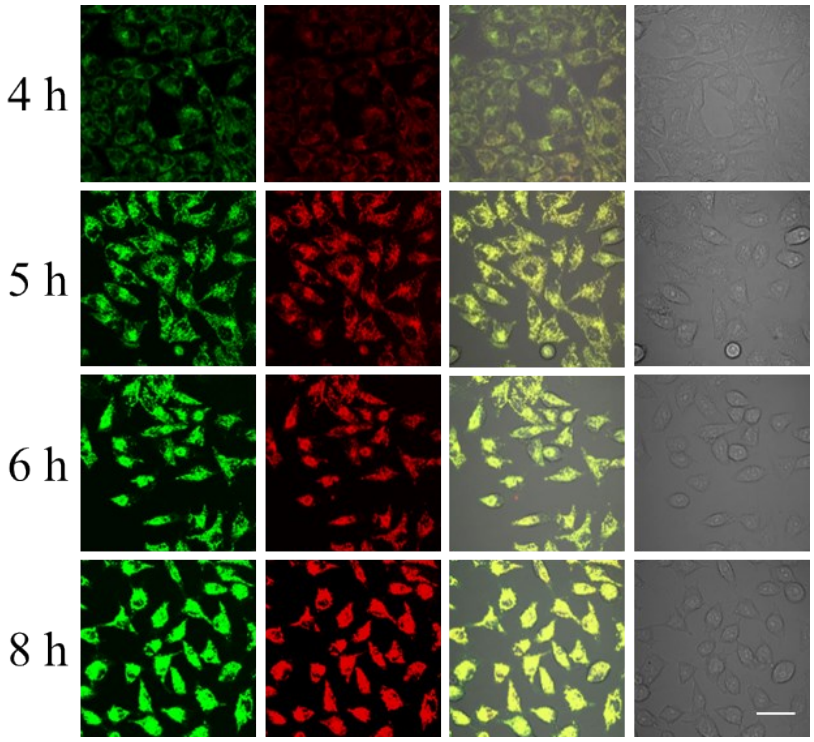


Figure S3. Fluorescence images of MCF-7 cells incubated with NLa-1/NLa-2 mixture for different time. Scale bar is 50 μm.

5. Cytotoxicity of nanoprobes towards HEK-293 cells

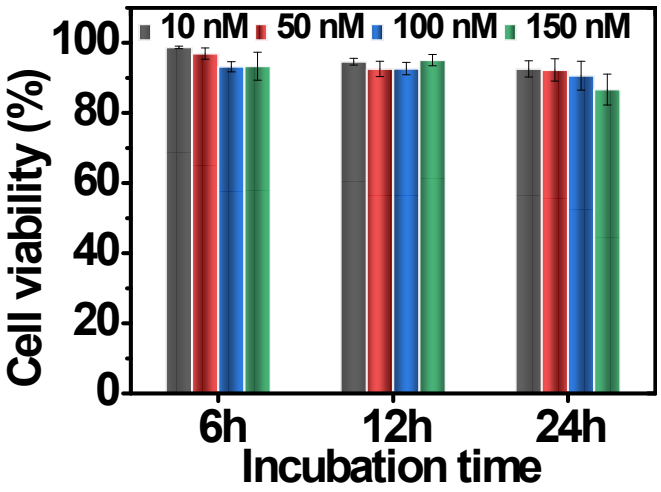


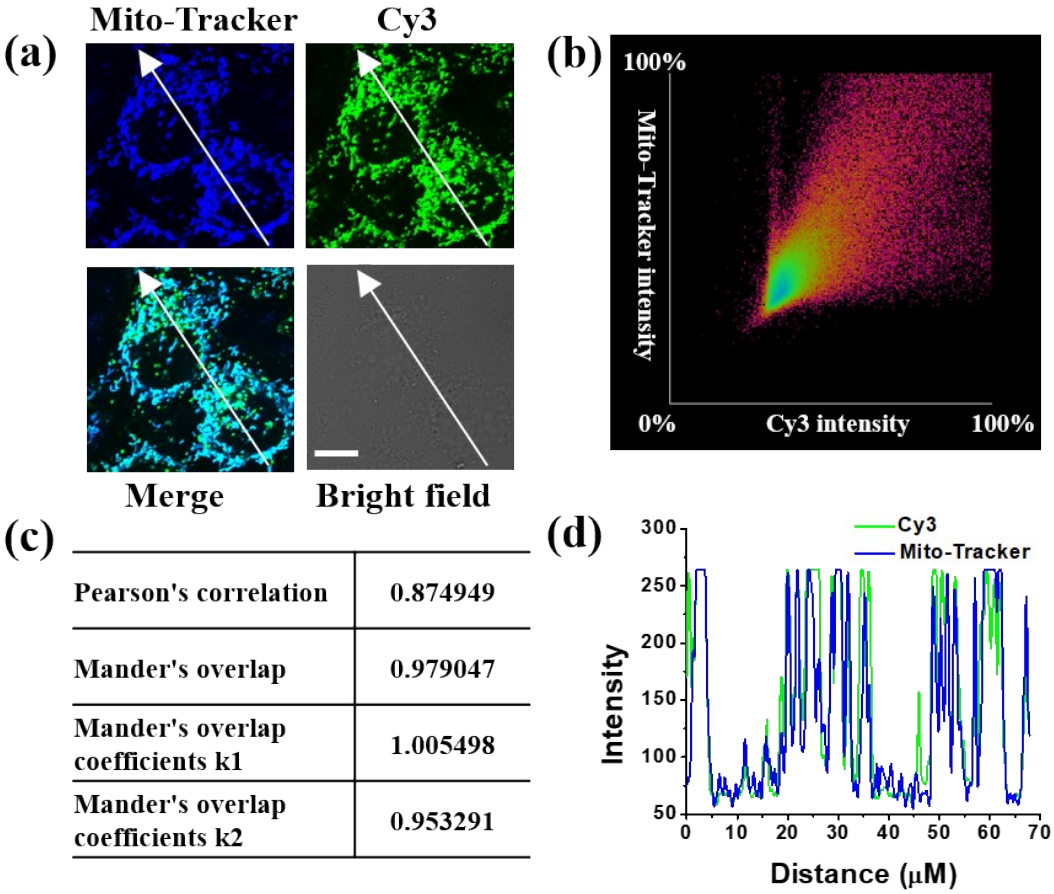
Figure S4. Cell viability of HEK-293 cells after incubation with different concentrations of NLa-1/NLa-2 mixture for different time.

6. Colocalization analysis between Mito-Tracker and Cy5

Table S2 Colocalization analysis parameters of the cell image in Figure 6

Pearson's correlation	0. 870249
Mander's overlap	0. 916618
Mander's overlap coefficients k1	0. 493518
Mander's overlap coefficients k2	1. 702448

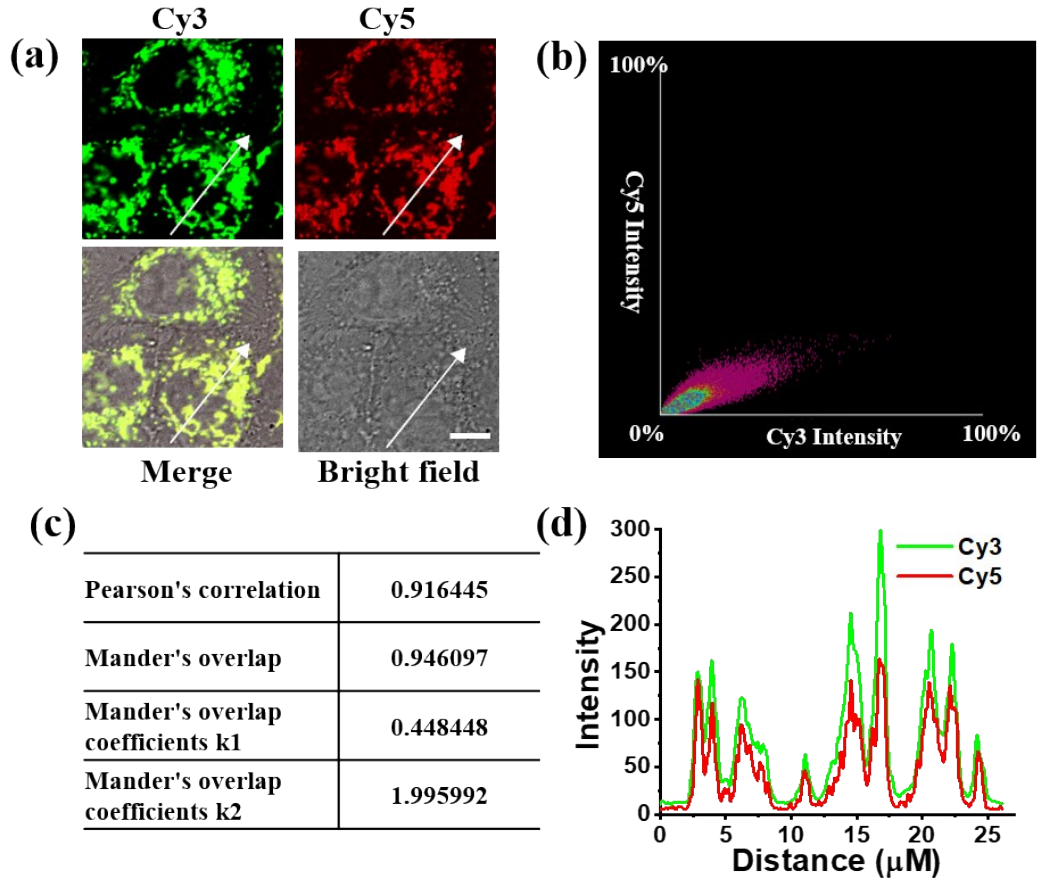
7. Colocalization analysis between Mito-Tracker and Cy3



Pearson's correlation	**0.874949**
Mander's overlap	**0.979047**
Mander's overlap coefficients k1	**1.005498**
Mander's overlap coefficients k2	**0.953291**

Figure S5. Intracellular colocalization image of Cy3 and Mito-Tracker Green fluorescence in MCF-7 cells. (a) Fluorescence images collected from Mito-Tracker Green and Cy3 channels and merged image. (b) Intensity scatter plot of Cy3 and Mito-Tracker Green. (c) Colocalization analysis parameters of the cell image. (d) Fluorescence intensity profile of the white arrow in (a). Scale bar is 20 μm.

8. Colocalization analysis between Cy3 and Cy5



Pearson's correlation	0.916445
Mander's overlap	0.946097
Mander's overlap coefficients k1	0.448448
Mander's overlap coefficients k2	1.995992

Figure S6. Intracellular colocalization image of Cy3 and Cy5 fluorescence in MCF-7 cells. (a) Fluorescence images collected from Cy3 and Cy5 channels and merged image. (b) Intensity scatter plot of Cy3 and Cy5. (c) Colocalization analysis parameters of the cell image. (d) Fluorescence intensity profile of the white arrow in (a). Scale bar is 20 μm.

9. Comparison of different methods for ATP sensing

Method	Sample	Detection range	Pros	Cons	Ref.
Graphene Oxide-Aptamer Beacon	Cytoplasm	0.125-2 mM	Stability	Extra synthesis and modification	1
Conjugated polymer	Solution	25-180 μM	Sensitivity, distinguish from UTP, GTP, ADP, AMP)	Require organic synthesis, time-consuming not suitable for intracellular analysis	2
Carbon Dots	Lysosomal	0.01-5 mM	Real-time monitoring; locate in lysosome	Require organic synthesis, cytotoxicity	3
Organic fluorescent probes (Rh6G−ACFPN)	Mitochondria	2-10 mM	Reversible detection; quantification analysis	Require organic synthesis, time-consuming, cytotoxicity	4
Upconversion Luminescence-Activated DNA Nanodevice	HeLa tumorbearing mice	1-10 mM	Living body ATP-analysis	Complex synthesis and modification, cytotoxicity	5
DNA Nanomachine	Lymphatic metastasis model	0.05-1 mM	Living body ATP-analysis	Complex synthesis and modification	6
AgNC-based ratiometric fluorescent nanosensor	Mouse cerebrospinal fluid	0.005-90 uM	Determination of ATP in the mouse cerebrospinal fluid with high accuracy	Not suitable for intracellular analysis	7
G-rich split aptamer-ThT based fluorescent ATP sensor	Human serum samples	50 nM-12 mM	High specificity (distinguish from UTP, GTP, ADP, AMP)	Not suitable for intracellular analysis	8
HCR-Amplified ATP Aptasenso	Cytoplasm	1-10 μM	Sensitivity	Trsnsfection step/ cytotoxicity	9
Photo-cleavable aptamer	Mitochondria	0.05-5 mM	Sensitivity, locate in mitochondria	Trsnsfection step/ cytotoxicity	10
ATP nanoflares	Cytoplasm	0.05-5 mM	Monitoring during the hyperthermia cell death process	Complex synthesis and modification, cytotoxicity	11
DNA Nanoprism	Cytoplasm	0.03 – 2 mM	Simple, high cell permeability and stability, low background	Carry a limited number of aptamers, cannot be used for quantitative detection of cellular ATP	12
DNA nanolantern-based split aptamer nanoprobe	Mitochondria	0.025-7 mM	Simple, high cell permeability and stability, low background, wide detection range/ high density of aptamers, locate in mitochondria	Cannot be used for quantitative detection of cellular ATP	this work

10. References

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