Electronic Supplementary Information

Triggered and catalytic self-assembly of hyperbranched DNA structures for logic operations and homogeneous CRET biosensing of microRNA

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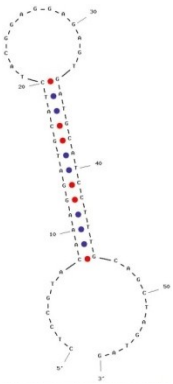
Experimental section

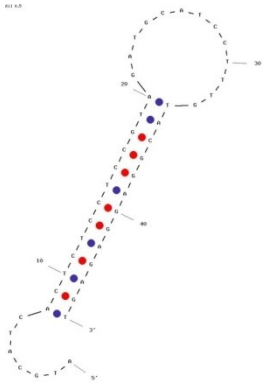
Materials and apparatus. All oligonucleotides used in this assay were synthesized and HPLC-purified by Sangon Biotech Co., Ltd. (Shanghai, China) (the sequences are listed in Table S1, and the secondary structures are predicted and specified in Fig. S1 in Supporting Information). Hemin and luminol were purchased from Aladdin (Shanghai, China). All the reagents were of analytical grade and used as received without further purification. Double-distilled deionized ultrapure water was used throughout the experiments.

Table S1. Sequences of oligonucleotides used in this study

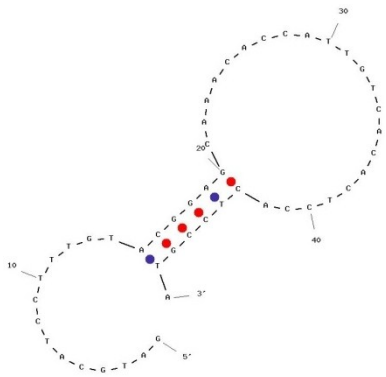
name	Sequences (from 5′ to 3′)
miR-122	UGGAGUGUGACAAUGGUGUUUG
miR-let-7a	UGAGGUAGUAGGUUGUAUAGUU
miR-let-7b	UGAGGUAGUAGGUUGUGUGGUU
miR-let-7c	UGAGGUAGUAGGUUGUAUGGUU
C	GATGCAT-CCTTTG-TACGGAG
HP1	CTCCGTA-CAAAGG-ATGCATC-TACGGAG-GAGAGT-GATGCAT-CCTTTG-CAGCTAGTAG
HP2	ATGCATC-ACTCTC-CTCCGTA-GATGCAT-CCTTTG-TACGGAG-GAGAGT
HP3	CAGCTAGTAG-TACCGT-CTACTAGCTG-CAAAGG
HP4	ACGGTA-CTACTAGCTG-CCTTTG-CAGCTAGTAG
HP3′	FAM-CAGCTAGTAG-TGG-TACCGT-CTACTAGCTG-CAAAGG-FAM
HP4′	TGGT-ACGGTA-CCA-CTACTAGCTG-CCTTTG-CAGCTAGTAG-TGG-GTGGGCGGGA
HP5	GATGCAT-CCTTTG-TACGGAG-CAAACACCATTGTCACACTCCA-CTCCGTA



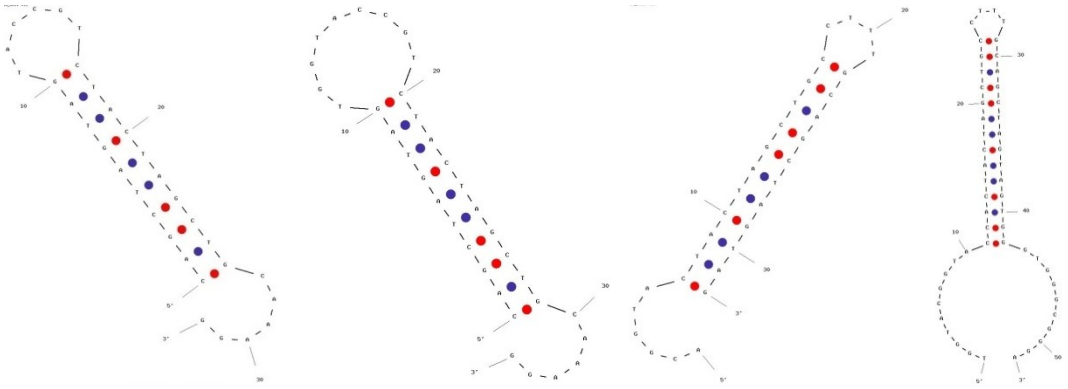
HP1



HP2



HP5



HP3 **HP3'** **HP4** **HP4'**

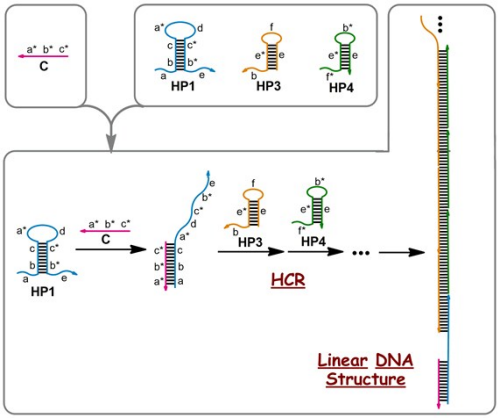
Fig. S1. Secondary structures of DNA hairpins used in the study, which are predicted using the IDT DNA software (www.idtdna.com).

Treatment of DNA hairpins. All oligonucleotides were prepared using TE buffer (10 mM Tris-HCl, 1mM EDTA-2Na) supplemented with 12.5 mM $MgCl_2$ (pH 8.0). To ensure the hairpin probes form desirable secondary structure as shown in Figure S1, all the hairpin strands were separately heated at 95 ^oC for 5 min, slowly cooled to 25 ^oC at rate of 0.1 $^oC/s$, and stood at 25 ^oC for 2 h at least before use.

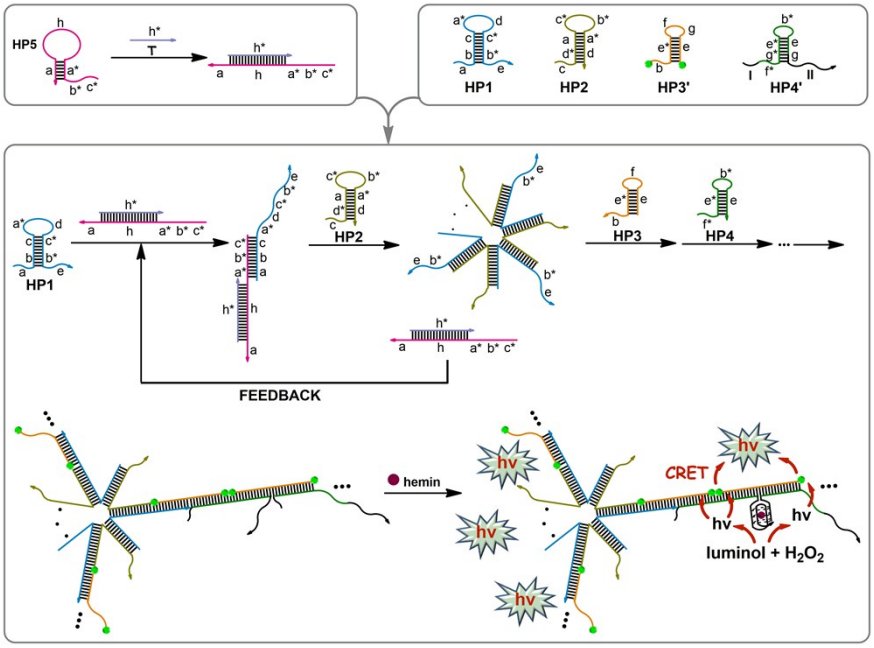
Atomic force microscopy (AFM) imaging. The mixture of (HP1 + HP2) (1 μM each) and (HP3 + HP4) (10 μM each) was reacted with miR-122 (0.1 μM) (the volume of each species is 2 μL). After incubation at 37 ^oC for 30 min, the resulting products were washed by centrifugation with double-distilled water. The AFM imaging was taken on a Being Nano-Instruments CSPM-4000 (Benyuan, China) in air under tapping mode.

CRET detection of microRNA by the proposed DNA circuitry. The mixtures of (HP1 + HP2) (10^{-7} M each), (HP3′ + HP4′) (10^{-6} M each) and HP5 (10^{-8} M) were reacted with miR-122 with various concentrations, respectively (the volume of each species is 2 μL). After incubation at 37 ^oC for 2 h, the resulting products were incubated with 100 μL of hemin (0.1 μM) at 25 ^oC for 30

min. And then, 50 μL of luminol (0.5 mM) was added into each sample. The CRET measurements were recorded on a Centro LB940 luminometer (Berthold, Germany) upon introduction of 50 μL of H_2O_2 (0.5 mM) through an automatic injector.



Scheme S1. Graphical representation of HCR. The system consists of HP1, HP3 and HP4, which is triggered by C.



Scheme S2. Schematic illustration of the reaction pathways of Fig. 3 for CRET biosensing of target microRNA (T).

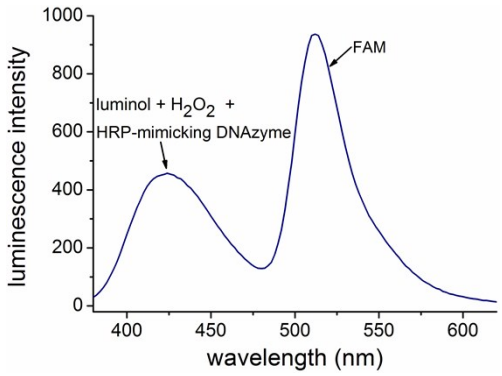
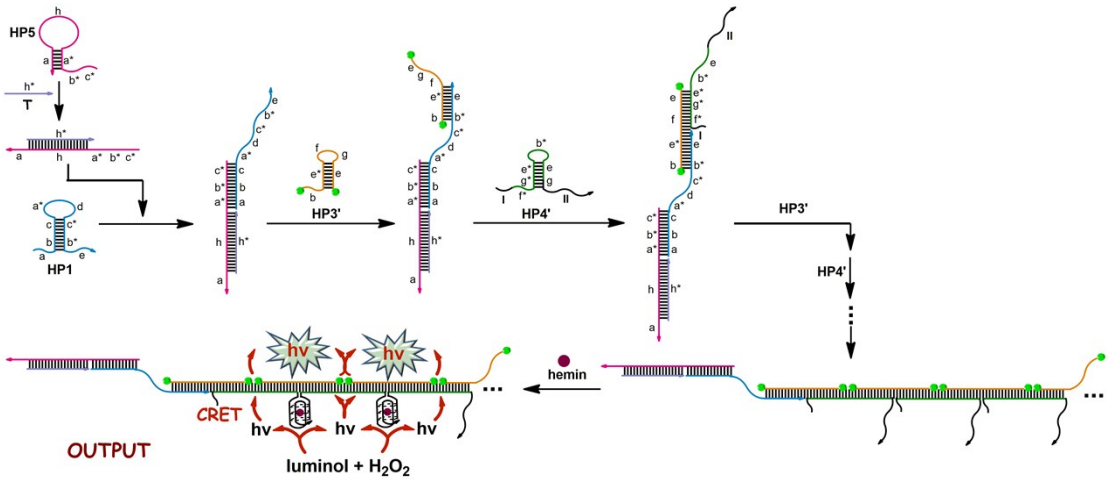


Fig. S2 Luminescence spectrum of the CRET system.

From Fig. S2, for the CRET system, there are two maxima of emission: one is around 425 nm that is corresponding to HRP-mimicking DNAzyme-catalyzed luminol-H_2O_2 CL system and the other is around 510 nm that is corresponding to FAM, which thus verifies the occurrence of CRET.



Scheme S3. Schematic illustration of HCR amplification for CRET detection of target (T) using one hairpin probe (HP1), two functional hairpin structures (HP3' and HP4') and one helper hairpin (HP5).

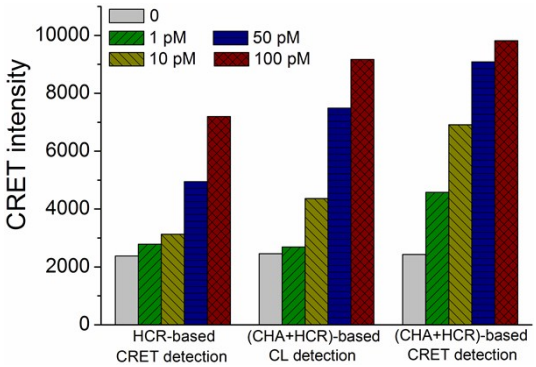


Fig. S3 Comparison of HCR-based CRET detection and the proposed logic circuitry (CHA+HCR)-based CL and CRET detection of miR-122 at different concentrations.