

Supplementary Material

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

Meixi Liu ^{1,2,†}, Yunjie Tan ^{1,2,†}, Chen Zhou ^{1,2,†}, Zhaoming Fu ^{1,2}, Ru Huang ^{1,2,*}, Jin Li ^{3,*} and Le Li ⁴

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

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

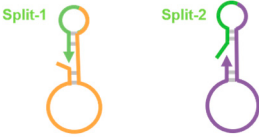
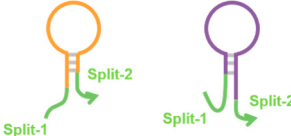


³ Department of Painology, Hainan Cancer Hospital, Haikou 570311, China

⁴ NHC Key Laboratory of Tropical Disease Control, Hainan Medical University, Haikou 571199, China

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

† The authors contribute equally in this study.

Table S1. Different design of two HP based self-assembly systems[§].

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

[§] 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-methyl 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>TAGTAGGGATGATGCGGCAGTGGGCT</u> <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> GAAAGTCT <u>CGGAGATGAAGATGAAGC</u> CATCGT <u>GCTTCATCTTCATCT-CCG</u>
4H-H2	<u>GCTTCATCTTCATCTCCGTTT</u> TGCGGAGATGAAGATGAAGCACGATG
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> GAAAGTCT <u>CGGAGATGAAGATGAA</u> ACTCGATCTCATC <u>TTCATCTTC</u> <u>ATCTCCG</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> TTCTGACAGGGG ACACTTAACG <u>TTCATCTTCATCTCCG</u>
FAC-H2(16)	<u>TTCATCTTCATCTCCG</u> AGACTTC <u>CGGAGATGAAGATGAA</u> CGTTAAGTGT AGGGATGATGCGGCAGTGGGCTT- GTCAGAA
FAC-H1(18)	GAAGTCT <u>CGGAGATGAAGATGAA</u> GCCTGACAGGGG ACACTTCA <u>GCTTCATCTTCATCTCCG</u>
FAC-H2(18)	<u>TTCATCTTCATCTCCG</u> AGACTTC <u>CGGAGATGAAGATGAA</u> GCTGAAGTGT AGGGATGATGCGGCAGTGGGCTT- GTCAGGC
FAC-H1(20)	GAAGTCT <u>CGGAGATGAAGATGAA</u> GCTGGACAGGGG ACACTT <u>CAGCTTCATCTTCATCTCCG</u>
FAC-H2(20)	<u>TTCATCTTCATCTCCG</u> AGACTTC <u>CGGAGATGAAGATGAA</u> GCTGAAGTGT AGGGATGATGCGGCAGTGGGCTT- GTCCAGC
ARP	<u>GAAGTCTC</u> / idSp / <u>GAG</u> CTTCATCTTCAT <u>CTCCGAGACTTC</u>
ARP-C	<u>GAAGTCTCGGAG</u> CTTCATCTTCAT <u>CTCCGAGACTTC</u>
Cy5-DNA	Cy5 -TATAGTACCTTCGACAACGAGCCCTTGTCGAAGGT

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

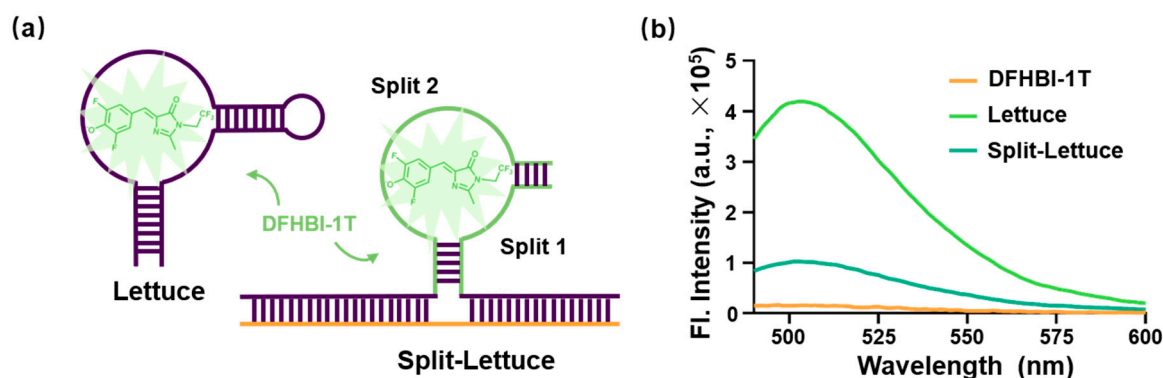


Figure S1. The structure and fluorescence ability of Lettuce and split-Lettuce. (a) The structure of Lettuce and the split-Lettuce. Lettuce can directly bind with DFHBI-1T and emit fluorescence. Split-Lettuce is formed by two DNA fragments, split-1 and split-2. Only when the split-1 and split-2 bind 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 μ M, all of the Lettuce, the split-L, the split-R, the substrate were 5 μ M.

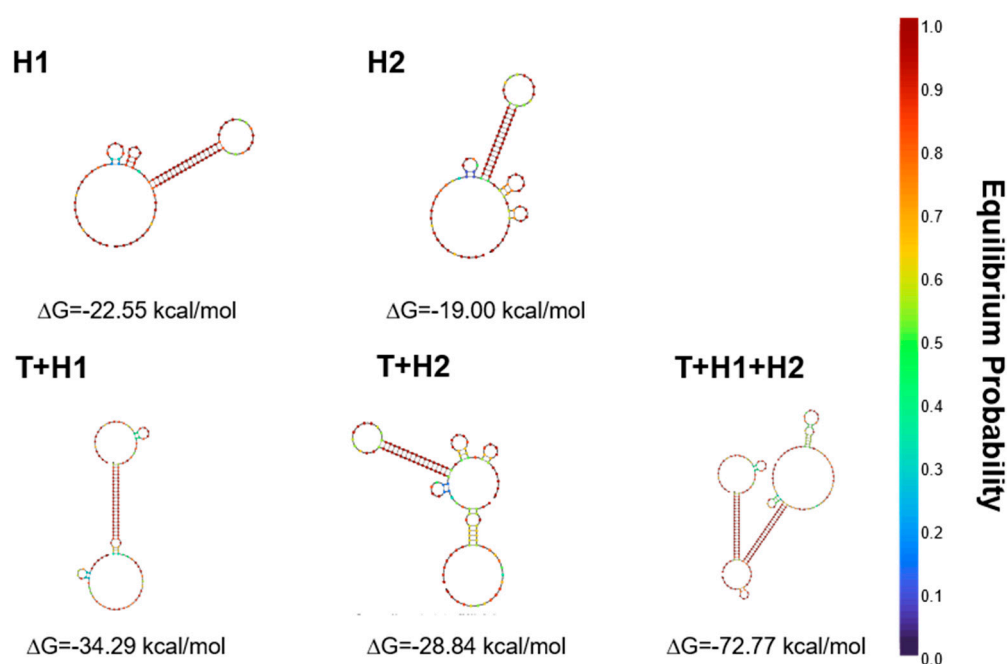


Figure S2. Secondary structures and Gibbs free energy change of 2H-H1 & 2H-H2 simulated by NUPACK.

References

1. Karunanayake Mudiyanse, 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 Mudiyanse, 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.