Electronic Supplementary Information (ESI)

Backbone-Modified Oligonucleotides for Tuning the Cellular Uptake Behavior of Spherical Nucleic Acids

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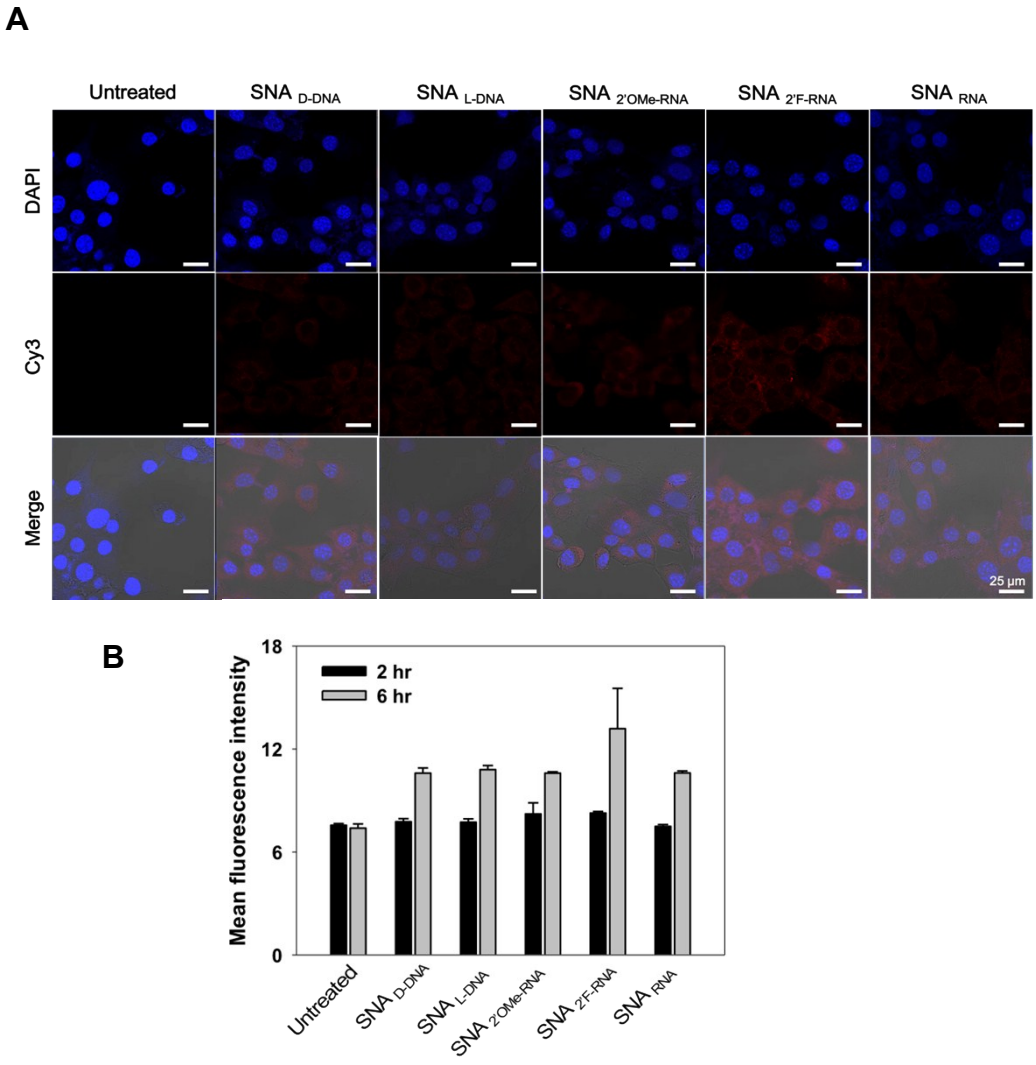

Figure S1. The cellular uptake efficiencies of SNAs in NIH3T3 cells. (A) The confocal fluorescence microscopic images of the NIH-3T3 cells treated with Cy3-labeld SNAs (6 h treatment). (B) Time-course analysis of the NIH-3T3 cells treated with Cy3-labeled SNAs by a flow cytometer (n = 3).

Table S1. The summary for the colloidal characteristics of SNAs.

	Size (nm)	PDI	Zeta potential (mV)	SPR peak (nm)	Loading capacity (strand/particle)
AuNP	11.35±0.50	0.35	-13.33±0.20	518	-
SNA_{DNA}	14.21±1.55	0.72	-27.29±0.75	523	134±15
$SNA_{L\text{-}DNA}$	16.81±0.38	0.70	-27.54±0.37	523	130±13
SNA_{RNA}	16.13±1.52	0.68	-27.99±1.55	523	131±8
$SNA_{2'\text{-}F\text{-}RNA}$	14.49±2.93	0.65	-26.48±0.55	523	132±7
$SNA_{2'\text{-}OMe\text{-}RNA}$	16.48±0.80	0.61	-27.82±0.13	523	129±17

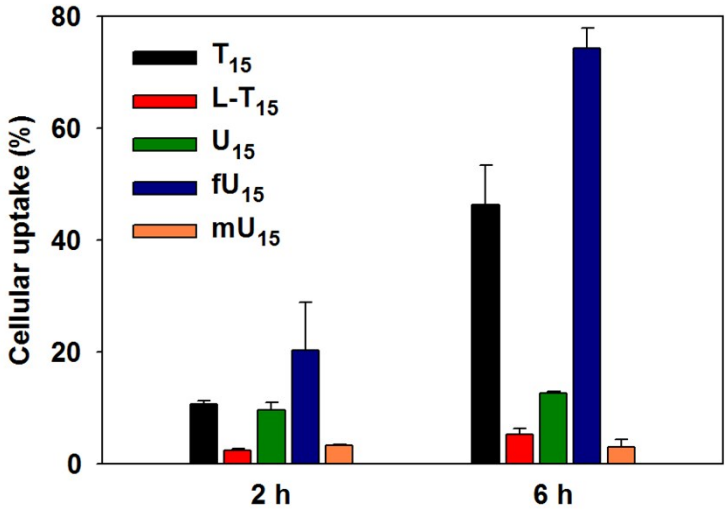

Figure S2. Cellular uptake efficiency of the single stranded oligonucleotides.

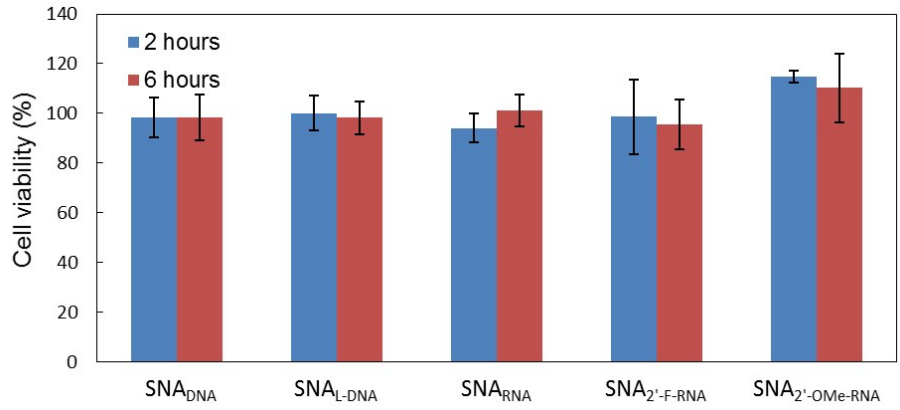

Figure S3. The cytotoxicity of SNAs in HeLa cells (n = 3).

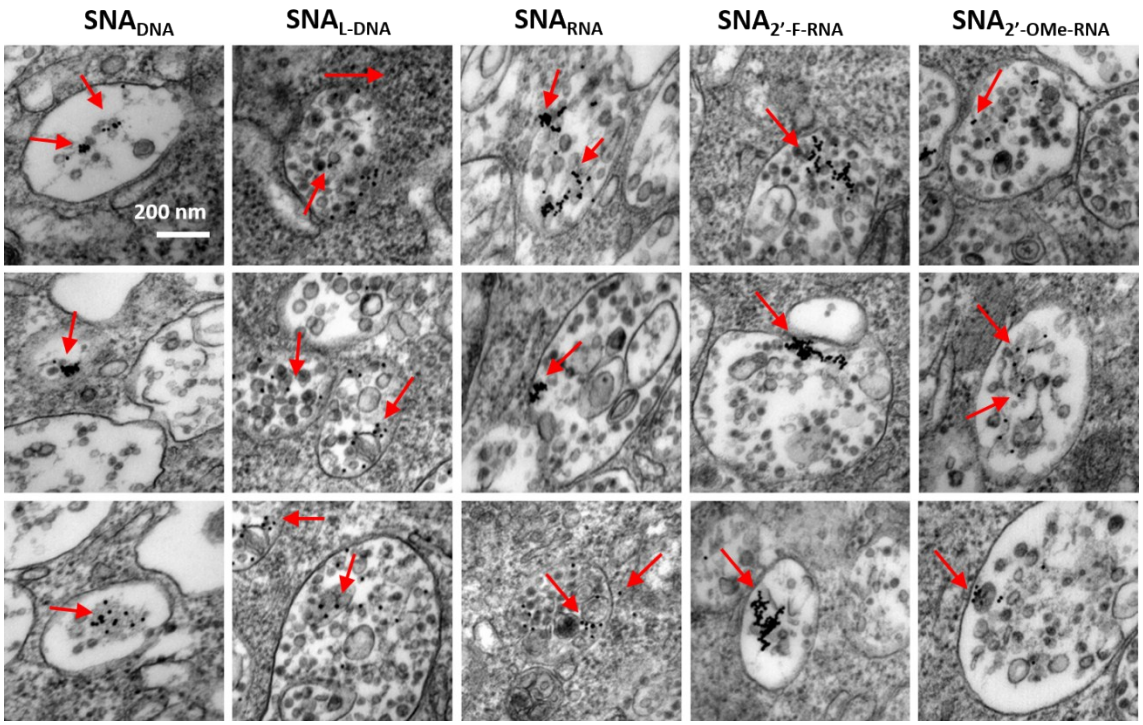

Figure S4. Additional TEM images showing the intracellular distribution of SNAs (red arrows).

Experimental Section

GNP synthesis

Citrate-stabilized GNPs were synthesized using previously reported method.[1,2] Briefly, $HAuCl_4$ solution (1mM, 100 mL) was heated up to 95 °C, and sodium citrate (33.8 mM, 10 mL) was added to the solution very quickly. The color of solution was changed from orange-yellow to black and then to red within 5 minutes, indicating typically polarized optical properties of GNPs. The solution was further kept for 10 minutes at 95 °C and then slowly cooled down to 4 °C. The synthesized GNPs were characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS) analysis.

Oligonucleotide synthesis and purification

Oligonucleotides were synthesized with a MerMade4 DNA synthesizer (BioAutomation, USA) by following the standard protocol for solid phase oligonucleotide synthesis using phosphoramidites. DNA, RNA, 3'-thiol-modifier phosphoramidites, and controlled pore glass (CPG) were purchased from Glen Research (USA). 2'-OMe-RNA, 2'-F-RNA, and L-DNA phosphoramidites were purchased from ChemGenes (USA), and other chemical reagents were from Sigma-Aldrich. Purification of oligonucleotides was performed using Agilent 1200 series HPLC equipped with semi-preparative DNAPac PA-100 anion-exchange column (Thermo Scientific, USA).

GNP functionalization with oligonucleotides

3'-thiolated oligonucleotides were reacted with tris(2-carboxyethyl)phosphine (TCEP) (100 equiv.) and desalted three times using Amicon ultra centrifugal filter unit of 3,000

MWCO (Merck Millipore, Germany). Bare GNPs were incubated with oligonucleotides (300 equiv.) in 10 mM sodium phosphate buffer (pH 7.4). The ionic strength of incubating solution was increased incrementally (0.1 M per hour) by adding 5 M sodium chloride solution until the final salt concentration reached to 0.5 M. After additional 16 h incubation, functionalized GNPs were centrifuged (15,000 g for 15 min) and the supernatant was removed. The particles were resuspended in distilled water. This centrifuge-based washing step was repeated three times. The concentration of finally obtained SNAs were measured by a UV-VIS spectrometer.

Dynamic light scattering

The size and surface charge of GNPs and SNAs were measured by using a Zetasizer Nano ZS (Malvern, UK).

Transmission electron microscopic (TEM) analysis of SNAs

The TEM bright-field images of nanoparticles were acquired using a TecnaiF20-cryo (FEI) operated at 200 kV. For element analysis, energy dispersive spectrum (EDS) mapping and spectrum analysis were carried out using a Talos TEM (FEI; Talos F200X) equipped with super-X EDS system with four silicon drift detectors (SDDs) (Bruker).

Determination of the number of loaded oligonucleotide strands per GNP

SNAs were treated with equal volumetric amount of 1,4-dithiothreitol (DTT) solution (1 M). After 16 h incubation (15,000g for 1 hour), the supernatant was mixed with SYBR Gold nucleic acid staining dye (ThermoFisher Scientific, USA), and fluorescence intensity of the mixture was measured. Oligonucleotides were quantified based on a

standard curve of fluorescence intensity obtained with known amount of oligonucleotides stained with SYBR Gold.

Cellular uptake of SNAs

In principle, SNA cellular uptake experiments for cytotoxicity assay, fluorescence microscopy, flow cytometry, TEM analysis and induced coupled plasma mass spectrometry (ICP-MS) were based on same procedures. HeLa cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) at 37 °C and 5 % CO_2. For SNA uptake experiments, cells were seeded into culture plates or dishes in advance and incubated until the cell's confluency reached to 80 %. Then, cells were washed with DPBS and incubated with serum free DMEM media containing desirable SNA concentrations. After incubation for 2 or 6 h, cells were vigorously washed with DPBS five times and subjected to proceed further analysis. Detailed SNA cellular uptake conditions were summarized in Table S2. To examine uptake mechanisms, cells were pre-incubated under each inhibitory condition for 30 min before uptake experiments.

Fluorescence-based analysis of cellular uptake of SNAs

For fluorescence microscopic analysis of SNAs, Cy3-labeled SNAs were used. Prior to SNA cellular uptake, cells were stained with Hoechst 33342 nuclei staining dye in order to prevent staining of SNAs by Hoechst 33342. After cellular uptake, the cells were fixed with 4% formaldehyde solution for 30 min at room temperature. The images were obtained under an LSM 700 confocal microscopy (Carl Zeiss, Germany) and analyzed by Image J software. The cells treated with Cy3-labeled SNAs were also analyzed by a

Guava easyCyte Flow Cytometer (Merck Millipore, UK). The obtained data were analyzed by FlowJo software.

TEM analysis of internalized SNAs

Preparation of cell specimens for TEM analysis was performed by following regular sequences of fixation, dehydration and resin-infiltration steps. All processes were conducted at 4 °C otherwise mentioned separately. Firstly, primary fixation for SNA-treated HeLa cells was conducted using Karnovsky's fixative solution (2.5 % glutaraldehyde and 4 % paraformaldehyde solution in 0.1 M sodium cacodylate buffer pH 7.4) for 2 h. After three washing steps with 0.05 M sodium cacodylate buffer, post fixation was performed using 2% osmium tetroxide solution and 0.1 M sodium cacodylate buffer for 2 h, followed by washing with distilled water. Then, specimens were immersed within 0.5 % uranyl acetate solution for 16 h. Dehydration was performed by exposing the specimens to 30%, 50%, 70%, 80%, 90% and 100% ethanol for 10 min, respectively. For resin-infiltration, the specimens were incubated within propylene oxide solution and Spurr's resin for 2 h and embedded within 100 % Spurr's resin for 16 h at room temperature. After that, incubating temperature was adjusted to 70 °C for resin polymerization. Solidified resin-infiltrated specimens were subjected to an EM UC7 ultramicrotome (Leica, Germany) for preparation of ultrathin sections, which were analyzed by a JEM1010 TEM (JEOL, Japan) operated at 80 kV.

Induced coupled plasma mass spectrometry (ICP-MS)

All glass wear was rinsed with optimal aqua regia, which is aqueous acidic mixture of hydrochloric acid and nitric acid with molar ratio of 3 to 1, and washed three times with

distilled water. After cellular uptake experiments, every sample's cell numbers were counted using hemacytometer. Less than 0.1 mL of samples were digested with 0.5 mL of optimal aqua regia at room temperature for 16 hours, followed by volume-up to 10 mL using distilled water. The digested gold contents were measured by Varian 820-MS mass spectrometer and the average GNP numbers internalized by one HeLa cell were calculated.

Table S2. The summary for the experimental conditions for the cellular uptake of SNAs.

Experiment	*Culture plate*	*Seeding density (cells/well)*	*Media vol. (mL)*	*SNA conc. (nM)*
Cell viability assay	*96-well*	*$7X10^3$*	*0.1*	*10*
Fluorescence microscopy	*35-ø confocal dish*	*$2X10^5$*	*0.2*	*3*
Flow cytometry	*24-well*	*$5X10^4$*	*0.25*	*3*
TEM	*100-ø culture dish*	*$2.2X10^6$*	*6*	*10*
ICP-MS	*24-well*	*$5X10^4$*	*0.25*	*10*

References

1. J. Turkevich, P. C. Stevenson, J. Hillier, *Discussions of the Faraday Society* **1951**, *11*, 21
2. G. Frens, *Nature Physical Science* **1973**, *241*, 3.