Bioorthogonal Regulation of DNA Circuit for Smart Intracellular MicroRNA Imaging

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Experiment section

**Reagents and materials.** 4-(2-Hydroxyethyl)piperazine-1 ethanesulfonic acid sodium salt (HEPES), sodium chloride and magnesium chloride were of analytical grade and all were bought from Sigma-Aldrich (MO, USA). The GelRed, lipofectamine 3000 transfection reagent and fetal bovine serum (FBS) were obtained from Invitrogen (Carlsbad, CA). All DNA oligonucleotides as shown in Tables S1 and S2 were synthesized and HPLC-purified by Sangon Biotech. Co., Ltd. (Shanghai, China). All solutions used in the study were prepared using ultrapure water with the help of Millipore Milli-Q water purification system. Human apurinic/apyrimidinic endonuclease 1 (APE1) (5 U/mL), homing endonuclease I-SceI (I-SceI) (5 U/mL), lambda exonuclease (λ Exo) (20 U/mL), Nb.BbvCI nicking endonuclease (Nb.BbvCI) (20 U/mL), HpaII restriction endonuclease (HpaII) (20 U/mL), and T4 polynucleotide kinase (T4 PNK) (20 U/mL) were purchased from New England BioLabs (Ipswich, MA).

**DNA pre-probe activation and fluorescence assay.** The DNA hairpin (4 μM) used in this work was heated at 95 °C for 5 min, and then slowly cooled down to 25 °C within 130 min in reaction buffer (10 mM HEPES, 100 mM NaCl, 10 mM MgCl2, and pH 7.2). APE1 cleavage assays were conducted in a batch of 50 μL 1 × NEBuffer 4 buffer (20 mM Tris-acetate, pH 7.9, 10 mM magnesium acetate, 50 mM potassium acetate, 1 mM DTT) containing 400 nM H2-II-P with the addition of 5 U/mL APE1, and then heated to 37 °C for 5 h. I-SceI cleavage assays were performed in 50 μL 1× Thermo Scientific Tango Buffer (33 mM Tris-acetate, pH 7.9, 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA) containing 400 nM H2-III-P by adding 5 U/mL I-SceI, and then heated to 37 °C for 6 h. For the amplified detection of miRNA by using the HCR-amplifier, various concentrations of target miR-21 were added into the samples of H1 (300 nM) and H2-II-P (100 nM), or H1 (300 nM) and H2-III-P (100 nM) with or without the presence of enzyme activation to initiate self-assembly process at 25 °C for 5 h. The control experiments were also proceeded without changing the concentration of DNA hairpin. All in vitro fluorescence experiments were carried out using a Cary Eclipse spectrometer (Varian Inc) at 25 °C. The fluorescence spectra were measured from 550 to 700 nm with excitation at 532 nm. Fluorescence emission ratio of acceptor to donor (FA/FD) was recorded for further data analysis.

**Cell culture and transfection for confocal microscopy imaging.** Human breast cancer cells (MCF-7) and cervical cancer cells (HeLa) were cultured in Dulbecco's Modified Eagle Medium.
(DMEM) containing 10% FBS and 1% penicillin/streptomycin at 37 °C in 5% CO2 atmosphere. Human breast normal cell line MCF-10A was cultivated under the same condition except that DMEM was replaced with mammary epithelial basal medium (MEBM) supplemented with the MEGM SingleQuots Supplements. These cells were seeded onto a 35 mm laser confocal culture dish at a density of 2×10^5 per well, and cultured overnight for the following transfection. In brief, Cy3- or Cy5-labeled DNA hairpins (0.1 nmol each, 200 μL) in fresh Opti-MEM were mixed with lipofectamine 3000 (5 μL) in Opti-MEM (200 μL) for 5 min, and then transferred into the plated cells. After incubation for 4 h, all treated cells were washed with PBS to remove the noninternalized probes, followed by confocal microscopy imaging. The anti-miR-21 antisense inhibitor experiment was implemented by transfecting MCF-7 cells with inhibitor oligonucleotide (0.1 nmol) using lipofectamine 3000 (300 μL) for 2 h firstly, followed by the DNA hairpins transfection as above.[1-3]

**In vivo imaging.** The 6-8-week old female BABL/c nude mice were used for *in vivo* imaging experiment. The animal experiments were approved by the Animal Care and Use Committee of Wuhan University and complied with all relevant ethical regulations. All animals were housed with a 12 h light/dark cycle at 22 °C, 40% relative humidity and food and water ad libitum. The MCF-7 tumor-bearing BALB/c nude mice were randomly divided into four groups, and injected with lipofectamine 3000-loaded oligonucleotide (APE1-regulated HCR system, \(H_1^* + H_{2-II-P^*}\); APE1-blunt HCR system, \(H_1^* + H_{2-III-P^*}\); the APE1-regulated HCR system after miR-21 inhibitor-pretreatment, miR-21 inhibitor + \(H_1^* + H_{2-II-P^*}\); the APE1-blunt HCR system after miR-21 inhibitor-pretreatment (5 nmol/kg), miR-21 inhibitor + \(H_1^* + H_{2-III-P^*}\), 5 nmol/kg each), through intratumor or tail intravenous injection. Then the in vivo imaging was performed by IVIS Spectrum (Ex, 640 nm; Em, 680 nm) at 0, 3, 8, 12 h after intratumor injection and intravenous injection respectively. The ex vivo imaging of tumors and main organs (heart, liver, spleen, lung, and kidney) were immediately carried out after mice were euthanized. [4]
| No.       | Sequence (5’→3’)* |
|-----------|------------------|
| H1        | (Cy5) ACT GAT GTT GAA CAG CTC AAC TAT CAA CAT CAG TCT GAT AAG CTA |
| H2-I-P    | GCG ATT ACC CTG TTA T dSpacer CCC TAT AGT TGA GCT GTT CAA CAT CAG TTA GCT TAT CAG ACT GAT GTT GAA CTA TTA TTT CTT AGG GAT AA dSpacer CAG GGT AAT CGC |
| H2-II-P   | GCG ATT ACC CTG TTA T dSpacer CCC TAT AGT TGA GCT GTT CAA CAT CAG (Cy3) TTA GCT TAT CAG ACT GAT GTT GAA CTA GGG ATA A dSpacer CAG GGT AAT CGC |
| H2-I      | CCC TAT AGT TGA GCT GTT CAA CAT CAG TTA GCT TAT CAG ACT GAT GTT GAA CTA TTA TTT CTT AGG GAT AA |
| H2-II     | CCC TAT AGT TGA GCT GTT CAA CAT CAG TTA GCT TAT CAG ACT GAT GTT GAA CTA GGG ATA A |
| H2-III-P  | GCG ATT ACC CTG TTA T ↓ CC CTA TAG TTG AGC TGT TCA ACA TCA G (Cy3) TTA GCT TAT CAG ACT GAT GTT GAA CTA GGG ATA A ↓ CA GGG TAA TCG C |
| H2-III    | CCC TAT AGT TGA GCT GTT CAA CAT CAG TTA GCT TAT CAG ACT GAT GTT GAA CTA GGG ATA A |
| AP-1      | GCG ATT ACC CTG TTA |
| AP-2      | CAG GGT AAT CGC |
| miR-21    | UAG CUU AUC AGA CUG AUG UUG A |
| anti-miR-21 | TCA ACA TCA GTC TGA TAA GCT A |
| miR-199a  | ACA GUA GUC UGC ACA UUG GUU A |
| let-7a    | UGA GGU AGU AGG UUG UAU AGU U |
| miR-155   | UUA AUG CUA AUC GUG AUA GGG GU |
| miR-429   | UAA UAC UGU CUG GUU AAA CCG U |
| miR-141   | UAC AGU AUA GAU GAU GUU CU |
| 1-mutant  | UAG AUU AUC AGA CUG AUG UUG A |
| 2-mutant  | UAG AUU AUC AGA UUG AUG UUG A |
| 3-mutant  | UAG CUU AUU UCA CUG AUG UUG A |

* The sequences ATT ACC CTG TTA T ↓ CC CTA and TAG GGA TAA ↓ CAG GGT AAT underlined are the specific recognition sites of a homing endonuclease (I-SceI). The dSpacer represents abasic site, which is the specific recognition site of enzyme APE1. The nucleic acid bases marked in blue are the mutant sites.
### Table S2. The DNA sequences for the in vivo imaging system

| No.   | Sequence (5’→3’)* |
|-------|-------------------|
| **H1** | (BHQ2) ACT GAT GTT GAA CAG CTC AAC TAT CAA CAT CAG (Cy5) TCT GAT AAG CTA |
| **H2-II-P** | GCG ATT ACC CTG TTA T dSpacer CCC TAT AGT TGA GCT GTT CAA CAT CAG TTA GCT TAT CAG ACT GAT GTT GAA CTA GGG ATA A dSpacer CAG GGT AAT CGC |
| **H2-III-P** | GCG ATT ACC CTG TTA TCC CTA TAG TTG AGC TGT TCA ACA TCA GTT AGC TTA TCA GAC TGA TGT TGA ACT AGG GAT AAC AGG GTA ATC GC |
| **miR-21 inhibitor** | mU*mC*mA* mAmCmA mUmCmA mGmUmC mUmGmA mUmAmA mG*mC*mU*mA |

The dSpacer represents abasic site.

* represents phosphorothioate.

mN = 2’-O-Me RNA base.
Scheme S1. Illustration of the sequential activation mechanism of cancer-specific DNA repairing enzyme-recognized DNA pre-probe for HCR circuit.
Figure S1. (A) Denatured PAGE analysis of APE1-mediated H\textsubscript{2-II-P} cleavage in different doses at 37 °C for 5 h (1 × NEBuffer 4 buffer), lane 1, H\textsubscript{2-II-P} (400 nM), 0 U, lane 2, cleaved product H\textsubscript{2-II}, lane 3, 1 U/mL, lane 4, 2.5 U/mL, lane 5, 5 U/mL, lane 6, 10 U/mL. (B) The substrate cleavage efficiency with different doses of APE1 was quantified by PAGE. (C) PAGE analysis of APE1-mediated H\textsubscript{2-II-P} cleavage for different times at 37 °C (5 U/mL, 1 × NEBuffer 4 buffer), lane 1, H\textsubscript{2-II-P} (400 nM), 0 h, lane 2, cleaved product H\textsubscript{2-II}, lane 3, 1 h, lane 4, 2 h, lane 5, 3 h, lane 6, 5 h, lane 7, 8 h, lane 8, 12 h. (D) The substrate cleavage efficiency for different incubation times was quantified by PAGE. Data were means ± SD (n = 3).
Figure S2. Theoretical simulation of the secondary hairpin structures of (A) H2-I-P and APE1-exposed H2-I. (B) H2-II-P and APE1-exposed H2-II. Abasic site is abbreviated as AP site, which is the specific recognition site of enzyme APE1.
Figure S3. Native gel electrophoresis characterization of APE1-regulated HCR amplifier. (A) Lane 1: miR-21 (300 nM), lane 2: H1 (600 nM), lane 3: H2-I-P (200 nM), lane 4: H2-I (200 nM), lane 5: AP-1-2, obtained by mixing AP-1 (200 nM) with AP-2 (200 nM) and then heated at 95 °C for 5 min, and slowly cooled down to 25 °C within 2h. lane 6: H1 (600 nM) + H2-I-P (200 nM), lane 7: H1 (600 nM) + H2-I (200 nM), lane 8: miR-21 (50 nM) + H1 (600 nM) + H2-I-P (200 nM), lane 9: miR-21 (50 nM) + H1 (600 nM) + H2-I-P (200 nM) + APE1 (5 U/mL), reacted for a fixed time interval of 3 h. (B) Lane 1: miR-21 (300 nM), lane 2: H1 (600 nM), lane 3: H2-II-P (200 nM), lane 4: H2-II (200 nM), lane 5: Waste, the wasted cleaved product, obtained by mixing AP-1 (200 nM) with AP-2 (200 nM) and then heated at 95 °C for 5 min, and slowly cooled down to 25 °C within 2h. lane 6: H1 (600 nM) + H2-II-P (200 nM), lane 7: H1 (600 nM) + H2-II (200 nM), lane 8: miR-21 (50 nM) + H1 (600 nM) + H2-II-P (200 nM), lane 9: miR-21 (50 nM) + H1 (600 nM) + H2-II-P (200 nM) + APE1 (5 U/mL), reacted for a fixed time interval of 3 h. lane 10: miR-21 (50 nM) + H1 (600 nM) + H2-II-P (200 nM) + APE1 (5 U/mL), reacted for a fixed time interval of 5 h. 10 μL of Each sample was mixed with loading buffer and transferred into the 9% gel for running at 120 V (4 h). After staining, the gel was imaged by using the Fluorchem FC3 system (ProteinSimple, U.S.A).
Figure S4. (A) Optimization of the molar ratio between hairpins $H_1$ and $H_{2-II-P}$ by fluorescence experiment with the help of APE1 (5 U/mL) at a fixed time interval of 100 min (10 nM miR-21), measured by fluorescence emission ratio of acceptor to donor ($F_A/F_D$). Data were means ± SD (n = 3). (B) Evaluation of the sequential specific activation of HCR amplifier by fluorescence experiments, fluorescence emission ratio of acceptor to donor ($F_A/F_D$) was calculated, a, $H_1+H_{2-II-P}+$APE1; a’, miR-21+$H_1+H_{2-II-P}+$APE1; b, $H_1+H_{2-II-P}$; b’, miR-21+$H_1+H_{2-II-P}$; c, $H_1+H_{2-II-P}+$APE1; c’, miR-21+$H_1+H_{2-II-P}+$APE1. (C) Feasibility of miR-21 imaging in MCF-7 cells by incubating cells with hairpins $H_1+H_{2-II-P}$ (with abasic site) or $H_1+H_{2-III-P}$ (without abasic site) for 4 h. Bright field was abbreviated as BF. All scale bars correspond to 20 mm. The corresponding statistical histogram analysis (in the forms of $F_A/F_D$) were also acquired. Data were means ± SD (n = 3).
Figure S5. (A) Fluorescence spectra of the APE1-regulated HCR amplifier upon analyzing varied concentrations of miR-21 for a fixed time interval of 100 min. (B) Fluorescence spectra of the blocked HCR system with different concentrations of miR-21 in the absence of APE1.

Figure S6. (A) Fluorescence spectra for cleavage specificity of APE1, which were recorded by comparing the specific recognition capability of APE1 (5 U/mL) with several interfering nucleases, lambda exonuclease (λ Exo) (20 U/mL), T4 polynucleotide kinase (T4 PNK) (20 U/mL), homing endonuclease I-SceI (I-SceI) (5 U/mL), Nb.BbvCI nicking endonuclease (Nb.BbvCI) (20 U/mL), and HpaII restriction endonuclease (HpaII) (20 U/mL). (B) Summary of fluorescence emission ratio of acceptor to donor (F_A/F_D) from Figure S6A. Data were means ± SD (n = 3).
Figure S7. (A) Fluorescence spectra of the selectivity evaluation by comparing (10 nM) miR-21, with mutant miR-21, one-base mutant target (1-Mut), two-base mutant target (2-Mut), three-base mutant target (3-Mut). Inset: The corresponding fluorescence emission ratio of acceptor to donor (FA/FD) calculated from Figure S7A. (B) Fluorescence spectra of specificity evaluation by analyzing miR-21 and several interfering miRNAs (10 nM), miR-141, miR-199a, let-7a, miR-155, and miR-429. Inset: The corresponding fluorescence emission ratio of acceptor to donor (FA/FD) calculated from Figure S7B. Data were means ± SD (n = 3).

Figure S8. (A) Fluorescence spectra of anti-interference capability evaluation generated by analyzing 50 nM of miR-21 in different reaction buffer for a fixed time interval of 2 h: HEPES buffer without (a) and with (a’) miR-21, 5% serum without (b) and with (b’) miR-21, 10% serum without (c) and with (c’) miR-21. (B) Summary of fluorescence emission ratio of acceptor to donor (FA/FD) calculated from Figure S8B. Data were means ± SD (n = 3).
Figure S9. Optimization of incubation time of APE1-regulated HCR sensing system in MCF-7 cells. Confocal laser scanning microscopy (CLSM) characterization and corresponding statistical histogram analysis (in the forms of $F_A/F_D$) were acquired by incubating with cells for different time-intervals at 37 °C. Bright field was abbreviated as BF. All scale bars correspond to 20 μm. Data were means ± SD (n = 3).
Figure S10. Selective miR-21 imaging analysis in living cells. AP site refers to that cells are treated with APE1 regulated groups, hairpins H₁ and H₂-II-P (with abasic site), no AP site refers to that cells are treated with APE1-blunt groups, hairpins H₁ and H₂-III-P (without abasic site). Hairpin H₂-II-P with abasic site can be specifically recognized and cleaved by APE1, while hairpin H₂-III-P without abasic site cannot be recognized and cleaved. Bright field was abbreviated as BF. All scale bars correspond to 20 μm.
Figure S11. (A) CLSM imaging of miR-21 in MCF-7 cells with different miR-21 levels. MCF-7 cells treated with miR-21 mimic obtained improved miR-21 level, MCF-7 cells treated with anti-miR-21 antisense oligonucleotide inhibitor were for purpose of downregulating miR-21 level. Bright field was abbreviated as BF. All scale bars correspond to 20 μm.
**Figure S12.** APE1-regulated miR-21 imaging in vivo by tail vein injection. (A) Illustration of whole-body fluorescence imaging by tail vein injection. (B) Whole-body fluorescence imaging of MCF-7 tumor-bearing mice at representative time points after administration of HCR systems, a, APE1-regulated HCR system, $H_{1}^{*}+H_{2}^{*}$, b, APE1-blunt HCR system, $H_{1}^{*}+H_{2}^{*}$, c, the APE1-regulated HCR system after miR-21 inhibitor-pretreatment, $H_{1}^{*}+H_{2}^{*}+\text{miR-21 inhibitor}$, d, the APE1-blunt HCR system after miR-21 inhibitor-pretreatment, $H_{1}^{*}+H_{2}^{*}+\text{miR-21 inhibitor}$. (C) Quantification of fluorescence intensity at the tumor sites in Figure S12B. (D) Ex vivo imaging of tumor and major organs (He: heart, Lu: lung, Li: liver, Sp: spleen, and Ki: kidney) from mice upon intravenous injection at 12 h post-administration. (E) Quantification of fluorescence intensity in Figure S12D. Data were means ± SD (n = 3).
Hemolysis experiment was done by incubating various concentration of DNA sensor (hairpins $H_1$ and $H_2$-I-P) in 2 % blood cells at 37 °C for 30 min. No obvious hemolytic reaction was observed in Figure S13, indicating the good compatibility of our activatable HCR sensing system.[5]

**Figure S13.** Hemolysis test of the APE1-regulated HCR sensing system. Data were means ± SD ($n = 3$).
Hematology and biochemical analyses were carried out to evaluate the influence of activatable HCR system on important liver and kidney function. As depicted in Figure S14, indexes of important liver and kidney function markers were within the normal ranges for APE1-regulated HCR system treated group, which was in accordance with the PBS-treated control group, indicating negligible damage of our proposed HCR circuit.

**Figure S14.** Hematology and biochemical analyses of the APE1-regulated HCR sensing system after their intravenous injection into mice for 48 h. The tested indexes include white blood cells (WBC), lymphocyte (Lymph), monocyte (Mon), granulocyte (Gran), blood cells (RBC), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), mean platelet volume (MPV), glutamate pyruvate transaminase (ALT), aspartate aminotransferase (AST), albumin (ALB), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and creatinine (CRE). Data were means ± SD (n = 3).
Figure S15. SDS-PAGE for characterization of I-SceI expression in HeLa cells. Lane 1: Thermo Scientific PageRuler prestained protein ladder (10 to 180 kDa), lane 2: commercial homing endonuclease I-SceI (10 U), lane 3: lysate from non-transfected cells, lane 4: lysate from cells transfected with 0.5 μg plasmid pCBASceI and incubated for 12 h, lane 5: lysate from cells transfected with 1 μg plasmid pCBASceI and incubated for 12 h, lane 6: mixture of commercial I-SceI (10 U) and lysate from non-transfected cells as a positive control, lane 7: lysate from cells transfected with 0.5 μg plasmid pCBASceI and incubated for 24 h, lane 8: lysate from cells transfected with 1 μg plasmid pCBASceI and incubated for 24 h (this transfection condition was selected as the optimized one), lane 9: commercial homing endonuclease I-SceI (10 U). Lipofectamine 3000 was selected as the transfection reagent for plasmid pCBASceI. I-SceI was expressed in HeLa cells by incubating with pCBASceI plasmid (Addgene plasmid#26477) in 300 μL Opti-MEM at 37 °C for 12 h or 24 h. For the control groups, cells in each well were treated with Opti-MEM and lipofectamine 3000 in the absence of plasmid. Then the cells were washed with PBS for three time and lysed. The concentration of protein was measured by BCA assay (Beyotime). All proteins were separated by 15% SDS-PAGE.[6]
Figure S16. (A) Denatured PAGE analysis of I-SceI-mediated \( \text{H}_2\text{III-P} \) cleavage in different doses at 37 °C for 8 h (1× Thermo Scientific Tango Buffer), lane 1, \( \text{H}_2\text{III-P} \) (400 nM), 0 U/mL I-SceI, lane 2, cleaved product \( \text{H}_2\text{III} \), lane 3, 0.5 U/mL I-SceI, lane 4, 1 U/mL I-SceI, lane 5, 2.5 U/mL I-SceI, lane 6, 5 U/mL I-SceI, lane 7, 10 U/mL I-SceI. (B) The substrate cleavage efficiency with different doses of I-SceI quantified by PAGE. (C) PAGE analysis of I-SceI-mediated \( \text{H}_2\text{III-P} \) cleavage for different times at 37 °C (5 U/mL, 1× Thermo Scientific Tango Buffer), lane 1, \( \text{H}_2\text{III-P} \) (400 nM), 0 h, lane 2, cleaved product \( \text{H}_2\text{III} \), lane 3, 2 h, lane 4, 4 h, lane 5, 6 h, lane 6, 8 h, lane 7, 10 h. (D) The substrate cleavage efficiency for different incubation times quantified by PAGE. Data were means ± SD (n = 3).
Figure S17. Time-dependent FRET signal response of I-SceI-regulated HCR system in the absence and presence of target.
Figure S18. (A) Fluorescence changes of I-SceI-regulated HCR system after treatment with different concentrations of miR-21 (0–10 nM). (B) Corresponding calibration curve of fluorescence emission ratio of acceptor to donor (FA/FD) obtained from Figure S18A. A limit of detection of 40.7 pM was obtained from this linear correlation curve. (C) Fluorescence changes of the inactivated HCR system with various concentrations of miR-21 in the absence of I-SceI. (D) Corresponding calibration curve of fluorescence emission ratio of acceptor to donor (FA/FD) obtained from Figure S18C. Inset: The resulting linear correlation curve of Figure S18D ranging from 0–20 nM. Data were means ± SD (n = 3).
Figure S19. (A) Illustration of the I-SceI-expressed HeLa cells imaging by the I-SceI-controlled DNA pre-probe. (B) MiR-21 imaging in HeLa cells and statistical histogram analysis of the FRET signals (F_A/F_D). For group a, HeLa cells were treated with plasmid pCBASceI to allow intracellular expression of I-SceI before transfection of the HCR system (I-SceI-regulated group, hairpins H1 and H2-III-P), group b, HeLa cells without I-SceI expression were directly transfected with hairpins H1 and H2-III-P, group c, HeLa cells were treated with plasmid pCBASceI to allow intracellular expression of I-SceI, before transfection of anti-miR-21 and subsequent HCR system (hairpins H1 and H2-III-P), group d, HeLa cells without I-SceI expression were transfected with anti-miR-21, and then treated with the HCR system (hairpins H1 and H2-III-P) for 4h. Bright field was abbreviated as BF. All scale bars correspond to 20 μm. Data were means ± SD (n = 3).
References

(1) Zoya Cheglakov, Timothy M. Cronin, Chuan He, and Yossi Weizmann. Live Cell MicroRNA Imaging Using Cascade Hybridization Reaction. J. Am. Chem. Soc. 2015, 137, 6116–6119.

(2) Jie Wei, Huimin Wang, Xue Gong, Qing Wang, Hong Wang, Yangjie Zhou and Fuan Wang. A Proteinase-Free DNA Replication Machinery for in Vitro and in Vivo Amplified MicroRNA Imaging. Nucleic Acids Res. 2020, 48 (10), No. e60.

(3) Jie Wei, Xue Gong, Qing Wang, Min Pan, Xiaowei Liu, Jing Liu, Fan Xia, and Fuan Wang. Construction of an Autonomously Concatenated Hybridization Chain Reaction for Signal Amplification and Intracellular Imaging. Chem. Sci. 2018, 9, 52–61.

(4) Han Wu, Ting-Ting Chen, Xiang-Nan Wang, Yonggang Ke, and Jian-Hui Jiang. RNA Imaging in Living Mice Enabled by an in Vivo Hybridization Chain Reaction Circuit with a Tripartite DNA Probe. Chem. Sci. 2020, 11, 62–69.

(5) Meijuan Liang, Yanbing Wang, Kang Ma, Shanshan Yu, Yingying Chen, Zhao Deng, Yi Liu, and Fuan Wang. Engineering Inorganic NanoFlares with Elaborate Enzymatic Specificity and Efficiency for Versatile Biofilm Eradication. Small 2020, 16, 2002348.

(6) Yao Lin, Zhenglin Yang, Ryan J. Lake, Chengbin Zheng, and Yi Lu. Enzyme-Mediated Endogenous and Bioorthogonal Control of a DNAzyme Fluorescent Sensor for Imaging Metal Ions in Living Cells. Angew. Chem. Int. Ed. 2019, 58, 17061–17067.