

Supplementary Material

# Fluorogenic Aptamer-Based Hybridization Chain Reaction for Signal-Amplified Imaging of Apurinic/Apyrimidinic Endonuclease 1 in Living Cells

Meixi Liu <sup>1,2,†</sup>, Yunjie Tan <sup>1,2,†</sup>, Chen Zhou <sup>1,2,†</sup>, Zhaoming Fu <sup>1,2</sup>, Ru Huang <sup>1,2,\*</sup>, Jin Li <sup>3,\*</sup> and Le Li <sup>4</sup>

<sup>1</sup> State Key Laboratory of Digital Medical Engineering, School of Biomedical Engineering, Hainan University, Sanya 572024, China

<sup>2</sup> Key Laboratory of Biomedical Engineering of Hainan Province, One Health Institute, Hainan University, Sanya 572024, China

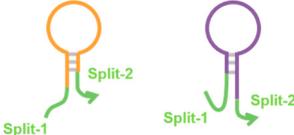
<sup>3</sup> Department of Painology, Hainan Cancer Hospital, Haikou 570311, China

<sup>4</sup> NHC Key Laboratory of Tropical Disease Control, Hainan Medical University, Haikou 571199, China

\* Correspondence: huangru@hainanu.edu.cn (R.H.); hnszlyytk@163.com (J.L.)

† The authors contribute equally in this study.

**Table S1.** Different design of two HP based self-assembly systems<sup>§</sup>.

Methods	FA	Probes' Design	Features	Ref.
CHARGE	Broccoli		The split FA fragments are set at the end of the two HP and are blocked by the extended stem respectively.	[1]
INSIGHT	Broccoli		The split FA fragments are set at the two ends of the two HP and one of the split is blocked by the extended stem.	[2]
EXO-HCR	Aptamer for NMM		The split FA fragments are set at the two ends of one HP and one of the split is blocked by the extended stem.	[3]
FAC-HCR	Lettuce		One of the split is set at the loop of a HP, and the other split is set as a toehold of the other HP.	Our work

<sup>§</sup> CHARGE: Catalytic Hairpin Assembly RNA circuit that is Genetically Encoded. INSIGHT: IN Situ Genetic Hybridization amplification Technique. EXO-HCR: Exonuclease III-assisted DNA cycling and hybridization chain reaction. NMM: N-nethyl mesoporphyrin IX.

**Table S2.** The sequences of the complete or split FA.

Name	Sequence(5'→3')
Lettuce	<u>GTCTTAGT</u> AGGGATGATGCGGCAGTGGGCT <u>TCATCGAA</u> CAGTGTTTA <u>TTCGATGA</u> GGGG <u>ACTAAGAC</u>
Substrate	<u>CCAGGAACTAATCAGACAAG</u> G <u>AACTGATTACAAACA</u>
Split-1	<u>TAGT</u> AGGGATGATGCGGCAGTGGGCT <u>TCATC</u> <u>CTTGCTGATTAGTTCCTGG</u>
Split-2	<u>TGTTTGTAATCAGTT</u> <u>TGATGA</u> GGGG <u>ACTA</u>

Note: The underlines indicate the complementary base among the oligonucleotides.

**Table S3.** The sequences of the probes for 4H-HCR.

Name	Sequence(5'→3')
Trigger	GAGCTTCATCTTCATCTCCGAGACTTC
4H-H1	<u>TAGTAGGGATGATGCGGCAGTGGGCTTCATC</u> GGAAGTCT <u>CGGAGATGAAGATGAAGC</u> CATCGT <u>GCTTCATCTTCATCT-CCG</u>
4H-H2	<u>GCTTCATCTTCATCTCCG</u> TTTTGCGGAGATGAAGATGAAGCACGATG
4H-H3	CAAAAC <u>CGGAGATGAAGATGAAGC</u> TTGCCT <u>GCTTCATCTTCATCTCCG</u> <u>TGATGAGGGGACTA</u>
4H-H4	<u>GCTTCATCTTCATCTCCG</u> AGACTTC <u>CGGAGATGAAGATGAAGC</u> AGGCAA

Note: The underlines indicate the intramolecular complementary base of the probes. The green fonts represent the split Lettuce sequences.

**Table S4.** The sequences of the probes for 2H-HCR.

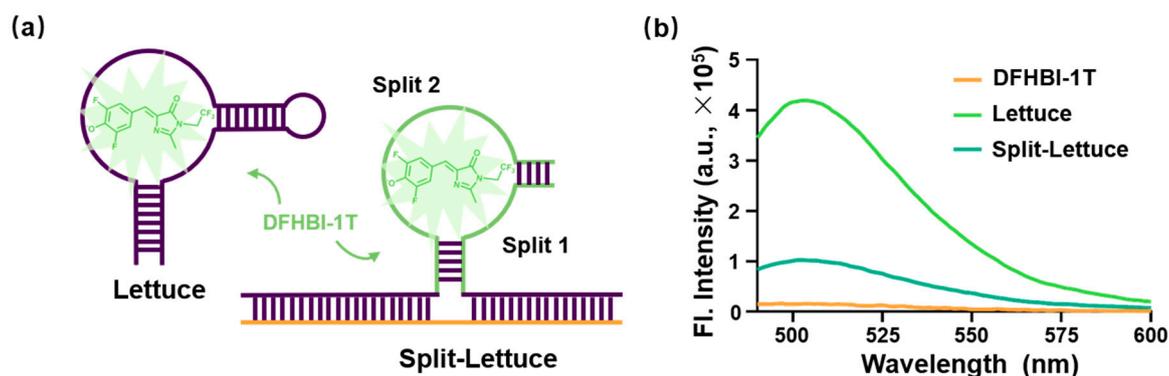
Name	Sequence(5'→3')
2H-H1	<u>TAGTAGGGATGATGCGGCAGTGGGCTTCGGA</u> GGAAGTCT <u>CGGAGATGAAGATGAA</u> ACTCGATTCATC <u>TTCATCTTC</u> <u>ATCICCG</u> AGGGGACTATTTT
2H-H2	<u>TAGTAGGGATGATGCGGCAGTGGGCTTCATC</u> TTCATCTTCATC TCCGAGACTTC <u>GATGAAGATGAAGATGA</u> GATCGAG- TAGATGAGGGGACTATTTT

Note: The underlines indicate the intramolecular complementary base of the probes. The green fonts represent the split Lettuce sequences.

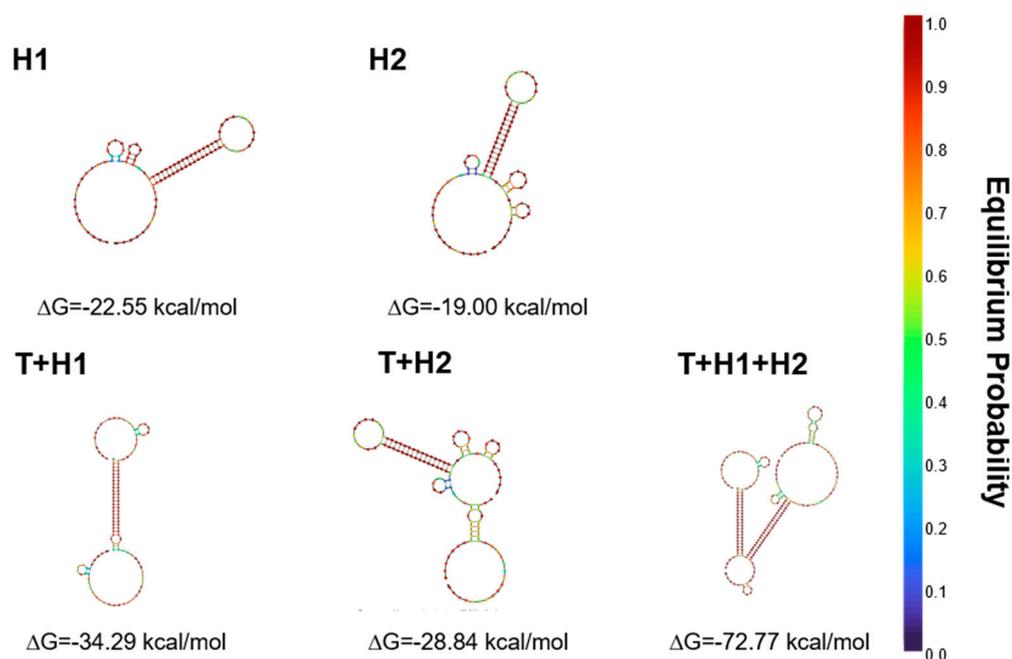
**Table S5.** The sequences of the probes for FAC-HCR.

Name	Sequence(5'→3')
FAC-H1(16)	GAAGTCT <u>CGGAGATGAAGATGAA</u> <b>TTCTGACAGGGG</b> ACACTTAACG <u>TTCATCTTCATCTCCG</u>
FAC-H2(16)	<u>TTCATCTTCATCTCCG</u> AGACTTC <u>CGGAGATGAAGATGAA</u> CGTTAAGTGT <b>AGGGATGATGCGGCAGTGGGCTT-</b> <b>GTCAGAA</b>
FAC-H1(18)	GAAGTCT <u>CGGAGATGAAGATGAA</u> <b>GCCTGACAGGGG</b> ACACTTCA <u>GCTTCATCTTCATCTCCG</u>
FAC-H2(18)	<u>TTCATCTTCATCTCCG</u> AGACTTC <u>CGGAGATGAAGATGAA</u> GCTGAAGTGT <b>AGGGATGATGCGGCAGTGGGCTT-</b> <b>GTCAGGC</b>
FAC-H1(20)	GAAGTCT <u>CGGAGATGAAGATGAA</u> <b>GCTGGACAGGGG</b> ACACTT <u>CAGCTTCATCTTCATCTCCG</u>
FAC-H2(20)	<u>TTCATCTTCATCTCCG</u> AGACTTC <u>CGGAGATGAAGATGAA</u> GCTGAAGTGT <b>AGGGATGATGCGGCAGTGGGCTT-</b> <b>GTCCAGC</b>
ARP	<u>GAAGTCTC/idSp/GAG</u> CTTCATCTTCAT <u>CTCCGAGACTTC</u>
ARP-C	<u>GAAGTCTCGGAG</u> CTTCATCTTCAT <u>CTCCGAGACTTC</u>
Cy5-DNA	<b>Cy5</b> -TATAGTACCTTCGACAACGAGCCCTGTGCGAAGGT

Note: The underlines indicate the intramolecular complementary base of the probes. The green fonts represent the split Lettuce sequences. idSp represents an apurinic/aprimidinic (AP) site. The 5'-end of the probe was functionalized with a cyanine-5 dye, represented by Cy5.



**Figure S1.** The structure and fluorescence ability of Lettuce and split-Lettuce. (a) The structure of Lettuce and the split-Lettuce. Lettuce can directly bind with DFHBI-1T and emit fluorescence. Split-Lettuce is formed by two DNA fragments, split-1 and split-2. Only when the split-1 and split-2 bind a single substrate simultaneously, DFHBI-1T can be recognized and emit fluorescence. (b) Fluorescence detection results of free DFHBI-1T, DFHBI-1T + Lettuce, and DFHBI-1T + split-Lettuce + Substrate. Wherein the DFHBI-1T was 2  $\mu\text{M}$ , all of the Lettuce, the split-L, the split-R, the substrate were 5  $\mu\text{M}$ .



**Figure S2.** Secondary structures and Gibbs free energy change of 2H-H1 & 2H-H2 simulated by NUPACK.

## References

1. Karunanayake Mudiyansele, A.P.K.K.; Yu, Q.; Leon-Duque, M.A.; Zhao, B.; Wu, R.; You, M. Genetically encoded catalytic hairpin assembly for sensitive RNA imaging in live cells. *J Am Chem Soc* **2018**, *140*, 8739-8745.
2. Ren, K.; Wu, R.; Karunanayake Mudiyansele, A.P.K.K.; Yu, Q.; Zhao, B.; Xie, Y.; Bagheri, Y.; Tian, Q.; You, M. In Situ genetically cascaded amplification for imaging RNA subcellular locations. *J Am Chem Soc* **2020**, *142*, 2968-2974.
3. Sun, J.; Jiang, W.; Zhu, J.; Li, W.; Wang, L. Label-free fluorescence dual-amplified detection of adenosine based on exonuclease III-assisted DNA cycling and hybridization chain reaction. *Biosens Bioelectron* **2015**, *70*, 15-20.