Supporting Information

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SI Materials and Methods

Nanoplex Preparation and Analysis. The GNRs were synthesized as previously described (1, 2). The nanoplex formulation was prepared just before each experiment by electrostatically attaching 1 μg of cationic GNR to 1.2 μg of the appropriate RNA (5′PPP-ssRNA, CIAP-ssRNA, or Capped-ssRNA) in Opti-MEM medium (Invitrogen) and incubating at room temperature for 5 min. The size of the nanoparticles ranged from 35 to 70 nm as described earlier (1). The electrophoretic assessment of nanoplex formation was done according to standard procedures (3), using a 1.5% agarose gel in a Tris acetate EDTA buffer system. For TEM, transfected cells were fixed as described (4), sectioned (70–100 nm), stained with lead citrate, and viewed with a Tecnai-12 electron microscope (Phillips) at 120 kV. ζ potential measurements of GNRs in the presence and absence of RNA molecules were acquired at 25 °C using a 90-Plus particle size analyzer (Brookhaven Instrument Corp.).

Biofunctional Analysis Following Viral Infections. The A549, human respiratory epithelial, and Madin–Darby canine kidney cell lines (ATCC) were grown according to the distributor's instructions and infected according to standard protocols (5). For transfections using nanoplexes, A549 cells were seeded in six-well plates to achieve 30–50% confluence (3.5×10^5 cells/well). Three micrograms of RNA as GNR-RNA nanoplexes was added to each well in Opti-MEM. The efficiency of transfection was quantified using spectrophotometric measurements with excitation at 488 nm and emission at 510 nm from the lysed cells. At designated time points, cellular protein and RNA were harvested from duplicate wells for Western and quantitative (q)RT-PCR analyses. Total proteins were resolved on 4–15% SDS/PAGE gels, transferred to nitrocellulose membranes, and probed with commercial antibodies purchased from Sigma (actin) or Santa Cruz Biotechnology (RIG-I, MDA5, IPS1, and NS1). qRT-PCR was done with the SuperScript III Platinum SYBR Green One-Step kit (Invitrogen) in a Stratagene MX3000P thermal cycler according to the manufacturer's instructions. Primer sets used for these studies are as follows:

IFNβ: forward, *5′-TGG GAG GCT TGA ATA CTG CCT CAA-3′*; reverse, 5′-TCT CAT AGA TGG TCA ATG CGG CGT-3′.

RIG-I: forward ‚*5′-AAA CCA GAG GCA GAG GAA GAG CAA-3′*; reverse, 5′-TCG TCC CAT GTC TGA AGG CGT AAA-3′.

β-actin: forward, *5′-ACC AAC TGG GAC GAC ATG GAG AAA -3′*; reverse, 5′-TAG CAC AGC CTG GAT AGC AAC GTA-3′.

PCR-Array data were collected using IFN-α, -β Response PCR Array plates and analyzed using the RT^2 Profiler PCR Array Data Analysis software (SA Biosciences). Quantification of secreted IFN-β was performed using the Verikine Human IFN-β ELISA Kit (PBL IFN Source) and the Synergy 4 plate reader (Biotek).

Statistical Analysis. To determine the statistical significance between the 5′PPP-ssRNA, CIAP-ssRNA, and Capped-ssRNA treated and untreated groups, we used analysis of variance and a value of $P < 0.05$ was considered significant. All data points were included in the analysis and there were no outliers.

Studies of Nanoplexes Surface Charge. GNRs were complexed with RNAs and ζ potential was acquired at 25 °C using a 90-Plus particle size analyzer (Brookhaven Instrument Corp.).

Studies of Nanoplexes Distribution in Vitro. The A459 cellular uptake of the nanoplexes (GNR-siRNA^F), siPORT-siRNA^F, and free siRNA^F distribution was monitored using dark-field and fluorescence microscopy. The siRNA^F used in this study was purchased from Ambion (AM4620). The light-scattering images were recorded using an upright Nikon Eclipse 800 microscope with a high numerical dark-field condenser (N.A. 1.20–1.43, oil immersion) and a 100/1.4 N.A. oil Iris objective (Cfi Plan Fluor). In the dark-field configuration, the condenser delivers a narrow beam of white light from a tungsten lamp and the high N.A. oil immersion objective collects only the scattered light from the samples. The dark-field imaging was captured using a QImaging Micropublisher 3.3 RTV color camera. The Qcapture software was used for image acquisition. Fluorescence microscopy images were acquired using a Nikon Eclipse 800 upright microscope 100/1.4 N.A. oil Iris objective (Cfi Plan Fluor) and QImaging Micropublisher 3.3 RTV color camera for image acquisition (1). The signal from siRNA^F was acquired using a 488ex/510em filter, and for acquiring the signal from the nuclear dye Hoechst a 405ex/460em filter was used.

Fluorescence Studies from A549 Cell Lysates. A459 cells were incubated with 50 pmol of free siRNA^F, GNR-siRNA^F, and siPORTsiRNA^F nanoplexes and 24 h later cells were processed for fluorescence measurements. The medium was removed and the cells were lysed using mammalian protein extraction reagent (M-PER) (Pierce Chemical Co.), and the PL spectrum was analyzed using a Horiba Jobin Yvon Fluorolog-3 spectrofluorometer.

MTT Cell Viability Assay. The viability of A459 cells was investigated up to 96 h after treatment with GNR complexes with RNAs. The cell viability assay measures the reduction of a tetrazolium component [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)] into an insoluble formazan product by the mitochondria of viable cells. (6) Cells, in a 24-well plate (10,000 cells/well), were incubated with the MTT reagent for 3 h, followed by addition of a detergent solution to lyse the cells and solubilize the colored crystals. The samples were read using an ELISA plate reader at 570 nm wavelength.

Agarose Gel Electrophoresis. GNRs were complexed with 5′PPP-ssRNA and equivalent ssRNA that was free of 5′PPP (0.9 μg). The nanoplexes were added in individual wells in a 1.5% agarose gel casted in Tris acetate–EDTA (TAE) buffer (7). The gel was run for 1.5 h at 100 V and stained with EtBr. Images of the gel were obtained using an LM-20E UV benchtop transilluminator (UVP) in conjunction with an Olympus C-4000 zoom color digital camera with a UV filter.

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2. Bonoiu A, et al. (2009) MMP-9 gene silencing by a quantum dot-siRNA nanoplex delivery to maintain the integrity of the blood brain barrier. *Brain Res* 1282: 142–155.
3. Bonoiu AC, et al. (2009) Nanotechnology approach for drug addiction therapy: Gene silencing using delivery of gold nanorod-siRNA nanoplex in dopaminergic neurons. *Proc Natl Acad Sci USA* 106:5546–5550.
4. Xie FY, Woodle MC, Lu PY (2006) Harnessing in vivo siRNA delivery for drug discovery and therapeutic development. *Drug Discov Today* 11:67–73.

5. Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD (2005) Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small* 1:325–327.
6. Plumb J (2004) Cell sensitivity assays: The MTT assay. *Methods Mol Med* 88:165–169.
7. Bartlett DW, et al. (2007) Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality in vivo imaging. *Proc Natl Acad Sci USA* 104 (39):15549–15554.

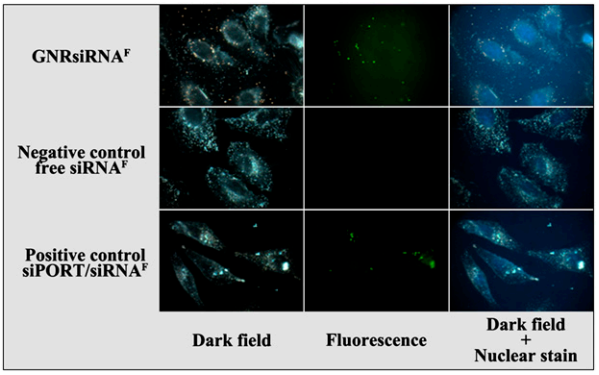

Fig. S1. Study of nanoplex distribution in A459 cells. Dark-field and fluorescence images were acquired on cells following treatment with GNR-siRNA^F nanoplex, free siRNA^F (negative control), and siPORT-siRNA^F (positive control). Fluorescence images show robust uptake of the GNR-siRNA^F and siPORT-siRNA^F as opposed to free siRNA^F. The dark-field images of GNRs corresponding to the longitudinal surface plasmonic enhancement in the red region can be clearly visualized in the samples treated with GNR-siRNA^F.

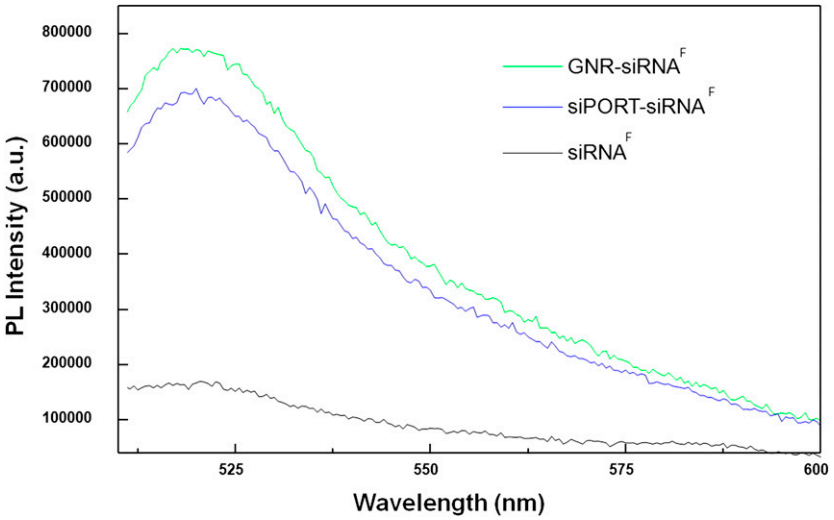

Fig. S2. Fluorescence spectra of siRNA^F from A549 lysates. Data show the highest values of fluoresecence intensity in the samples treated with GNR-siRNA^F as compared with samples transfected with siRNA^F alone or siPORT-siRNA^F.

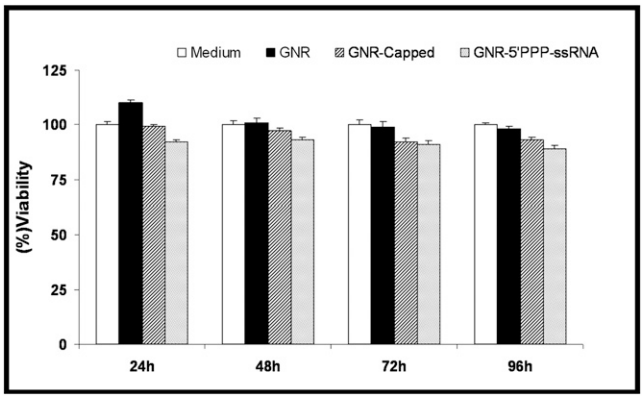

Fig. S3. Cell viability (MTT) assay of A459 cells following treatment with GNR, GNR-Capped, and GNR-5′PPP-ssRNA nanoplexes. Results show minimal toxic effects on the cells following treatment with the nanoplexes, which were observed up to 96 h posttreatment. The results are the mean ± SD of three separate experiments.

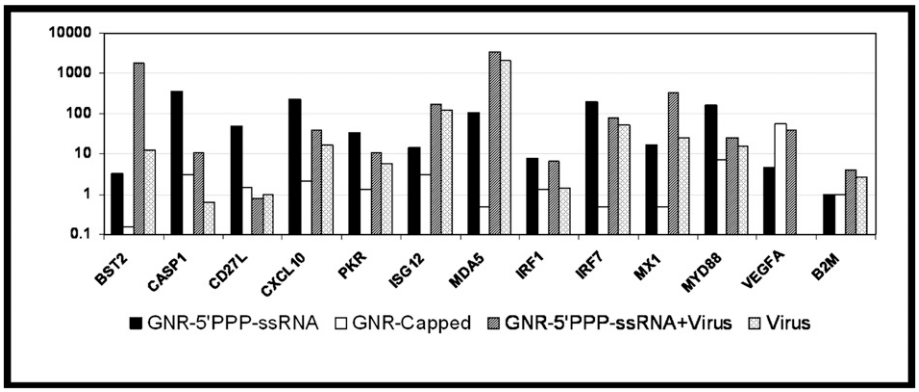

Fig. S4. RT-PCR-Array analysis of IFN-stimulated genes. A459 cells were mock transfected or transfected with 3 μg of RNA complexed with 2.5 μg of GNR per well for 48 h and then either mock infected or infected with A/California/08/09 at an MOI of 1 for 24 h. Columns represent the fold differences in the mRNA levels of selected IFN-stimulated genes compared to the mock-transfected and uninfected controls.

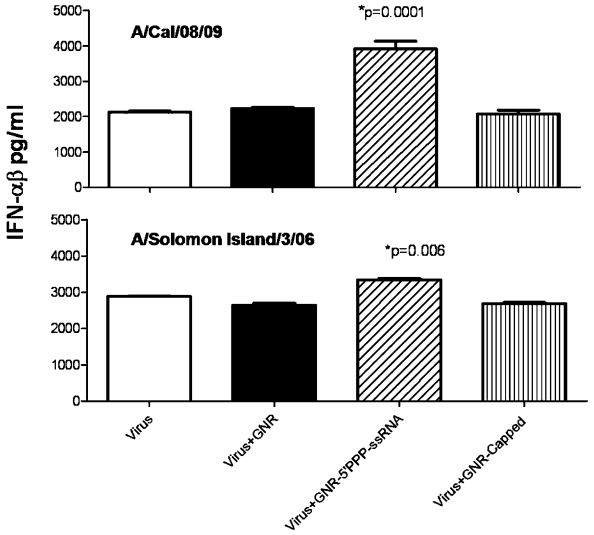

Fig. S5. Quantification of secreted IFN-β. A459 cells were mock transfected or transfected with 3 μg of RNA complexed with 2.5 μg of GNR per well for 48 h and infected with A/California/08/09 or A/Solomon Islands/03/06 at an MOI of 1 for 24 h. Secreted IFN-β levels in cell culture supernatants were determined by ELISA.

Table S1. ζ potential measurement of GNR in presence and absence of various RNAs at 25 °C

	Surface charge of GNR after complexation with RNAs			
Nanoplexes	GNR	GNR-CIAP	GNR-5PPP-ssRNA	GNR-Capped
Surface charge*	+20.71	−9.61	−9.91	−8.23
SD	(±)2.64	(±)3.22	(±)1.84	(±)1.29

SD, standard deviation.
*Measured as the ζ potential (mM).