Supporting Information

Oligonucleotides with consecutive alkylated phosphate units: Aggregation characteristics and drug transport into living cells

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Experimental Section

General Procedures. All starting materials and reagents were purchased from Tokyo Kasei Kogyo (Tokyo, Japan), Wako (Tokyo, Japan) and Aldrich Chemical (Milwaukee, WI). All other solvents, purchased from Wako, were GR grade or dry grade and used without further purification. The ESI-MS spectra were recorded on a Exactive (Thermo) spectrometer. The organic reactions were carried out in oven-dried glassware under an argon atmosphere with magnetic stirring. Fluorescence spectra were recorded on a SHIMADZU RF-5300PC spectrofluorophotometer with a 1cm quartz cell. Hypoxic cell culture conditions was made by means of Russkin Invivo 400. ODN 1 was synthesized as described previously (ESI-MS: found 1840.93, calcd for $[M–H]^{2–}$ 1840.93) (K. Tanabe, Y. Ando and S. Nishimoto, *Tetrahedron Lett.*, 2011, **52**, 7135–7137).

Click reaction (Huisgen reaction) of ODN 1. ODN 1 (50 μM) was added to the aqueous solution of benzyl azide (500 μM), sodium ascorbate (50 mM) containing t-BuOH (20%). After the mixture was purged with argon for 15 min, the aqueous solution of $CuSO_4$ (30 mM) and TBTA (30 mM) was added, and the resulting mixture was

stirred at ambient temperature for 21 h. After the reaction, the crude products were purified by HPLC to give ODN 2. ESI-MS; found 2174.08, calcd for $[M–H]^{2–}$ 2174.09.

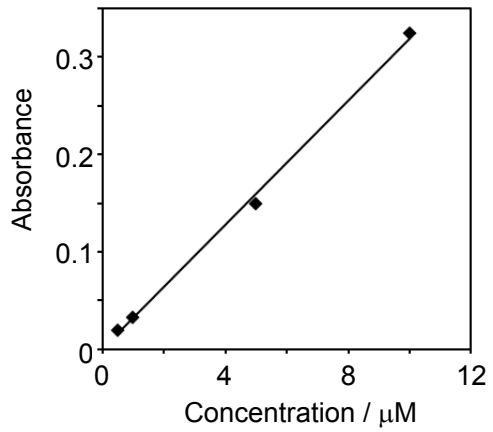
Measurement of Fluorescence Spectra. To form the aggregate, indicated concentration of ODNs in phosphate buffer (10 mM, pH 7.0) were added to nile red (10 μM) in acetonitrile. After the removal of the solvent *in vacuo*, the resulting mixture was dissolved in water to form the aggregate and measure the fluorescence spectra. We measured the fluorescence spectra by means of the excitation wavelength at 555 nm.

Mesurement of Dynamic Light-Scattering (DLS). The size distribution of aggregates was determined by Nano series Nano-ZS Zetasizer (Malvern Instrument). The aggregates consisted of ODNs (200 μM) were prepared in aqueous solution containing phosphate Na buffer (10 mM, pH 7.0) as described above. After the filtration using 0.45 μm filter, the measurements of DLS were conducted at 25 ˚C.

General procedure of fluorescence observation in living cells. To form the aggregate,

an aqueous solution of ODNs (200 μM) in was added to nile red (1 μM) in acetonitrile. After the removal of the solvent *in vacuo*, the resulting mixture was dissolved in Dulbecco's modified Eagle's minimum essential medium (DMEM) to assess fluorescence emission in the living cells.

A549 cells were cultured in DMEM containing 10% fetal bovine serum (FBS). The cells were seeded into 96-well plates (10000 cells/well) and cultured at 37 ˚C in a well-humidified incubator with 5% CO_2 and 95% air for 24 h. The cells were then incubated with the aggregate encapsulating nile red for 2 h. After the incubation, the medium containing the aggregate was replaced by Dulbecco's Phosphate Buffered Saline (DPBS), and the cells were were observed by the fluorescence microscope.



Oligonucleotides	Absorbance at 563 nm	Amount of nile red in aggregate (μM)
ODN 1	0.022	0.68
ODN 2	0.061	1.91

Figure S1. Estimation of the amount of nile red encapsulated in the aggregates. To form the aggregate, ODN 1 (200 μM) or ODN 2 (200 μM) in phosphate buffer (10 mM, pH 7.0) were added to nile red (10 μM) in acetonitrile. After the removal of the solvent in vacuo, the resulting mixture was dissolved in water to form the aggregate and measure the absorption spectra. Amount of encapsulated nile red was quatified by using the linear fit (solid line) given based on A_{563} of nile red (0.5, 1.0, 5.0 and 10 μM) in acetonitrile-H_2O (1:1) solution.