Supporting Information

Metastable Dumbbell Probe-Based Hybridization Chain Reaction for Sensitive and AccurateImaging of Intracellular-Specific MicroRNAs *In Situ* in Living Cells

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Content

S1. Reagents and Materials

Table S1 Sequences of the Used Oligos

Description	Name	Sequences (5'-3')
	miR-27a	UUCACAGUGGCUAAGUUCCGC
	miR-27b	UUCACAGUGGCUAAGUUCUGC
	Block Probe	GCGGAACTTAGCCACTGTGAA
Probes for anti-digestibility tests	BHQ1 labeled LP	TTCACAGTGGCTAAGTTCCGC(**BHQ1**)
	FAM labeled LP	(**FAM**)GCGGAACTTAGCCACTGTGAA
	HP	(**FAM**)CGCGCGGAACTTAGCCACTGTGAACGCGCG(**BHQ1**)
	M_1DP1 precursor	TCGGCCACACACCCGCGGAAC(**BHQ1**)TTAGCCACTGTGAAAATGGCTAAGT(**FAM**)
Probes for M_xDPHCR	DP1 precursor	TCCGCCACACACCCGCGGAACTTAGCCACTGTGAAAATGGCTAAGT
	DP2 precursor	AAGTGTGTGGCGGAACTTAGCCAAATTCACAGTGGCTAAGTTCCGC
	M_1DP1 precursor	TCGGCCACACACCCGCGGAACTTAGCCACTGTGAAAATGGCTAAGT
	M_1DP2 precursor	AAGTGTGTGGCGGAACTTAGCCAAATTCACAGTGGCTAAGTTCCGC
	M_2DP1 precursor	TCGGCCACACACCCGCGGAACTTAGCCACTGTGAAAATGCCTAAGT
	M_2DP2 precursor	AAGTGTGTGGCCGAACTTAGGCAAATTCACAGTGGCTAAGTTCCGC
Probes for dynamic tests	HP1	TCAACATCAGTCTGAT(**BHQ1**)AAGCTACACACACTAGCTTATCAGACTG(**FAM**)
	HP2	TAGCTTATCAGACTGATGTTGACAGTCTGATAAGCTAGTGTGTG
	DP1 precursor	TCCGCCACACACCCGCGGAAC(**BHQ1**)TTAGCCACTGTGAAAATGGCTAAGT(**FAM**)
	DP2 precursor	AAGTGTGTGGCGGAACTTAGCCAAATTCACAGTGGCTAAGTTCCGC
	M_1DP1 precursor	TCGGCCACACACCCGCGGAAC(**BHQ1**)TTAGCCACTGTGAAAATGGCTAAGT(**FAM**)
	M_1DP2 precursor	AAGTGTGTGGCGGAACTTAGCCAAATTCACAGTGGCTAAGTTCCGC
Probes of M_1DPHCR for FRET imaging	M_1DP1 precursor	TCGGCCACACACCCGCGGAACTTAGCCACTGTGAAAATGGCT(**Cy3**)AAGT
	M_1DP2 precursor	AAGTGTGTGGCCGAACTT(**Cy5**)AGCCAAATTCACAGTGGCTAAGTTCCGC
Probes of HPHCR for FRET imaging	HP1	TCAACATCAGTCTGATAAGCT(**Cy3**)ACACACACTAGCTTATCAGACTG
	HP2	TAGCTTATCAGACTGATGTTGACAGTCTGATAAGCTAGTGTGTG(**Cy5**)

S2 Calculation of Standard Free Energy for M_xDPHCR

S2.1 Activation of M_xDPHCRby Target MiRNA

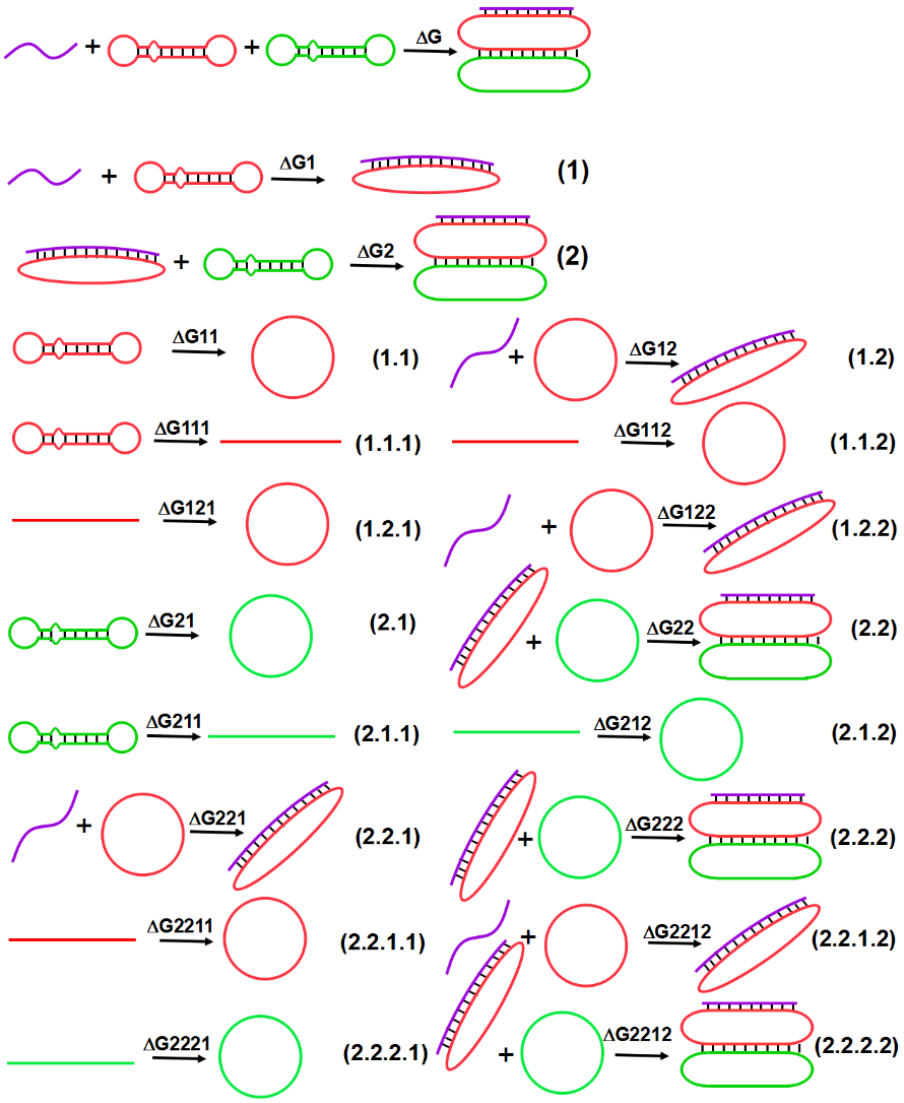

Figure S1. The activation process consists of two reactions: (1) hybridization of target miRNA with the M_xDP1, and (2) hybridization of Reaction product (1) with the M_xDP2. The two reactions were further decomposed into four separate reactions of (1.1.1), (1.1.2), (1.2.1), (1.2.2) (2.1.1), (2.1.2), (2.2.1.1), (2.2.1.2), (2.2.2.1) and (2.2.2.2). The standard free energy (ΔG) of reaction was calculated on the ViennaRNA website under conditions as follows: temperature: 37 °C, salt concentration: 10 mM Na^+, 5 mM Mg^{2+}, M_xDP1: 200 nM, M_xDP2: 200 nM, miRNA: 1 nM. The "dangles" parameter was set to "on both sides of a helix in any case". The bending energies of M_1DPs were omitted as single stranded DNA, because it is very flexible and the ratio of duplex length of miRNA-M_xDP1 is less than 40% of the full length.

Table S2 ΔG of HCR on M_xDPs

template	G/KJ mol^{-1}										
	G_{111}	G_{112}	G_{121}	G_{122}	G_{211}	G_{212}	G_{2211}	G_{2212}	G_{2221}	G_{2222}	ΔG
DP	23	6.12	6.12	–39.73	23.7	6.12	6.12	–39.73	6.12	–2.08	–4.24
M_1DP	16.6	6.12	6.12	–39.73	15.5	6.12	6.12	–39.73	6.12	–2.08	–18.84
M_2DP	10.2	6.12	6.12	–39.73	9.31	6.12	6.12	–39.73	6.12	–2.08	–37.56

S2.2 Self-Assembly between M_xDPs

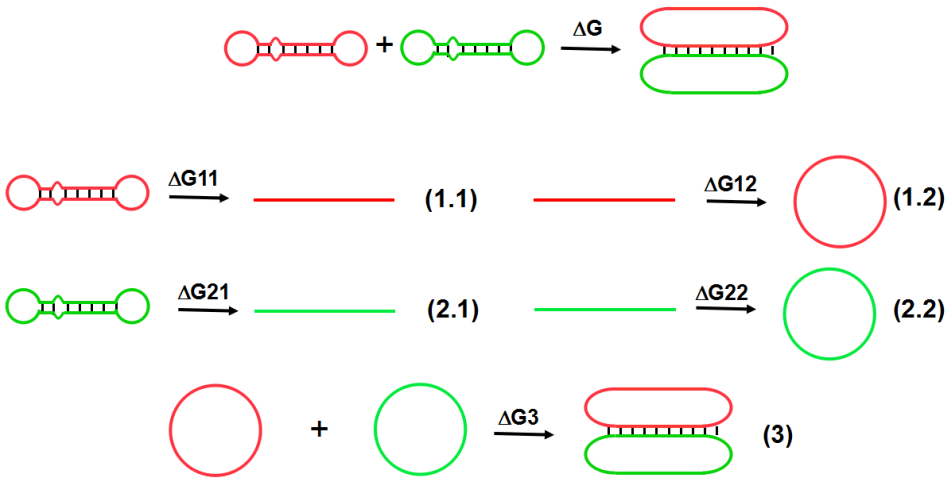

Figure S2. The reactions were decomposed into five separate reactions of (1.1), (1.2), (2.1), (2.2) and (3). The ΔG of reaction was calculated on the ViennaRNA website under conditions as follows: temperature: 37 °C, salt concentration: 10 mM Na^+, 5 mM Mg^{2+}, M_1DP1: 200 nM, M_1DP2: 200 nM. The "dangles" parameter was set to "on both sides of a helix in any case".

Table S3 ΔG of Self-Assembly between M_xDPs

Template	G/KJ mol^{-1}					
	G_{11}	G_{12}	G_{21}	G_{22}	G_3	ΔG
DP1/DP2	23	6.12	23.7	6.12	–39.73	19.2
M_1DP1/M_1DP2	16.6	6.12	15.5	6.12	–39.73	4.61
M_2DP1/M_2DP2	10.2	6.12	9.31	6.12	–39.73	–7.91

S3. Characterization of M_1DP1

Native 15% polyacrylamide gel electrophoresis was performed on the prepared gel in TBE at 200 V for 30 min. The non-ligated, non-digested, and well prepared M_1DP1 in a 15 μL volume containing 2 μL of 6×gel loading buffer were used for gel electrophoresis, respectively. After electrophoresis, the gel was stained with SYBR Gold and visualized via DigiGenius gel system (Syngene, UK). As shown in Figure S3, the M_1DP1 precursor showed one bright band (lane 1). After the ligation of the M_1DP1 precursor by T4 DNA ligase, the resultant mixture showed two bands (lane 2). The mixture was further digested by Exonuclease I and Exonuclease III, and the resultant product showed only a band (lane 3), suggesting that pure sealed M_1DP1 was obtained.

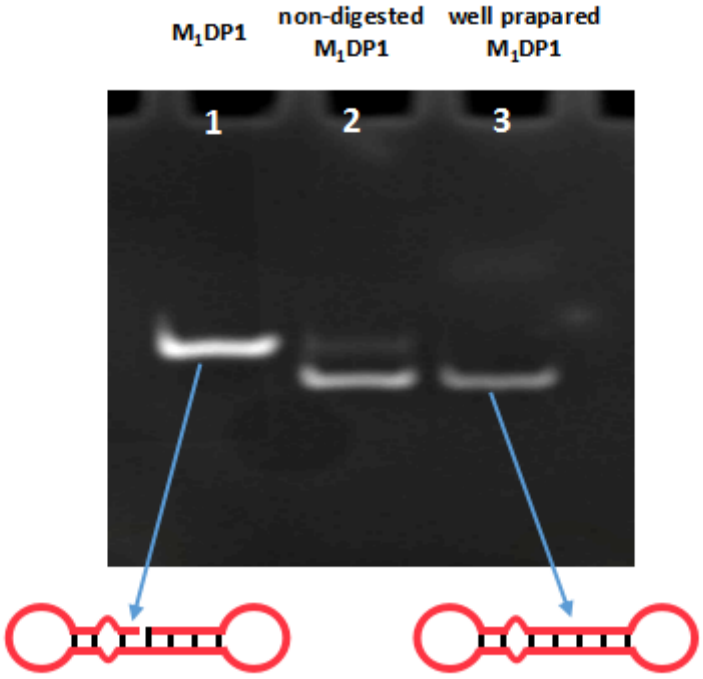

Figure S3. Native polyacrylamide gel electrophoresis of non-ligated M_1DP1 (M_1DP1 precursor, lane 1), non-digested M_1DP1 (M_1DP1 precursor after ligation by T4 DNA ligase, lane 2), and well prepared M_1DP1 (the ligated M_1DP1 after digestion by Exonuclease I and Exonuclease III, lane 3).

S4. Metastability Property of M_xDPs in HCR

Native 15% polyacrylamide gel electrophoreses were performed on the prepared gel in 0.5×TBE buffer at 150 V for 45 min. M_xDPs individually or in the absence or presence of target miR-27a were used for gel electrophoreses.After electrophoresis, the gel was stained with SYBR Gold and visualized via DigiGenius gel system (Syngene, UK).

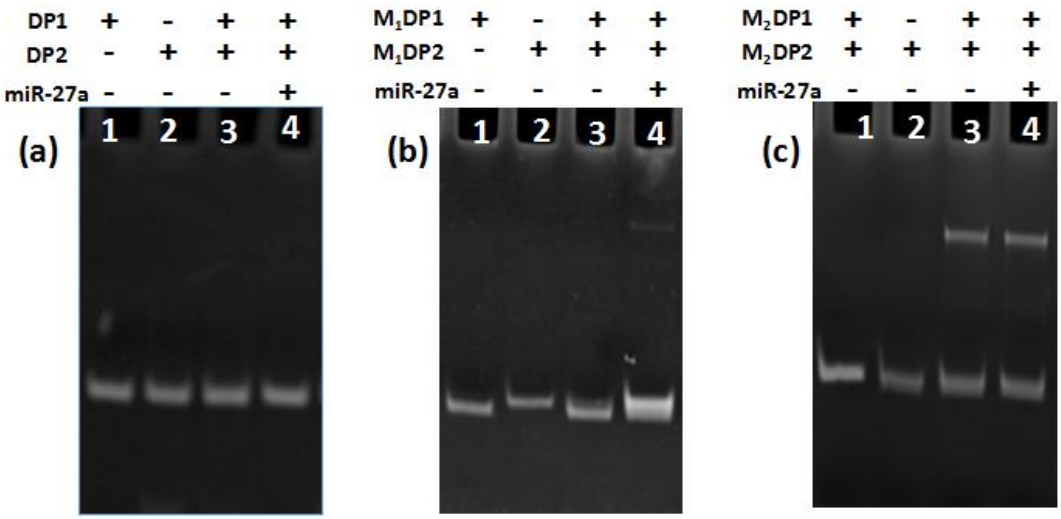

Figure S4. Agarose gel-electrophoresis results demonstrate the effect of the stem region on the metastability properties of the M_xDPs. The M_xDPHCR was based on (a) DP1 and DP2, (b) M_1DP1 and M_1DP2, (c) M_2DP1 and M_2DP2, respectively.

S5. Anti-Digestibility of M_1DP1 in Cell Lysate

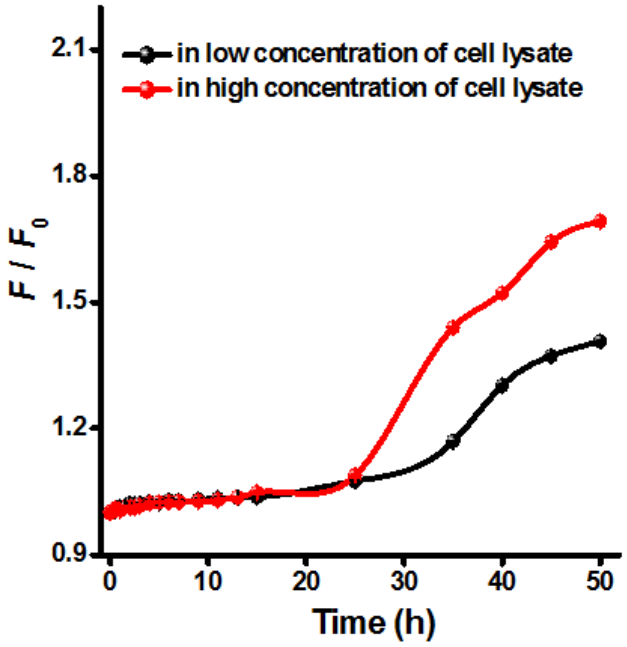

Figure S5. Fluorescence intensity of FAM/BHQ1 labeled M_1DP1 in 50 μL of 20 mM Tris-HCl buffer solutions (pH 7.4, 100 mM NaCl, 5 mM KCl, 4.5 mM $MgCl_2$) containing cell lysate from ~1×10^4 or ~2×10^4MCF-10A cells at different times.

S6. Analysis of MiR-27a in Cell Lysate by HPHCR

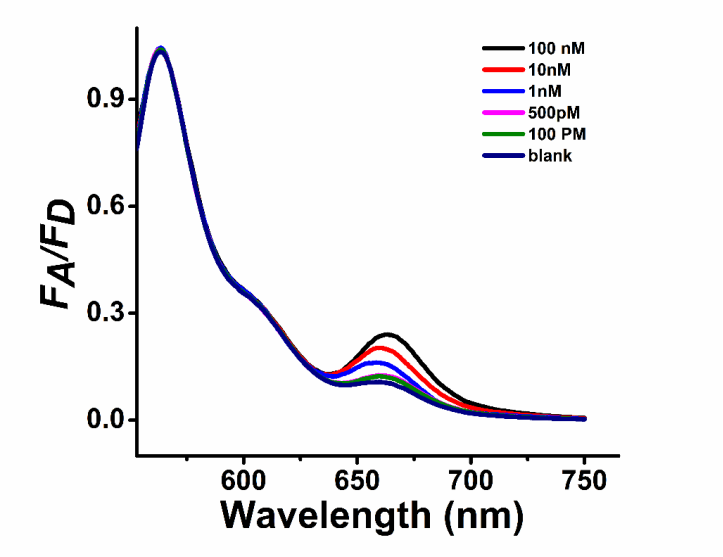

Figure S6. Fluorescence emission spectra of HPHCR in 50 μL solutions containing ~2×10^4 MCF-10A cellslysate in response to different miR-27a concentrations of 0, 0.1, 0.5, 1, 10, and 100 nM.

S7. Analysis of MiR-27a in Buffer by HPHCR

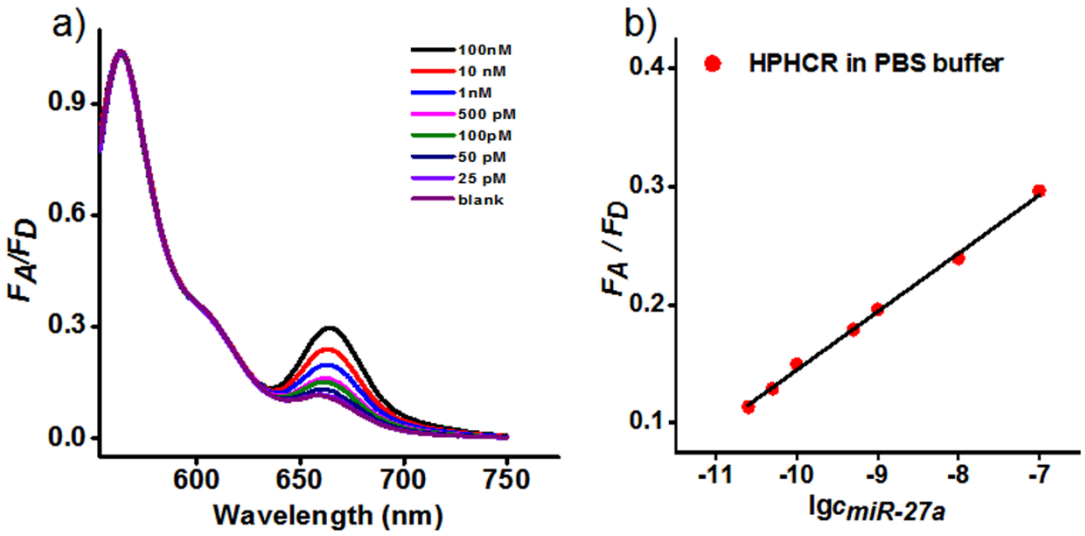

Figure S7. a) Fluorescence emission spectra of HPHCR in 1× PBS buffer in response to different miR-27a concentrations of 0, 0.025, 0.05, 0.1, 0.5, 1, 10, and 100 nM. b) Plot between FRET efficiency of HPHCR versus logarithmic miR-27a concentration.

S8. *In Situ* Imaging of A549 Cells by M_1DPHCR and HPHCR

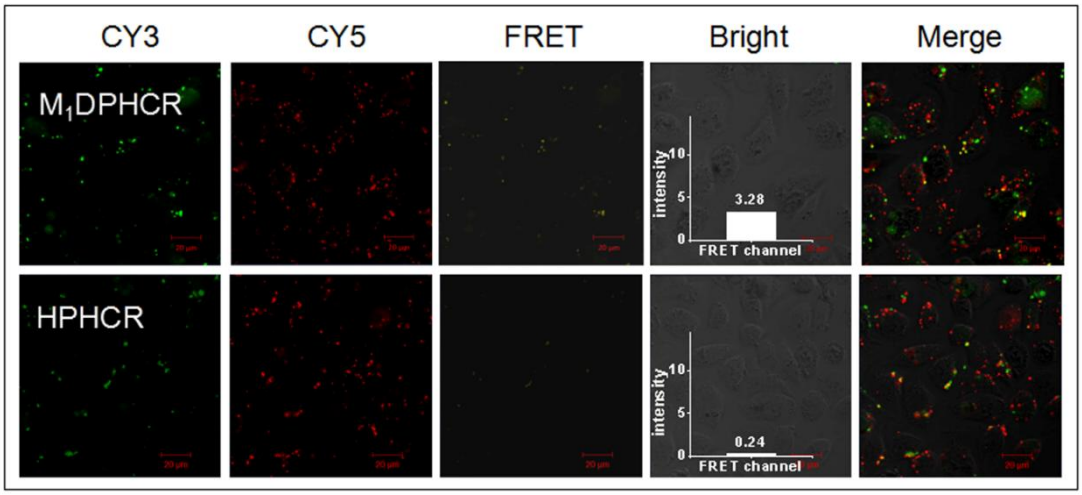

Figure S8. Confocal microscopy images of A549 cells based on M_1DPHCR and HPHCR. The cells were transfected with the probes by Lipofectamine 3000 and were incubated at 37 °C for 2 h. Insets: the arithmetic mean intensity of FRET channel.