Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Electronic Supporting Information

Target-Directed Enzyme-Free Dual-Amplification DNA Circuit for Rapid Signal Amplification

Chao Xing,*[a] Ziyi Chen,[b] Cheng Zhang,[a] Jun Wang*[a] and Chunhua Lu*[b]

a. Fujian Key Laboratory of Functional Marine Sensing Materials, Center for Advanced Marine Materials and Smart Sensors, Minjiang University, Fuzhou 350108, P. R. China; E-mail: chaoxing@mju.edu.cn; wjnaf@163.com.

b. College of Chemistry, Fuzhou University, Fuzhou 350116, P. R. China; E-mail: chunhualu@fzu.edu.cn

Results and Discussion

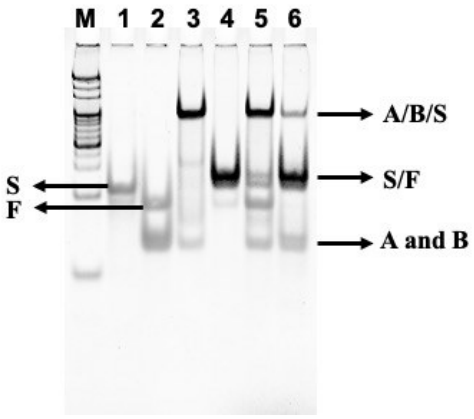

Figure S1. Characterization of EDC product using native PAGE: lane M, 20bp marker; lane 1, S; lane 2, mixture of A, B, F; lane 3, A/B/S; lane 4, S/F; lane 5, mixture of A/B/S + F, lane 6, mixture of A/B/S + F +I. In the presence of target DNA, three-strand complexes were converted to duplex strand S/F with obvious bands and by-product strand (lane 6), indicating the successful initiation of the EDC.

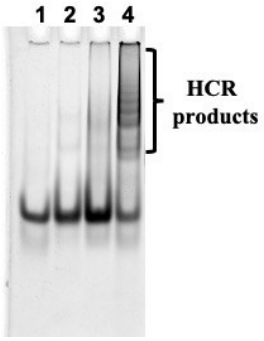

Figure S2. Characterization of HCR product using native PAGE: lane 1, H1; lane 2, H2; lane 3, mixture of H1 and H2; lane 4, mixture of H1+ H2+ target DNA. With the mix of H1 and H2 together, no side reaction was observed (Lane 3). Once target DNA was introduced into the mixture of H1 and H2, large products with pronounced retarded mobility were yielded, indicating the successful initiation of the HCR (Lane 4).

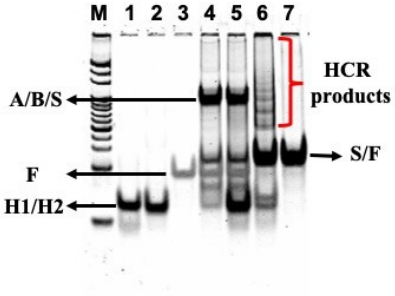

Figure S3. Characterization of EDC-HCR process using native PAGE: lane M, 20bp marker; lane 1, H1; lane 2, H2; lane 3, F; lane 4, mixture of A/B/S + F; lane 5, mixture of A/B/S + F + H1 + H2; lane 6, mixture of A/B/S + F + H1 + H2 + I; lane 6, S/F. In absence of target DNA, no new bands appeared (lane 5), indicating that no occurrence of EDC-HCR reaction. There is some trailing can be observed in lane 5, which can be due to the spurious hybridization events. However, incubating target DNA with A/B/S, F, H1 and H2, many newly formed bands with large molecular weight were observed, while almost all fuel strand F disappeared (lane 6), indicating the success of HCR-EDCR operation.

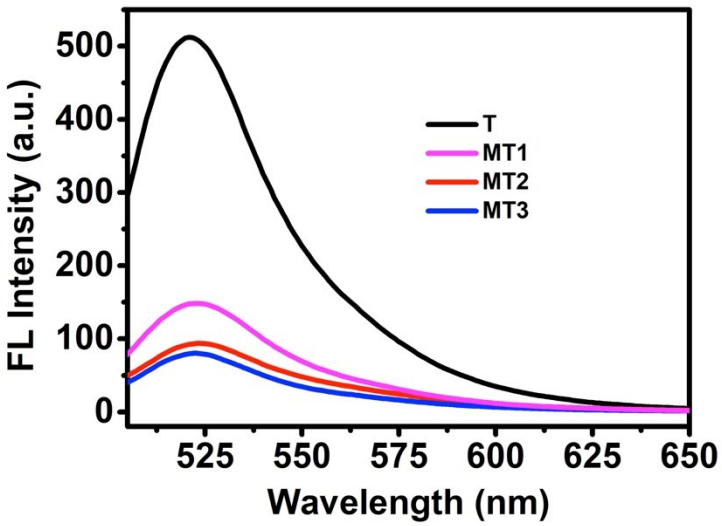

Figure S4. Fluorescent spectra of the EDC-HCR system in the presence of perfectly matched target (T), single-base mismatched oligonucleotides (MT1), single-base deleted oligonucleotides (MT2), single-base inserted oligonucleotides (MT3).

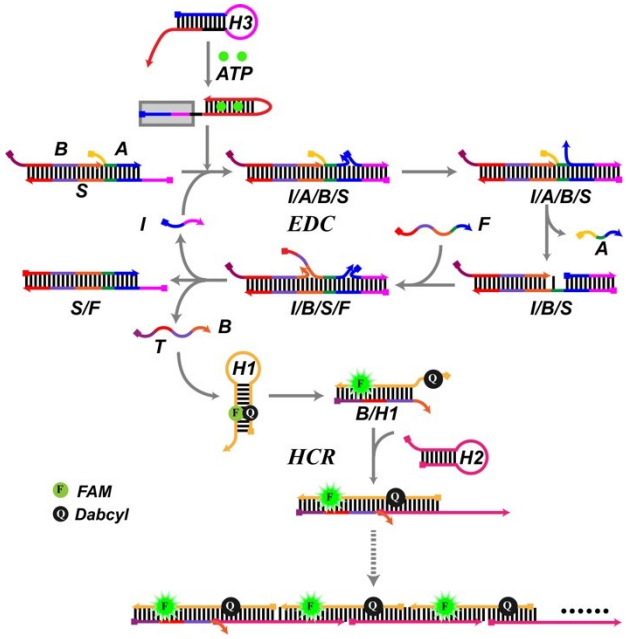

Figure S5. Schematic of the EDC–HCR circuit for an accurate ATP detection.

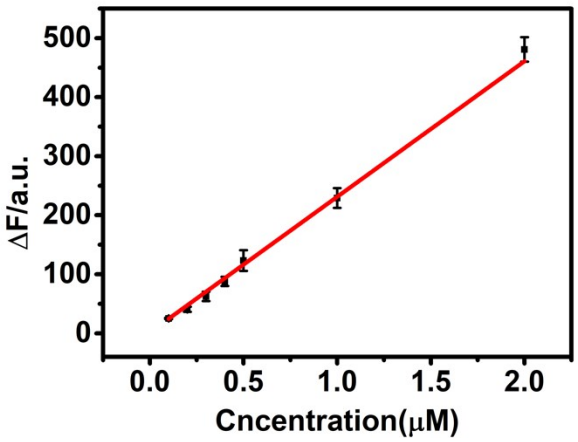

Figure S6. A plot of fluorescent decrease versus ATP concentration in healthy human serum in the range of 0.1 μM to 2 μM, $R^2 = 0.991$.

Table S1. The DNA strands used in tile self-assembly.

S	TGGAGACGTAGGGTAACCTGAGGGCCGTAAGAGAGCTGTAGATTGGATCG
A	CCACATACATCATATTCCCTCAGGTTACCCTACG
B	GTCACTCGATCCAATCTACAGCTCTCTTACGG
F	CGATCCAATCTACAGCTCTCTTACGGCCCTCATTCAATACCCTACG
I	CAGGTTACCCTACGTCTCCA
H1	CGATCCAA(FAM)TCTACAGCAGATGTGTAGCTGTAGA(Dabcyl)TTGGATCGAGTGAC
H2	TACACATCTGCTGTAGATTGGATCGGTCACTCGATCCAATCTACAGC
Aptamer-I	CAGGTTACCCTACGTCTCCATAGGGTAACCTGGGGGAGTATTGCGGAGGAAGGT
MT1	CAGATTACCCTACGTCTCCA
MT2	CAG-TTACCATACGTCTCCA
MT3	CAGGATTACCATACGTCTCCA

The red domain is initiator sequence and the green domain is ATP aptamer sequence.

Table S2. Comparison of different signal amplification systems for nucleic acid detection

System	Signal readout	Reaction time	Detection limit	Ref.
Exonuclease III-aided target recycling	Fluorescence	30 min	10 pM	[1]
Catalyzed hairpin assembly (CHA)	Fluorescence	1.5 h	19 pM	[2]
Hybridization chain reaction (HCR)	Colorimetric	1h	50 pM	[3]
CHA-DNAzyme cascade	Fluorescence	2 h	20 pM	[4]
CHA	Colorimetric	1.5 h	9 pM	[5]
CHA-HCR cascade	Fluorescence	5 h	2 pM	[6]
entropy-driven circuit	Fluorescence	3 h	5 pM	[7]
EDC-HCR	Fluorescence	45 min	7 pM	This work

Table S3. Comparison of different signal amplification systems for ATP detection

System	Signal readout	Reaction time	Detection limit	Ref.
G-quadruplex/hemin complex	Colorimetric	3h	40 nM	[8]
DNAzyme	Fluorescence	3h	10 nM	[9]
Self-replicating CHA	Colorimetric	25 min	48 nM	[10]
DNA/Ag nanoclusters-based flourescent aptasensor	Fluorescence	2.5 h	91.6 nM	[11]
Three-way DNA junction -based aptasensor	Fluorescence	4 h	40 μM	[12]
Dual-amplified fluorescence anisotropy assay	Fluorescence	2 h	100 nM	[13]
EDC-HCR	Fluorescence	45 min	38 nM	This work

1. X. L. Zuo, F. Xia, Y. Xiao and K. W. Plaxco, *J. Am. Chem. Soc.*, 2010, **132**, 1816-+.
2. Z. J. Duan, Z. Li, J. Y. Dai, H. F. He and D. Xiao, *Talanta*, 2017, **164**, 34-38.
3. P. Liu, X. H. Yang, S. Sun, Q. Wang, K. M. Wang, J. Huang, J. B. Liu and L. L. He, *Analytical Chemistry*, 2013, **85**, 7689-7695.
4. F. A. Wang, J. Elbaz, C. Teller and I. Willner, *Angewandte Chemie-International Edition*, 2011, **50**, 295-299.
5. H. F. He, J. Y. Dai, Z. J. Duan, Y. Meng, C. S. Zhou, Y. Y. Long, B. Z. Zheng, J. Du, Y. Guo and D. Xiao, *Biosens. Bioelectron.*, 2016, **86**, 985-989.
6. H. Wang, C. Li, X. Liu, X. Zhou and F. Wang, *Chem. Sci.*, 2018, **9**, 5842-5849.
7. Y. F. Lv, L. Cui, R. Z. Peng, Z. L. Zhao, L. P. Qiu, H. P. Chen, C. Jin, X. B. Zhang and W. H. Tan, *Analytical Chemistry*, 2015, **87**, 11714-11720.
8. L. B. Zhang, S. J. Guo, J. B. Zhu, Z. X. Zhou, T. Li, J. Li, S. J. Dong and E. K. Wang, *Analytical Chemistry*, 2015, **87**, 11295-11300.
9. Y. S. Guo, J. Liu, G. X. Yang, X. F. Sun, H. Y. Chen and J. J. Xu, *Chemical Communications*, 2015, **51**, 862-865.
10. J. Dai, H. He, Z. Duan, Y. Guo and D. Xiao, *Anal Chem*, 2017, **89**, 11971-11975.
11. Y. Zhu, X. C. Hu, S. Shi, R. R. Gao, H. L. Huang, Y. Y. Zhu, X. Y. Lv and T. M. Yao, *Biosens. Bioelectron.*, 2016, **79**, 205-212.
12. J. B. Zhu, L. B. Zhang, Z. X. Zhou, S. J. Dong and E. K. Wang, *Analytical Chemistry*, 2014, **86**, 312-316.
13. B. Yang, X. B. Zhang, L. P. Kang, G. L. Shen, R. Q. Yu and W. H. Tan, *Analytical Chemistry*, 2013, **85**, 11518-11523.