Supporting Information

Modular plasmonic nanocarriers for efficient and targeted delivery of siRNA

Xiao Huang[1], Alessia Pallaoro[1], Gary B. Braun[2], Demosthenes P. Morales[1], Maria Olu Ogunyankin[3], Joseph Zasadzinski[3], Norbert O. Reich[1]*

[1] Department of Chemistry and Biochemistry, University of California, Santa Barbara, CA 93106, USA

[2]Cancer Research Center, Sanford-Burnham Medical Research Institute, La Jolla, CA 92037, USA

[3]Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN 55455, USA

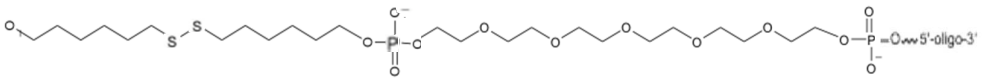
*Address correspondence to: reich@chem.ucsb.edu

Oligonucleotides

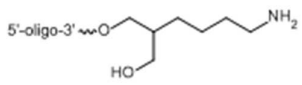
Table S1. Oligonucleotides used.

Oligos	5'	Sequence (5'→3')	3'	Use
RNA-1	Thiol-PEG18	GGGCGGCUUUGCCAAGUGCUU	NH_2	Short RNA anchor (sense)
RNA-2	-	AAGCACUUGGCAAAGCCGCCCUU	-	Knockdown control (anti-sense)
RNA-3	-	GGGCGGCUUUGCCAAGUGCUU	-	Knockdown control (sense)
RNA-4	Quasar 570	AAGCACUUGGCAAAGCCGCCCUU	-	Knockdown control/ Quantification/Imaging
RNA-5	-	UGGGACUUCAAGUAGACGUGGUGGCUU AAGCACUUGGCAAAGCCGCCCUU	-	Modular design test
RNA-6	-	UGGGACUUCAAGUAGACGUGGUGGCUU AAGGGCGGCUUUGCCAAGUGUUU	-	Modular design test
RNA-7	Quasar 570	GGGCGGCUUUGCCAAGUGCUU	-	Modular design quantification/knockdown
RNA-8	Quasar 570	GCACUUGGCAAAGCCGCCCUU	-	Modular design quantification/knockdown
DNA-1	Thiol-PEG18	ACCCTGAAGTTCATCTGCACCACCG	NH_2	Short DNA anchor
DNA-2	FAM	CGGTGGTGCAGATGAACTTCAGGGT	-	Knockdown control/ Quantification
DNA-3	Thiol-PEG18	ACCCTGAAGTTCATCTGCACCACCG	Quasar 570	Anchoring quantification
DNA-4	NH_2	GCCACCACGTCTACTTGAACTCCCA	Thiol-PEG18	Modular design anchor

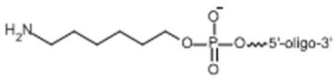
5' Thiol-PEG18:



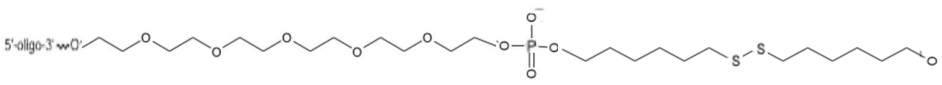
3' NH_2:



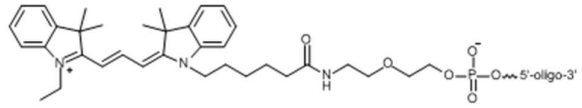
5' NH_2:



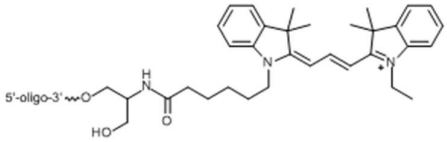
3' Thiol-PEG18



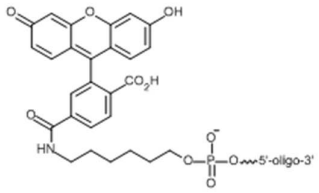
5’ Quasar 570:



3’ Quasar 570:



5’ FAM:



Materials and Methods

Au Nanoshell Synthesis. Hollow gold nanoshells (HGN) were synthesized as described previously.[1,2] Silver seed particles were prepared by reducing a stirred solution of 500 mL of 0.2 mM $AgNO_3$ with 0.5 mL of 1.0 M $NaBH_4$ in the presence of 0.5 mM sodium citrate in deionized water. The solution was stirred for two hours at 60°C to allow $NaBH_4$ to fully hydrolyze. Larger silver nanoparticles to be used as sacrificial templates for HGN were grown from the silver seed solution by adding 0.75 mL of 2 M NH_2OH HCl and 1.75 mL of 0.1 M $AgNO_3$ and stirring overnight. The galvanic replacement of the silver template particles with gold was optimized to have an absorbance peak at around 800 nm by quickly mixing 3.2 mL of 25 mM $HAuCl_4$ at 60°C. The solution was cooled to room temperature (RT) and kept in dark for at least several days to allow the excess Ag^+ that formed AgCl to settle to the bottom. HGN was then placed in a 3500 MWCO Slide-A-Lyzer (Thermo) in 3500 mL

of 500 μM citrate buffer at pH 5.5. 0.1% of diethylpyrocarbonate (DEPC, Sigma) was added to neutralize any RNase activity, and stirring for 2 days at RT allows the DEPC to decompose. TEM imaging was performed using a FEI Tecnai G2 Sphera microscope (200 kV). Size distribution analysis was done by dynamic light scattering (DLS) with a Malvern Nano ZS with autotitrator instrument. Optical characterization was performed by UV-VIS spectrophotometry with a UV-1700, PharmaSpec instrument. Particle concentration was estimated using the nanoparticle-tracking analysis system Nanosight LM10HS (Nanosight, Amesbury, UK).

Anchoring DNA or RNA Preparation. The siRNA sequence (sense: RNA-3, Table S1; anti-sense: RNA-2, Table S1) was used previously to knockdown *plk1* gene expression.[3] Modified sense RNA strand RNA-1 (Table S1), control DNA strand DNA-1 (Table S1) and anchoring DNA strand for modular design DNA-4 (Table S1) were purchased from Biosearch Technologies. The RNA strand was dissolved in water (RNase-free) to a concentration of 100 μM and aliquoted at 200 μL volume per tube (1.5 mL RNase-free Eppendorf) followed by vacuum evaporation. The dried RNA was treated with 100 μL of 100% ethanol and stored at -80^{o}C. On thawing, RNA disulfide protection was removed by first vacuum-evaporating the ethanol and then adding 200 μL of water and 5 μL Tris(2-carboxyethyl)phosphine HCl at pH 7.0 (TCEP, 0.5 M, #646547-10 X 1mL, Sigma). After 10 min, $CHCl_3$ (800 μL) was mixed to extract and remove the mercaptohexanol, carefully discarding the organic phase, and repeating for a total of four extractions. The aqueous layer was transferred to a new tube and used immediately.

HGN-Anchoring DNA or RNA Assembly. Thiol-modified sense RNA or DNA strands were assembled onto the HGN using a fast pH-induced self-assembly method.[4] ~0.1 nM HGN were combined with 9 μM freshly reduced thiol-modified

DNA or RNA (100 μM in 12.5 mM TCEP pH7.0) and 10 mM sodium citrate-HCl (Na_3Cit-HCl, 500 mM, pH 3.0, RNase free), sonicated, and incubated at RT for 20 minutes. Thereafter the solution was pH-neutralized by adding 130 mM HEPES buffer (1 M, RNase free, pH 7.5, Ambion), then salted to 1 M Na^+ in steps, using 3.0 M NaCl, 0.3 M Na_3Cit pH 7.0 (20X SSC, RNase free, Promega), in ~300 mM Na^+ increase per step, waiting 5 minutes between each step. 1 mM $MgCl_2$ (1 M, RNase free, TEKNOVA) and 0.01% Tween-20 (RNase free, Promega) were added and incubated overnight at RT to increase DNA or RNA density on the HGN. The next day HGN-Anchoring DNA or RNA were washed by centrifuging twice at ~7000×g for 10 min, each time keeping the pellet and redispersing with washing buffer (1 mM $MgCl_2$, 0.01% Tween-20, 300 mM NaCl, 30 mM Na_3Cit pH 7.0).

HGN-dsDNA or siRNA Duplex Hybridization and Targeting Peptide Conjugation. Anti-sense strand RNA-2 or RNA-4 (all RNA, Biosearch) (Table S1), complementary DNA strands for control DNA-2 (all DNA, Biosearch) or complementary RNA strands for modular design RNA-3 and RNA-5 (all RNA, Biosearch) were added at 3 μM to a washed stock of 1 mL HGN-anchoring DNA or RNA (0.1 nM) and incubated at 70˚C for 2 minutes followed by 45˚C for 30 minutes. Excess DNA or RNA was removed with conjugation washing buffer (100 mM HEPES pH 7.5, 1mM $MgCl_2$, 0.01% Tween-20). Thiol-PEG-amine (3 kDa, Rapp Polymere GmbH) in ethanol was added at 100 μM to backfill any large exposed surface sites. After 1 h, excess thiol-PEG-amine was removed by centrifugation at ~7000×g for 10 minutes and pellet was resuspended in conjugation wash buffer. 6-mercapto-1-hexanol (MCH, Sigma) in ethanol was added at 5 μM to further passivate HGN surface sites. After 3 h, HGN-dsDNA or HGN-siRNA were centrifuged (at ~7000×g for 10 minutes) and washed with conjugation wash buffer to remove excess

MCH. Thereafter, a large excess (~1 mg/mL) of MAL-dPEG$_4$-NHS linker (Quanta Biodesign, CAS# 756525-99-2) for conjugation of the HGN-dsDNA or siRNA and the targeting peptide was added to functionalize the 3' end of anchoring the DNA or RNA. The solution was sonicated briefly and incubated for 15 minutes at RT, followed by centrifugation at ~7000×g for 10 minutes at 4˚C and pellet resuspension with conjugation wash buffer twice to remove excess linker. 20 μM of FAM-Cys-X-RPARPAR-OH peptide (RP) (LifeTein LLC, X is aminohexanoic linker, FAM is fluorescein attached to the N-terminus, C-terminus is free carboxyl) was then added, the solution was briefly sonicated and incubated at RT for 1 h. The dsDNA or siRNA-RP coated HGN were centrifuged twice at ~7000×g, the pellet redispersed in conjugation wash buffer to remove any unreacted peptide, and sterile-filtered through a 0.22 μm syringe filter (Millpore). The solution was then concentrated ~5 fold (10 nM HGN) by centrifugation at ~7000×g for 10 minutes with resuspension of the pellet in conjugation wash buffer. Product was stored at 4˚C before adding to cells.

Cell Culture. The human prostate cancer cells (PPC-1) were a generous gift from Erkki Ruoslahti (Sanford-Burnham Medical Research Institute, La Jolla, San Diego, CA). They were grown in DMEM/high glucose medium with phenol red (Hyclone), supplemented with 10% FBS (Hyclone). Noncancerous prostate epithelial cells (RWPE-1) (ATCC) were grown in keratinocyte serum free medium (Invitrogen) supplemented with bovine pituitary extract (0.05 ng/mL) and recombinant EGF (5 ng/mL). Both cell lines were maintained at 37 ^{o}C in 5% CO_2 atmosphere and grown in 6-well or 96-well plates (BD Falcon) for experiments.

siRNA transfection with Lipofectamine RNAiMAX. For the typical control transfection experiment with non-HGN conjugated siRNA duplex, cells were plated in 96-well plates at a concentration of 3000 cells per well. The following day the

reagents were prepared for transfection according to the manufacturer's protocol, with the following quantities intended per well in a 96-well plate format: 0.1 μL of Lipofectamine RNAiMAX and 0.1 μL of 5 μM siRNA were each diluted in 10 μL of OPTI-MEM reduced serum medium (Invitrogen), then combined and incubated at RT for 15 minutes. The transfection mix was then added to the wells containing the plated cells and complete growth medium. The following day the medium was replaced and cells imaged, scored for viability or allowed to grow up to 48 h or 72 h after transfection.

HGN Transfection and Femtosecond Laser Irradiation. Cells were plated in 6-well plates (24 hours before experiment) and harvested by incubation with 500 μL of non-enzymatic cell dissociation buffer (CDB, Invitrogen) at 37˚C in 5% CO_2 atmosphere for 10-15 minutes, after one wash in calcium and magnesium free Dulbecco's phosphate buffered saline (D-PBS, Invitrogen). Complete growth medium was added and cells centrifuged at 1000×g to remove the CDB. The cell pellet was diluted in the appropriate amount of medium to obtain a concentration of 1×10^6 cells/mL. 6.5 pM of coated HGN were added to 200 μL of cell suspension and incubated in 1.5 mL Eppendorf tubes at room temperature (RT) for 2h on a rotator. 1.2 mL of cold Hank's balanced saline solution (HBSS, with Ca^{2+} and Mg^{2+}, pH 6.7-7.8, Invitrogen) was added and the tube was centrifuged at 55×g for 8 minutes at 4˚C. The supernatant containing free particles was removed and 45 μL HBSS were added and mixed with the cell pellet. Tubes were irradiated with 2.4 W/cm^2 pulsed NIR laser for 10 s by the output of femtosecond (fs) Ti:sapphire regenerative amplifier (Spectraphysics Spitfire) running with 1 kHz repetition rate. The laser beam with ~4 mm diameter was directed onto the sample by a system of mirrors without any focusing optics. The pulsed duration was monitored by a home-built single-shot

optical autocorrelator and was kept at about 130 fs. The spectral range of laser irradiation was ~12 nm centered around 800 nm, and the energy of the optical pulse was controlled by Schott neutral density glass filters. A thermopile power meter (Newport Inc., Irvine, CA) was used to measure the incident optical power. Cells were then either collected for fluorescence intensity measurement by flow cytometry, or plated in 96-well plates for cell viability assay and in 6-well plates for Western blot.

Cell Fluorescence Intensity Measurements. Cells with internalized particles in HBSS buffer with or without femtosecond laser treatment were collected and injected into a BD Accuri C6 flow cytometer with a flow rate of 14 μL/min. The gate was based on the lineage area of forward and side scatter plots, and 10,000 events were collected for each sample. The increase in fluorescence intensity after particle internalization or laser treatment was assessed by the population of the cells having intensity higher than 99% of the control cells.

Confocal Microscopy. Cells were plated on a 8-well chamber glass slide (Lab-Tek II), and incubated with 6.5 pM coated HGN at 37˚C in 5% CO_2 atmosphere for 2 h followed by two washes with HBSS to remove any excess HGN. An Olympus Fluoview 1000 MPE Microscope with a 25x water immersion objective (NA 1.05) was used for live cell imaging. The microscope is equipped with a mode-locked titanium-sapphire femtosecond tunable pulsed laser (MaiTai HP, Newport-Spectra Physics), that was used to irradiate the sample to induce cargo release from the HGN, a 473 nm blue laser diode, used to image the FAM signal from the peptide, and a 559 nm green laser diode and a 633 nm HeNe laser that were not used. Images were collected at 12 bit with 512 X 512 pixels. The MaiTai laser was tuned to 800 nm and used to irradiate the sample area with a scan speed of 125k Hz for up to 35 repetitions. The specimen was imaged in a single-photon confocal mode with the blue laser diode

at a scan speed of 80k Hz before and after the exposure to the MaiTai fs laser to compare the cell fluorescence intensity difference caused by the laser treatment.

Cell Viability Assay. The PrestoBlue (Invitrogen) assay was used to determine the effect of transfection on cell proliferation for both laser treatment and controls with Lipofectamine RNAiMAX. For laser experiments, cells, with or without particles and treated or untreated with the laser, were plated in 96-well plates at a density of 5000 cells per well. 100 μL of complete medium were added per well and cells were incubated for 0 h, 24 h, 48 h and 72 h, before determining cell viability. For Lipofectamine control experiments cells were tested for viability 24 h, 48 h, or 72 h after transfection. The PrestoBlue assay was used according to the manufacturer's instructions: 10 μL of reagent were added to cells with 90 μL of fresh complete medium; the plate was incubated at 37˚C, 5% CO_2 atmosphere for 2 h; the fluorescence signal was recorded in a Tecan Infinite 200 Pro reader in bottom-read mode. Excitation and emission wavelengths were set at 560 nm (9 nm bandwidth) and 600 nm (20 nm bandwidth) respectively. 4 replicates for each treatment were averaged and analyzed based on a calibration curve to determine then number of cells in each sample. All treatments were repeated at least 3 times and reported as mean ± standard deviation (SD). One-way ANOVA analysis was performed to determine the statistical significance of changes in cell viability for each treatment; $p<0.001$ was considered statistically significant.

Western Blot. Cells, with or without particles and treated or untreated with laser, were plated in 6-well plates with 2×10^5 cells per well in 2 mL medium and cultured for 48 h and 72 h. Cells were harvested with trypsin/EDTA (0.25%, Sigma) and about 1×10^6 cells were lysed in 100 μL of RIPA lysis buffer (50 mM Tris, 150 mM NaCl, 1% Triton, 0.5% deoxycholate, and 2 mM EDTA) supplemented with

protease inhibitor cocktail (Promega) on ice for 30 minutes. The cell lysate was centrifuged at ~12400×g, at 4˚C for 20 minutes. The supernatant was collected and combined with loading buffer (6X, 300 mM Tris-HCl, 0.01% w/v bromophenol blue, 15% v/v glycerol, 6% w/v SDS and 1% v/v beta-mercaptoethanol) and kept at 95˚C for 10 minutes. 40 μL of this solution and 10 μL of pre-stained molecular weight standard (BioLabs) were loaded and separated by electrophoresis through Precast 10% SDS-PAGE gel (Bio-rad). Proteins were electro-transferred onto a nitrocellulose membrane (Bio-rad). The membrane was blocked with 5% bovine serum albumin (BSA, Sigma) in PBST (PBS, Fisher; 0.1% Tween-20) at RT for 30 minutes, then incubated overnight at 4˚C with primary antibodies diluted in 5% BSA-PBST buffer: mouse anti-PLK1 (monoclonal, 1:500 dilution, EMD) and rabbit anti-β-actin (monoclonal, 1:1000 dilution, Abcam). The membrane was subsequently washed thrice for 15 minutes using PBST and then incubated for 3 h in 5% BSA-PBST with secondary antibodies including Alexa Fluor 488 labeled goat anti-mouse IgG (1:10,000 dilution, Invitrogen) and Alexa Fluor 647 labeled goat anti-rabbit IgG (1:10,000 dilution, Invitrogen), followed by wash thrice for 15 minutes using PBST. Images were acquired using a GE Healthcare Typhoon 9400 scanner system and the bands were analyzed using the scanner control software.

Particle concentration determination. Particle concentration was estimated using the nanoparticle-tracking analysis system Nanosight LM10HS (Nanosight, Amesbury, UK), equipped with a sample chamber NanoSight LM14 with a 638 nm laser and a Viton Fluoroelastomer O-ring. The samples are injected in the sample chamber at room temperature with sterile syringes (Kendall Monoject, Mansfield, MA) until the liquid reached the tip of the nozzle. The software used for capturing and analyzing the data is the NTA 2.3 Build 0025. The samples are measured for 60 s

with manual camera level adjustments. The Nanosight system shows excellent accuracy by calibrating with monodisperse 50 nm and 60 nm diameter solid gold nanoparticles with known concentrations (Ted Pella Inc, CA). HGN with maximum absorption at 2.0 optical density (1.0 cm path length) was determined by Nanosight to contain 3.9×10^{9} particles per milliliter. A linear correlation is observed when measuring the maximum absorption of HGN with a serial of dilutions, suggesting that HGN follows the Beer-Lambert Law. Particle concentration after coating was estimated to have an extinction coefficient of 3.1×10^{10} M^{-1} cm^{-1} at the NIR plasmon peak maximum absorbance (Figure S1).

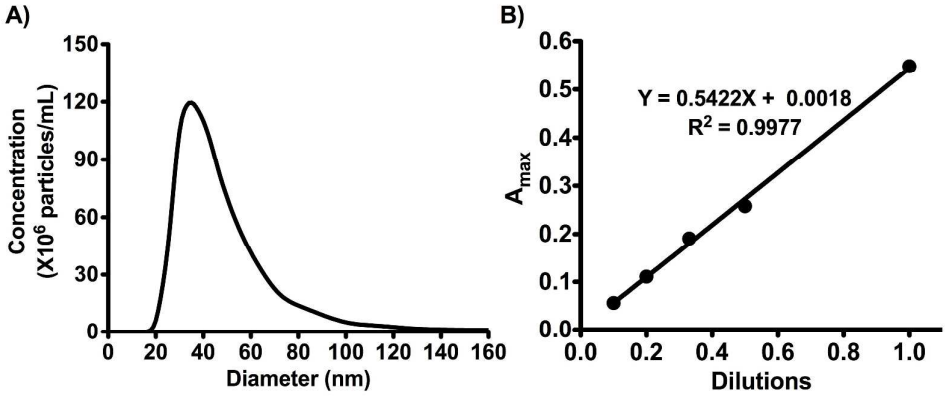


Figure S1. Determination of HGN particle concentration. A) Size distribution and total concentration of HGN by particle tracking analysis using a Nanosight LM10HS (Nanosight, Amesbury, UK). B) Correlation between measured HGN concentration and optical density at maximum absorption wavelength.

Nanocarrier (HGN-SD-RP) optical characterization at different coating steps. Sense RNA strand modified with thiol-PEG on the 5' and with amine on the 3' end was assembled on HGN through a fast pH-induced self-assembly method. Thereafter anti-sense RNA strand was hybridized onto HGN-ssRNA to form functional *plk1*-siRNA duplex. Targeting peptide was Cys-FAM-RP (FAM-Cys-X-

RPARPAR-OH, X being aminohexanoic linker, and FAM is fluorescein coupled to the N-terminus through an amide bond). Cys-FAM-RP with free thiol was tethered to the 3' end of siRNA sense strand with the help of a NHS-$(PEG)_4$-MAL linker (CAS# 756525-99-2, Figure S2A). We monitored the diameter of the construct during assembly by DLS. Citrate-stabilized HGN, as synthesized, had an average diameter of 56 (Figure S2B). The addition of the anchoring strand and subsequent hybridization of RNA caused a radial increase of ~8 nm (Figure S2B). The value was in agreement with the theoretical length of dsRNA (8 nm), and was consistent with a compactly arranged layer perpendicular to the gold surface. The PEG and peptide coating step further increased the particle diameter from 73 to 89 nm (Figure S2B). A small amount of aggregation was observed from the comparatively wider particle size distribution peak (Figure S2B).

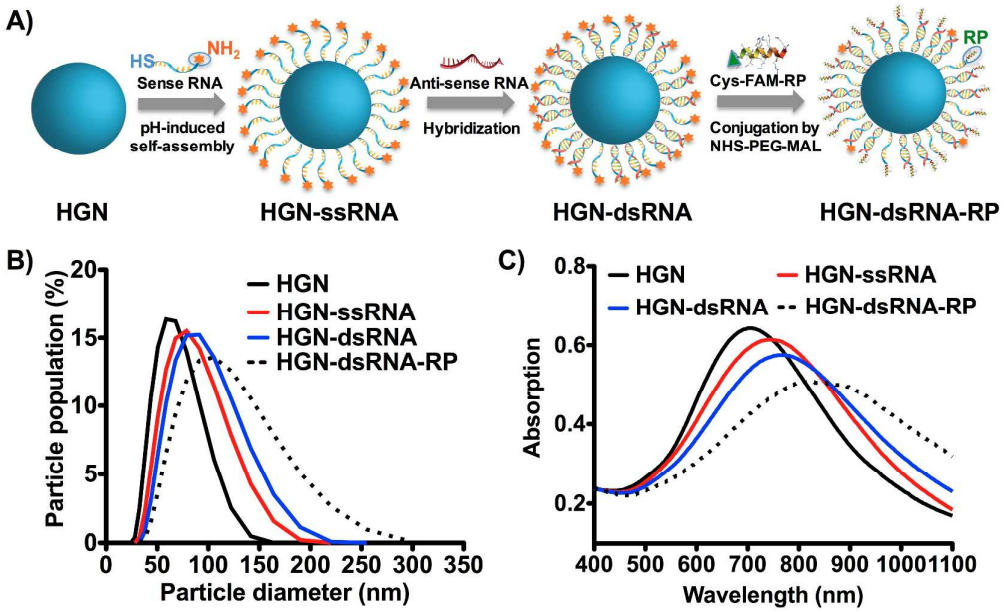


Figure S2. HGN-SD-RP synthesis and characterization. A) Schematic of the HGN-SD-RP synthesis steps. B) Size distribution of nanoparticles for the steps during coating. HGN-citrate has a Z-average diameter of 56 nm; HGN-ssRNA 66 nm; hybridized HGN-dsRNA 73 nm; final construct HGN-dsRNA-RP 89 nm. C)

Absorption spectrum of HGN broadens and red-shifts along with the increase of particle size and size distribution range at each coating step. The plasmon peak shifted from original ~710 nm to ~800 nm after RNA and peptide coating.

Quantification of siRNA coating density on HGN and NIR laser-dependent release. The coating density of the siRNA short duplex (SD) on HGN was estimated by native-PAGE. The siRNA-coated nanoparticles were etched by KCN to completely release the oligonucleotides (*Caution: KCN solution pH should not be acidic due to potential for toxic cyanide gas formation; prepare all solutions in 0.1 M NaOH*). The chemically released siRNA from HGN was loaded into a native-PAGE gel. The gel was then stained in SYBR Gold (Invitrogen), followed by densitometry analysis of the gel-scanning image. A calibration curve was generated by loading a known concentration gradient of siRNA in the gel and performing densitometry analysis of the bands (Figure S3). Using the reported calibration curve and the measured HGN concentration, the number of siRNA short duplex strands per HGN was estimated as 2300±600.

The coating density of the long duplex (LD) siRNA on HGN was estimated using a fluorescence-based method, relatively to the SD siRNA. 5' Quasar570 (Q)-labeled antisense strand (Table S1, RNA-4, Biosearch Technologies) and 5' Q-labeled sense strand (Table S1, RNA-7, Biosearch Technologies) were hybridized to form SD and LD respectively. The siRNA (SD and LD) coated nanoparticles were etched by KCN to completely release the oligonucleotides. The fluorescence intensity of the SD solution after KCN etching was measured and the fluorescence per HGN was calculated and considered to be 1. The fluorescence intensity of the LD solution after KCN etching was measured and the contribution per HGN found to be 60% of the SD intensity. Similarly, the efficiency of NIR laser release of siRNA from HGN surface

was assayed by measuring the fluorescence in solution after laser release, in comparison with that from KCN etching.

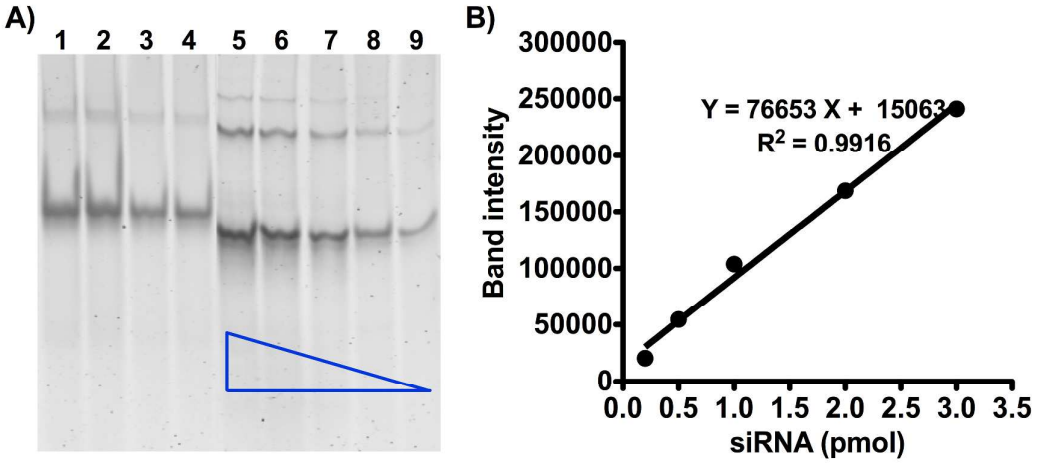


Figure S3. Native-PAGE and densitometry analysis of chemically released siRNA (SD) from HGN. A) Native-PAGE gel of siRNA KCN-released from HGN (lanes 1-4, replicates from the same sample), and calibration siRNA concentration gradient (lanes 5-9). Concentration gradient used, from lane 5 to lane 9: 3, 2, 1, 0.5, 0.2 pmol. B) Calibration curve correlating known siRNA concentration and band intensity from densitometry measurement. The number of siRNA short duplex strands per HGN is estimated from the linear fit equation to be 2300±600.

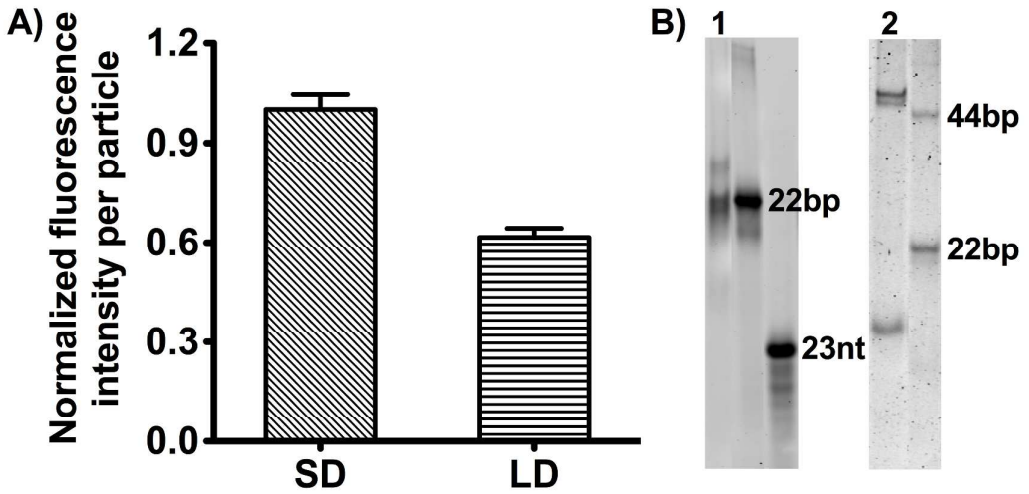


Figure S4. Comparison of siRNA coating density between SD and LD on HGN. A) Fluorescence intensity of KCN-released SD siRNA was measured and the contribution per HGN calculated. This value was set to 1. KCN-released LD per HGN was estimated as ~60% of the SD. B) Native-PAGE of KCN-released siRNA from HGN. Lane 1: KCN-released SD; lane 2: KCN-released LD. Lack of ssRNA (23 nucleotides) in lane 1 suggests ~100% hybridization efficiency for SD, however, an apparent band of ssRNA in lane 2 shows less hybridization efficiency for LD formation, supporting the fluorescence-based data.

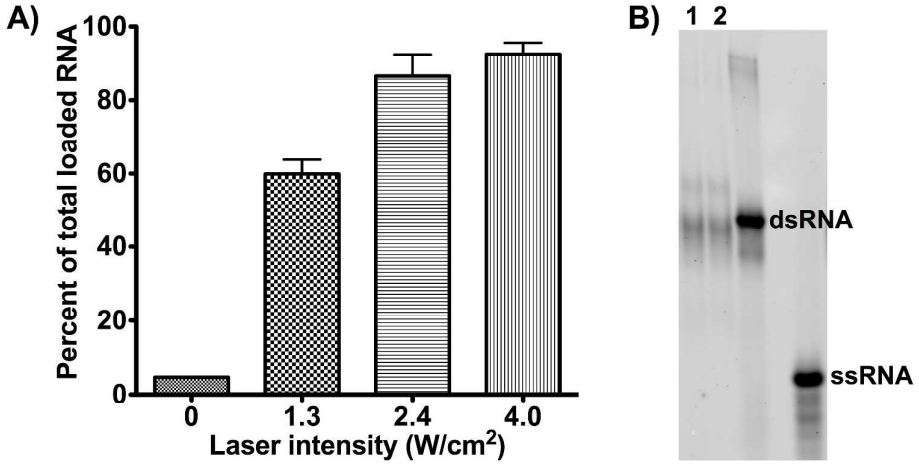


Figure S5. NIR laser-dependent release of siRNA from HGN. A) Laser release efficiency of HGN-SD-RP with 10 s pulsed laser treatment (1.3, 2.4, 4.0 W/cm^2) compared to KCN chemical release (taken to be 100%). Q dye is on the 5' of the anti-sense strand. 2.4 W/cm^2 for 10 s laser irradiation was found to release ~85% SD on HGN. B) Native PAGE analysis of released RNA. Lanes 1 and 2 show dsRNA released with laser treatment at 4.0 W/cm^2 for 10 s. The lack of ssRNA (23 nucleotide length) upon laser treatment suggests the duplex RNA remains hybridized.

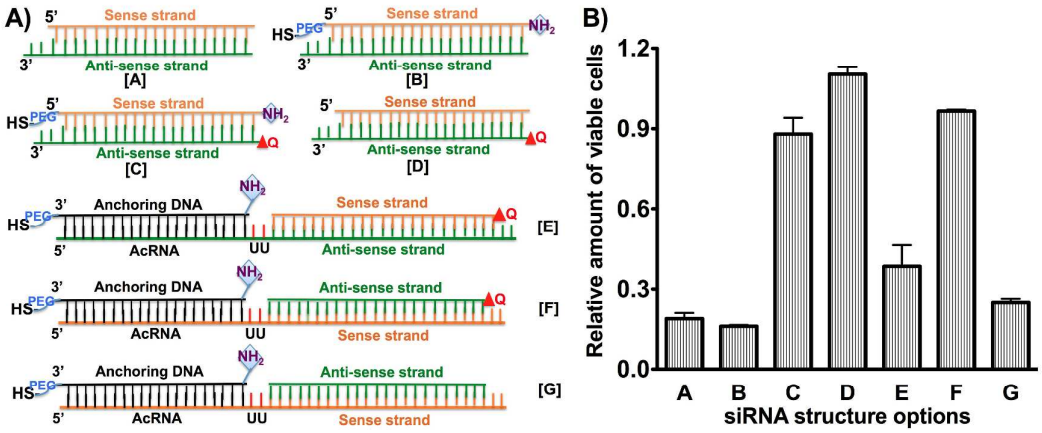


Figure S6. Q modification on 5' end of anti-sense strand in PLK1-siRNA blocks the siRNA knockdown activity, as indicated by cell viability. A) Different siRNA structures with or without Q at different sites on the strands. B) Cell viability assay 72 h after Lipofectamine transfection of different siRNA options. Second generation of siRNA architecture shows similar knockdown efficiency as original short duplex structure. Q modification on 5' end of siRNA anti-sense strand blocks the siRNA knockdown activity (C, D and F). However, both thiol-PEG and Q modifications on 5' end of siRNA sense strand do not (B, E and G), indicating the importance of anti-sense strand 5' site for siRNA functionality.

Minimum NIR laser power and HGN-SD-RP dosage for effective knockdown. Different NIR laser power intensity and irradiation duration combinations were applied on the PPC-1 cells internalized by HGN-SD-RP or HGN-dsDNA-RP. Cell viability after 72 h was tested to separate the released-siRNA biological effect from nanoparticle local heating damage. Cell death from HGN-dsDNA-RP would indicate particle local heating damage to the cells, since the construct is not biologically active. On the other hand, cell death from HGN-siRNA-RP is the result of siRNA

knockdown. 2.4 W/cm^2 for 10 s was the optimal laser condition for effective siRNA knockdown with minimum local heating damage.

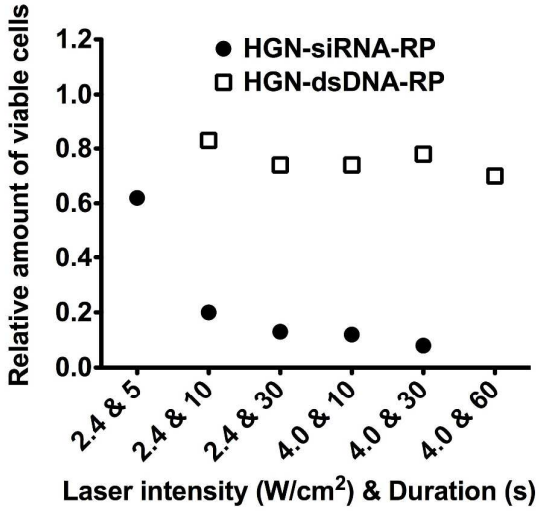


Figure S7. NIR laser power optimization for effective siRNA release from HGN surface with minimum cell damage caused by particle local heating. PPC-1 cells incubated with either HGN-SD-RP or HGN-dsDNA-RP are exposed to different laser power intensity and irradiation duration combinations. Cell viability is assayed 72 h after laser treatment. 2.4 W/cm^2 for 10 s was chosen as optimal for effective siRNA knockdown. Minimal loss of cell viability was found for all HGN-dsDNA-RP conditions, indicating minimal damage induced by the local heating.

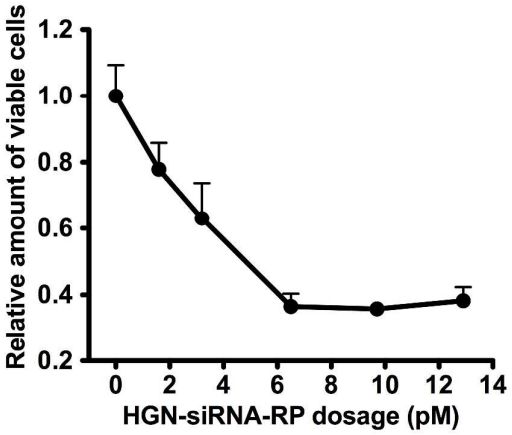


Figure S8. HGN-siRNA-RP dosage titration for effective siRNA knockdown in PPC-1 cells. Various concentrations of HGN-siRNA-RP were incubated with PPC-1 cells, treated with laser, and then plated to 96-well plate to assay cell viability after 72 h. We found 6.5 pM (for 2×10^5 cells) to be the minimal dosage for effective siRNA knockdown and cell apoptosis.

References

(1) Prevo, B. G., Esakoff, S. A., Mikhailovsky, A., Zasadzinski, J. A. *Small* **2008**, *4*, 1183-1195.

(2) Wu, G. H., Milkhailovsky, A., Khant, H. A., Fu, C., Chiu, W., Zasadzinski, J. A. *J. Am. Chem. Soc.* **2008**, *130*, 8175-+.

(3) Dassie, J. P., Liu, X. Y., Thomas, G. S., Whitaker, R. M., Thiel, K. W., Stockdale, K. R., Meyerholz, D. K., McCaffrey, A. P., McNamara, J. O., 2nd, Giangrande, P. H. *Nat. Biotechnol.* **2009**, *27*, 839-849.

(4) Zhang, X., Servos, M. R., Liu, J. W. *J. Am. Chem. Soc.* **2012**, *134*, 7266-7269.