

Supplementary Information

Self-assembled DNA tetrahedron as a carrier for in vivo liver delivery of siRNA

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Table S1. The sequences used for assembly of Td, siRNA, Td-siSC and Td-siApoB1

	Sequence (5'to3')
S1	[Cy5]CCAGGCAGTTGAGACGAACATTCCTAAGTCTGAAATT TATCACCCGCCATAGTAGACGTATCA
S2	CTTGCTACACGATTCAGACTTAGGAATGTTTCGACATGCGA GGGTCCAATACCGACGATTACAG
S3	GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGA GCATGCCCATCCACTACTATGGCG
S4	CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCA TGCTCTTCCCGACGGTATTGGAC
S4-SS (siApoB1)	CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCA TGCTCTTCCCGACGGTATTGGAC TTTTT rGrUrCrArUrCrArCrArCrUrGrArArUrArCrCrArA*rU
SS (siApoB1)	rGrUrCrArUrCrArCrArCrUrGrArArUrArCrCrArA*rU[Cy5]
AS (siApoB1)	rArUrUrGrGrUrArUrUrCrArGrUrGrUrGrArUrGrAmC*mA*rC
S4-SS (scrambled)	CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCA TGCTCTTCCCGACGGTATTGGAC TTTTT rGrUrGrArUrCrArGrArCrUrCrArArUrArCrGrArArU*[Cy5]
AS (scrambled)	rArUrUrCrGrUrArUrUrGrArGrUrCrUrGrArUrCrArC*mA*mC

m : 2'OMe-RNA, * : phosphorothioate linkage

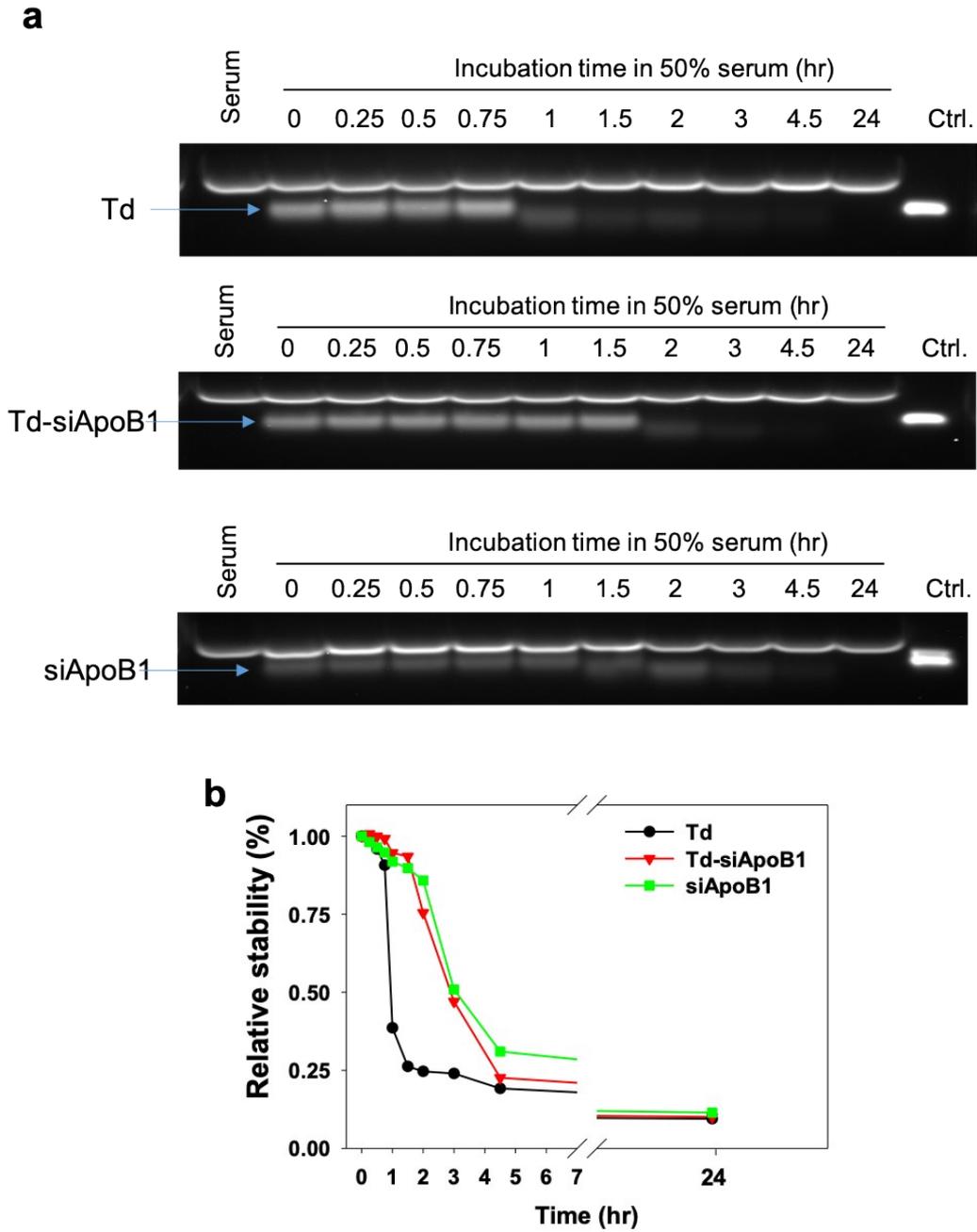


Fig. S1 (a) Agarose gel (1%) images showing serum stability tested in 50% mouse serum solution in a time course manner. Ctrl indicates untreated DNA or RNA sample. (b) The decrease of relative serum stability of Td, Td-siApoB1 and siApoB1 depending on time was illustrated in the graph.

Experimental Section

Self-assembly of Td, Td-siRNA and siRNA duplex

All DNA oligonucleotides were purchased from IDT. Td, Td-siRNA and siRNA duplex were assembled by mixing each strand (S1, S2, S3 and S4 for Td, S1, S2, S3, S4-SS and AS for siRNA labeled Td). Four or five sequences were mixed in TM buffer (10 mM Tris-HCl, 5 mM MgCl₂, pH 8). The mixture was denatured at 95°C for 5 min and then cooled to 4°C for 1 hour using RT-PCR machine (Applied Biosystems, USA).

Gel electrophoresis

The construction of Td and Td-siRNA was verified on non-denaturing polyacrylamide gel (6%). Gels were run in TBE buffer with 100V at 4°C for 50 min. After electrophoresis, gel was stained with SYBR gold solution and the image was visualized using iBright FL1000 (ThermoFisher Scientific, USA)

DLS

The hydrodynamic sizes of Td and Td-siRNA were measured with Zetasizer (Malvern, UK) by following the previously reported procedure. The concentration of samples used for the DLS analysis was 250 nM.

AFM

The size and morphology of Td and Td-siRNA were observed with atomic force microscopy (AFM; XE-100, Park Systems, Korea). Prior to sample treatment, mica was coated with NiCl₂ (50 μL, 4 mM) for 1 min. After cleaning the surface of mica with DW, Td and Td-siRNA (50 μL, 100 nM) in TM buffer was dropped onto mica and incubated for 1 min followed by Mg(OAc)₂ (50 μL, 2 mM) treatment. AFM images were obtained using a non-contact cantilever (PPP-NCHR, Park Systems, Korea) in non-contact mode and analyzed with XEI software (Park Systems, Korea).

Cellular uptake (confocal microscopic image and flow cytometer analysis)

To examine the cellular uptake of Td, Td-siRNA and siRNA duplex, HepG2 cells (Korean Cell Line Bank, Seoul, Korea) were seeded (5×10^4 cell/well) onto 24-well plate. After 24 h, the cells were then washed two times with PBS, and incubated with Td, Td-siRNA and siRNA duplex (labeled with Cy5, Final conc.=100 nM) in serum free DMEM at 37°C in a 5% CO₂ incubator. After 6 h, the cells were washed two times with PBS, trypsinized, then washed with cold PBS two times, resuspended in ice cold PBS (500 µL), and analyzed by means of a flow cytometry (Guava, Millipore, USA). Samples of at least 10,000 cells were analyzed in triplicate.

For confocal image of cellular internalization of structures, HepG2 (25000 cells) were seeded onto glass bottomed dish and incubated with Td, Td-siRNA and siRNA duplex(labeled with Cy5, final conc. was 100 nM) for 6 hr. The cells were then stained with Hoechst 34580 (1 µg/mL for 5 min, Invitrogen, USA) and washed with PBS two times. The intracellular fluorescence of the cells was imaged using a fluorescence microscopy (LSM 700, Carl Zeiss, Germany).

In vivo bio-distribution of Td, Td-siRNA and siRNA duplex

All experiments with live animals were performed in compliance with the relevant laws and institutional guidelines of KIST, and the institutional committees (the committee chair: Dr. Key-Sun Kim, the head of Research Animal Resource Center, KIST) have approved the experiments (2017-110). For in vivo imaging, Balb/c mice (male, 5 weeks old) were purchased from Orient Bio Inc.(Korea). Each sample was administered with Cy5 labeled Td, Td-siRNA and siRNA duplex (1 µM, 200 µL) by intraperitoneal injection. Fluorescence imaging was performed using the IVIS imaging Spectrum System (emission at 670 nm and excitation at 620 nm filter) and analyzed by IVIS Living Imaging 3.0 software.

Organ lysate analysis

At 2 h post injection of the constructs, the mice were sacrificed, and organs were harvested. The excised tissues were homogenized under liquid nitrogen and lysed with RIPA buffer. The lysed tissues were centrifuged (12,000 rpm, 10 min, 4 °C), and the supernatant of each

sample was analyzed using a fluorescence spectrophotometer (F7000, Hitachi, Japan). After excitation at 640 nm, the maximum emission intensity was acquired at 680 nm in the profile measured at 650–750 nm.

Western blot

Proteins in the cell or tissue lysate (20 µg) were separated by SDS-PAGE (8%), transferred to a polyvinylidene difluoride (PVDF) membrane, and incubated with solutions (5% BSA/TBST) containing an antibody (anti-ApoB antibody, 1:500, ThermoFisher Scientific, USA and anti-β-actin 1:1,000, Cell Signaling Technology, USA) at 4°C for overnight. Then, they were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:10,000, Santa Cruz Biotechnology, USA) at room temperature for 1 h. After washing, the protein bands were visualized using Super Signal™ West Pico Chemiluminescent (ThermoFisher Scientific, USA) and imaged by iBright FL1000 (ThermoFisher Scientific, USA)

qPCR

1. Sample treatment to cells and animals

- Transfection (Cell) : HepG2 cells were seeded onto 6 well plate . To measure siRNA mediated knockdown, cells were transfected with Td, siRNA and Td-siRNA were treated. siRNA transfection using Lipofectamine™ RNAi MAX(Invitrogen) was used as a positive control. After 48-hour post transfection cells were isolated and mRNA knockdown was measured as described below.
- Administration (Animal) : Balb/c mice (female, 8 weeks old) were purchased from Orient Bio Inc.(Korea). Each sample (PBS, Td, siApoB1 duplex, Td-siApoB1) was administered by intraperitoneal injection. At 48-hour post injection, livers were harvested.

2. Isolation of RNA and cDNA synthesis (Cells and animals)

: RNA was extracted from HepG2 cells (or mouse liver) using the RNeasy mini prep kit (Qiagen, , UK), 48 hours post-transfection. 2 µg total RNA was used for cDNA

synthesis with Top script™ Reverse Transcriptase and random hexamers primers in cDNA synthesis kit (Enzynomics).

3. RT-qPCR

: cDNA was mixed with 2 × SYBR Green master Mix (Applied Biosystems), ApoB1 primer pair (Forward: GGCACTGTGGGTCTGGAT, Reverse: TTCTTCTCTGGAGGGGACTG), or β-actin primer pair (Forward: AGAGCTACGAGCTGCCTGAC, Reverse: AGCACTGTGTTGGCGTACAG for cell, Forward: GCCATGTACGTAGCCATCCA, Reverse: ATGGCGTGAGGGAGAGCATA for animal sample) and then amplified by a StepOnePlus real-time PCR systems (Applied Biosystems). The amounts of amplified ApoB1 DNA were normalized against those of amplified β-actin DNA.

Td distribution in sectioned-liver tissue.

The harvested liver tissues were fixed in 4% formaldehyde solution, embedded in OCT, and cut into 15 μm sections. The sectioned liver tissues were analyzed using a fluorescence confocal microscopy (LSM700, Carl Zeiss, Germany) after immunostaining which was performed by same manner used for western blot. Human serum albumin antibody (for detection of hepatocyte, 1:200, ThermoFisher Scientific, USA) and FITC-labeled secondary antibody (1:1000, ThermoFisher Scientific, USA) was used for imaging.

Serum lipid analysis

For analysis of serum lipid levels in mice, Balb/c mice (female, 8 weeks old) were purchased from Orient Bio Inc.(Korea). Each sample was administered with Td, Td-siApoB1, Td-siSC and siApoB1 duplex (1 μM, 200 μL) by intraperitoneal injection. At 48 h post injection of the constructs, blood was collected by heart puncture after complete anesthesia was achieved.

The blood lipid level (Total cholesterol, HDL cholesterol, LDL cholesterol and Triglyceride) examined by enzymatic colorimetric tests using the reagents OSR6116, OSR6187, OSR6183 and OSR6133 (Olympus, Hamburg, Germany) with the automatic biochemistry analyzer according to the manufacturer's protocol.

Serum nuclease resistance

For the serum stability test, mouse serum (final conc.=50%, Sigma Aldrich, USA) were added to the DNA solutions (Td, Td-siApoB1 and siApoB1, 250 nM), and the mixtures were incubated at 37 °C. At each time point (0, 15, 30, 45 min, 1, 1.5, 2, 3, 4.5, 24 h), the solutions were quenched by adding the stop solution composed of EDTA (final conc.= 50 mM), proteinase K and 5% SDS (final conc.=2.5%) then, incubated at 37 °C. After 1 hr., samples were mixed with loading buffer (formamide, 0.5M EDTA), denatured at 95 °C for 10 min and then analyzed on SYBR gold-stained agarose gel (1%).