Supporting Information

Upconversion Luminescence-Controlled DNA Computation for Spatiotemporally Resolved, Multiplexed Molecular Imaging

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Materials and Methods

Materials: All reagents were used as received without further purification. Rare earth oxides, oleylamine (OM, 90%), and 1-octadecene (ODE, 95%) were purchased from Acros. Oleic acid (OA, 90%) and trifluoroacetic acid (99%) were bought from Sigma-Aldrich. The poly-L-lysine (PLL) was bought from Sangon Biotech Co. (Shanghai, China). All DNA primers were synthesized and purified by Sangon Biotech Co. (Shanghai, China). LysoTracker Green DND-26 was obtained from YEASEN. CCK-8 assay kit was purchased from Transgene Biotech Co. (Beijing, China). Hoechst 33342, MitoTracker Green and ATP assay kit were purchased from Beyotime Institute of Biotechnology (Shanghai, China). The water used throughout the experiments was Millipore water (18.2 MΩ).

Instrumentations: Fluorescence spectra were collected on a Hitachi F-4600 fluorimeter (Hitachi Co. Ltd., Japan) with Xe lamp as the excitation source. The upconversion luminescence (UCL) spectra were obtained using a Hitachi F-4600 spectrometer (Hitachi Co. Ltd., Japan) with 980 nm NIR laser as the excitation source. Transmission electron microscopic (TEM) images were captured on a Hitachi HT-7700 (Hitachi Co. Ltd., Japan) transmission electron microscope. The hydrodynamic diameter and zeta potentials of nanomaterials were tested on a Malvern nano ZS90 instrument. Confocal microscopic images were obtained using a Zeiss LSM 710 confocal microscope at 63× magnification. The flow cytometric assays were carried out using a BD Accuri C6 Flow cytometry. Cell viability data were measured on a Biotech Epoch2 microreader. MicroRNA expression level was quantified by Bio-Rad iQ™5 Real-Time PCR (Bio-Rad Laboratories, Inc, USA). ATP level was measured using ATP kit on Enspire multimode detector (PerkinElmer Inc., USA). The in vivo fluorescence images were acquired with IVIS® Spectrum in vivo imaging system (PerkinElmer Inc., Waltham, Massachusetts).

Synthesis of NaGdF₄: UCNPs were synthesized using the thermal decomposition method combined with a seed-mediated shell growth strategy. Trifluoroacetate precursors including CF₃COONa (1 mmol) and Gd(CF₃COO)₃ (1 mmol) were added to a 100 mL three-neck flask. A mixed solution [OA (10 mmol), OM (10 mmol) and ODE (20 mmol)] was added to the flask. Then, the mixed solution was stirred vigorously and heated to 120 °C under vacuum to remove the dissolved oxygen and low-boiling solvent. Next, the mixture was heated to 310 °C under the atmosphere of nitrogen (N₂). After 50 min of reaction, the mixture was cooled to room temperature slowly. The solid was precipitated with ethanol, collected through centrifugation, and dispersed in 10 mL cyclohexane. Then, 5 mL of the as-prepared solution
was introduced into a mixture of CF$_3$COONa (0.5 mmol) and Gd(CF$_3$COO)$_3$ (0.5 mmol), followed by addition of OA (20 mmol) and ODE (20 mmol). The mixture was stirred vigorously and heated to 120 °C under vacuum to remove cyclohexane, water and oxygen. After 30 min, the mixture was heated to 310 °C and maintained for 50 min under the atmosphere of nitrogen (N$_2$). Then, the mixture was cooled to room temperature slowly. The solid was precipitated with ethanol, collected through centrifugation, and dispersed in 10 mL cyclohexane.

**Synthesis of NaGdF$_4$@NaGdF$_4$@Yb,Tm**: 5 mL of the as-prepared NaGdF$_4$, CF$_3$COONa (1 mmol), Gd(CF$_3$COO)$_3$ (0.29 mmol), Yb(CF$_3$COO)$_3$ (0.70 mmol), Tm(CF$_3$COO)$_3$ (0.01 mmol) were introduced into a solution of OA (20 mmol) and ODE (20 mmol). The mixture was stirred vigorously and heated to 120 °C under vacuum to remove oxygen and low-boiling component. After 30 min, the mixture was heated to 310 °C and maintained for 50 min under the atmosphere of nitrogen (N$_2$). Then, the mixture was cooled to room temperature slowly. The solid was precipitated with ethanol, collected through centrifugation, and stored in 10 mL cyclohexane.

**Synthesis of NaGdF$_4$@NaGdF$_4$@Yb,Tm@NaGdF$_4$ (sandwich-structure UCNPs)**: 5 mL of the as-prepared NaGdF$_4$@NaGdF$_4$@Yb,Tm, CF$_3$COONa (0.5 mmol), Gd(CF$_3$COO)$_3$ (0.5 mmol) were introduced into a solution of OA (20 mmol) and ODE (20 mmol). The mixture was stirred vigorously and heated to 120 °C under vacuum to remove oxygen and low-boiling component. After 30 min, the mixture was heated to 310 °C and maintained for 50 min under the atmosphere of nitrogen (N$_2$). Then, the mixture was cooled to room temperature slowly. The sandwich-structure UCNPs were precipitated with ethanol, collected through centrifugation, and stocked in 10 mL cyclohexane. The oleic-acid (OA) molecules on the UCNPs were removed by the following steps. The OA capped UCNPs was first dispersed in 2 mL water/ethanol (v/v = 1/1) solution (containing HCl, 50 mM). The mixture solution was ultrasonicated for 10 min followed by vigorous stirring at room temperature for 2 h. The OA-free UCNPs were collected by centrifugation, washed with acidic ethanol solution (containing HCl, 10 mM) and MQ water for three times, and re-dispersed in 1 mL MQ water.

**Quantification of the DNA circuit density on UCNP**: The number of DNA circuits on the surface of UCNP is calculated by measuring the quantitative concentration changes of DNA circuits before and after assembly on UCNPs. Typically, only Cy3 labeled AMC was used to prepare AMC/UC. During the loading and washing processes, the supernatant was collected. The amount of Cy3-AMC in the collected solution was calculated using a
fluorescence standard curve. Then, the loading amount of Cy3-AMC could be calculated from the difference of the initial and collected solution. Finally, the surface density of Cy3-AMC on the UCNP could be calculated through the used molar concentration of the UCNPs and the loading amount of Cy3-AMC.

**Native polyacrylamide gel electrophoresis (PAGE):** The assembly of DNA circuits (AMC and O-A) were confirmed by native polyacrylamide gel electrophoresis (15%). Electrophoresis was carried out at 90 V for 2 h at room temperature. The gels were stained with GelRed (Sangon Biotech) for 30 min. Finally, the images of the gels were captured by using a CHEMIDOC™ TOUCH Imaging System (Bio-Rad, USA).

**Cell uptake analysis:** HeLa cells were seeded in 35 mm glass-bottom confocal dish (Cellvis) and incubated in DMEM for 24 h to reach 70-80% confluence. Cells were then treated with Cy3-AMC or Cy3-AMC/UC (DNA equivalent 100 nM) in Opti-MEM medium. Then, cells were incubated for another 2 h and washed with PBS to remove uninternalized materials. Nuclei was stained with Hoechst 33342 before imaged on confocal laser scanning microscope. For study the localization of Cy3-AMC/UC in cells, LysoTracker Green DND-26 (YEASEN) was used for specifically stain the endo/lysosomes according to the manufacturer protocol.

**Cell viability test:** Cells were seeded in 96-well plate (1 × 10^4 cells/well) and incubated for 24 h beforehand. Then, cells were treated with different materials (UCNPs, AMC, or AMC/UC) for 2 h, and then washed and replaced with fresh culture medium. For NIR light-treated groups, 980 nm laser irradiation was performed on cells for 20 min (1.2 W cm^2, 5 min break after 1.5 min irradiation). After incubation for another 24 h, cell culture media was replaced with 100 μL fresh culture medium containing 10 % CCK-8. The absorbance was measured at 450 nm after 1 h incubation on a microplate reader. Cells treated with PBS were served as a negative control.

**Measure of ATP levels in cells:** An ATP bioluminescent assay kit (Beyotime Institute of Biotechnology) was used to measure the ATP levels within cells. Typically, cells were seeded into a 6-well plate (NEST Biotechnology) and incubated in DMEM for 24 h. Then, treated cells were washed thoroughly with PBS and counted with a hemocytometer. After that, we determined the molar quantities of ATP according to the manufacturer’s instructions. At last, the concentration of ATP in a single cell were calculated from the quantity and the volume of cells.
Quantification of miR21 expression through qPCR: Typically, total RNA was extracted from cells with the SanPrep Column microRNA Mini-Preps Kit (Sangon Biotech), eluted with RNase-free water and quantified by spectrometry. The first strand cDNA was reversely transcribed from miR21 RT-primer sequence (CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG TCA ACA TCA) using the PrimeScript™ II 1st strand cDNA Synthesis Kit (Takara Biotech) according to the manufacturer protocol. The TransStart Green qPCR SuperMix (Transgene Biotech) were used as instructed by the manufacturer. The qPCR reactions were conducted on a Bio-Rad iQ™5 real-time PCR detection system. Human U6 was used as an internal normalization standard. Expression levels of miR21 were then estimated following the standard ΔΔCt method.
Figure S1. Construction and characterization of AMC. (a) Schematic showing the sequence of DNA used for the synthesis of AMC. (b) Native PAGE gel (15%) electrophoresis results confirmed the formation of AMC. The components are labeled on the lanes.
Figure S2. Characterization of AMC using fluorescence method. (a) Fluorescence spectra and (b) fluorescence intensity at 572 nm of AMC responding to different concentration of ATP in the presence of miR21 (20 nM) after 365 nm light irradiation (5 mW cm\(^{-2}\), 5 min). (c) Fluorescence spectra and (d) fluorescence intensity at 572 nm of AMC responding to different concentration of miR21 in the presence of ATP (5 mM) after 365 nm light irradiation (5 mW cm\(^{-2}\), 5 min).
Figure S3. TEM images of nanoparticles. (a) TEM images of NaGdF₄ (Gd), NaGdF₄@NaGdF₄:Yb,Tm (Gd@Tm), NaGdF₄@NaGdF₄:Yb,Tm@NaGdF₄ (Gd@Tm@Gd) UCNPs. Scale bars: 50 nm. (b) TEM images of OA-free UCNP, UCNP@PLL, AMC/UC in water. Scale bars: 50 nm.
Figure S4. Characterization of AMC/UC. (a) The hydrodynamic size distribution and (b) zeta potential of nanoparticles during the synthesis. The hydrodynamic size of UCNP, UCNP@PLL and AMC/UC was ~114 nm, ~126 nm and ~162 nm, respectively. Zeta potential measurement revealed that the particles become more positively charged after PLL coating and negatively charged after DNA loading. The zeta potential of UCNP, UCNP@PLL and AMC/UC was +38 mV, +43 mV and -27 mV, respectively. Data are represented as means ± SD (n = 3).
Figure S5. The stability test of AMC/UC. The fluorescence of AMC and AMC/UC was measured in 10% fetal bovine serum at different time intervals.
Figure S6. The cumulative release curve of AMC from AMC/UC in the HEPES buffer with or without 10% FBS, respectively. The release of AMC from the NPs in the buffer containing 10% FBS is much faster than that in HEPES buffer due to the competition of the polyanions. Data are represented as means ± SD (n = 3).
Figure S7. UCL spectra of UCNPs before and after AMC loading. The UCL was measured under 980 nm NIR irradiation.
**Figure S8.** Cellular uptake of AMC/UC. Confocal fluorescence images of HeLa cells treated with only Cy3-labeled AMC (upper) and AMC/UC (UCNPs loaded with only Cy3-labeled AMC) (below). Scale bars: 20 μm.
Figure S9. Intracellular distribution of AMC/UC. Confocal fluorescence images of HeLa cells treated with lysosome tracker Green and AMC/UC (UCNPs loaded with only Cy5-labeled AMC) after 3 hours incubation. Scale bars: 20 μm.
Figure S10. Evaluation of non-photoactive nanocircuit (nAMC/UC) in vitro. Confocal images of HeLa cells treated with nAMC/UC upon 980 nm light irradiation (below) or not (upper). Scale bars: 20 μm.
Figure S11. Cell viability of HeLa cells with different treatments. (a) UCNPs with different concentration. (b) 1: saline, 2: 980 nm, 3: AMC, 4: AMC + 980 nm, 5: AMC/UC, 6: AMC/UC + 980 nm. Data are represented as means ± SD (n = 3).
**Figure S12.** Relative levels of ATP and miR21 in HeLa cells with different treatments. The (a) ATP and (b) miR21 levels were measured using ATP kit and q-PCR, respectively. HeLa cells were treated with IAA (ATP inhibitor), miR21 inhibitor or the combination of the two. Data are represented as means ± SD (n = 3).
Figure S13. Evaluation of non-photoactive nanocircuit (nAMC/UC) in vivo. (a) The representative images of fluorescence output in mice after injection of nAMC/UC in the HeLa tumor and normal tissue followed by 980 nm light irradiation (upper) or not (below). (b) Quantification of fluorescence intensity of the injection sites in (a). Data are represented as means ± SD (n = 4).
**Figure S14.** Construction and characterization of O-A. (a) Schematic showing the sequence of DNAs used for the synthesis of O-A. (b) PAGE gel (15%) electrophoresis results confirmed the formation of the O-A. The components are labeled on the lanes.
**Figure S15.** Characterization of O-A using fluorescence method. (a) Fluorescence spectra of O-A responding to different nucleoside triphosphates in the presence of miR21 (20 nM). [Analytes] = 5 mM. (b) Fluorescence spectra of O-A responding to different concentration of ATP in the presence of miR21 (20 nM). (c) Fluorescence spectra of O-A responding to different types of miRNAs in the presence of ATP (5 mM). [Analytes] = 20 nM. (d) Fluorescence spectra of O-A responding to different concentration of miR21 in the presence of ATP (5 mM).
Figure S16. In vivo OR-AND-gated DNA computation. (a) Representative images showing fluorescence output of O-A/UC in mice. The same amount of O-A/UC was directly injected into the tumor site (T) and normal tissue (N). (b) Quantification of fluorescence intensity of the tumor and normal tissue in (a). Data are represented as means ± SD (n = 4). *P < 0.05, **P < 0.01.
Figure S17. In vivo OR-AND-gated DNA computation. (a) Representative time course images showing fluorescence output of O-A/UC in vivo through tail vein injection. To downregulate the level of ATP, mice were pretreated with 3-BP (+3-BP). Tumors are indicated with red dashed circles. (b) Quantification of fluorescence intensity at tumor sites in (a). Data are represented as means ± SD (n = 5). (c) Representative ex vivo fluorescence images and (d) quantification of major organs and tumor from mice 4 h after tail vein injection. Data are represented as means ± SD (n = 5). **P < 0.01.