Electronic Supplementary Information

Rapid Self-Disassembly of DNA Diblock Copolymer Micelles *via* Target Induced Hydrophilic-Hydrophobic Regulation for Sensitive MiRNA Detection

Xin Peng,[†] Ze-Zhou Yang,[†] Peng Yang,[†] Ya-Qin Chai,[†] Wen-Bin Liang,[†] Zhao-Hui Li,[*,‡] and Ruo Yuan[*,†]

[†]*Laboratory of Luminescence Analysis and Molecular Sensing (Southwest University), Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P.R. China.*

[‡]*College of Chemistry, Henan Joint International Research Laboratory of Green Construction of Functional Molecules and Their Bioanalytical Applications, Zhengzhou University, Zhengzhou 450001, P.R. China.*

[*]Corresponding authors at: Tel.: +86-371-67780037, +86-23-68252277.
Fax: +86-371-67781556, +86-23-68253172.

E-mail addresses: zhaohui.li@zzu.edu.cn (Z. H. Li), yuanruo@swu.edu.cn (R. Yuan).

Table of Contents

Materials and Reagents. All oligonucleotides (Table S1) were custom-synthesized by Takara Biotechnology Co., Ltd. (Dalian, China) and used without further purification. Perylene, Mg $(OAc)_2$, acetic acid and Tris were all purchased from Sigma Chemical Co. (St. Louis, MO, USA). TAM buffer (pH = 8.0) was prepared by 12.5 mM Mg^{2+}, 20 mM acetic acid and 45 mM Tris to dissolve the DNA monomer. The methylbenzene was acquired from Chengdu Kelong Company Ltd. (Chengdu, China) for dissolution of Perylene. The mica was purchased from the Zkbaice Company (Beijing, China). The deionized water with the electrical resistance of 18.2 MΩ·cm was used in this detection system.

Table S1. Nucleic acid sequences used in the experiments.

Name	Sequence from 5′ to 3′
H1	TCA ACA TCA GTC TGA TAA GCT ACA TTG GAT GCT CTA GCT TAT CAG ACT G
H2	TAA GCT AGA GCA TCC AAT GTA GCT TAT CAG ACT GCA TTG GAT GCT CAC GAC G
H3	ATG CTC ACG AC CTGGACA CGT CGT GAG CAT CCA ATG TTT TT
sp-H3	ATG CTC ACG AC CTGGACA CGT CGT GAG CAT CCA ATG TTT TT - $(Spacer\ C12)_{10}$
H4	ACG ACC TGG ACA GCT ATG TGT CCA GGT CGT GAG CAT
H5	CAT AGC TGT CCA GGT CGT ATG CTC ACG ACC TGG ACA
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A
miRNA-203a	AGU GGU UCU UAA CAG UUC AAC AGU U
miRNA-122	UGG AGU GUG ACA AUG GUG UUU G

miRNA-126	CAU UAU UAC UUU UGG UAC
miRNA-141	UAA CAC UGU CUG GUA AAG AUG G

Apparatus and Measurements. The fluorescence signal responses were measured by F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). Gel Doc XR$^+$ System (Bio-Rad, USA) was adapted to take gel-imaging. DNA micelles were characterized by atomic force microscopy (AFM, Nanoscope V Multi-mode 8, Bruker Company, Santa Barbara, USA).

Preparation of the DNA Micelles and Fluorescence Experiments. The preparation of DNA micelles was according to literatures with some modifications.[1,2] Firstly, sp-H3 was annealed from 95 ℃ to 25 ℃ and kept at 25 ℃ for 10 min to acquire the hairpin structure. Next, the fluorescence dyes perylenes and the amphiphilic sp-H3 were mixed to form the DNA micelles by sonication. Then, the annealed DNA hairpins containing H1, H2, H4 and H5 were introduced to obtain the fluorescence biosensor. After the target miRNA-21 incubated with the prepared biosensor, the fluorescence response was measured by the fluorescence spectrophotometer with the largest excitation wavelength at 440 nm.

Native Polyacrylamide Gel Electrophoresis (PAGE). The practicability of the proposed strategy was approved by PAGE characterization, where the H3 unlabeled with the alkane group (Spacer C12)$_{10}$ was selected to replace the sp-H3 in the PAGE experiment section, because the sp-H3 could assemble into high molecular weight of micelles to influence the judgement of the DNA hybridization process. As shown in Figure S1, lane 1-6 represented miRNA-21, H1, H2, H3, H4 and H5, respectively. Lane

7 was hybrid of H1 and miRNA-21. The brightest band in lane 8 was the H1-H2 hybrid, which proved the CHA reaction between H1 and H2 has been successfully generated by miRNA-21. Compared with lane 8, the band with lower mobility in lane 9 was the H1-H2-H3 hybrid, indicating that the H3 was successfully opened by the released sticky end of H2 of H1-H2 hybrid. The top band in lane 10 was the HCR product of H4 and H5, which was initiated by the sticky end of H3 of H1-H2-H3 hybrid. As a contrast, lane 11 was the mixture of DNA substrates including H1, H2, H3, H4 and H5. In absence of miRNA-21, the CHA and HCR reaction couldn't be generated, resulting in the unobvious product band in the lane 11. The PAGE result demonstrated that the detection system could be triggered by miRNA-21.

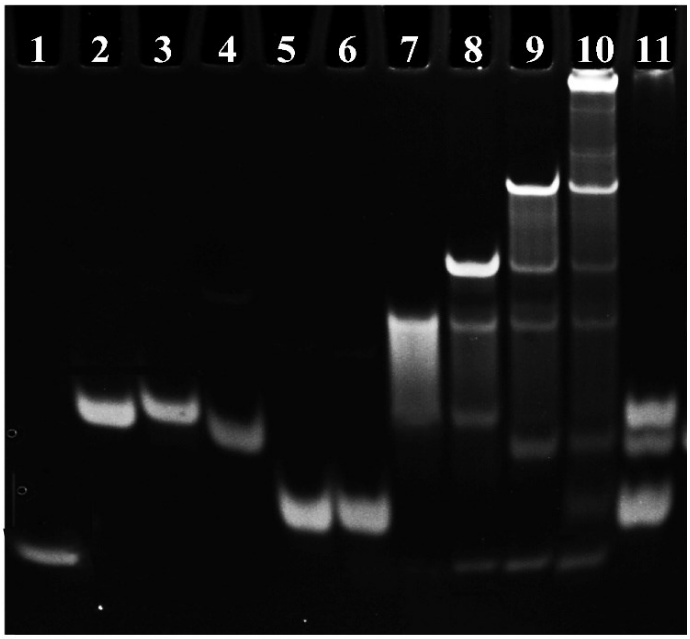

Figure S1. 16% PAGE analysis of the proposed strategy. Lane 1: miRNA-21; lane 2: H1; lane 3: H2; lane 4: H3; lane 5: H4; lane 6: H5; lane 7: hybrid of H1 and miRNA-21; lane 8: hybrid of H1 and H2; lane 9: hybrid of H1, H2 and H3. Lane 10 and lane 11 were the proposed strategy with and without target, respectively.

Optimum of the Biosensor. In order to confirm the optimum conditions for the determination of miRNA-21, the concentration of sp-H3, H4 and H5, and the dosage of perylene were investigated. As shown in Figure S2A, the fluorescence intensity of aqueous continuously increased with the concentration of sp-H3 raising from 0.5 μM to 1.25 μM, while the fluorescence response almost remained unchanged when the concentration of sp-H3 was higher than 1.25 μM, indicating that 1.25 μM of sp-H3 was the optimum concentration for the assembly of DNA micelles. At the same time, the ratio value of total volume (V_{total}) to saturated perylene's volume ($V_{perylene}$) was performed in the range from 75 to 200. According to Figure S2B, when the ratio value of V_{total} to $V_{perylene}$ was decreased from 200 to 100, the fluorescence intensity of aqueous was gradually increased, and then the growth trend was flattened out. Therefore, the V_{total} :$V_{perylene}$ = 100 was the optimal condition for this experiment. Moreover, the concentration of the H4 and H5 was investigated in the presence of 2 nM miRNA-21. As shown in Figure S2C, the fluorescence response of aqueous continuously increased in the range of 0.4 μM to1.0 μM H4 and H5, and trended to level off after 1.0 μM, proving that the optimal concentration of H4 and H5 for this detection system was 1.0 μM.

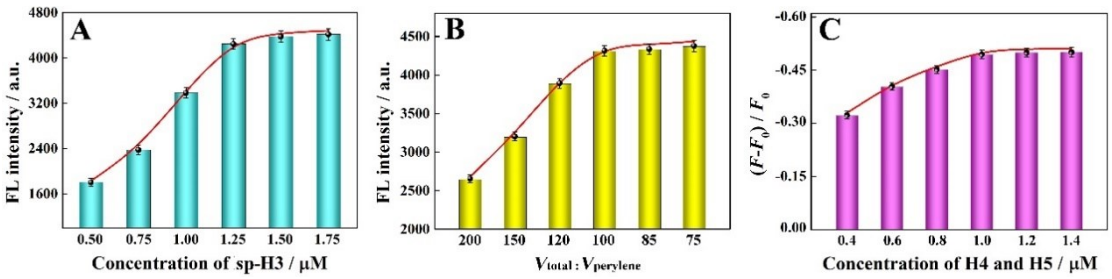

Figure S2. The investigation of the optimum reaction conditions, (A) the concentration of sp-H3, (B) the ratio of V_{total} to $V_{perylene}$ and (C) the concentration of H4 and H5.

Table S2. Comparison of this work with previous literatures for miRNA-21 detection

Target	Detection method	LOD	Linear range	References
miRNA-21	Fluorescence assay	47 pM	0-16 nM	3
miRNA-21	Fluorescence assay	680 pM	2-60 nM	4
miRNA-21	Fluorescence assay	8 pM	20 pM-10 nM	5
miRNA-21	Fluorescence assay	34 pM	0-4 nM	6
miRNA-21	Fluorescence assay	130 pM	0.15-37.5 nM	7
miRNA-21	Electrochemistry	40 pM	0.14 nM-10 nM	8
miRNA-21	Photoacoustic Imaging	11.69 pM	10 pM-100 nM	9
miRNA-21	Mass Spectrometry	41 pM	0-22.5 nM	10
miRNA-21	Fluorescence assay	6.9 pM	20 pM-20 nM	This work

The Stability of DNA Micelles in Human Serum. The human serum is a relatively complicated system that contained different kinds of hormones, enzymes and antibodies, where the nucleases may degrade the DNA nanostructures to influence the detection system.[11-13] In this strategy, the stable DNA micelles structure was attributed to the amphiphilic sp-H3 composed by the hydrophilic DNA head and the hydrophobic alkane tail, which means if the DNA have been degraded, the stability of DNA micelles would be destroyed. As shown in Figure S3, the fluorescence intensity almost keep stable from 4 h to 24 h after ultrasonic mixing, indicating that the DNA micelles would not been degraded by the nucleases within 24 h.

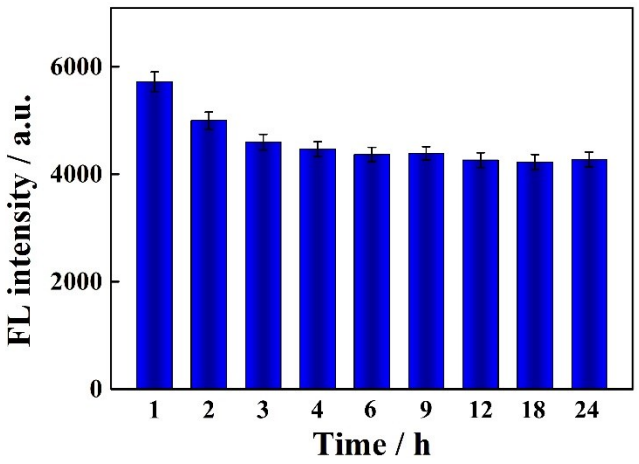

Figure S3. The investigation of the stability of DNA micelles in human serum from 1 h to 24 h (1h, 2h, 3h, 4h, 6h, 9h, 12h, 18h and 24h).

The Detection Specificity of Our Biosensor. The detection specificity is very important in the diagnostic application of such methods. Therefore, we have investigated 100 groups of negative samples to testify the detection specificity of this biosensor, and the fluorescence intensity distribution interval of those samples were shown in Figure S4 with the average value $\bar{x} = \frac{\sum_{i=1}^{100} x_i}{100} =$ 4143, and the Standard Deviation (S_D) $= \sqrt{\frac{(x_i - \bar{x})^2}{n-1}} = 44$. Due to the signal-off detection models of our strategy, we defined indicators of positive judgment as the value of CUT OFF = $\bar{x}$ - 3×S_D=4143-3×44=4011, so samples with fluorescence intensity lower than 4011 were false positive.[14-16] The Figure S4 exhibited that the samples with fluorescence intensity lower than 4011 were distributed in the interval from 3950 to 4050, and the fluorescence intensity were 3970, 3987, 3993, 4024, 4029, 4033, 4037, 4039, respectively, where only 3 group of samples (fluorescence intensity with 3970, 3987 and 3993) were the false positive. Therefore, according to the previous literatures,[17] we have calculated the

samples of false positive (FP) = 3, the samples of true negative (TN) =97, the specificity (Sp) = $\frac{TN}{FP+TN}$ ×100% =97% and the false detection = $\frac{FP}{FP+TN}$ ×100% = 3%. Those results demonstrated our biosensor presented a good detection specificity.

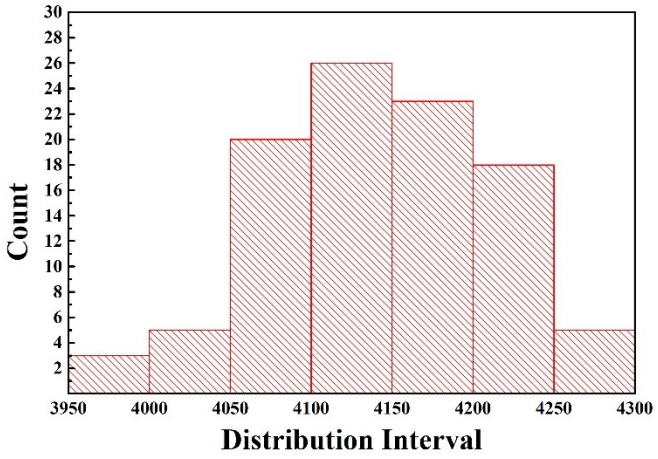

Figure S4. The 100 groups of sample distribution histogram used to investigate the detection specificity of our proposed strategy.

The Detection Performance of Our Biosensor in Serum. We have utilized the serum of healthy human to examine whether those DNA micelles could remain stable to bind with DNA substrates in a relatively complex biological and physiological system. Due to the low expression of miRNA-21 in the serum of healthy human, we have introduced different concentrations of miRNA-21 in the human serum buffer. As depicted in Figure S5, the fluorescence response (F-F_0) / F_0 changed obviously with the miRNA-21 concentrations ranging from 50 pM to 20 nM, which demonstrated the miRNA-21 could generated the CHA reaction between H1 and H2 to output a lot of H1-H2 hybrids to hybridize with DNA micelles and induced the rapid disassembly of DNA micelles with the assistance of H4 and H5 in human serum. Those results indicated the DNA diblock copolymer micelles could remain stable to bind with the reaction substrates in

the human serum buffer.

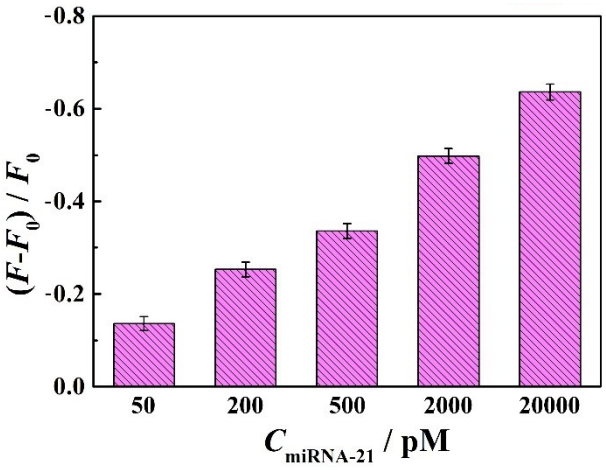

Figure S5. The fluorescence response of our biosensor in human serum with different concentration of miRNA-21 (50 pM, 200 pM, 500 pM, 2 nM, 20 nM).

References

(1) X. L. Li, H. Li, G. Q. Liu, Z. W. Deng, S. L. Wu, P. H. Li, Z. S. Xu, H. B. Xu and P. K. Chu, *Biomaterials*, 2012, **33**, 3013-3024.

(2) D. Bousmail, P. Chidchob and H. F. Sleiman, *J. Am. Chem. Soc.*, 2018, **140**, 9518-9530.

(3) S. J. Zhen, X. Xiao, C. H. Li and C. Z. Huang, *Anal. Chem.*, 2017, **89**, 8766-8771.

(4) J. T. Yi, T. T. Chen, J. Huo and X. Chu, *Anal. Chem.*, 2017, **89**, 12351-12359.

(5) C. P. Liang, P. Q. Ma, H. Liu, X. G. Guo, B. C. Yin and B. C. Ye, *Angew. Chem. Int. Ed.*, 2017, **56**, 9077-9081.

(6) H. M. Fang, N. L. Xie, M. Ou, J. Huang, W. S. Li, Q. Wang, J. B. Liu, X. H. Yang and K. M. Wang, *Anal. Chem.*, 2018, **90**, 7164-7170.

(7) J. Su, F. B. Wu, H. P. Xia, Y. F. Wu and S. Q. Liu, *Chem. Sci.*, 2020, **11**, 80-86.

(8) S. Campuzano, R. M. Torrente-Rodriguez, E. Lopez-Hernandez, F. Conzuelo, R.

Granados, J. M. Sanchez-Puelles and J. M. Pingarron, *Angew. Chem. Int. Ed.*, 2014, **53**, 6168-6171.

(9) K. Zhang, X. D. Meng, Z. Yang, Y. Cao, Y. R. Cheng, D. D. Wang, H. T. Lu, Z. J. Shi, H. F. Dong and X. J. Zhang, *Adv. Mater.*, 2019, **31**, 1807888-1807894.

(10) C. X. Shi, S. X. Li, Z. P. Chen, Q. Liu and R. Q. Yu, *Anal. Chem.*, 2019, **91**, 2120-2127.

(11) S. Matysik and G. Liebisch, *J. Chromatogr. A*, 2017, **1526**, 112-118.

(12) M. Harel, A. Aharoni, L. Gaidukov, B. Brumshtein, O. Khersonsky, R. Meged, H. Dvir, R. B G Ravelli, A. McCarthy, L. Toker, I. Silman, J. L Sussman and D. S Tawfik, *Nat. Struct. Mol. Biol.*, 2004, **11**, 412-419.

(13) Y. C. Wang, Z. G. Ju, B. R. Cao, X. Gao, Y. Zhu, P. H. Qiu, H. Xu, P. T. Pan, H. Z, Bao, L. Wang and C. B. Mao, *ACS Nano*, 2015, **9**, 4475-4483.

(14) N. Tofta, E. Jørgensenb and S. Højsgaard, *Prev. Vet. Med.*, 2005, **68**, 19-33.

(15) A. Rutjes, J. B. Reitsma, A. Coomarasamy, K. S. Khan and P. Bossuyt, *Health Technol. Asses.*, 2007, **11**(50).

(16) S. Ryan, A. J. Kell, H. V. Faassen, L. L. Tay,| B. Simard, R. MacKenzie, M. Gilbert and J. Tanha, *Bioconjugate Chem.*, 2009, **20**, 1966-1974.

(17) N. L. Sim, P. Kumar, J. Hu, S. Henikoff, G. Schneider and P. C. Ng, *Nucleic Acids Res.*, 2012, **40**, 452-457.