

Supporting Information

Localized catalytic hairpin assembly-based DNA tetrahedral sensor for sensitive and rapid miRNA detection in living cells

Chunhui Zhao,^{*a} Shenzhi Lai,^a Jiao He,^b Yuexiang Sun,^b Haoxiao Wang,^b Feng Chen,^{*b} Chunyan Chen,^b Hang Gong^{b,c} and Changqun Cai^b

^aHunan Chemical Vocational Technology College, Zhuzhou 412000, China

^bKey Laboratory of Environmentally Friendly Chemistry and Application, College of Chemistry, Xiangtan University, Xiangtan 411105, China

^cCollege of Chemistry and Chemical Engineering, Yunnan Normal University, Kunming, 650500, China.

* E-mail addresses: ZhaoCH127@163.com (C. Zhao); chenfeng@xtu.edu.cn (F. Chen)

Contents

Table S1. Nucleic acid sequences

Table S2. Comparison of the analytical performances of DNA-based hydrogels

Figure S1. The effect of reaction buffers on relative fluorescence intensity of the sensor

Figure S2. The effect of Mg²⁺ concentration on relative fluorescence intensity of the sensor

Figure S3. The effect of reaction temperature on relative fluorescence intensity of the sensor

Figure S4. Cytotoxicity evaluation of the DNA tetrahedral sensor

Figure S5. Confocal micrographs of MCF-7 cells incubated with the DNA tetrahedral sensor at different temperature

Reference

Table S1. Nucleic acid sequences used in the experiment

Oligonucleotide	Sequence (5'-3')
S1	TTC AGA CTT AGG AAT GTG CTT CCC ACG TAG TGT CGT TTG TAT TGG ACC CTC GCA TTT GGT TGA ATG AGG G
S2	TCA ACT GCC TGG TGA TAA AAC GAC ACT ACG TGG GAA TCT ACT ATG GCG GCT CTT CTT GTA TTG AAT GAG G
S3	ACA TTC CTA AGT CTG AAA CAT TAC AGC TTG CTA CAC GAG AAG AGC CGC CAT AGT A
S4	TAT CAC CAG GCA GTT GAC AGT GTA GCA AGC TGT AAT AGA TGC GAG GGT CCA ATA C
H1	FAM-CAC AAA TAC CCT GTA GAA CCG AAT TTG TG/BHQ1/T TTT TTC CCT CAT TCA ACC
H2	CAC AAA TTC GGT TCT ACA GGG TAT TTG TGT ACC CTG TAG AAC CGA ATT TTT TCC TCA TTC AAT AC
H1-C	FAM-CAC AAA TAA CCT ATA AAA CCAAAT TTG TG/BHQ1/T TTT TTC CCT CAT TCA ACC
H2-C	TAC TAA TTT GGT TTT ATA TGG TAT TTG TGT ACC CTG TAG AAC CGA ATT TTT TCC TCA TTC AAT AC
miRNA-10b	UAC CCU GUA GAA CCG AAU UUG UG
miRNA-122	UGG AGU GUG ACA AUG GUG UUU G
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A
miRNA-141	UAA CAC UGU CUG GUA AAG AUG G

Table S2. Comparison of the analytical performances of CHA-based DNA sensor for miRNAs detection.

Signal	Target	LOD	Detectio	Reference
Fluorescence	miRNA-21	22.9 pM	150 min	[1]
Fluorescence	miRNA-21	5 nM	180 min	[2]
Fluorescence	miRNA-156	89 pM	120 min	[3]
Fluorescence	miRNA-146a	1 fM	120 min	[4]
Fluorescence	miRNA-21	65 fM	180 min	[5]
Fluorescence	miRNA-21	3.4 pM		[6]
Fluorescence	miRNA-451	0.24 fM	90 min	[7]
Fluorescence	miRNA-21	14 pM		[8]
Fluorescence	miRNA-10b	3.8 pM	100 min	This work

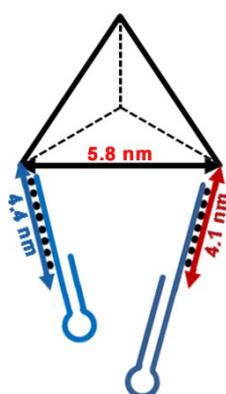


Figure S1. Schematic illustration of the DNA tetrahedral sensor structure. The motion trajectories of CHA hairpin H1 and H2 on the DNA tetrahedron was estimated according to the circumcircle diameter model.

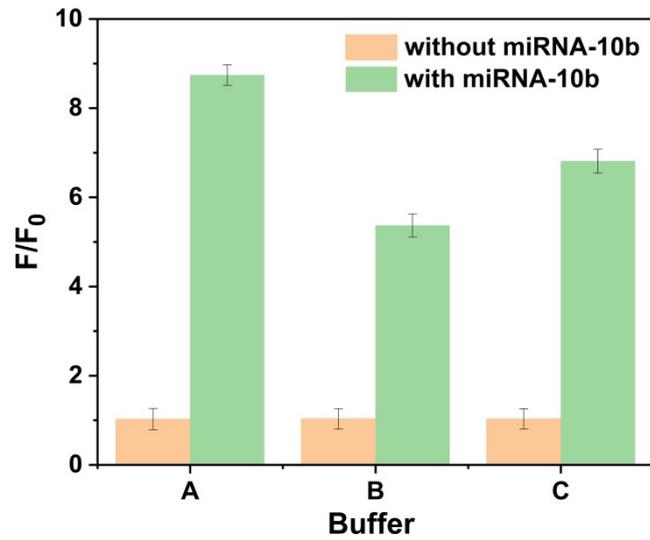


Figure S2. The relative fluorescence intensity of the DNA tetrahedral sensor in different reaction buffers with or without target miRNA-10b. Buffer A: TAMg buffer (45 mM of Tris containing 12 mM of magnesium acetate). Buffer B: PBS buffer. Buffer C: HEPES buffer (10 mM of HEPES containing 12 mM of magnesium acetate).

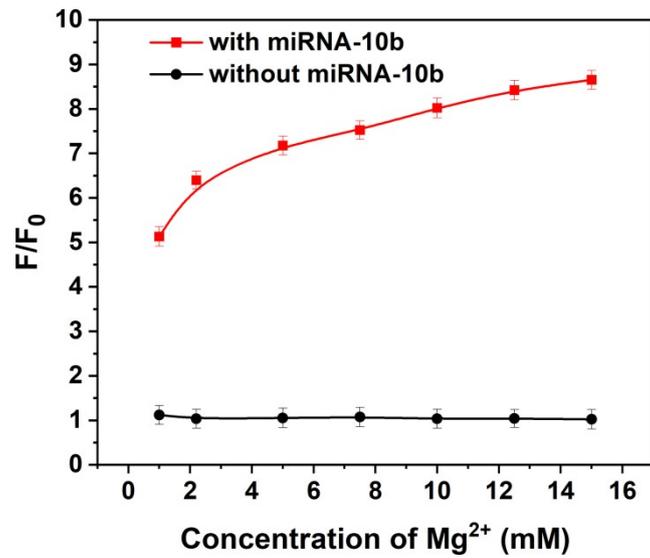


Figure S3. The relative fluorescence intensity of the DNA tetrahedral sensor in different Mg²⁺ concentration with or without target miRNA-10b.

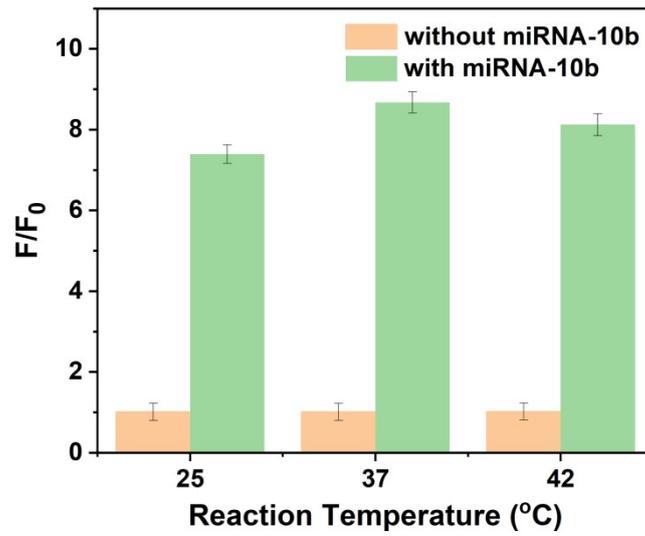


Figure S4. The relative fluorescence intensity of the DNA tetrahedral sensor in different reaction temperature with or without target miRNA-10b.

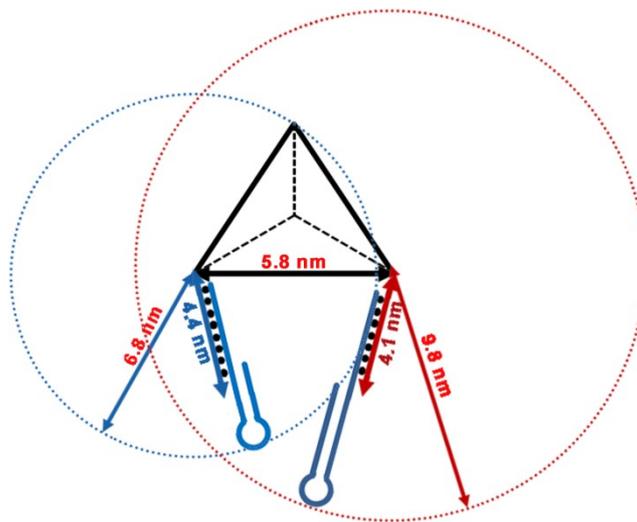


Figure S5. Schematic illustration of the motion trajectories of CHA hairpins H1 and H2 on the DNA tetrahedron was estimated according to the circumcircle diameter model.

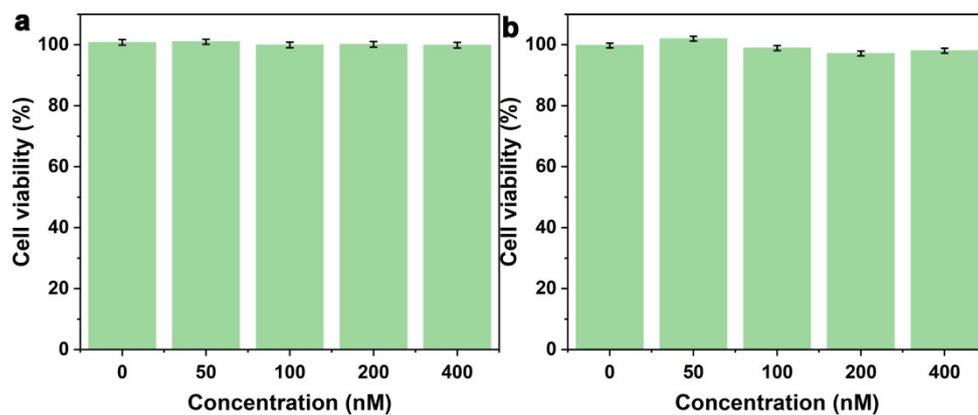


Figure S6. Cytotoxicity evaluation of the DNA tetrahedral sensor at different concentrations in MCF-7 cells (a) and MCF-10A cell (b) for 24 h at 37 °C.

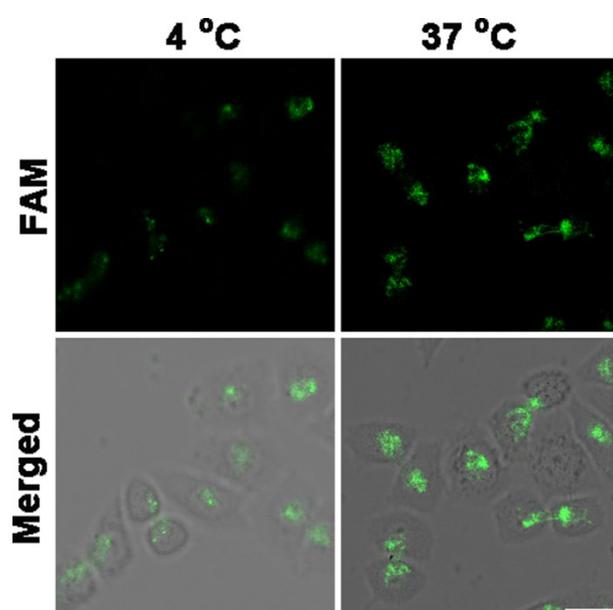


Figure S7. Confocal micrographs of MCF-7 cells incubated with the DNA tetrahedral sensor at 4 °C and 37 °C, respectively. Scale bar: 25 μ m.

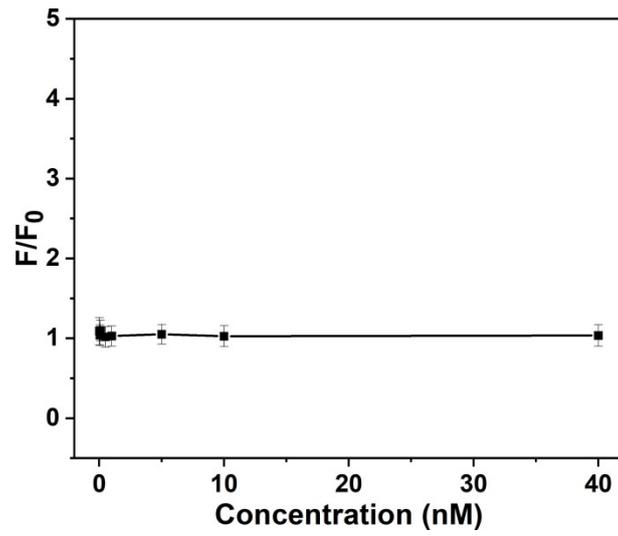


Figure S8. Fluorescence intensity of the Control in response to different concentration of miRNA-10b.

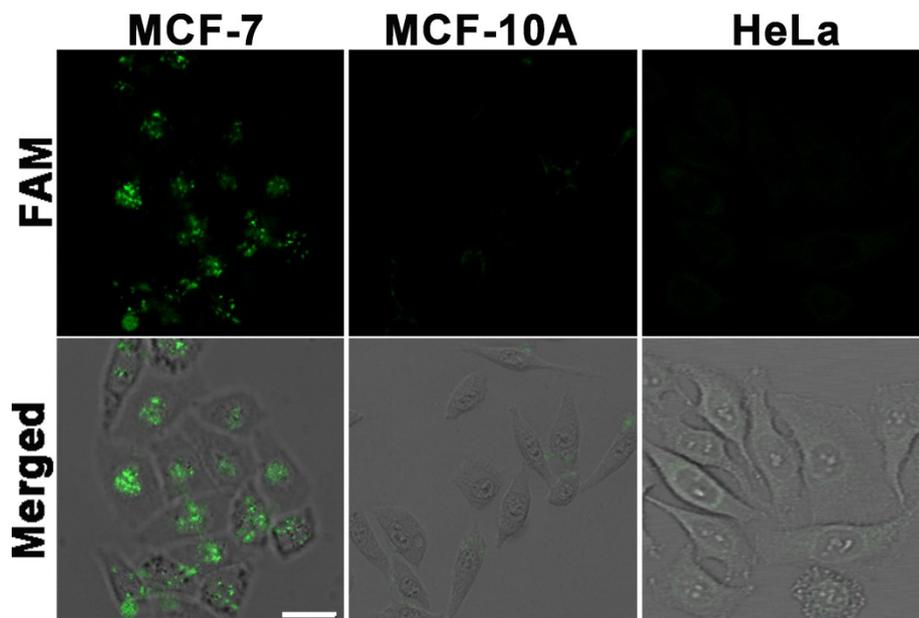


Figure S9. Fluorescence images of different kinds of cells after incubation with the DNA tetrahedral sensor. Scale bar: 25 μm .

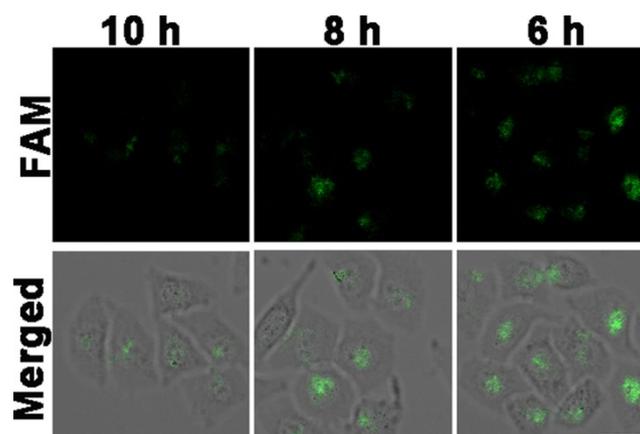


Figure S10. Fluorescence images of MCF-7 cells after treated with miR-10b inhibitors at different time. Scale bar: 25 μm .

References

- [1] Y. Jiang, C. Xie, Y. Chen, H. Wang, J. Shang, X. Liu, F. Wang, Endogenous enzyme stimulation of localized catalytic DNA assembly for high-contrast microRNA imaging and reliable evaluation of drug resistance. *Anal. Chem.*, 2025, **97**, 19719.
- [2] S. Wang, J. Guang, Y. Gao, B. Fan, L. Yan, J. Pan, L. Li, W. Meng, F. Hu, Fluorescent DNA tetrahedral probe with catalytic hairpin self-assembly reaction for imaging of miR-21 and miR-155 in living cells. *Microchim. Acta*, 2024, **191**, 462.
- [3] L. Chen, X. Ding, Y. Dong, H. Chen, F. Gao, B. Cui, X. Zhao, H. Cui, X. Gu, Z. Zeng, Integration of catalytic hairpin assembly probes into microneedles for detection of MicroRNA in plants. *Sensor. Actuat. B-Chem.*, 2024, **404**, 135277.
- [4] X. Bao, L. Ma, L. Yang, X. Li, L. Feng, T. Zhong, A leakage-resistant catalytic hairpin assembly for ultrasensitive detection of miRNA-146a in systemic lupus erythematosus, *Microchem. J.*, 2024, **200**, 110479.
- [5] X. Huang, Y. Zhang, J. Chen, L. Zhang, Y. Xu, W. Yin, Y. Shi, S. Liu, X. Zou, Z. Dai, Dual-locked DNAzyme platform for in vitro and in vivo discrimination of cancer cells. *Anal. Chem.*, 2022, **94**, 12221.
- [6] Z. Yang, R. Qin, D. Ruan, C. Hu, W. Li, J. Zhou, F. Zhang, B. Guo, L. Huang, D. Jaque, Y. Shen, F. Wang, Ce6-DNAzyme-laded metal-organic framework theranostic agents for boosting miRNA imaging-guided photodynamic therapy in breast cancer. *ACS Nano*, 2025, **19**, 27873.
- [7] J. Xu, X. Luo, H. Chen, B. Guo, Z. Wang, F. Wang, Machine learning-aided intelligent monitoring of multivariate miRNA biomarkers using bipolar self-powered sensors, *ACS Nano*, 2025, **19**, 8812.
- [8] Z. Yang, C. Hu, W. Li, D. Ruan, B. Guo, Y. Zhao, L. Huang, Y. Shen, F. Wang, Specified simultaneous monitoring of miRNA and telomerase based on fluorescence sensor triggered by H_2O_2 -GSH. *Sensor. Actuat. B-Chem.* 2025, **439**, 137855.