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Supporting Information

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for

Gene Silencing in Mammalian Cells with Light-Activated Antisense Agents

Douglas D. Young, Hrovje Lusic, Mark O. Lively,
Jeffrey A. Yoder, and Alexander Deiters*

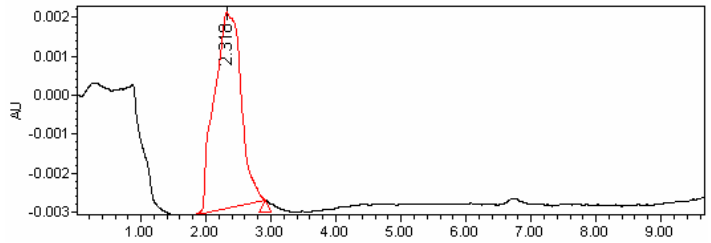
DNA Synthesis Protocol. DNA synthesis was performed using an Applied Biosystems (Foster City, CA) Model 394 automated DNA/RNA Synthesizer using standard ß-cyanoethyl phosphoramidite chemistry. All caged PS DNA oligomers were synthesized using 1 µmol scale, low-volume solid-phase supports obtained from Glen Research (Sterling, VA). Reagents for automated DNA synthesis were also obtained from Glen Research. Standard synthesis cycles provided by Applied Biosystems were used for all normal bases using 2 min coupling times. The coupling time was increased to 10 min for the positions at which the caged-T modified phosphoramidites were incorporated. Each synthesis cycle was monitored by following the release of dimethoxy trityl (DMT) cations after each deprotection step. No significant loss of DMT was noted following the addition of the caged-T for any of the phosphorothioates, so 10 min was sufficient to allow maximal coupling of the caged-T. Sulfurization of each base position was performed following each coupling step using 3H-1,2-benzodithiol-3-one 1,1-dioxide, known as Beaucage Reagent (R.P. Iyer, W. Egan, J.B. Regan, and S.L. Beaucage, *J. Am. Chem. Soc.* **1990**, *112*, 1253-1254), obtained from Glen Research. The reagent was dissolved in acetonitrile at a concentration of 1 g / 100 mL. Following the final coupling step and sulfurization, the DMT-protecting

group was left on the completed oligonucleotide. After deprotection and cleavage from the resin, the DMT-containing phosphorothioates were isolated using a C-18 reversed-phase solid phase extraction column (Waters Sep-Pak Classic C18 cartridge WAT051910). Removal of the last DMT group was accomplished by treatment of the oligonucleotide bound to the Sep-Pak column with 2% (*v/v*) trifluoroacetic acid in water and the fully deprotected oligo was eluted with 40% (*v/v*) MeOH in water.

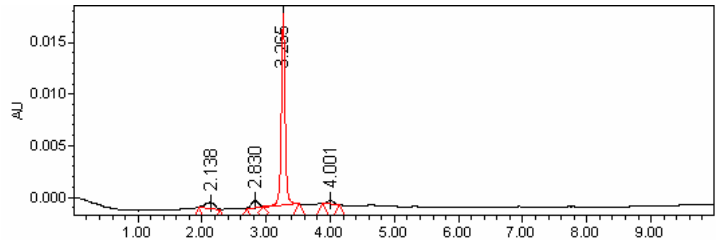
Hybridization assay. Complementary RNA was purchased from IDT DNA. Melting curves were measured on a BioRad MyiQ RT-PCR thermocycler by conducting a sequence of 3 heating and cooling cycles (10 μM of both PS DNA and complementary RNA with 12.5 μL iQ SYBR Green Supermix to a total volume of 25 μL; 30 to 80 °C with a 0.5 °C/min ramp). The non-caged PS DNA, 3-caged PS DNA, 4-caged PS DNA, and control PS DNA were analyzed in triplicate, and samples were either irradiated for (365 nm, 5 min, 23 W), or were not irradiated prior to measurement of the melting temperature.

HPLC assessment of decaging. To ensure effective decaging, samples were analyzed on a Hamilton reverse phase preparatory column (10 μM, 250 x 4.1 cm, PRP-1) via HPLC on a Waters 2796 HPLC coupled with a Waters 2996 photodiode array. A 10 μM sample of non-caged PS DNA was initially analyzed to establish optimal conditions (90% H_2O/ 10% acetonitrile isocratic 10 min, ramp to 90% acetonitrile 10 min; each solvent with 0.1% TFA). The 3-caged PS DNA was then run (10 μM) and the same sample was then irradiated with a hand-held UV lamp (365nm, 5min, 23W) and injected again. The original peak at 3.3 min completely disappeared and the chromatogram was similar to the wild type chromatogram with a peak at approximately 2.3 min.

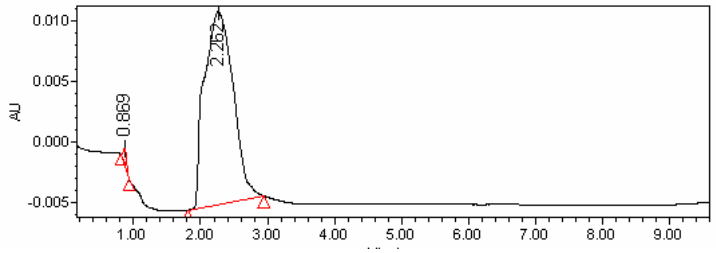
WT PS DNA



3-Caged PS DNA –UV



3-Caged PS DNA +UV



Spatial control of PS DNA activity. Mouse fibroblast cells (NIH/3T3) were grown at 37 ^{o}C and 5% CO_2 in Dulbecco's modified Eagle's media (DMEM; Hyclone); supplemented with 10% Fetal Bovine serum (FBS; Hyclone) and 10% streptomycin/ampicillin (MP Biomedicals). Cells were passaged into a 6-well plate (2 mL per well; ~1×10^4 cells) and grown to ~80% confluence within 24 h. The media was changed to OPTI-MEM (Invitrogen), and the cells were transfected with pRL-TK (0.5 µg, Promega), and the phosphorothioate DNA (250 pmol) using X-Treme GENE (3:2 reagent/DNA ratio; Invitrogen). The following conditions were used: no phosphorothioate oligomer, a sense-strand control phosphorothioate oligomer, and the phosphorothioate with either 3 or 4 caging groups. All transfections were performed in triplicate. Cells were incubated at 37 ^{o}C for 6 h and the transfection media was removed. A mask was made using aluminum foil, and cells were irradiated from underneath for 3 min with a hand-held UV lamp (365 nm, 25 W). The media was then replaced with standard growth media and the cells were incubated for an additional 24 h. After the 24 h incubation, the cells were observed and no changes in growth or morphology were visible when comparing the irradiated areas with the nonirradiated areas. Following the visi-

ble inspection, the media was removed and replaced with 1 mL of fresh media and 4 µg/mL of coelenterazine and incubated at room temperature for 10 min. The 6 well plate was then imaged on a Xenogen Lumina in vivo imaging system with a 60 s exposure time.

Cell viability assay. In order to assess the cellular toxicity of UV irradiation, 3T3 cells were either irradiated (hand held UV lamp, 365 nm, 5 min, 25W) or not irradiated (3 wells each in a 96-well plate). Cultures were allowed to grow for 24 h (a comparable time to the luciferase experiment), and then assayed using Cell Titer Blue Assay (Promega). A control experiment with dead cells was achieved via dessication of three wells. To each well 20 µL of Cell Titer Blue reagent was added and incubated with the cells for 4 h. Fluorescence measurements (λ_{ex} = 530 nm/λ_{em} = 590 nm) were taken on a Labsystems Fluoroskan Ascent FL plate reader. Non irradiated cells: 240 ± 34 RFU; irradiated cells: 233 ± 34 RFU; dead cells: 13 ± 1 RFU. Thus, no measurable cytotoxicity was observed as a direct effect of UV irradiation.