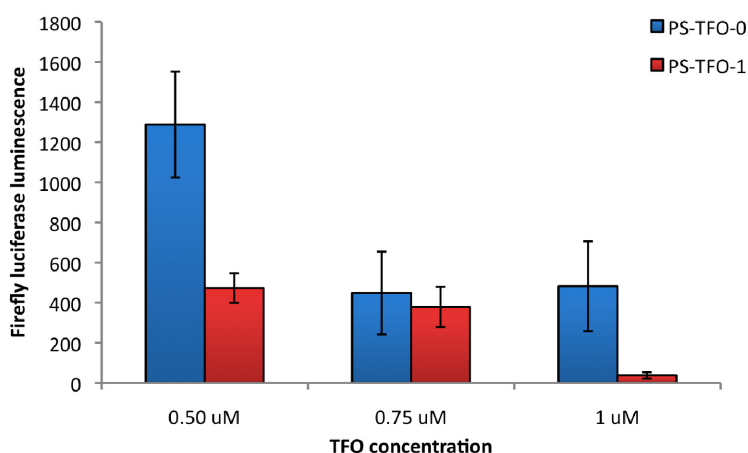


## Supporting Information

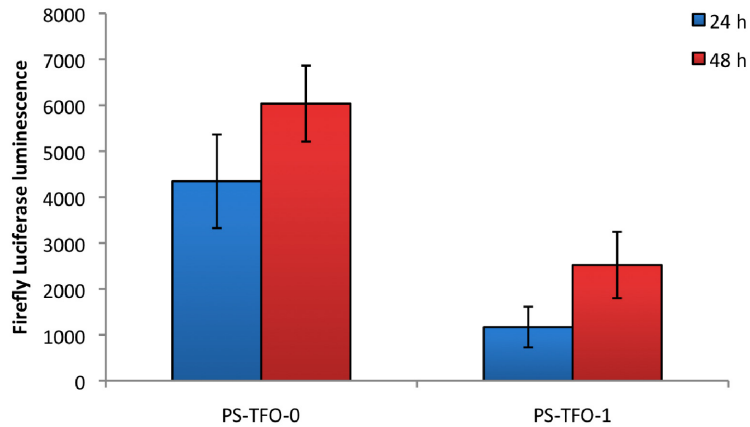
### Regulation of Transcription through Light-Activation and Light-Deactivation of Triplex-Forming Oligonucleotides in Mammalian Cells

Jeane M. Govan<sup>1</sup>, Rajendra Uprety<sup>1</sup>, James Hemphill<sup>1</sup>, Mark O. Lively<sup>2</sup>,  
and Alexander Deiters<sup>1,\*</sup>

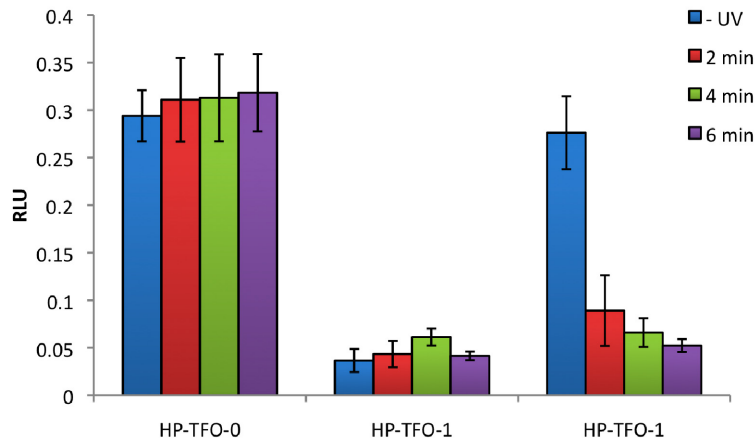
<sup>1</sup>North Carolina State University, Department of Chemistry,  
Raleigh, NC 27695, and <sup>2</sup>Wake Forest University School of Medicine,  
Center for Structural Biology, Winston-Salem, NC 27157  
[alex\\_deiters@ncsu.edu](mailto:alex_deiters@ncsu.edu)



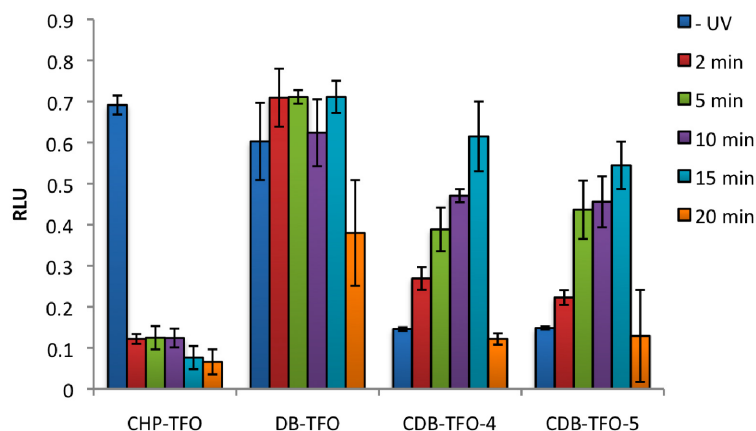
**Supporting Figure 1.** Optimization of TFO concentration. HEK 293T cells were transfected with pCyclin-D1  $\Delta$ -944 and increasing concentration of TFOs. After 24 h incubation, the cells were assayed with a Bright Glo Assay system (Promega). Error bars represent standard deviations from three independent experiments.



**Supporting Figure 2.** Analysis of TFO inhibition after 24h and 48h incubation in mammalian cells. HEK 293T cells were transfected with pCyclin-D1  $\Delta$ -944 and 0.5  $\mu$ M TFO. After 24 h and 48 h incubations, the cells were assayed with a Bright Glo Assay system (Promega). Error bars represent standard deviations from three independent experiments.



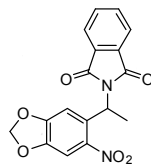
**Supporting Figure 3.** Irradiation time course of caged hairpin TFO. HEK 293T cells were co-transfected with pCyclin-D1  $\Delta$ -944, pRL-TK, and 0.5  $\mu$ M TFO. After transfection, cells were irradiated for 2, 4, or 6 min with a transilluminator (365 nm, 25W). After a 24 h incubation, the cells were assayed with a Dual-Luciferase Reporter Assay system. Error bars represent standard deviations from three independent experiments.



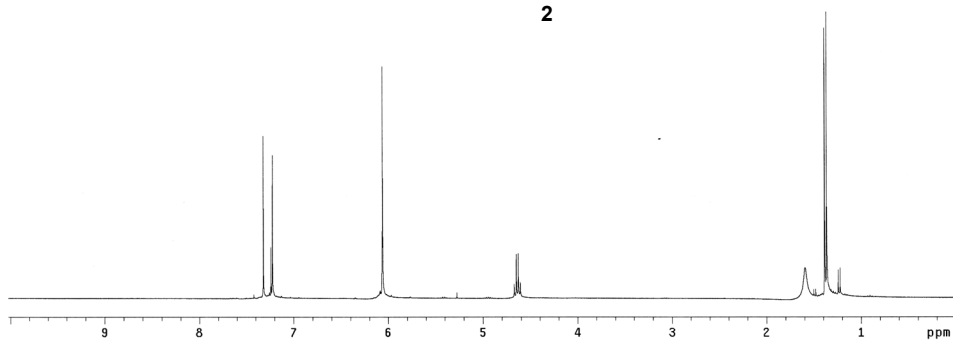
**Supporting Figure 4.** Irradiation time course of caged dumbbell TFOs. HEK 293T cells were co-transfected with pCyclin-D1  $\Delta$ -944, pRL-TK, and 0.5  $\mu$ M TFO. After transfection, cells were irradiated for 2, 5, 10, 15, or 20 min with a transilluminator (365 nm, 25W). After a 24 h incubation, the cells were assayed with a Dual-Luciferase Reporter Assay system. Error bars represent standard deviations from three independent experiments.

ru-120-IV-NPamine  
Pulse Sequence: s2pu1  
Solvent: CDCl3  
Ambient temperature  
File: ru-120-IV-ONB-ET-NH2  
Mercury-300BS "ncsumerc300"

Relax. delay 1.000 sec  
Pulse 35.0 degrees  
Acq. time 1.995 sec  
Width 4506.5 Hz  
16 repetitions  
OBSERVE H1, 299.7918160 MHz  
DATA PROCESSING  
FT size 32768  
Total time 0 min, 49 sec

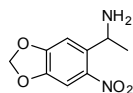


2

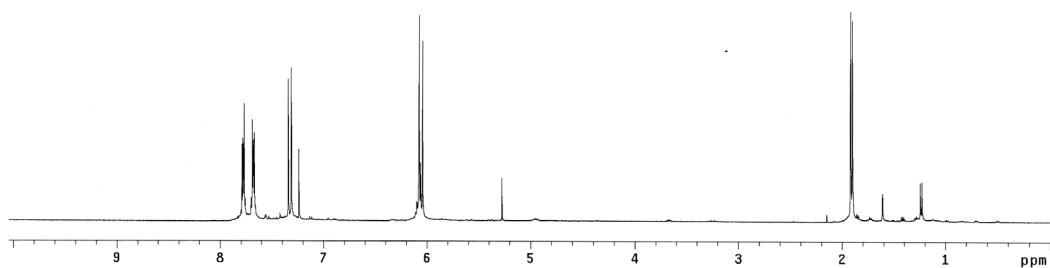


ru-119-IV-NPamide  
Pulse Sequence: s2pu1  
Solvent: CDCl3  
Ambient temperature  
File: ru-119-IV-ONB-ET-NH2  
Mercury-400BS "ncsumerc400"

Relax. delay 1.000 sec  
Pulse 35.0 degrees  
Acq. time 1.993 sec  
Width 6006.0 Hz  
16 repetitions  
OBSERVE H1, 400.1352012 MHz  
DATA PROCESSING  
FT size 32768  
Total time 0 min, 50 sec

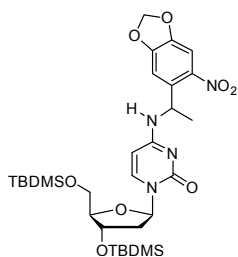


3

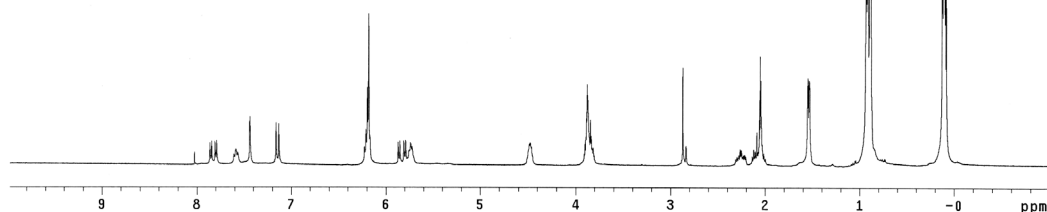


STANDARD 1H OBSERVE

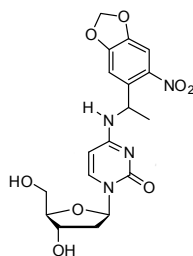
Pulse Sequence: s2pul  
 Solvent: Acetone  
 Ambient temperature  
 File: ru-122-IV-NP-2dC-OTBDMS  
 Mercury-400B6 "ncsumerc400"  
 Relax. delay 1.000 sec  
 Pulse 35.0 degrees  
 Acq. time 1.853 sec  
 Width 6006.0 Hz  
 16 repetitions  
 OBSERVE H1, 400.1372713 MHz  
 DATA PROCESSING  
 FT size 32768  
 Total time 0 min, 50 sec



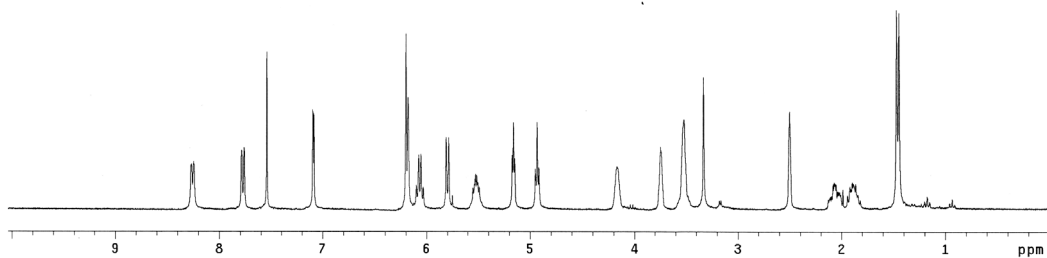
5



ru-126-IV-NP-2dC-alco  
 Pulse Sequence: s2pul  
 Solvent: DMSO  
 Ambient temperature  
 Mercury-300B6 "ncsumerc300"  
 Relax. delay 1.000 sec  
 Pulse 35.0 degrees  
 Acq. time 1.895 sec  
 Width 4506.5 Hz  
 16 repetitions  
 OBSERVE H1, 299.7932297 MHz  
 DATA PROCESSING  
 FT size 32768  
 Total time 0 min, 49 sec

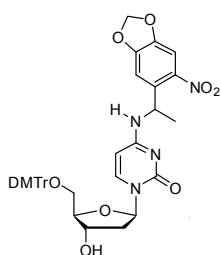


6

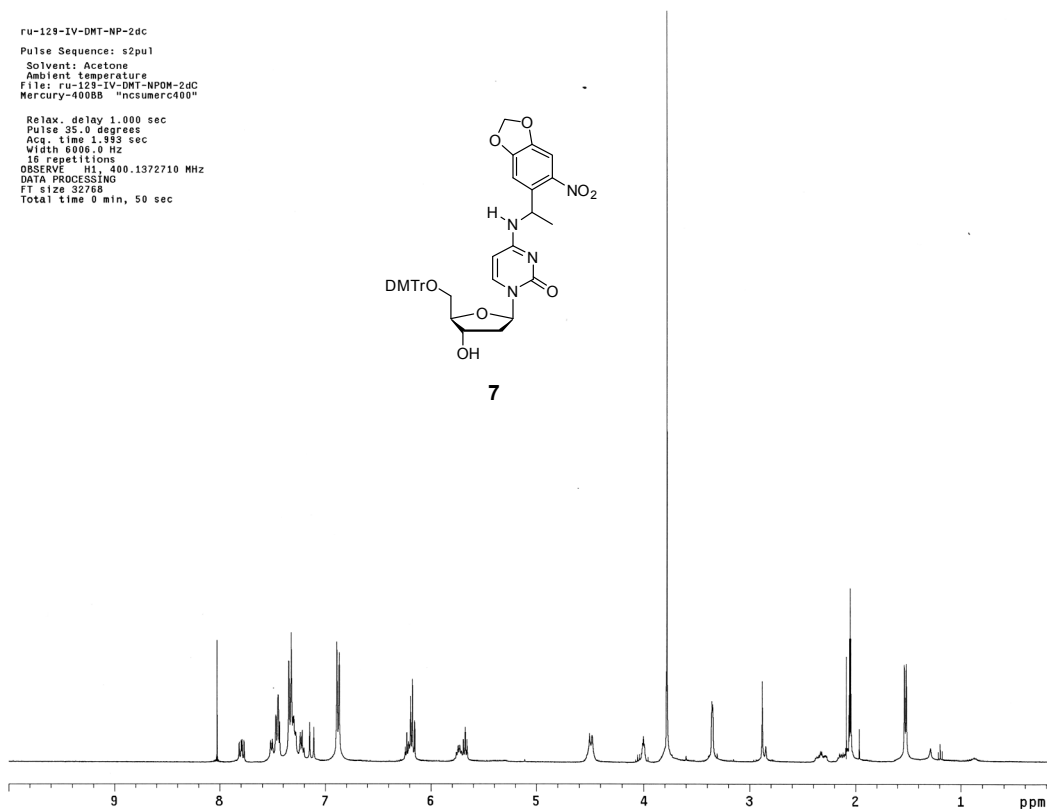


ru-129-IV-DMT-NP-2dc  
Pulse Sequence: s2pul  
Solvent: Acetone  
Ambient temperature  
File: ru-129-IV-DMT-NPDM-2dc  
Mercury-400BB "ncsumerc400"

Relax. delay 1.000 sec  
Pulse 35.0 degrees  
Acq. time 1.993 sec  
Width 6906.0 Hz  
16 repetitions  
OBSERVE H1 400.1372710 MHz  
DATA PROCESSING  
FT size 32768  
Total time 0 min, 50 sec

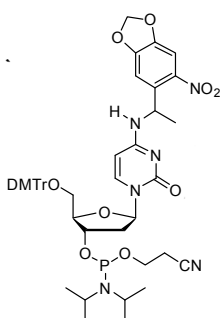


7



ru-130-IV-DMT-NP-2dc-Phos  
Pulse Sequence: s2pul  
Solvent: Acetone  
Ambient temperature  
File: ru-130-IV-DMT-NPDM-2dc-phospho  
Mercury-300BB "ncsumerc300"

Relax. delay 1.000 sec  
Pulse 36.0 degrees  
Acq. time 1.995 sec  
Width 4506.5 Hz  
16 repetitions  
OBSERVE H1 299.7933664 MHz  
DATA PROCESSING  
FT size 32768  
Total time 0 min, 49 sec



8

