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Electronic Supplementary Information

Photoresponsive spherical nucleic acid: spatiotemporal control of assembly circuit and intracellular microRNA release

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EXPERIMENTAL METHODS

Preparation of Sterile, DNase/RNase-Free Solution. If the operation involves in the synthesis of miR-34a SNA nanocarriers, this section is very important to prevent RNA degradation. 0.2M Phosphate (NaH_2PO_4/Na_2HPO_4) buffer, 4M NaCl solution, 1XPBS buffer (137mM NaCl, 10mM Phosphate, 2.7mM KCl, pH 7.4, Hyclone), Nanopure water (18.2 MΩ) and Au nanoparticles colloidal were treated by 1‰ diethylprocarbonate (DEPC; Sigma-Aldrich) at room temperature (RT) overnight, and then autoclaved. The tips and tubes also were sterile, and DNase/RNase-free.

Synthesis of photoreponsive SNA conjugates. The 13±1nm citrate-capped AuNPs were prepared as previously described[1]. For the self-assembly scheme in PBS buffer, thiol-terminated capture DNA was reduced by excess tris (2-carboxyethyl) phosphine hydrochloride (TCEP) for one hour, and the purified via size-exclusion chromatography with a NAP5 Column (GE Healthcare). The resultant capture DNA were added to gold nanoparticle colloids at a final concentration of 3.5 μM in AuNP colloids solution of 10nM, and the mixture was incubated overnight. Then, the mixture was brought to 10 mM sodium phosphate (PH = 7.4) by dropwise adding 0.2 M concentrated buffer and allowed to hold for another 12 hours. The solution was then salted to final concentration of 0.3 M NaCl by dropwise adding 4 M buffer in three times at least 8 h intervals. Unbound DNA strands were removed via centrifugation (15000 rpm, 30 min, 3X), resulting in capture-AuNP complexes. The PC linker-modified hairpin precursors were prepared through slow annealing process from 95 °C. Finally, the photoresponsive SNA conjugates were formed by incubating hairpin precursors with capture-AuNP complexes at a 25:1 molar ratio (Fig. S8) overnight at room temperature. Similarly, the free hairpin strands were removed by centrifugation. As for the photoresponsive miR-34-functionalized SNA nanocarriers, the DNA-RNA duplexes were prepared in the same annealing process first. Then miR-34a SNA nanocarriers were prepared in the same manner as abovementioned. The resultant SNA solution was quantified by measuring the extinction at 520 nm ($\varepsilon=2.7\times10^8$ L mol^{-1} cm^{-1}). The resultant SNA solution was stored in 4°C for future use.

Real-time detection of SNA self-assembly. Photoresponsive SNA (SNA-1, 4 nM), SNA-2 (4 nM) and the trigger strands were added to a plastic tube in sequence. Then the mixture was treated under different conditions, such as varied trigger concentrations and durations of UV irradiation as indicated in article. The light source is a UV lamp (LUYOR-365, China) with output power of ~ 30

mW/cm^{-2} at 365 nm. Finally, the samples were transferred to the cuvette and the kinetics of the SNA assembly were monitored by tracking the absorption value of the sample at 520 nm at 10 min intervals using an Agilent Cary 300 UV-vis spectrophotometer (Santa Clara, CA, USA).

Transmission electron microscopy (TEM). The sample was prepared by drop casting an aqueous nanoparticle solution onto a carbon coated copper grid, followed by slow removal of the remaining solution with a filter paper after 10 min. TEM images were obtained on a Hitachi 7650 transmission electron microscope.

Oligonucleotide Loading Quantification. In this section, the hairpin and miR-34a were tagged with Cy5 fluorophore. The SNA colloid (400μL, 1 nM) was treated with KCN solution (0.1 M) to dissolve the gold core and release the oligonucleotides. After centrifugation, the fluorescence intensity of supernatant was measured and compared to a standard curve. The Cy5 is excited at 633 nm and the emission peak is in a range of 660-665 nm. The extracellular fluorescence measurements were performed on Jobin Yvon Fluorolog FL3-22.

Cell Culture. MCF-7 cell lines were obtained from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), and grown in Dulbecco's modified Eagles medium (DMEM, Hyclone) with 10% heat inactivated fetal bovine serum (FBS, Hyclone) and 1% Penicillin/Streptomycin (Gibco) in 5% CO_2 at 37 °C.

Cell Confocal Fluorescence Imaging. Cells were seed on coverglass bottom dish (diameter 35 mm, SPL Lifescience Co., Ltd.). After 1 day, the cells were treated with photoresponsive miR-34a nanocarriers (1 nM) in Opti-MEM (Gibco). After a 4h incubation period, cells were washed with PBS buffer. To achieve the spatial control of miR-34a release, we discretionarily choose a small area of the culture dish to be exposed to UV irradiation (~10 min) and the other part is shielded with tinfoil. Then, cells were cultured for another 12 h and imaged under Lecia SP5 confocal microscope with HeNe laser excitation at 633 nm (Cy5).

Flow Cytometry Analysis. The experimental group was prepared as described above. The additional control groups include blank control and cells treated with UV light or SNA conjugates alone. Then cells were trypsinized, collected, and suspended in PBS buffer. Flow cytometry was performed with BD Analyses were performed on BD FACSCanto II system with BD FACSDiva software.

Total RNA Extraction and Quantitative RT-PCR. Cells were cultured with photoresponsive miR-34a SNA nanocarriers (3 nM) for 4h, followed by PBS wash and UV irradiation ~10 min. After a final 72h culture period, cells were harvested and total RNA was extracted by using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. For miR-34a expression analysis, quantitative RT-PCR was performed with the miRcute miRNA First-Stand cDNA Synthesis Kit and miRcute miRNA qPCR Detection Kit (SYBR Green; Tiangen). U6 was used as a control. For survivin mRNA expression analysis, total RNA was isolated from cells and cDNA was prepared by using the transcriptor First-Stand cDNA Synthesis Kit, following the indicated protocol. Quantitative RT-PCR was performed with LightCycler 480 SYBR Green I Master on a LightCycler 480 system (Roche). The relative expression of survivin mRNA was normalized to the internal control of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and compared to the untreated cells. All relative expression levels were calculated by the following equation: $Relative\ gene\ expression = 2^{-(\Delta Ct\ sample - \Delta Ct\ control)}$. Finally, primers sequences were listed in Table S2.

Cell Viability Assay. Cell viability assay was measured by using Cell Counting Kit (CCK-8). Cells were seeded in a 96-well plate at 3000 cells per well. Cells were treated with SNA nanocarriers (3 nM) 4 hours, followed by PBS wash. After a final 120h incubation period, 10 μL CCK-8 solution was added to each well and incubated for 2 hours. The absorbance of each well was recorded at 450 nm.

Cell Apoptosis Measurement. MCF-7 cells were treated with SNA nanocarriers (3 nM) for 4 hours, followed by PBS wash. After a final 72h incubation period, apoptosis was measured by treating cells with FITC-Annexin V and PI Cell Apoptosis Detection Kit (Invitrogen), according to the manufacturer's protocol. Analyses were performed on BD FACSCanto II system with BD FACSDiva software.

Table S1 DNA sequences used in SNA self-assembly.

Name	**Sequences (5' to 3')**
Capture DNA-1	ATAGGGAGAGAAGGTGTTTTTTTTTTTTTT-SH
Capture DNA-2	CATCACCCTATCACCCTTTTTTTTTTTTTT-SH
PC-Hairpin	CACCTTCTCTCCCTATCCCTATCACCTTCATTACCCTACA C-PC-GGTAATGAAGGTGATAGG

Fuel DNA	GGGTGATAGGGTGATGTTAGGGTAATGAAGGTGATAGG
Trigger DNA	GTGTAGGGTAATGAAGGTGA

Table S2 DNA/RNA/primers sequence used for intracellular miR-34a release.

Name	**Sequence (5'→3')**
Thiol-strand of miR-34a SNA	SH-TTTTTT-ACAAC(-PC-)CAGCTA(-PC-)AGACAC
miR-34a mimics	UGGCAGUGUCUUAGCUGGUUGU
miR-34a forward	AAGGCCACGGATAGGTCCATA
miR-34a reverse	CGCTTTGGTGGTTCTGAAAGG
U6 forward	AAGGCCACGGATAGGTCCATA
U6 reverse	CGCTTTGGTGGTTCTGAAAGG
Survivin forward	ATGGGTGCCCCGACGTTG
Survivin reverse	AGAGGCCTCAATCCATGG
GAPDH forward	TGCACCACCAACTGTTTAGC
GAPDH reverse	GGCATGGACTGTGGTCATGAG

Additional data

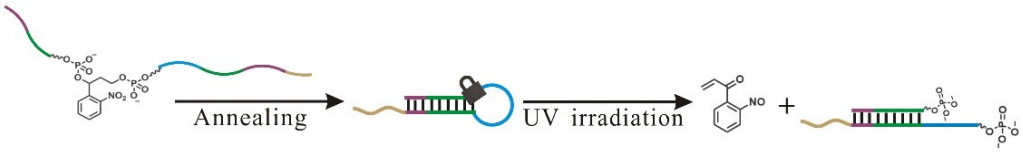

Fig. S1 Details of hairpin and PC linker.

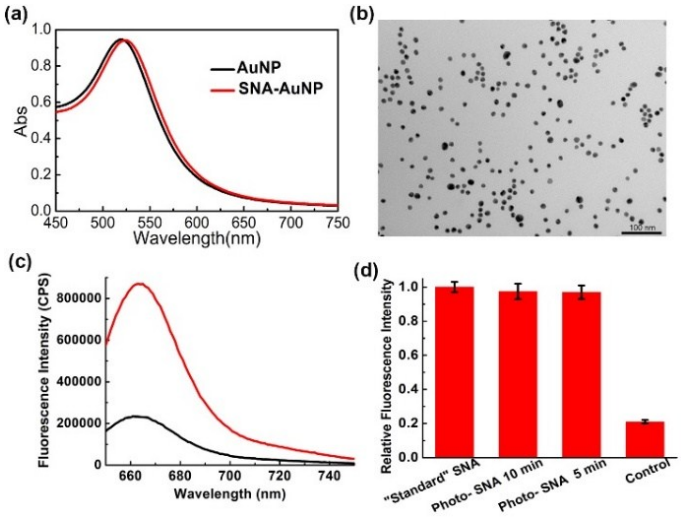

Fig. S2 The characterizations of photoresponsive SNA conjugates. (a) The UV-vis absorption of AuNP and SNA-AuNP. The absorption peak emerges at ~520 nm. After functionalizing with DNA strands, a slight red (~522 nm) shift occurs; (b) The TEM images of SNA-AuNP conjugates; (c) The fluorescence emission spectrum of photoresponsive SNA conjugate. The protector is labeled with Cy5 dye. After UV treatment, the PC linker is cleaved and toehold is exposed. Then, the protector is released from the AuNP surface by adding the trigger, leading remarkable fluorescence signal (red). Without UV treatment, adding trigger strand cannot cause significant fluorescence intensity (black); (d) Estimation of PC linker cleavage efficiency. The standard SNA means the linear double-stranded DNA-AuNP complex with an exposed toehold. Then trigger strands are added to displace Cy5-labeled protector. By comparing the florescence intensity, the PC linker cleavage efficiency is estimated as ~97.5% and ~97% for 10 min and 5 min UV irradiation, respectively.

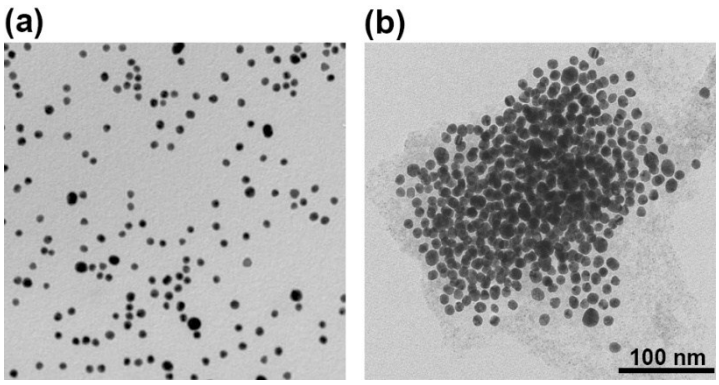

Fig. S3 TEM images of SNA conjugates before/after self-assembly. By introducing UV irradiation, the dispersed SNA nanoparticles (a) self-assemble into larger clusters (b).

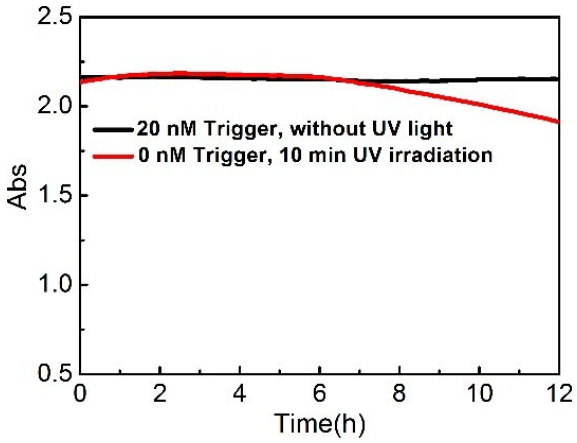

Fig. S4 The influences of UV irradiation and trigger strand on SNA self-assembly reaction. Without UV light, the aggregation reaction cannot happen even adding trigger strands. However, if the system is treated with UV light but no trigger strands, the system still undergoes detectable spontaneous aggregation.

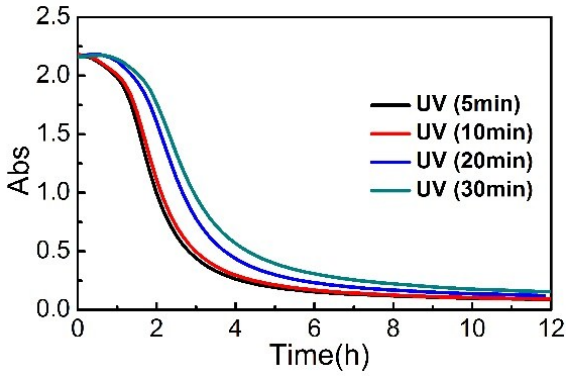

Fig. S5 When the exposure duration exceeds 10 min, the self-assembly rate becomes slower. This may be attributed to the DNA photodegradation caused by excess UV irradiation.

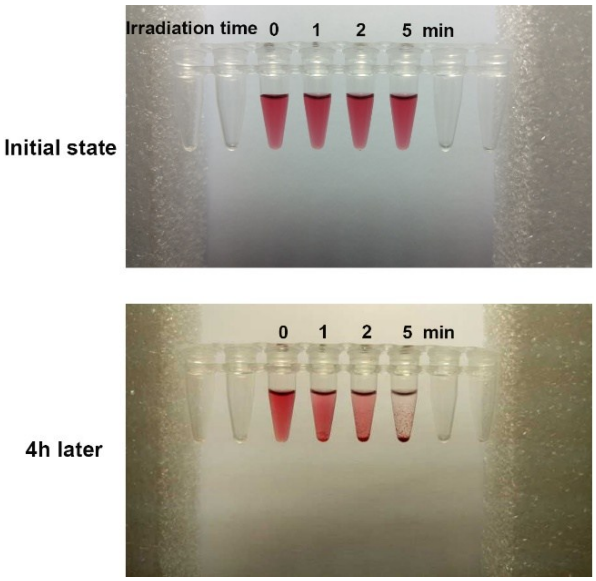

Fig. S6 The time-resolved SNA aggregation level can be observed by naked eye. Initially, the SNA-AuNP solution is homogeneous red color. However, the solution fades gradually as aggregation level is elevated by increasing exposure time.

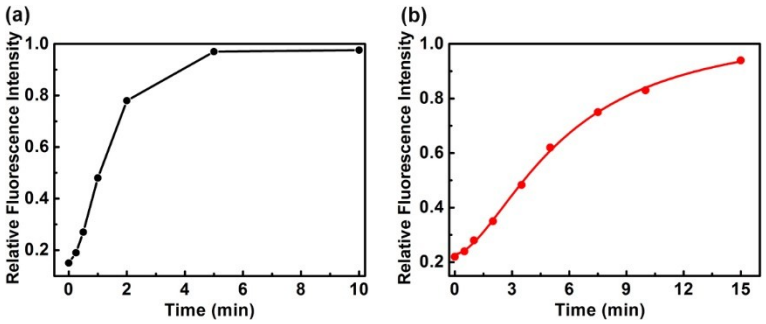

Fig. S7 Estimation of release rate of miR-34a strands in buffer solution (a) and MCF-7 cells (b). The miR-34a strands are labeled with Cy5 dye. When SNA nanocarriers are activated by UV exposure durations, fluorescence intensity is measured by using fluorescence spectrophotometer (buffer solution) or flow cytometer (MCF-7 cell). As indicated, the release rate of miR-34a strands in cell population is slower than that in buffer solution. For cell population, UV irradiations of 10 min and 15 min lead to 85% and 94% release rate, respectively. However, potential damage to MCF-7 cells could be caused by excess UV light, and therefore we adopted the UV treatment of 10 min.

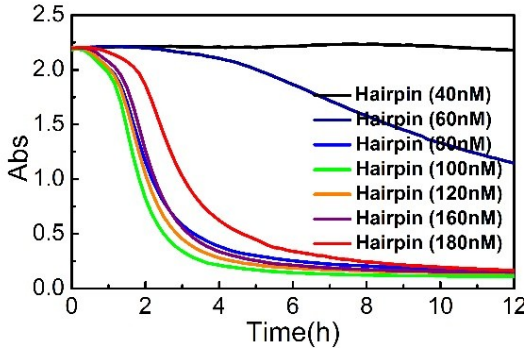

Fig. S8 Optimization of hairpin amounts. The capture-AuNP conjugates (4 nM) were incubated with hairpin strands at varied concentrations as indicated. The self-assembly kinetics is remarkably accelerated with the increase of hairpin concentration from 40 nM to 100 nM. However, when hairpin amount exceeds

100 nM, the aggregation rate decreases instead. So, 100 nM hairpin, a 25:1 molar ratio of hairpin/capture-AuNP achieves an optimal reaction rate.

1. J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, J. Am. Chem. Soc., 1998, 120, 1959-1964.