# **Electronic Supplementary Information**

# A DNA encoding loop program: the snowball effect enhanced microRNA visualization in living cells

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#### **Supplementary Methods**

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#### **Supplementary Figure S1**

UV-Vis absorbance spectra of a) bare GNPs, b) seed probes and c) fuel probes. d) TEM image of the 60 nm GNPs.

#### **Supplementary Figure S2**

Scattering spectra of the GNP dots in Figure 1b (left column, a-f corresponding to 0, 15, 30, 60, 90, 120 min).

#### **Supplementary Figure S3**

Dark field images of GNPs (mixed solution of seed and fuel) incubated a) with or b) without miRNA for different times (scale bar:  $1 \mu m$ ).

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UV-Vis absorbance spectra of the supernatant collected after incubating the mixed solution of seed and fuel with the cell extract for 3 h.

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Scattering spectra of the GNP dots in Figure 2b (dark field cell images, a-f corresponding to 5, 15, 30, 60, 90, 120 min).

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Enlarged dark field cell images in Figure 2b (selected GNP dots pointed by white arrows, scale bar: 10  $\mu$ m).

# **Supplementary Figure S8**

Microscopic images of HeLa cells treated with seed probe (400  $\mu$ L) for different times (scale bar: 10  $\mu$ m). From top to bottom: bright field (BF); dark field (DF); fluorescence of FAM (FL-FAM).

#### **Supplementary Figure S9**

Enlarged dark field cell images in Figure 3a (selected GNP dots pointed by white arrows, scale bar: 10  $\mu$ m).

# **Supplementary Figure S10**

Dark field images revealing the miRNA-21 expression in different cellular extracts (scale bar: 1 µm).

# **Supplementary Figure S11**

Simulations setup by COMSOL. a) The 2 D component model of GNPs dotted cell. b) Boundary conditions setting for the heat transport. c) Mesh building for the steady state study. d) The simulated temperature profile.

# **Supplementary Figure S12**

a) Microscopic images of HeLa cells treated with GNPs (mixed solution of seed and fuel) for 1.5 h and then exposed under 680 nm laser irradiation for different times. Scale bar: 10 µm. Bright field (BF); dark field (DF); fluorescence of FAM (FL-FAM). b) Annexin V-FITC/PI stained HeLa cells after NIR irradiation. Scale bar: 10 µm. Red dashed line: apoptosis vesicles Bright-field (BF), dark field (DF).

# **Supplementary Figure S13**

a) FAM (FL-FAM) fluorescence of anti-miRNA-21 treated HeLa cells. b) Annexin V-FITC/PI stained HeLa cells (treated with anti-miRNA-21) after NIR irradiation. Scale bar: 10 μm.

#### **Supplementary Methods**

Materials and Reagents. Chloroauric acid (HAuCl₄•3H<sub>2</sub>O ≥ 99.9%) and trisodium citrate (≥ 99%), were purchased from Sigma-Aldrich (St Louis, MO, USA). Sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Aladdin (Shanghai, China). Annexin V-FITC/propidium iodide (PI) apoptosis detection kit was obtained from KeyGen Biotech. Co. Ltd. (Nanjing, China). All solutions were prepared using 18 MΩ.cm ultrapure water (EMD Millipore, TONDINO, Shanghai). HeLa cervical cancer cells, MCF-7 breast cancer cells and RAW264.7 macrophage cells were obtained from Shanghai Moxi Boil Co., Ltd. DNA and RNA sequences were purchased from Shanghai Gene Pharma Co., Ltd. (Shanghai, China). The hairpin DNA sequences for preparation of the seed probe: 5'-HS-(CH<sub>2</sub>)<sub>6</sub>-CCGTTCTA T CAA CAT CAG TCT GAT AAG CTA TAGAACGG-FAM-3'; the DNA sequences for preparation of the fuel probe: 5'-HS-(CH<sub>2</sub>)<sub>12</sub>-CCGTTCTA TAG CTT ATC AGA CT-3'. MiRNA-21: 5'-UAG CUU AUC AGA CUG AUG UUG A-3'; mismatched miRNA-21: 5'-UAG CUU AUC AGA C<u>A</u>G AUG UU<u>C</u> A-3'; anti-miRNA-21: 5'-UCA ACA UCA GUC UGA UAA GCU A-3'.

**Apparatus.** A JEM-2100 high-resolution transmission electron microscope (TEM, JEOL, Japan) was used for the morphology characterization in the experiment. A USB 2000+ spectrometer (Ocean Optics Inc., USA) was used for UV-Vis characterization. An inverted microscope (eclipse TiU, Nikon, Japan) equipped with a dark-field condenser (0.8 < NA < 0.95), a  $40 \times$  objective lens (NA = 0.8) and a white light source (100 W halogen lamp) was used to obtain the plasmon resonance scattering images and spectra of GNPs. The fluorescence images were obtained by the same microscope using a mercury lamp as the light source (100 W Epi illuminator).

**Preparation of GNPs.** GNPs were prepared as previously reported.<sup>1</sup> Briefly, deionized water (100 mL) and HAuCl<sub>4</sub> solution (4.8 mL, 1.0 wt%) were added into a clean beaker and heated under continued stirring. As soon as the solution started boiling, trisodium citrate solution (10 mL, 1.0 wt%) was added, and the color changed from faint yellow to black and then wine red. Next, the mixture was kept boiling for additional 20 min and then cooled down to room temperature. The obtained solution was centrifuged (5600 rpm, 5 min) and resuspended in pure water. Afterwards, the GNP solution was stored in refrigerator (4  $\infty$ ) for the following experiments.

**Preparation of DNA-Functionalized GNP Probes (Seed Probe and Fuel Probe).** For the preparation of seed probe, 100  $\mu$ L FAM-tagged hairpin DNA (10  $\mu$ M) was mixed with 1 mL GNP solution and then stirred overnight at room temperature. The obtained solution was centrifuged, washed with PBS, and then resuspended in 1 mL PBS. The solution of fuel probe was obtained with the same method. Then

200  $\mu$ L seed probe solution and 200  $\mu$ L fuel probe solution were mixed and incubated with different cells for the following experiments.

**Cell Culture.** Hela cells were cultured in DMEM (GIBCO) with 10% fetal bovine serum (FBS, Sigma), streptomycin (100  $\mu$ g mL<sup>-1</sup>), and penicillin (100  $\mu$ g mL<sup>-1</sup>). MCF-7 and RAW264.7 cells were cultured by RPMI-1640 (GIBCO) with fetal bovine serum (10%, Sigma), streptomycin (100  $\mu$ g mL<sup>-1</sup>), and penicillin (100  $\mu$ g mL<sup>-1</sup>). The culture dishes were placed in a humid atmosphere at 37 °C with 5 % CO<sub>2</sub>.

**Detection of MiRNA-21 in Solution and in Living Cells.**<sup>2</sup> For the detection of miRNA-21 in solution, the mixed solution of seed and fuel (200  $\mu$ L each) was added into the miRNA-21 solution (20  $\mu$ M) for different times and then observed on ITO glasses under the dark-field microscope. For detection in living cells, the cells (1 mL, 1×10<sup>6</sup> mL<sup>-1</sup> per culture dish, dish size: 4 cm in diameter, 40-60 % confluency) were incubated with the mixed solution of seed and fuel (200  $\mu$ L each) for various times in cell incubator (in a humid atmosphere at 37 °C with 5 % CO<sub>2</sub>) and then observed under the microscope.

**Information Extraction from the Images.** The color dark field images are composed of three primary colors (R: red, G: green, and B: blue), and the RGB images are the combination of three channels (red channel, green channel, and blue channel). Each channel stored pixels that corresponded to a brightness intensity from 0 to 255 (0: no brightness, 255: full strength). From the dark field images, the red/green channel intensity of the GNPs was analyzed using Adobe Photoshop software. Matlab was used for the extraction of a two-dimensional matrix containing the intensity of the various channels at each pixel.

**Targeted Photothermal Therapy on Living Cells.** After cells  $(1 \text{ mL}, 1 \times 10^6 \text{ mL}^{-1} \text{ per culture dish, dish size: 4 cm in diameter, 40-60 % confluency) were cultured and incubated with the mixed solution of seed and fuel (200 µL each) for 1.5 h in cell incubator (in a humid atmosphere at 37 °C with 5 % CO<sub>2</sub>), the cells were irradiated with the 680 nm laser for 2 h at <math>4 \times 10^3$  W cm<sup>-2</sup> at 37 °C. The apoptosis process was visualized under a DFM/fluorescence microscope. Afterwards, the cells were stained with Annexin V-FITC/PI for 10 min following the manufacturer's instruction, washed with PBS and the cell apoptosis was observed under fluorescence microscope.

**Finite Element Simulations.** COMSOL Multiphysics 5.2a (COMSOL AB, Stockholm, Sweden) was used to perform the numerical simulations of the photothermal effect of the established DNA coded recycling programmed (DCRP) method.

#### Supplementary Note 1. Characterization of GNPs, Seed Probes and Fuel Probes

The 60 nm GNPs and DNA-Functionalized GNP Probes (Seed Probe and Fuel Probe) were characterized by UV-Vis spectra. The typical peak of DNA at 260 nm emerged after the modification. The TEM image of GNPs showed uniform sizes.



**Figure S1.** UV-Vis absorbance spectra of a) bare GNPs, b) seed probes and c) fuel probe, d) TEM image of the 60 nm GNPs (scale bar: 50 µm).

# Supplementary Note 2. Dark Field Scattering Spectrum of MiRNA-21 Treated GNPs

The mixed solution of GNP probes (seed and fuel) was incubated with miRNA-21 for different times. The spectral peak showed obvious redshift as the incubation time increased.



**Figure S2.** Scattering spectra of the GNP dots in Figure 1b (left column, a-f corresponding to 0, 15, 30, 60, 90, 120 min).

# Supplementary Note 3. Detection of MiRNA-21 in Solution

The mixed solution of seed and fuel (200  $\mu$ L each) was treated with or without miRNA-21 for different times and then observed under the dark-field microscope.



**Figure S3.** Dark field images of GNPs (mixed solution of seed and fuel) incubated a) with or b) without miRNA-21 for different times (scale bar:  $1 \mu m$ ).

# Supplementary Note 4. Stability of GNP Probes in Acidic Complex Sample

The mixed solution of seed and fuel (200  $\mu$ L each) was incubated with 20  $\mu$ L HeLa cell extract (pH value adjusted to 6.0) and incubated at 37 °C for 3 h, the supernatant was collected for UV-Vis detection, which did not show the characteristic peak of DNA at 260 nm.



**Figure S4.** UV-Vis absorbance spectra of the supernatant collected after incubating the mixed solution of seed and fuel with the cell extract for 3 h.

#### Supplementary Note 5. Demonstration of the Specific Recognition of the DCRP Method

The mixed solution of seed and fuel (200  $\mu$ L each) was treated with the mismatched miRNA-21 for different times and then observed under the dark-field microscope. The scattering light did not change during the 2 h incubation.



**Figure S5.** Dark field images of GNPs (mixed solution of seed and fuel) incubated with mismatched miRNA-21 for different times (scale bar: 1 μm).

# Supplementary Note 6. Dark Field Scattering Spectrum of GNP Dots in HeLa Cells

HeLa cells were treated with GNPs (mixed solution of seed and fuel) for different times. The scattering spectral peak of the intracellular GNP dots showed obvious redshift as the incubation time increased.



**Figure S6.** Scattering spectra of the GNP dots in Figure 2b (dark field cell images, a-f corresponding to 5, 15, 30, 60, 90, 120 min).

Supplementary Note 7. HeLa Cells Treated with the DCRP Method



**Figure S7.** Enlarged dark field cell images in Figure 2b (selected GNP dots pointed by white arrows, scale bar:  $10 \ \mu m$ ).

# Supplementary Note 8. HeLa Cells Treated with Only Seed Probe



**Figure S8.** Microscopic images of HeLa cells treated with seed probe (400  $\mu$ L) for different times (scale bar: 10  $\mu$ m). From top to bottom: bright field (BF); dark field (DF); fluorescence of FAM (FL-FAM).

Supplementary Note 9. Various Cells Treated with the DCRP Method



**Figure S9.** Enlarged dark field cell images in Figure 3a (selected GNP dots pointed by white arrows, scale bar:  $10 \mu m$ ).

# Supplementary Note 10. Analysis of MiRNA-21 in Cell Extracts

HeLa, MCF-7 and RAW264.7 cells (1 mL,  $1 \times 10^{6}$  mL<sup>-1</sup> each) were centrifuged respectively, and the cell extracts were incubated with the mixed solution of seed and fuel (200 µL each) for 1.5 h. After the treatment, the obtained solutions were dropped on the bottom surface of clean culture dishes for dark field imaging respectively. As shown in Fig. S10, most of the GNP dots from HeLa cell extract showed orange red scattering light, while GNP dots from RAW264.7 extract showed yellow green, confirming the highest miRNA-21 level in HeLa cells.



**Figure S10.** Dark field images revealing the miRNA-21 expression in different cellular extracts (scale bar: 1 µm).

#### Supplementary Note 11. Setup for Numerical Simulations

The model was established by COMSOL multiphysics 5.2a in this work. The heat transfer of GNP dots in a cell was solved using the Heat Transfer interface. The model showed an axisymmetric steady-state thermal analysis. Kelvin was used as the temperature unit. A 2D cylindrical system was used for the simulation in order to simplify the calculation steps, reduce the complicated 3D calculation and get optimized results quickly. Cell boundary temperature was set as 310 K (initial condition). Along with the NIR irradiation, a steady state solution could be calculated. The average cellular temperature was higher than 50 °C.



**Figure S11.** Simulations setup by COMSOL. a) The 2 D component model of GNPs dotted cell. b) Boundary conditions setting for the heat transport. c) Mesh building for the steady state study. d) The simulated temperature profile.

# Supplementary Note 12. Photothermal Treatment in MiRNA-21 Positive Cells

HeLa cells (1 mL,  $1 \times 10^{6}$  mL<sup>-1</sup>) were incubated with the mixed solution of seed and fuel (200 µL each) for 1.5 h. The cells were irradiated with the 680 nm laser for different times under  $4 \times 10^{3}$  W cm<sup>-2</sup> at 37 °C. After laser irradiation for 2 h, the cells were stained with Annexin V-FITC/PI for cell apoptosis analysis.



**Figure S12.** a) Microscopic images of HeLa cells treated with GNPs (mixed solution of seed and fuel) for 1.5 h and then exposed under 680 nm laser irradiation for different times. Scale bar: 10  $\mu$ m. Bright field (BF); dark field (DF); fluorescence of FAM (FL-FAM). b) Annexin V-FITC/PI stained HeLa cells after NIR irradiation. Scale bar: 10  $\mu$ m. Red dashed line: apoptosis vesicles Bright-field (BF), dark field (DF).

#### Supplementary Note 13. Photothermal Treatment in MiRNA-21 Silenced Cells

The anti-chain of miRNA-21 (anti-miRNA-21) was used for inhibiting the intracellular miRNA-21 level. HeLa cells (1 mL,  $1 \times 10^6$  mL<sup>-1</sup>) were treated with anti-miRNA-21 (final concentration: 1  $\mu$ M) for 4 h and then incubated with the mixed solution of seed and fuel (200  $\mu$ L each) for 1.5 h. The FAM fluorescence of GNPs was observed under microscope. Afterwards, the cells were irradiated with the 680 nm laser for 2 h at  $4 \times 10^3$  W cm<sup>-2</sup> at 37 °C. After laser irradiation, the cells were stained with Annexin V-FITC/PI for cell apoptosis analysis.



**Figure S13.** a) FAM (FL-FAM) fluorescence of anti-miRNA-21 treated HeLa cells. b) Annexin V-FITC/PI stained HeLa cells (treated with anti-miRNA-21) after NIR irradiation. Scale bar: 10 μm.

# References

- 1. P. C. Lee, D. Meisel, J. Phys. Chem. 1982, 86, 3391-3395.
- 2. R. C. Qian, Y. Cao, Y. T. Long, Anal. Chem. 2016, 88, 8640-8647.