Supplementary Information

Heteromultivalent scaffolds fabricated by biomimetic co-assembly of DNA-RNA building blocks for the multi-analysis of miRNAs

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Table S1. Nucleic acids used in this study.

Name	Sequences (from 5' to 3')
miR-342-3p (miR-1)	UCUCACACAGAAAUCGCACCCGU
miR-18b-5p (miR-2)	UAAGGUGCAUCUAGUGCAGUUAG
miR-30e-5p (miR-3)	UGUAAACAUCCUUGACUGGAAG
miR-143-3p (miR-4)	UGAGAUGAAGCACUGUAGCUC
Branch Linker-1	TTCTGTGTGAGAAGATGCACCTTAACGGGTGCGAT
Branch Linker-2	AGATGCACCTTATTCTGTGTGAGACTAACTGCACT
TAMRA modified Branch Linker-1	TTCTGTGTGAGAAGATGCACCTTAACGGGTGCGAT-TAMRA
FAM modified Branch Linker-2	AGATGCACCTTATTCTGTGTGAGACTAACTGCACT-FAM
M1[a]	UCUCACACAAAAAUCGCACCCGU
M2	UCUCACACAAGAAUCGCACCCGU
M3	UCUCACACAAGGAUCGCACCCGU

[a]The mutant sequence of miR-342-3p, 1-3 represent the number of the mutant nucleotides. The mutant region is marked in red.

Optimization of the experimental condition.

Since the FRET signal can be calculated by the ratio of the fluorescence intensity of the FRET donor and acceptor, the concentration ratio between the FRET donor, BL-1 and FRET acceptor BL-2 may have a significant influence on the FRET efficiency. Therefore, the ratio of BL-1/BL-2 have been optimized. As shown in Figure S1A, the F_A/F_D value increases with increasing ratio and reaches a plateau when the ratio approaches 2. Therefore, this ratio was selected as the optimized ratio for the next experiments. Then, the time for the hybridization of the dendrimer was optimized. The result (Figure S1B) shows a fast increase in the F_A/F_D value in the first hour and a slight change thereafter, which means that 1 hour of hybridization is enough for the effective analysis of the targets. Therefore, in the subsequent experiment, the hybridization time was set as 1 hour.

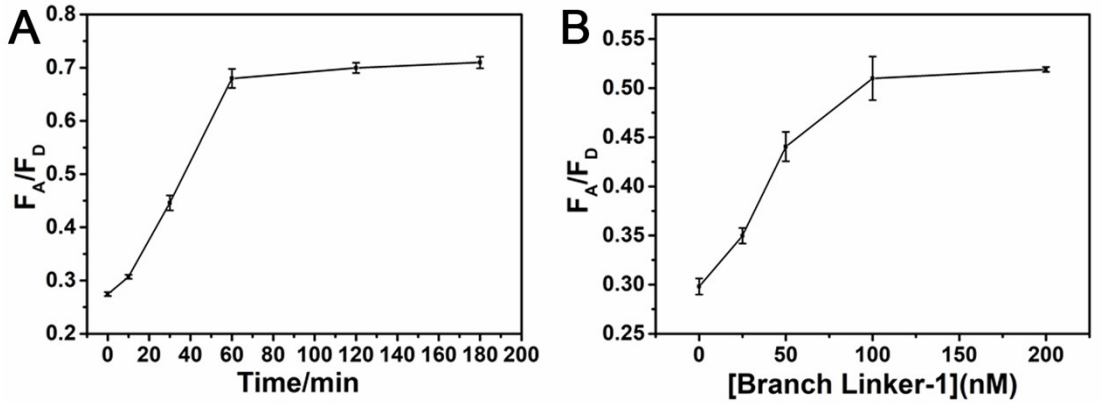

Fig. S1 (A) Optimization of the hybridization time of the miRNAs and the Branch Linkers. The concentrations of miRNAs and Branch Linkers are 100 nM and 50 nM respectively. (B) Optimization of BL-1/BL-2 ratio. In all of the experiments, the concentrations of miRNAs are 75 nM. The concentration of BL-2 is 50 nM.

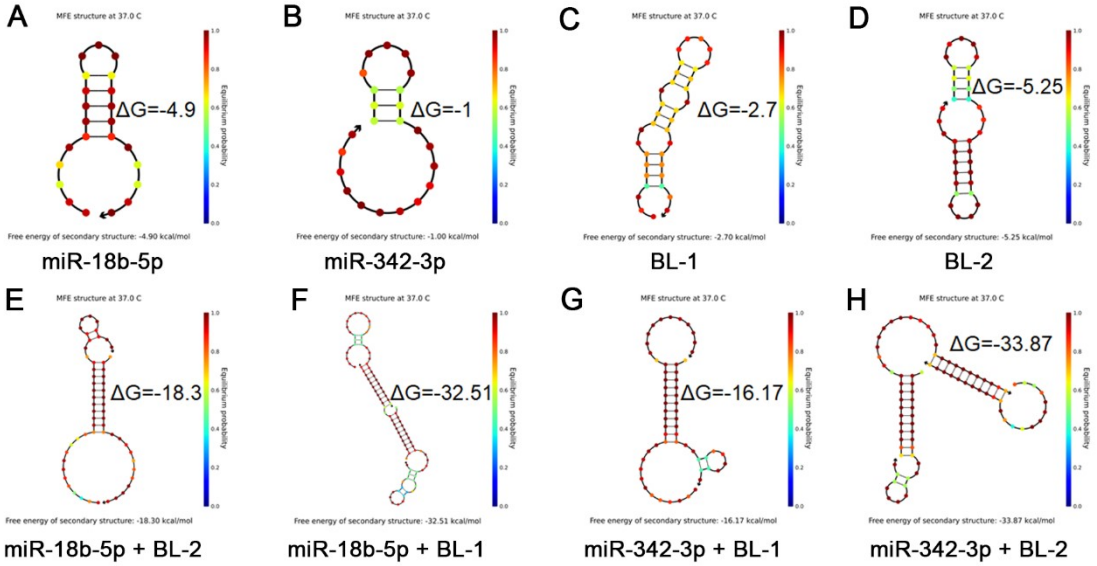

Fig. S2 Predicted secondary structure of miR-18b-5p (A), miR-342-3p (B), BL-1 (C), BL-2 (D), hybridization product of miR-18b-5p and BL-2 (E), miR-18b-5p and BL-1 (F), miR-342-3p and BL-1 (G), miR-342-3p and BL-2 (H).

Table S2. The recovery tests of the proposed method.

Sample	Kind of sample	Dose of spiked miRNA (nM)	Found (nM)	Recovery (%)	CV (%, n=3)
1	10% serum	0.1	0.098	98%	0.34%
2	10% serum	1	1.103	110.3%	1.1%
3	10% serum	10	8.945	89.45%	6.9%
4	aCSF	0.1	0.091	91%	0.25%
5	aCSF	1	0.852	85.2%	0.16%
6	aCSF	10	10.123	101.23	0.57%