Supporting Information for

Silver Nanoscale Antisense Drug Delivery System for Photoactivated Gene Silencing

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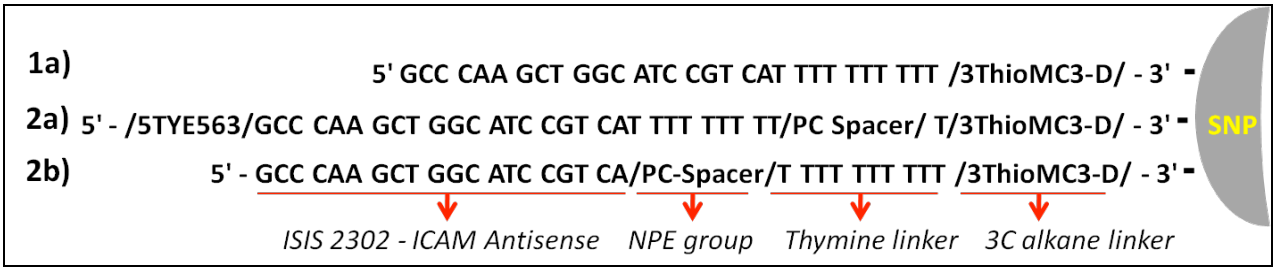
Design of Oligonucleotides

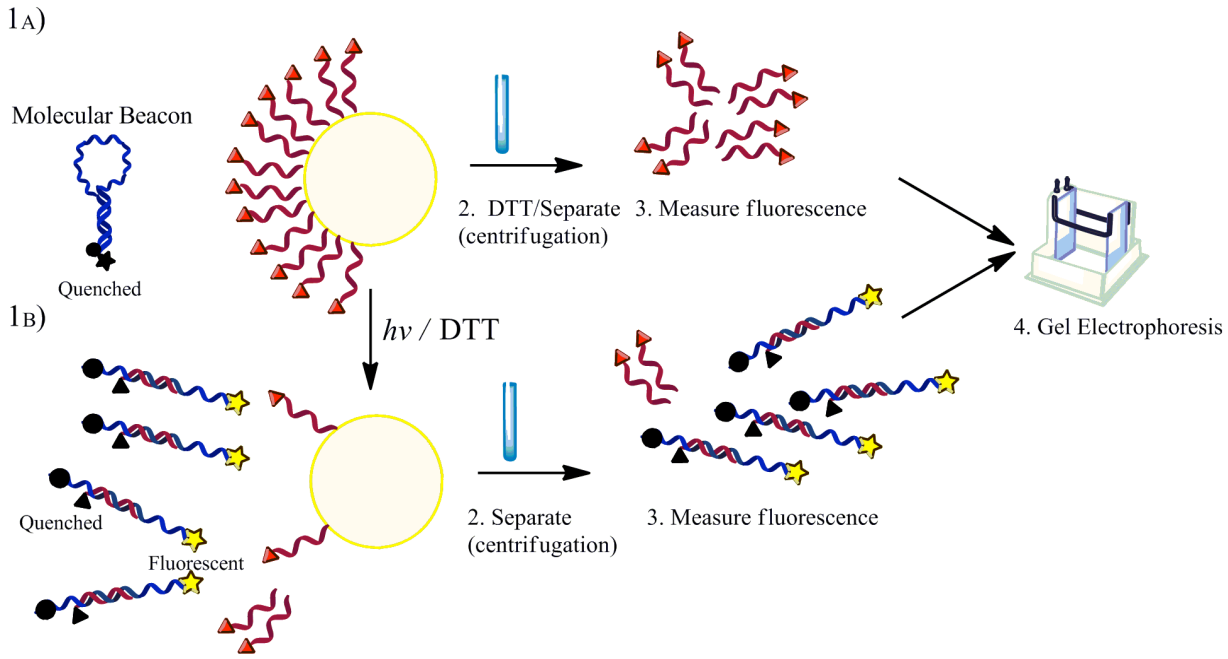
Provided is a visual schematic of particle-bound oligonucleotides used for in vitro and intracellular characterization of SNP-oligo conjugates:



Supplementary Figure 1 – Design of oligonucleotides for SNP functionalization. 1a) Thiol-modified oligonucleotide 2a) Fluorophore-labeled thiol-modified oligonucleotide with 1-thymine linker (***TYE-NPE(1n)-oligo***). 2b) Non-labeled thiol-modified oligonucleotide with 10-thymine linker (***NPE(10n)-oligo***)

Hybridization Assay Schematic

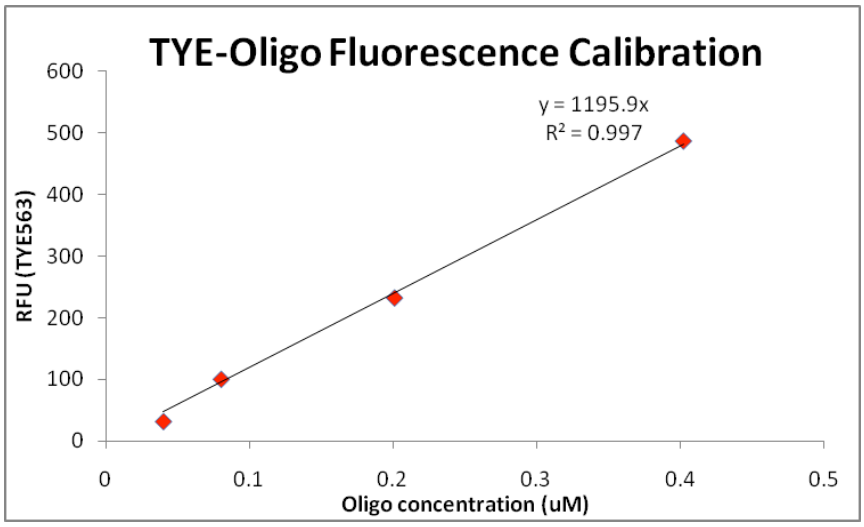
Provided is a schematic of the hybridization assay experimental set-up:



Supplementary Figure 2 - Hybridization assay schematic. A) Quantification of hybridization of molecular beacon (MB) to *TYE-NPE(1n)-oligo* in particle-bound case. B) Quantification of hybridization of MB to TYE-oligo in DTT-released and photoreleased cases.

Surface coverage Quantification: TYE-oligo calibration

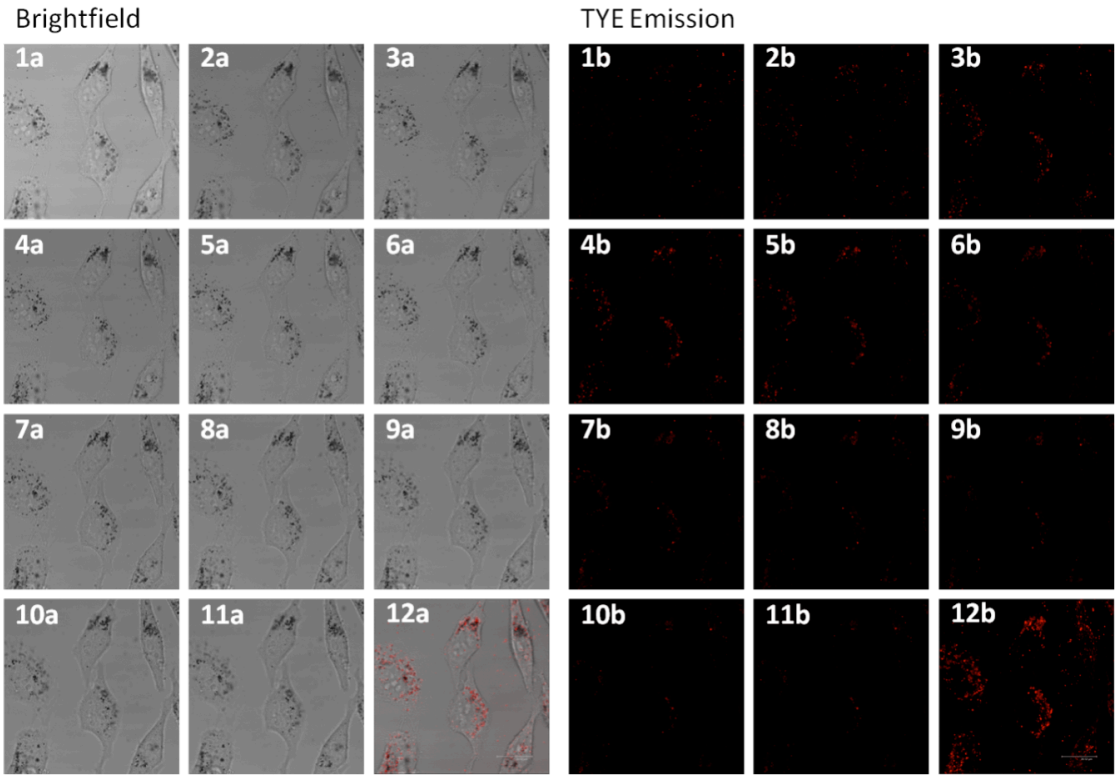
SNP-oligo conjugate surface coverage was quantified using fluorescence calibrations (Figure S-3) of *TYE-NPE(1n)*-oligo at known concentrations. Oligonucleotide at given concentrations in 100ul volumes were analyzed for fluorescence intensity values (540/563nm). These values were then used to evaluate experimental quantities of DTT-removed oligonucleotides from functionalized SNP surfaces.



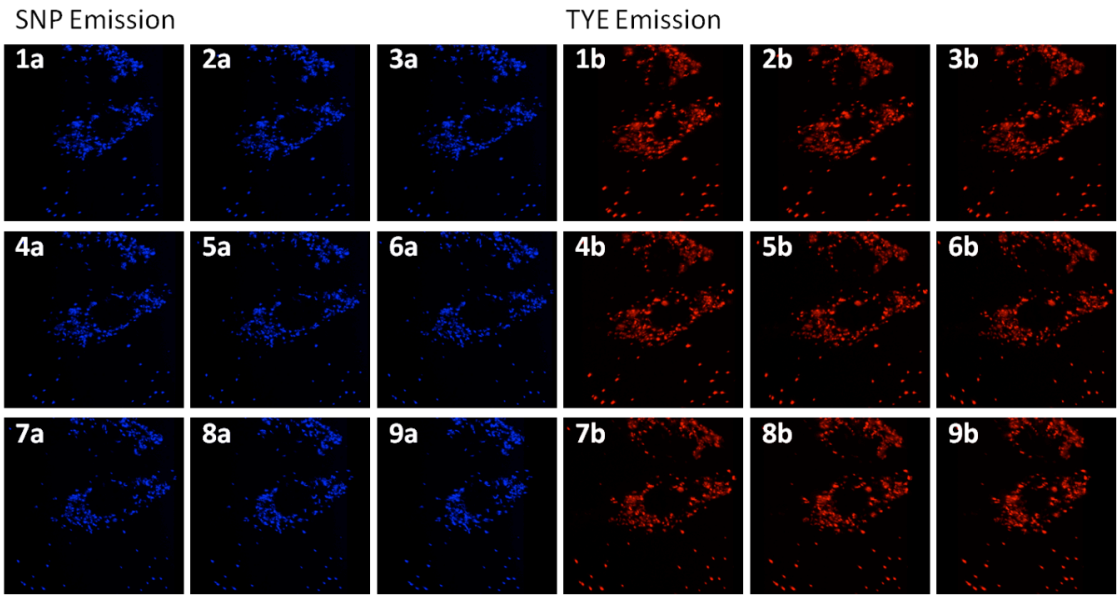
Supplementary Figure 3 – TYE-oligo calibration curve for surface coverage quantification

SNP-Conjugate Delivery: Confocal 3D Reconstruction

Confocal z-direction stacks were taken of SNP-TYE-NPE(1n)-oligo conjugate treated cells in order to verify intracellular localization. Select slices are shown (Supplementary Figure -4) which confirm that particles are located in the cytosolic environment of imaged cells. 3D reconstruction rotating images are also provided which reveal the three-dimensional SNP-oligo conjugate delivery throughout a treated HeLa cell (Figure S-5). Images were taken using a Leica DM IRE2 inverted microscope with a galvo-Z stage. Images were compiled and rendered into 3-dimensional projections using Leica Lite software.



Supplementary Figure 4 - Confocal stacks of HeLa cells treated with SNP-TYE-NPE(1n)-oligo. Shown are stack (1-11) and all-layer projection (12) images. Images depict a) brightfield and b) TYE emission (549/563nm) views.

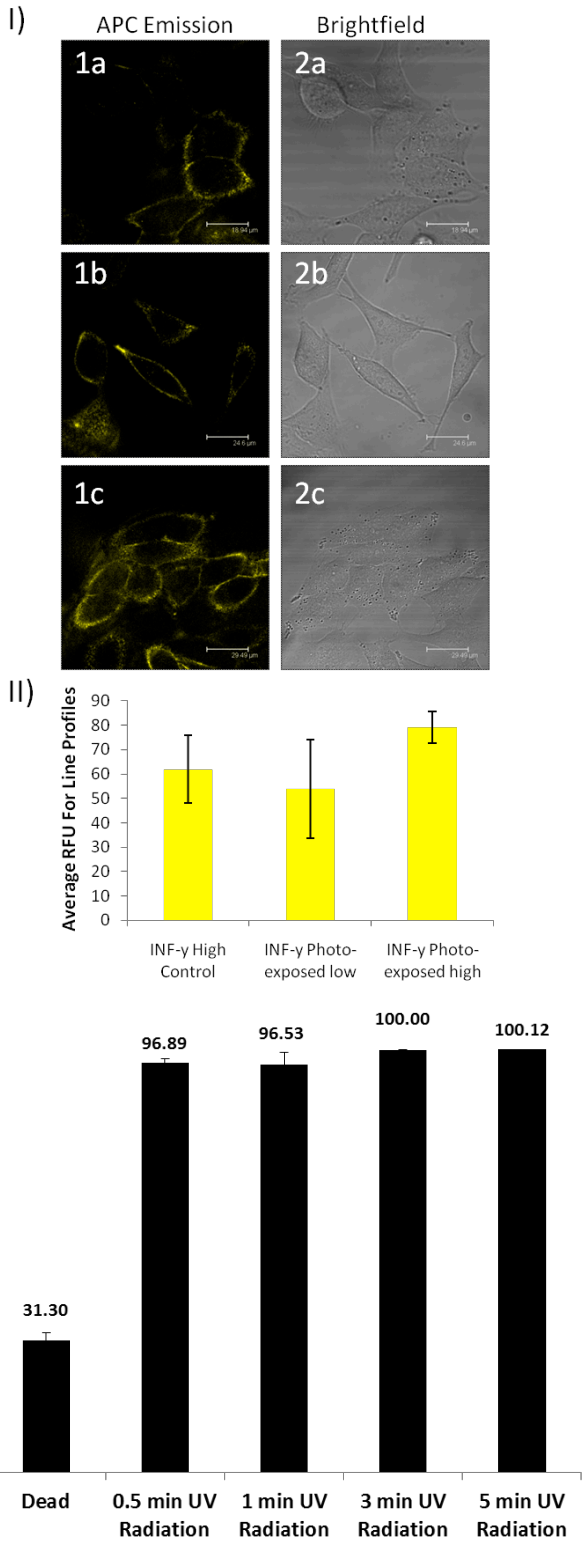


Supplementary Figure 5 - Confocal 3D images of a single HeLa cell treated with SNP-TYE-NPE(1n)-oligo. Shown are stack (1-9) images which depict a) SNP emission (480/520nm) and b) TYE emission (549/563nm) views.

HeLA Cell viability and ICAM-1 Expression Are Not Affected by Photoactivating Light Doses:

Confocal images were taken of ICAM-1 upregulated, photoexposed HeLa cells in order to verify that irradiation for the purpose of SNP-oligo photoactivation does not affect the anti-CD54 antibody labeling assay. Images are shown (*Figure 6 Panel (I)*) which confirm that low (image 1b) and high (image 1c) light doses at irradiation wavelengths of 365nm do not cause decreased ICAM-1 expression as compared to control (image 1a). Graphed means (+/- standard deviation) of average fluorescence (633/680nm emission) intensities over periphery line scans in Panel (II) confirms that ICAM-1 expression is not affected ($p > 0.05$) by light doses employed for antisense photoactivation.

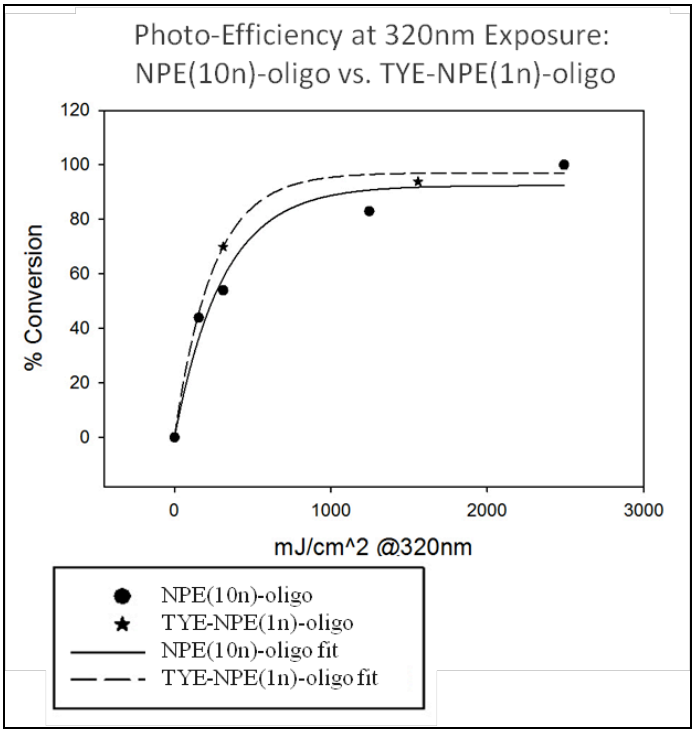
Panel (III) shows the results of HeLa cell viability when treated with doses of light ranging beyond those used in the manuscript's cellular experiments. There were no siginificant differences between live cell controls and cells treated with 0.5-5 minutes of the UVA



Supplementary Figure 6 – UVA dose effects on HeLA ICAM-1 expression and viability.

illumination used in the other experiments. Brief methods of this assay follows. HeLa cells were maintained in 25 cm^2 flasks (BD Falcon, Franklin Lakes, NJ) with 5 ml of Dulbecco's Modified Eagle's medium-reduced serum (DMEM-RS) augmented with 10% fetal bovine serum (FBS) and incubated at 37ºC in 5% CO^2. Cells were plated at a density of $1x10^6$ cells cm^{-2} in 12-well culture plates (BD Falcon, Franklin Lakes, NJ) and were allowed to adhere and grow for 24 h prior to UVA treatment (photoexposure methods were conducted as described in Materials & Methods). For a necrotic control, HeLa cells were incubated for 24 hr with 2 mM hydrogen peroxide (H_2O_2). After treatment, non-adhered cells were washed with 1 ml D-PBS and pooled with trypsinized adherent cells (0.25% Trypsin) followed by addition of 0.6 ml DMEM-RS and centrifugation at 1800 rpm. Cells were re-suspended in 1 ml D-PBS and stained with 3 µl of 500 µM Sytox Red (Invitrogen Molecular Probes, Carlsbad, CA) for 15 mins in the dark, then centrifuged and fixed in 250 µl of 1% paraformaldehyde (PFA) solution in PBS for flow cytometric analysis of viability with a BD FACS Calibur cytometer (Franklin Lakes, NJ). For each sample, scatter and fluorescent data was collected for 30,000 cell events using Cellquest pro (BD Biosciences, San Jose, CA). Toxicity was evaluated for cells containing above-threshold levels of Sytox Red (FL3) fluorescence and evaluated in triplicate, with statistical Two-Sample Assuming Unequal Varaiances dependent t-Tests.

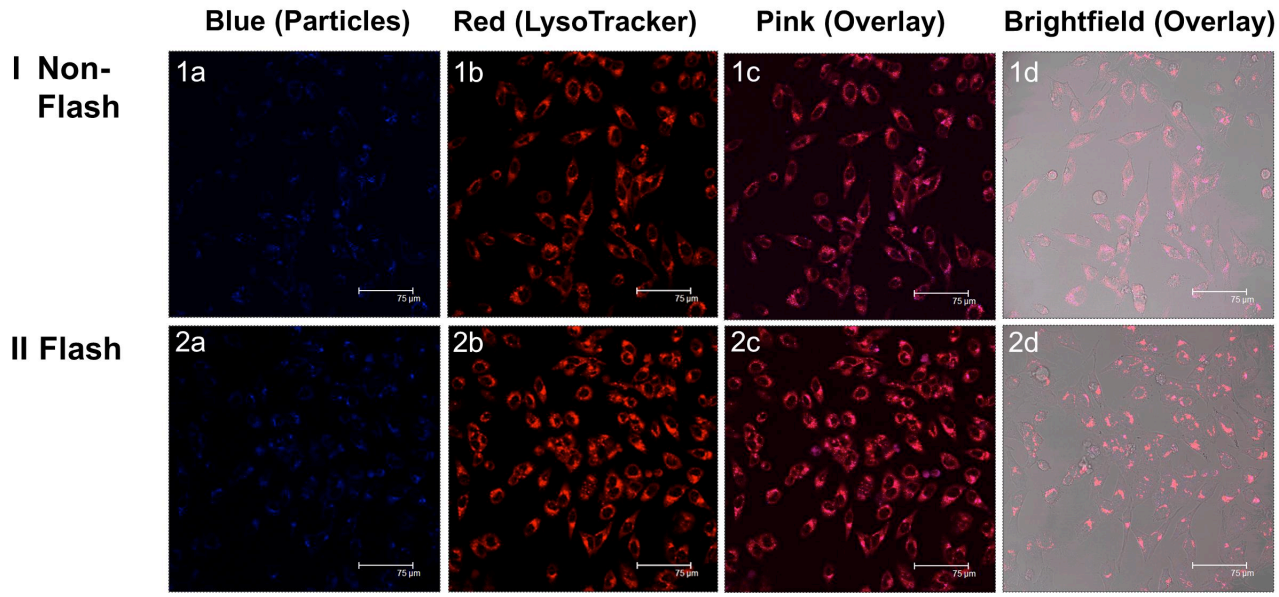
Silver Substrate Distance-Dependent Photochemistry:



Supplementary Figure 7 - Percent conversion of SNP-bound to photoreleased oligonucleotide states. a) Comparison of NPE(10n)-oligo and TYE-NPE(1n)-oligo photorelease from the SNP surface at a photo-irradiation wavelength of 320nm

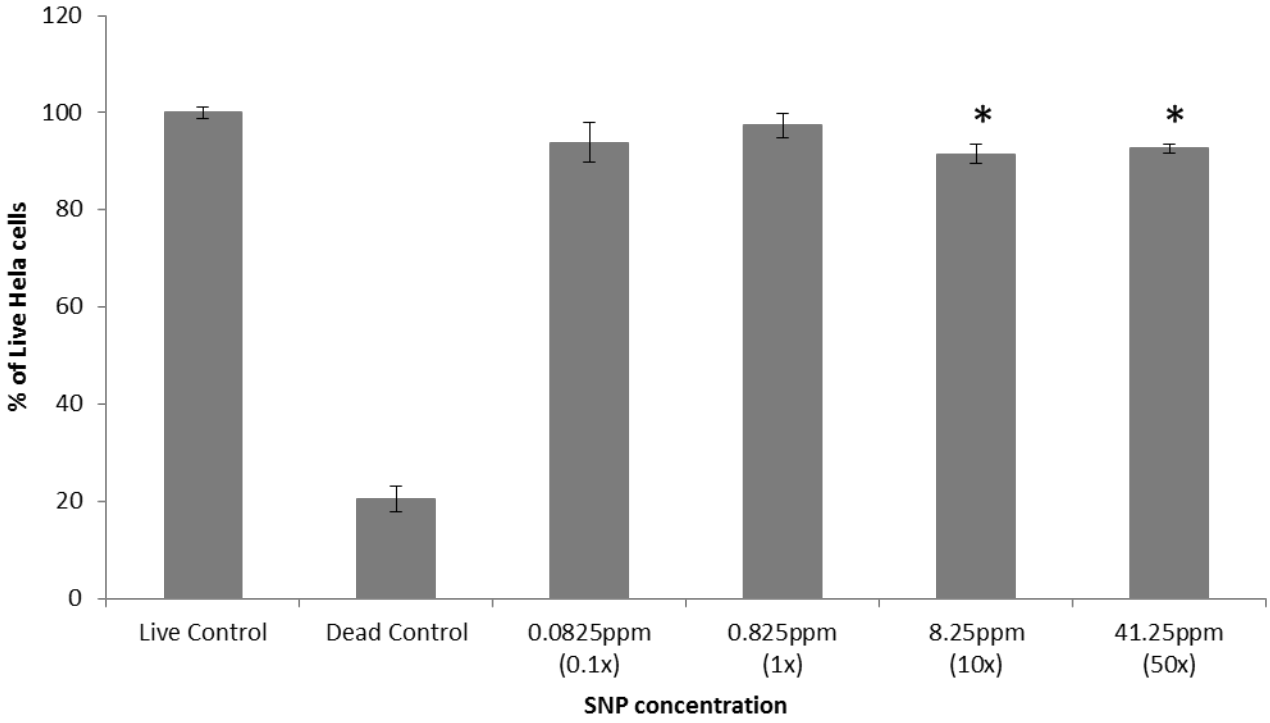
Supplementary Table 1 - Photocleavage properties of SNP-bound photolabile oligonucleotide

Condition/Oligonucleotide	Extinction (ε) M^{-1} cm^{-2} 320nm	Extinction (ε) M^{-1} cm^{-2} 365nm	Quantum Yield (ϕ) @320nm	Quantum Yield (ϕ) @365nm
Immobilized NPE(10n)-oligo	3520	660	0.16	0.04
Immobilized TYE-NPE(1n)-oligo	3520	660	0.09	---



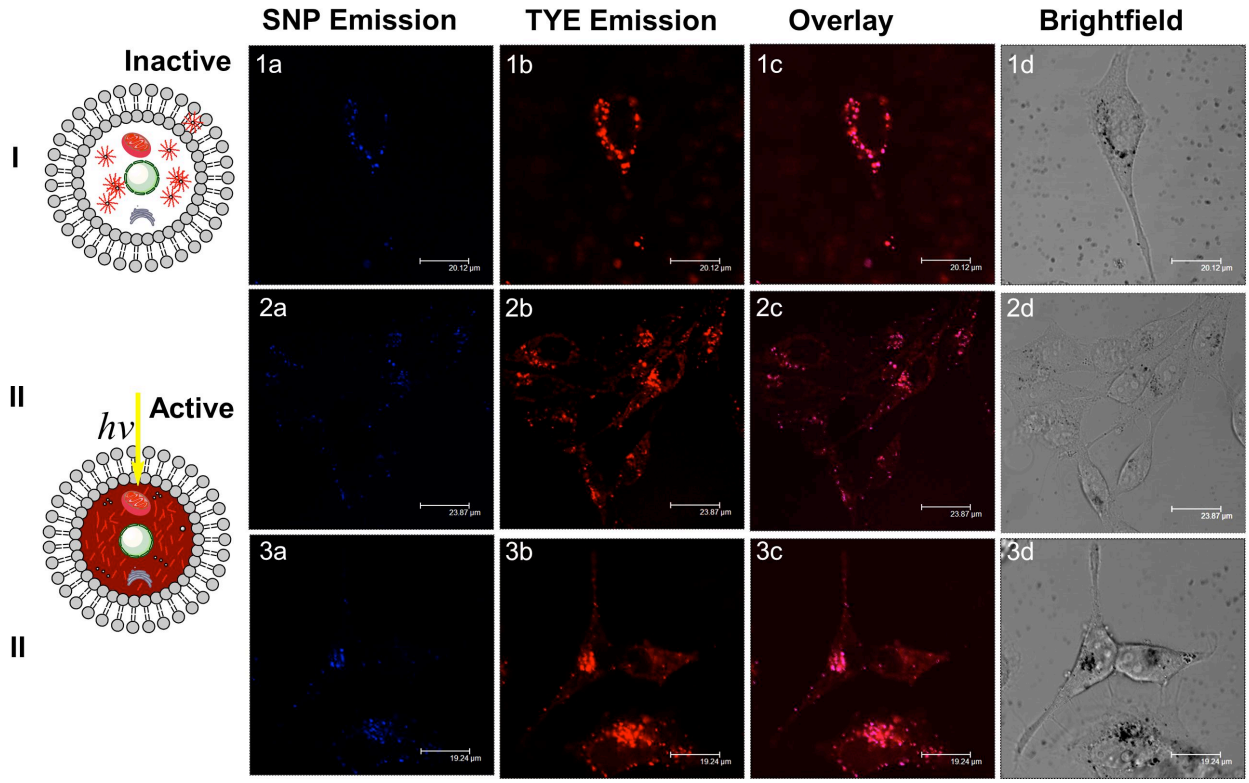
Supplementary Figure 8 – SNP-treated HeLa cells stained with the lysosomal probe LysoTracker Red does not show colocalization of these signals that would be indicative of lysosomal entrapment in the non-flashed or photoexposed cases.

SNP Cytotoxicity Assay:



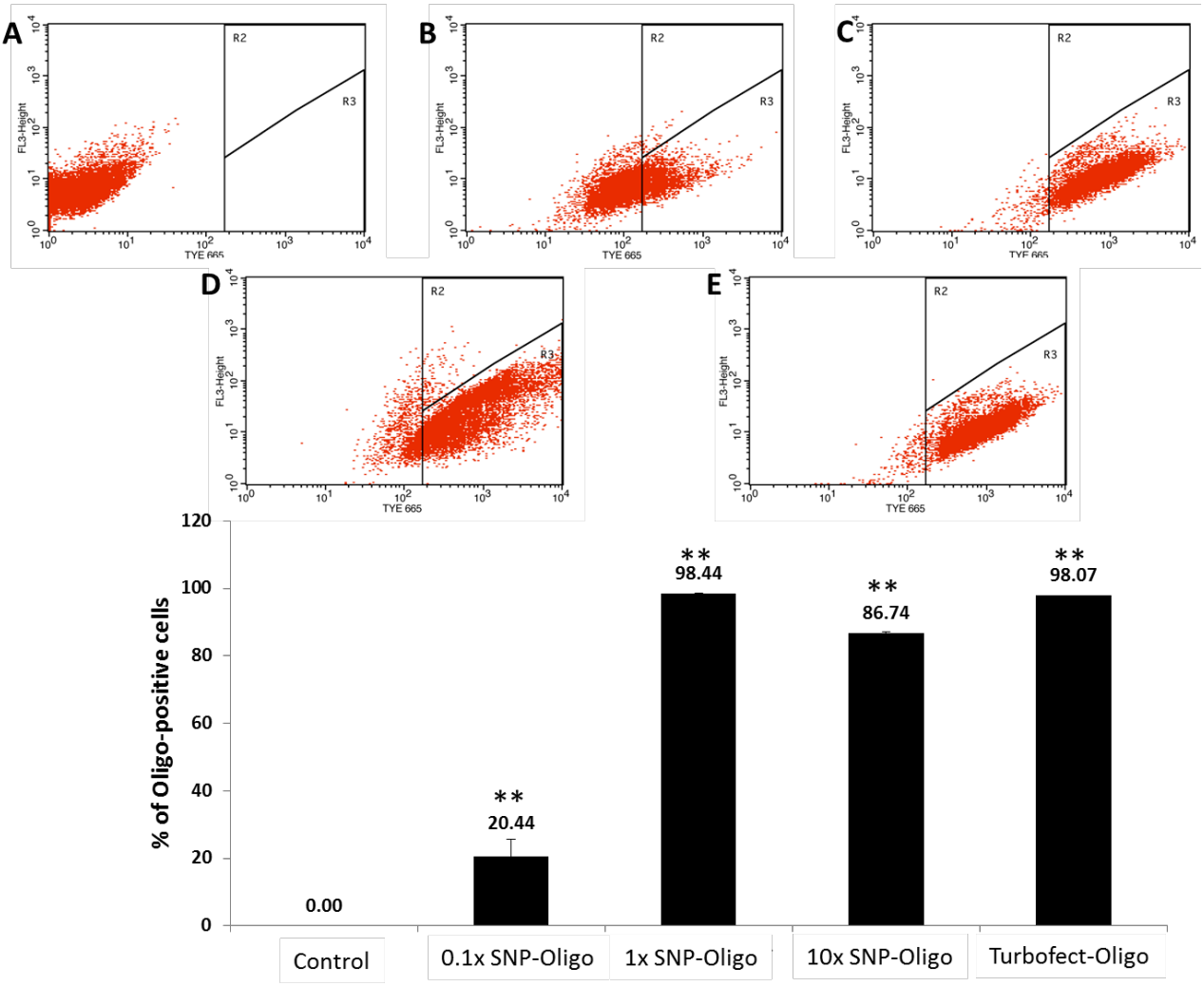
Supplementary Figure 9 – Percentage of live HeLa cells following 24h treatment with SNP show no toxicity (via Sytox Red) at the concentration used in the antisense experiments (0.825ppm). Concentrations of silver citrate particles (SNP) from 0.1x to 50x the doses used in the antisense experiments (0.0825 ppm, 0.825 ppm, 8.25 ppm, and 41.25 ppm) were evaluated. Methods of the toxicity assay are described in Supplemental Figure 6. Asterisk indicates significant difference from live control cells ($p < 0.05$).

Additional Examples of Diffuse Cytosolic Oligonucleotide Fluorescence following Photoactivation



Supplementary Figure 10 - Photoreleased SNP-TYE-NPE(1n)-oligo. I. Schematic of intracellular (a) particle-bound oligonucleotide ligand vs. (b) photoreleased ligand. II. (1) Non-released vs. (2,3) photoreleased SNP-TYE-NPE(1n)-oligo conjugates samples. Images depict (a) particle reflectance emission (488/520nm), (b) TYE fluorescence (549/563nm), (c) fluorescence overlay, and (d) brightfield views. Particle fluorescence is displayed in blue for ease of visualization of overlay regions in pink. Rows 1 and 2 are identical to Figure 5 in the main manuscript; Row 3 is provided as additional data for this experiment.

Flow Cytometric Analysis of SNP-Oligo Uptake in HeLa Cells



Supplementary Figure 11 – Flow cytometric analysis of HeLa cell fluorescence was used to assess the uptake of SNP-oligo conjugates. SNP-oligo conjugates identical to those used in other cellular experiments, except with a different fluorophore (TYE665, IDT, Coralville, IA) were used for detection via flow cytometry (Ex 633nm, Em 661/16nm; see Supplementary Figure 9 for cell treatment method details). HeLa cells were treated with 0.1x,1x or 10x the SNP-oligo conjugate doses used in the antisense experiments (A: control; B: 0.75 ul of SNP-oligo in 600ul media, C: 7.5ul, D: 75ul) and compared to free TYE665-oligonucleotide (E: 100ng/600ul) transfected with Turbofect polymer as in the antisense experiments. Supporting 2D plots of FL3 vs. FL4 fluorescence for representative samples illustrate the large increases in fluorescence detected in those cells containing SNP-oligo conjugates, similar to transfected oligo treatment. The percentage (mean ± std. error, n>3) of cells in the positive gate (R3) for each treatment are shown in the bar graph, where asterisks indicate significant differences from control.