

## Electronic Supplementary Information (ESI)

### A Splice-Switch Oligonucleotide Loaded Self-Cleavable DNA Nanogel

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*Materials and reagents.* N-bromosuccinimide, 2,3-dimethylmaleic anhydride, benzoyl peroxide, sodium azide, acrylamide/bis-acrylamide (40%) and other chemicals were purchased from Sigma. All oligonucleotides were purchased from Integrated DNA Technologies Inc. (Coralville, IA) and used without further purification. DNA sequences used for the construction of acidic-pH self-immolative DNA nanogel are:

a1: 5'- CTCTACCGTAGCTCGCAGTCGCTGTATGAACCCAATCCCAATCCCAATCCC -3'  
 a2: 5'- TCATACAGCGACTGCACTGATCCGAGTCCGAACCCAATCCCAATCCCAATCCC -3'  
 a3: 5'- TCGGACTCGGATCAGAGAGCTACGGTGAGAGACCCAATCCCAATCCCAATCCC -3'  
 l1: 5'- /5DBCON/CACGTCTGCGTCGAGCTGGTGATTGGGATTTGGATTGTG -3'  
 l2: 5'- /5DBCON/CAGCTCGACGCAGACGTGGTGATTGGGATTTGGATTGTG -3'  
 b1: 5'- CTCTACCGTAGCTCGGTTCATGAGCCTGCT -3'  
 b2: 5'- AGCAGGCTCATGACCACTGATCCGAGTCCGA -3'

*Synthesis and characterization of acid-labile chemical linker, azidomethyl-methylmaleic anhydride (AzMMMan).*

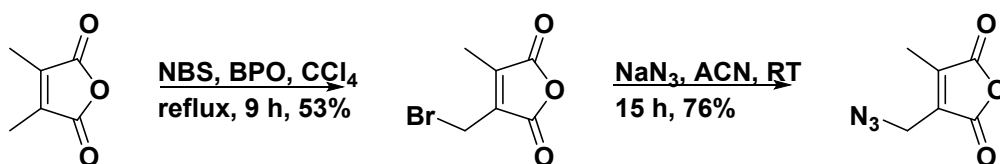
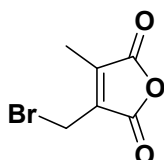
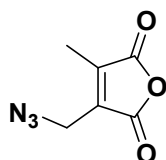


Figure S1. Synthesis of azidomethyl-methylmaleic anhydride (AzMMMan).



### 3-(bromomethyl)-4-methylfuran-2,5-dione

This compound was synthesized according to a reported protocol<sup>1-2</sup> with minor modifications. Dimethylmaleic anhydride (5.04 g, 40 mmol), N-bromosuccinimide (14.24 g, 80 mmol), and benzoyl peroxide (50%, 0.4 g, 0.83 mmol) were dissolved with CCl<sub>4</sub> (250 mL) in a 500-mL round bottom flask. This mixture was subjected to 4 rounds of vacuum and N<sub>2</sub> charge before gently refluxed for 5 h. The reaction was then cooled to room temperature, and a second batch of benzoyl peroxide (BPO, 50%, 0.4 g, 0.83 mmol) was added. The reflux was resumed and stopped after another 5 h. After cooling to room temperature, the reaction mixture was diluted with dichloromethane (DCM), and the resulting organic phase was washed twice with water (100 mL) and brine (100 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* to afford a crude product, which was purified by chromatography on a silica gel column and eluted with a mixture of hexane/ethyl acetate. Further purification was achieved by vacuum distillation to get a pure product (4.35 g, 53% yield). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 4.46 (q, J = 0.5 Hz, 2H), 2.9 (br, 3H).



### 3-(Azidomethyl)-4-methylfuran-2,5-dione

3-(Bromomethyl)-4-methyl-2,5-furandione (1.74 g, 8.5 mmol) was dissolved with acetonitrile (25 ml) in a 50-ml round bottom flask. Sodium azide (0.66 g, 10.2 mmol) was added in one portion. The suspension was stirred over night at room temperature. After filtration, the solvent was evaporated. The resulting residue was then purified by chromatographic purification over a silica gel column using hexane/ethyl acetate as mobile phase to give a pure product (1.08 g, yield 76%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 4.40 (q, *J* = 0.8 Hz, 2H), 2.10 (t, *J* = 0.9 Hz, 3H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ 165.84, 165.19, 144.31, 136.71, 42.74, 9.61.

*Modification of SSO with chemical linker AzMMMan.* The SSO was diluted into HEPES buffer (pH 9.0) with a concentration of 50 μM. Next, chemical linker (2 mM) was carefully added into the solution and reacted for 4 h at room temperature. The non-conjugated linker was removed, and the modified SSO was concentrated with Amicon Ultra centrifugal filter units (MWCO = 3 kDa, Millipore). The concentration of the modified SSO was measured by UV-vis spectroscopy. The product was kept under -20°C for future use.

*Click reaction-based linkage of SSO to DNA strands.* Copper-free cycloaddition between DBCO-modified DNA strands and linker-modified SSO (in an excess amount) was conducted in PBS buffer (pH 8.0) to get the conjugate SSO-I1 or SSO-I2. The excess SSO was removed by size exclusion chromatography using PBS buffer (pH 8.0) as the mobile phase.<sup>1</sup> The concentration of the conjugate was measured using UV-vis spectroscopy.

*Formation of DNA nanogel.* Briefly, stoichiometric quantities of the ssDNA strands for the formation of Ya (a1, a2, and a3), L (l1 and l2), and Yb (a3, b1, and b2) were separately added into three tubes containing 10 mM PBS buffer (pH 7.2, 10 mM MgCl<sub>2</sub>). Subsequently, each sample was heated to 95°C for 5 min and cooled to 4°C to form the desired building blocks. For the preparation of DNA nanogels, a stock solution of the building blocks was prepared, in which stoichiometric amounts of Ya, L, and Yb (4:6.5:1) were added in PBS buffer. The mixture was kept at 4°C overnight to form the DNA nanogels. To form the SSO-DNA nanogel, the conjugate SSO-I1 or SSO-I2 was used.

*PAGE analysis.* The 10% v/v PAGE gels were prepared and used in this study, which was run at a voltage of 180 V in 0.5× TBE buffer.

*Agarose gel electrophoresis.* The 4% w/v agarose gel was prepared and used, and it was run at a voltage of 80 V in 0.5× TBE buffer.

*Size and zeta potential measurements by dynamic light scattering (DLS).* The size and zeta potential characterizations of DNA nanogels were conducted with Zetasizer (Malvern Panalytical) or NanoBrook Omni particle size and zeta potential analyzer (Brookhaven).

*Atomic force microscopy (AFM).* The AFM images were taken in tapping mode (TAP 190-G, *k* ~ 48kHz) using a JPK Nanowizard 3.

*Fourier-transform infrared spectroscopy (FTIR).* The FTIR spectrum was collected with VERTEX 80v FT-IR Spectrometer (Bruker).

*Circular dichroism (CD) spectra.* The CD spectra of DNA strands at pH 7.2 and pH 5.0 were recorded in PBS buffer by using JASCO J-815 CD Spectrometer.

*High-performance liquid chromatography (HPLC) characterization.* HPLC analysis was performed with reverse-phase HPLC (Shimadzu, Japan) equipped with a Shim-pack GIST C18 column (5  $\mu$ m, 4.6  $\times$  150 mm i.d., Shimadzu, Japan) with a mobile phase of hexylammonium acetate (HAA) buffer and acetonitrile. UV absorbance was detected at 260 nm. The injection volume and flow rate were 50  $\mu$ l and 1 ml/min, respectively.

*In vitro studies.* To demonstrate the functions of SSO after encapsulation in DNA nanogel, *in vitro* studies were conducted. Huh7 cells (hepatocellular carcinoma) stably expressing the NATURA gene (unpublished data) were treated with 100 nM SSO-DNA nanogel or PBS, DNA nanogel, SSO. After 48 hours of treatment, the cells were collected and analyzed for fluorescence of the reporter gene using an Agilent Novocyte Pentecent flow cytometer.

The uptake of SSOs encapsulated in the DNA nanogel was evaluated in Huh7 cells expressing the NATURA reporter gene (unpublished data), designed to detect functional SSOs uptake. Under wild-type splicing conditions, the reporter gene produces eGFP. However, when targeted with SSOs, it produces tRFP. The degree of alternative splicing was assessed by measuring the protein percentage spliced index (pPSI), which was calculated by dividing the mean fluorescence of eGFP by the sum of the mean fluorescence of eGFP and tRFP. This index indicates the proportion of the wild-type transcript (eGFP) relative to the alternatively spliced transcript (tRFP). The value of pPSI ranges from 0 to 1, with wild-type conditions of 1, and decreases upon functional SSO uptake. Huh7 cells were treated with 100 nM of SSO-DNA nanogel targeting the reporter gene. As shown in Fig. S6, the Huh7 cells treated with SSO-DNA nanogel exhibited a decrease in pPSI compared to those treated with SSOs, DNA nanogel, or PBS.

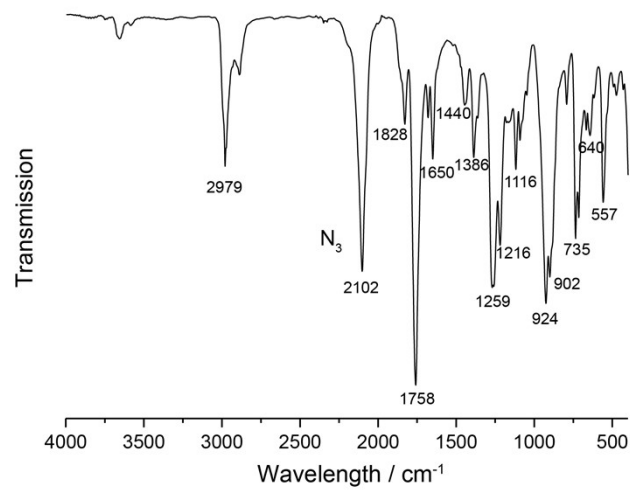


Fig. S1. FTIR spectrum of AzMMMan.

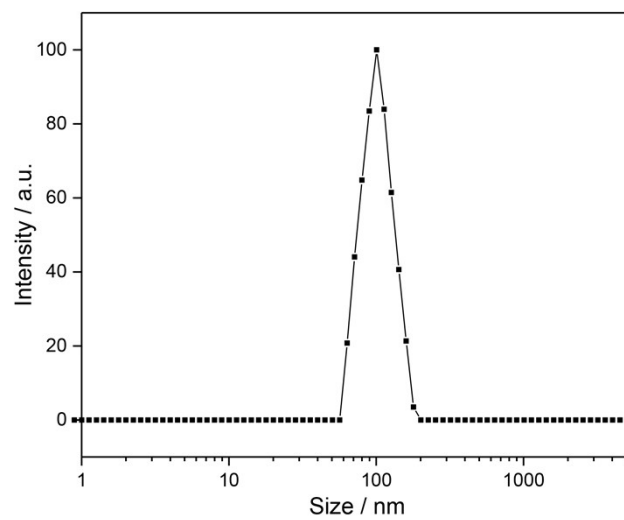


Fig. S2. Size distribution of DNA nanogels.

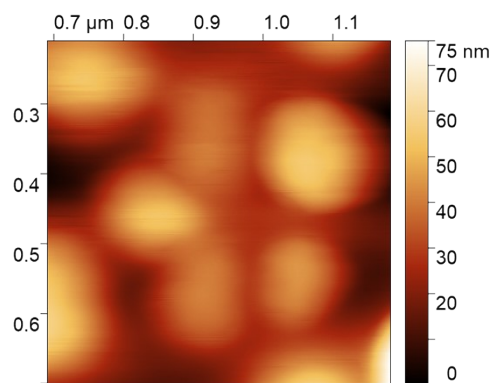


Fig. S3. AFM image of DNA nanogels.

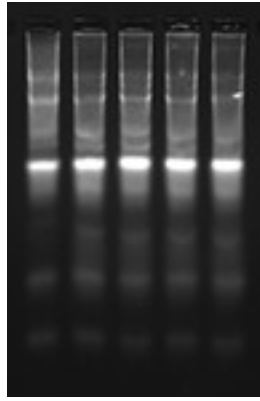


Fig. S4. Stability of DNA nanogels in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) for different durations (0, 1, 2, 4, and 8 h from left to right).

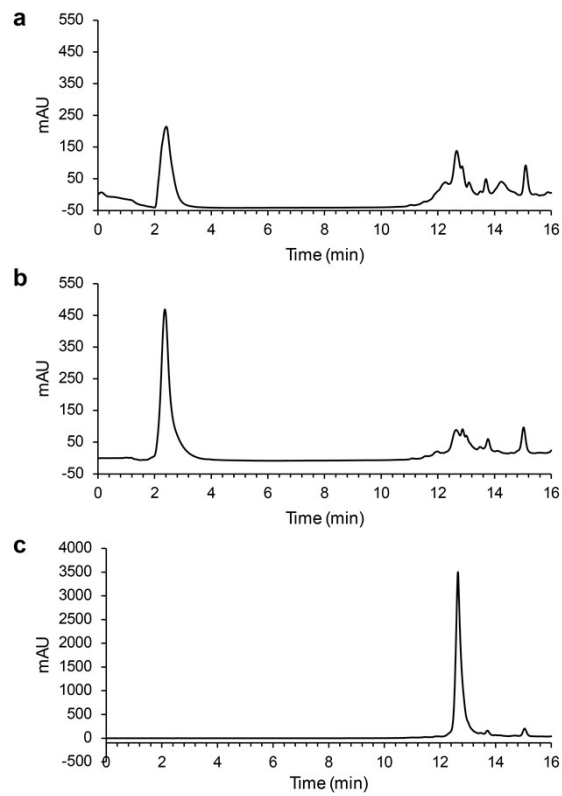


Fig. S5. HPLC characterizations of (a) SSO, (b) I1 and (c) SSO-I1 conjugate.

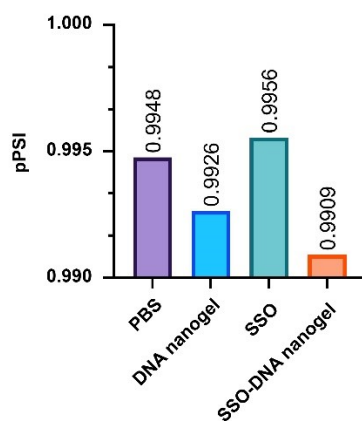


Fig. S6. pPSI values of NATURA-expressing Huh7 cells after 48 hours of treatment with 100 nM SSO-DNA nanogel, PBS, DNA nanogel or SSO.

References:

- 1 K. Maier and E. Wagner, *J. Am. Chem. Soc.* 2012, **134**, 10169-10173.
- 2 A. M. Deshpande, A. A. Natu and N. P. Argade, *J. Org. Chem.*, 1998, **63**, 9557-9558.