Supplemental Information

A cationic amphiphilic co-polymer as a carrier of nucleic acid nanoparticles (NANPs) for controlled gene silencing, immunostimulation, and biodistribution

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MATERIAL AND METHODS

PgP/nucleic acid complex formation. PgP was synthesized using PLGA (4 kDa, 50:50, Durect Corporation, Pelham, AL) and bPEI (MW 25 kDa, Sigma) as described previously (31). DNA duplex mimicking GFP siRNA (200 nM final) tagged with Alexa488 (DNA-Al488) was incubated for 30 minutes in the presence of PgP at various N/P (where N is the number of nitrogen atoms in PgP; P is the number of phosphorus atoms in the nucleic acids) ratios at 37 ^{o}C to allow for complexation. The samples were then run through an agarose gel (2% (w/v) agarose in 1 X TBE (pH 8.2)) for 15 minutes at 220 volts. The gel was imaged using a Bio-Rad ChemiDoc MP System (Bio-Rad, CA).

Nuclease protection assay of PgP/DNA duplex polyplexes. We employed a previously developed fluorescence-based approach suitable for different carriers (26,43-45). Briefly, DNA duplex (200 nM final) tagged with Alexa488 and Iowa Black quencher (PgP/DNA-Al488/IWB) was incubated with PgP at various N/P ratios at 37 ^{o}C for 30 minutes (30 µL final). Following complexation, 3 µL of RQ1 DNase (Promega, WI) was added to the solution. The fluorescence was measured every thirty seconds using a Bio-Rad C1000 Touch Thermal Cycler and CFX96 Real Time System (Bio-Rad, Hercules, California).

Preparation of NANPs. DNA templates and primers were purchased from Integrated DNA Technologies (Coralville, Iowa) and polymerase chain reaction (PCR) was performed using primers containing the T7 RNA polymerase promoter sequence to amplify the DNA template. To obtain RNAs, *in vitro* transcription was performed for four hours at 37 ^{o}C using T7 RNA polymerase in a buffer containing 80 mM HEPES-KOH (pH 7.5), 2.5 mM spermidine, 50 mM DTT, 2 mM $MgCl_2$, 25 mM rNTPs, 0.2 µM DNA templates and ~100 units/µL of T7 RNA polymerase enzyme (isolated in house). Transcription was stopped using RQ1 DNase (Promega, WI). RNAs were purified using a denaturing urea gel (PAGE, 10% acrylamide, 8 M urea). Gel pieces containing RNAs were excised from the gel and the RNA was eluted overnight at 4 ^{o}C in 1 X TBE buffer with 300 mM NaCl. The following day, RNAs were precipitated in 2.5 X volumes of ethanol, followed by a 90% ethanol wash. RNAs were dried using vacuum centrifugation and suspended in endotoxin-free double-deionized water. All NANPs were assembled using previously published protocols (14,36). Briefly, functional RNA rings and fibers were assembled by mixing individual monomers at an equimolar ratio, heating to 95 °C for 2 min, snap cooling on ice for 2 min, adding 20% volume of 5 X assembly buffer (final concentration: 89 mM TB (pH 8.2), 50 mM KCl, 2 mM $MgCl_2$), and further incubated for 30 min at 30 °C. Functional RNA cubes were assembled by mixing all constituent strands at an equimolar ratio and heating to 95 °C for 2 min. The samples were then snap cooled to 45 °C and incubated for 2 min. Finally, 5X assembly buffer was added and the assembly mixture was further incubated at 45 °C for an additional 30 min.

Nomenclature. For clarity purposes, RNA cubes, rings, and fibers with every monomer strand functionalized with Dicer Substrate (DS) RNAs designed to target either GFP or RhoA will be described as PgP/NANP(GFP or RhoA). As an example, a polyplex of PgP combined with a cube functionalized with six DS RNAs against GFP will be referred to as PgP/cube(GFP).

Atomic force microscopy (AFM). To evaluate the structure of assembled NANPs, 5 µL (50 nM) of each particle was deposited on APS-modified mica, incubated for ~2 min and air-dried, as described previously according to established protocols (46,47). Briefly, AFM was performed using a MultiMode AFM Nanoscope IV system (Bruker Instruments, Santa Barbara, CA) in tapping mode. The images were recorded with a 1.5 Hz scanning rate using a TESPA-300 probe from Bruker with a resonance frequency of 320 kHz and spring constant of about 40 N/m. Images were processed by the FemtoScan Online software package (Advanced Technologies Center, Moscow, Russia) (47,48).

Heparin competition assay. To assess whether the NANP structures stayed intact when released from the PgP polyplexes, we performed a heparin competition assay. Briefly, non-functionalized DNA cubes and RNA rings were mixed with PgP at an N/P ratio of 30:1 for 30 minutes at 37 ^{o}C. The solution was then treated with heparin (Sigma-Aldrich, MO) (heparin/DNA

or heparin/RNA ratio 6:1 w/w) for 30 minutes at 37 ºC. The samples were then run through native-PAGE (8% acrylamide, 37.5:1 acrylamide:bis-acrylamide).

Fluorescent (confocal) microscopy: All confocal images were obtained using a LSM710 confocal microscope (Carl Zeiss, Germany) equipped with a 63X/1.4 magnification lens. Images were taken with a pinhole adjusted to 1 airy unit.

In order to confirm an endosomal uptake pathway of the PgP complexed RNA, PgP/DS RNA-Al546 were transfected into MDA-MB-231 cells and incubated for 6 hours, at which point they were washed three times with PBS and fixed with 4% paraformaldehyde for 20 minutes at room temperature. For uptake analysis, Al546 was imaged by exciting the sample with a DPSS 561 laser and collecting emission at wavelengths of 566 and 640 nm, respectively. For co-localization, the cells were permeabilized with Tween 20 (0.2% for 30 minutes at room temperature), blocked with 1% BSA, and stained with an antibody directed against Rab5 (Santa Cruz, Dallas, TX) and a secondary antibody labeled with Alexa 647 (Molecular Probes, CA). For Alexa 647 imaging, a 633 nm Helium Neon laser was used for excitation at 638 nm and the emission was collected at a wavelength of 755 nm.

Effect of temperature on intracellular processing of polyplexes on MDA-MB-231 cells. MDA-MB-231 cells were seeded in a 24-well plate at 15,000 cells per well. DS RNAs and their DNA analogs labeled with Alexa 488 (100 nM final) were incubated with PgP (N/P 30:1) for 30 minutes at 37 ºC. Cells were then incubated with the polyplexes at either 37 ºC or 4 ºC for 16 hours. The cells were then suspended using cell dissociation buffer (Gibco, MA) and the fluorescence was measured using flow cytometry (Becton Dickinson, NJ). Fluorescence microscope images were taken at 6 hours using EVOS FL at 10X magnification with a GFP light cube, and over-lay with bright-field images.

Hemolysis assay. To evaluate the blood compatibility of various PgP/NANP polyplexes, a hemolysis assay was performed by adapting a protocol used by Aravindan and co-workers (49). Polyplexes were prepared by mixing PgP with various functionalized NANPs (cubes, rings, and fibers) carrying DS RNAs against GFP (an N/P ratio of 30:1) and individual DS RNAs against GFP (an N/P ratio of 30:1) for comparison. Briefly, rat blood (4 mL) was collected in a heparinized tube via cardiac puncture and centrifuged at 700 X *g* for 20 minutes at 4 ºC. The buffy coat and plasma was removed and then the erythrocyte (red blood cell) pellet was washed three times with PBS (pH 7.4) by centrifuging at 1000 X *g* for 10 minutes at 4 ºC. Erythrocytes were re-suspended in a 3% (w/v) solution in PBS. Equal volumes of erythrocyte solution and PgP/NANP(GFP) polyplexes (80 µL each) were mixed and incubated for 1 hour at 37 ºC. Following incubation, suspensions were centrifuged at 1000 X *g* for 10 minutes and the pellet and supernatant were separated. The pellet was resuspended in PBS and the morphology of erythrocytes was imaged using an inverted microscope (Zeiss Axiovert 200, Göttingen, Germany). The supernatant (100 µL) was transferred to a 96 well plate and absorbance was measured at 540 nm using a Synergy HT plate reader (Biotek, Winooski, VT). PBS and Triton X-100 treatments were used as controls for 0 and 100% hemolysis. Hemolysis was QUANTIfied using the following formula:

$$\frac{A_{Sample} - A_{PBS}}{A_{Triton-X} - A_{PBS}} \times 100\%$$

where A_{Sample}, A_{PBS}, A_{Triton} are the absorbances of the sample, PBS, and Triton X-100, respectively.

Immunostimulation of various PgP/NANP *polyplexes in hµglia cells in vitro***.** The human microglia-like cell line, hµglia, was generously provided from the laboratory of Dr. Jonathan Karn (Department of Molecular Biology and Microbiology, Case Western Reserve University). This cell line was generated from primary human microglia cells purchased from ScienCell and transformed with lentiviral vectors expressing SV40 and hTert antigens. These cells have been classified as microglia due to their microglia-like morphology, migratory and phagocytic activity,

presence of the surface markers CD11b, TGFβR, and P2RY12, and characteristic microglial RNA expression profile (50). Hμglia cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% FBS and 100 U/mL penicillin-100 μg/mL streptomycin at 37 °C 5% CO_2. PgP/NANP polyplexes were prepared by mixing PgP and various NANPs (1 μg/well) at an N/P ratio of 30:1. Lipofectamine 2000 (L2K, Invitrogen) was used for positive control and transfection was conducted according to the manufacturer's guidelines. NANPs were pre-incubated either with L2K plus OPTI-MEM medium or PgP plus OPTI-MEM for 30 minutes prior to transfection. Hμglia cells were transfected with NANPs plus L2K or NANPs plus PgP at a final concentration of 5 nM in DMEM supplement with 5% FBS. Cell culture media was changed to media additionally supplemented with 100 U/mL penicillin-100 μg/mL streptomycin 4 hours post transfection. Cell supernatants were collected 24 hours post transfection for analysis.

Enzyme-linked immunosorbent assay (ELISA). To quantify human IL-6 and IFN-β expression after transfection of polyplexes into hμglia cells, ELISAs were performed as previously described by our laboratory (51,52). IL-6 ELISAs were conducted using a rat anti-human IL-6 capture antibody (BD Pharmingen; Clone Mq2-13A5) and a biotinylated rat anti-human IL-6 detection antibody (BD Pharmingen; Clone MQ2-39C3). IFN-β ELISAs were conducted using a polyclonal rabbit anti-human IFN-β capture antibody (Abcam) and a biotinylated polyclonal rabbit anti-human IFN-β detection antibody (Abcam). Bound antibody was detected using streptavidin-horseradish peroxidase (BD Biosciences) followed by the addition of tetramethylbenzidine substrate. The reaction was stopped using H_2SO_4 and absorbance was measured at 450 nm. Dilution of recombinant cytokines for IL-6 (BD Pharmingen) and IFN-β (Abcam) were used to generate a standard curve. The concentration of each cytokine was determined by extrapolation of absorbance to the standard curve.

*Assessment of cellular immune signaling and human toll-like receptor (TLR) stimulation***.** THP1-Dual™ cells (Invivogen, CA) are monocytes engineered to express secreted alkaline phosphatase (SEAP) when NF-κB is stimulated and luciferase when the IRF pathway is stimulated. THP1-Dual™ cells were plated at 40,000 cells per well in a 96 well-plate and immediately transfected with PgP/NANP(GFP) complexes at 100 nM with a 30:1 N/P ratio. R848 (1 μg/mL) and poly I:C (0.5 μg/mL) were used as positive controls. After 24 hours of incubation, 20 μL of the suspension media was mixed with 50 μL of QUANTI-Luc™ (Invivogen) and the luminescence was immediately measured. Furthermore, 20 μL of supernatant media was then mixed with 180 μL of QUANTI-Blue™ (Invivogen, CA) and incubated for two hours at 37 ºC and the absorbance at 620 nm was measured.

HEK-Blue™ hTLR (Invivogen, CA) cells are HEK 293 cells engineered to express a single TLR with an NF-κB-inducible SEAP reporter gene. Both HEK-Blue™ hTLR -3 and -7 cells were maintained under selective antibiotics as recommended by the manufacturer and were plated in 96-well plates at 40,000 cells per well. The cells were then transfected with 100 nM PgP/NANP(GFP) at a 30:1 N/P ratio for 24 hours. R848 was used as a positive control for hTLR7, and poly I:C for hTLR3. Following incubation, 20 μL of the supernatant media was mixed with 180 μL of QUANTI-Blue™ detection media (Invitrogen, CA) for 2 hours and the absorbance was measured at 620 nm.

Knockdown efficiency and cytotoxicity of PgP/NANP(GFP) polyplexes under serum conditions in vitro. MDA-MB-231/GFP cells were cultured in DMEM supplemented with 10% FBS and 100 U/mL penicillin-100 μg/mL streptomycin at 37 °C 5% CO_2. Cells were seeded in 24-well plates at 20,000 cells per well and allowed to adhere overnight. The following day, 50 nM of NANP (GFP) was combined with PgP at an N/P ratio of 30:1 for 30 minutes at 37 ºC. The PgP/NANP(GFP) polyplexes were then added to the cells by mixing with FBS supplemented DMEM. After 72 hours of incubation, fluorescent imaging was conducted using an EVOS FL with 10X magnification, and QUANTItative fluorescence was measured using flow cytometry (BD Accuri C6) measuring 10,000 events per condition.

Knockdown efficiency and cytotoxicity of PgP/NANP(RhoA) polyplexes under serum conditions in vitro. B35 neuroblastoma cells (CRL-2754, ATCC, Manassas, VA) at a density of 8 × 10^4 cells/well were seeded in 24-well plates in 10% serum-supplemented medium and cultured overnight to allow cells adhere to the plate. PgP/NANP(RhoA) polyplexes (1 µg of various NANPs) were prepared by mixing PgP and NANPs(RhoA) at an N/P ratio of 30:1. PgP/NT-siRNA at an N/P ratio of 30:1 was prepared as a negative control and Lipofectamine 3000/DS RNA(RhoA) was prepared according to manufacturer's protocol and was used as a positive control. Non-transfected cells were also used as a control. The cells were transfected with polyplexes in medium containing 10% FBS, incubated for 24 hours, and then the media containing polyplexes was removed and replaced by fresh medium containing 10% FBS. The cells were then incubated for an additional 48 hours. At 72 hours post-transfection, the cells were lysed and total RNA was isolated using RNeasy mini kit (Qiagen, Germany). The isolated RNA quality and QUANTIty were evaluated by Take 3 using a BioTek synergy microplate reader (BioTek, Synergy HT). Complementary DNA (cDNA) was synthesized by reverse transcription reactions with isolated total RNA (1.0 µg) using MultiScribe™ reverse transciptase with random primers (High Capacity cDNA Reverse Transcription Kit; Applied Biosystems, Foster city, CA). Real-time PCR was performed using target-specific primers (final concentration: 0.5 µM) and SYBR Green PCR kit in a Rotorgene Q thermal cycler (Qiagen). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. Primers for RhoA: forward primer: 5'-CAA GGA CCA GTT CCC AGA GG -3', reverse primer: 5'-GCT GTG TCC CAT AAA GCC AAC-3'. Primers for GAPDH: forward primer: 5'- ATG GCC TTC CGT GTT CCT AC-3'; reverse primer: 5'-TAG CCC AGG ATG CCC TTT AG -3'. Relative mRNA expression levels of RhoA were calculated using the $2^{-\Delta\Delta Ct}$ method (53). The minus RT (reverse transcriptase) reactions were performed on a representative subset of samples to confirm no genomic DNA contamination. Reaction specificities were verified by melting curve analysis.

The cytotoxicity of polyplexes in B35 cells was analyzed by thiazolyl blue tetrazolium (MTT) assay in parallel experiments with knockdown. At 72 hours post-transfection, media was replaced with fresh media without serum and 250 µL MTT bromide (Sigma-Aldrich) solution in PBS (2 mg/mL) was added to each well. Plates were incubated for 4 hours at 37 ^{o}C and then washed with PBS. The formazan crystals formed by live cells were dissolved in dimethyl sulfoxide (DMSO) and absorbance was measured at 570 nm. Cell viability was calculated relative to non-transfected control according to the following equation:

Cell viability (%) = (A_{570} (sample)/ A_{570} (control)) X 100%

Biodistribution of PgP/NANP polyplexes after systemic injection. To visualize the biodistribution of PgP/NANP polyplexes, the hydrophobic fluorescent dye 1,1-dioctadecyl-3,3,3,3-tetramethyl indo tricarbocyanine iodide (DiR, PromoCell GmbH, Germany) was loaded in the core of PgP by the solvent evaporation method, followed by complexation with either cubes, rings, or fibers functionalized with DS RNAs against GFP or free DS RNAs. Briefly, DiR dye was dissolved in acetone and the DiR solution was added into a PgP solution (10 mg/mL) and then incubated for 4 hours at room temperature under constant stirring. After loading, the DiR-PgP solution was incubated overnight to evaporate acetone (final DiR concentration: 250 µg/mL). The DiR-PgP solution was filtered (0.2 µm pore size) to remove unloaded DiR dye. Various DiR-PgP/NANP (50 µg RNA) polyplexes at an N/P ratio 30:1 were prepared by mixing various NANP solutions with DiR-PgP and incubated at 37 °C for 30 min. Male CD-1 mice (7-8 weeks-old, Charles River Laboratories, MA) were anesthetized using isoflurane gas and various DiR-PgP/NANP polyplexes (2 mg/kg, RNA per body weight) were injected via tail vein. PgP complexed to DS RNA was used for comparison and untreated mice were used as a control group. At 1, 2, 6, and 24 hours post-injection, the animals were imaged by a live animal fluorescence imaging system (IVIS Luminar XR, Caliper Life Sciences) under anesthesia with isoflurane gas. At 24 hours post-injection, the animals were euthanized by CO_2 and their organs (liver, heart, lungs, spleen, kidneys, and brain) were harvested for *ex vivo* organ imaging and determination of the

fluorescence intensity of the region of interest (ROI). The % of organ distribution was calculated as the fluorescence of the organ normalized by the total fluorescence of the total organs. QUANTIfication of organ accumulation of polyplexes was compared for the five groups using analysis of variance with a significance level of 0.05.

Statistics. Experimental results are presented as the mean ± SEM. Statistical significance was determined using a one-way Anova using GraphPad Prism Software Version 7.

SEQUENCES USED IN THIS PROJECT

All sequences are shown 5'→3'

Six-stranded DNA cube without functionalization:

Strand A
GGCAACTTTGATCCCTCGGTTTAGCGCCGGCCTTTTCTCCCACACTTTCACG
Strand B
GGGAAATTTCGTGGTAGGTTTTGTTGCCCGTGTTTCTACGATTACTTTGGTC
Strand C
GGACATTTTCGAGACAGCATTTTTTCCCGACCTTTGCGGATTGTATTTTAGG
Strand D
GGCGCTTTTGACCTTCTGCTTTATGTCCCCTATTTCTTAATGACTTTTGGCC
Strand E
GGGAGATTTAGTCATTAAGTTTTACAATCCGCTTTGTAATCGTAGTTTGTGT
Strand F
GGGATCTTTACCTACCACGTTTTGCTGTCTCGTTTGCAGAAGGTCTTTCCGA

Six-stranded RNA ring functionalized with six DS RNAs against GFP

Strand A
GGGAACCGUCCACUGGUUCCCGCUACGAGAGCCUGCCUCGUAGC
Strand B
GGGAACCGCAGGCUGGUUCCCGCUACGAGAGAACGCCUCGUAGC
Strand C
GGGAACCGCGUUCUGGUUCCCGCUACGAGACGUCUCCUCGUAGC
Strand D
GGGAACCGAGACGUGGUUCCCGCUACGAGUCGUGGUCUCGUAGC
Strand E
GGGAACCACCACGAGGUUCCCGCUACGAGAACCAUCCUCGUAGC
Strand F
GGGAACCGAUGGUUGGUUCCCGCUACGAGAGUGGACCUCGUAGC

Six-stranded RNA cube functionalized with six DS RNAs against GFP:

Strand A
GGCAACUUUGAUCCCUCGGUUUAGCGCCGGCCUUUUCUCCCACACUUUCACGU
UCGGUGGUGCAGAUGAACUUCAGGGUCA
Strand B
GGGAAAUUUCGUGGUAGGUUUUGUUGCCCGUGUUUCUACGAUUACUUUGGUCU
UCGGUGGUGCAGAUGAACUUCAGGGUCA

Strand C
GGACAUUUUCGAGACAGCAUUUUUUCCCGACCUUUGCGGAUUGUAUUUUAGGU
UCGGUGGUGCAGAUGAACUUCAGGGUCA
Strand D
GGCGCUUUUGACCUUCUGCUUUAUGUCCCCUAUUUCUUAAUGACUUUUGGCCU
UCGGUGGUGCAGAUGAACUUCAGGGUCA
Strand E
GGGAGAUUUAGUCAUUAAGUUUUACAAUCCGCUUUGUAAUCGUAGUUUGUGUU
UCGGUGGUGCAGAUGAACUUCAGGGUCA
Strand F
GGGAUCUUUACCUACCACGUUUUGCUGUCUCGUUUGCAGAAGGUCUUUCCGAU
UCGGUGGUGCAGAUGAACUUCAGGGUCA

Six-stranded RNA ring functionalized with six DS RNAs against GFP:
Strand A
GGGAACCGUCCACUGGUUCCCGCUACGAGAGCCUGCCUCGUAGCUUCGGUGGU
GCAGAUGAACUUCAGGGUCA
Strand B
GGGAACCGCAGGCUGGUUCCCGCUACGAGAGAACGCCUCGUAGCUUCGGUGGU
GCAGAUGAACUUCAGGGUCA
Strand C
GGGAACCGCGUUCUGGUUCCCGCUACGAGACGUCUCCUCGUAGCUUCGGUGGU
GCAGAUGAACUUCAGGGUCA
Strand D
GGGAACCGAGACGUGGUUCCCGCUACGAGUCGUGGUCUCGUAGCUUCGGUGG
UGCAGAUGAACUUCAGGGUCA
Strand E
GGGAACCACCACGAGGUUCCCGCUACGAGAACCAUCCUCGUAGCUUCGGUGGU
GCAGAUGAACUUCAGGGUCA
Strand F
GGGAACCGAUGGUUGGUUCCCGCUACGAGAGUGGACCUCGUAGCUUCGGUGGU
GCAGAUGAACUUCAGGGUCA

RNA fiber functionalized with DS RNAs against GFP:
Strand A
GGGAAUCCAAGGAGGCAGGAUUCCCGUCACAGAAGGAGGCACUGUGAC
Strand B
GGGAACGUAAGCCUCCAACGUUCCCGGAUGCUAAGCCUCCAAGCAUCCUUUGG
UGGUGCAGAUGAACUUCAGGGUCA

GFP Sense
pACCCUGAAGUUCAUCUGCACCACCG
“p” denotes phosphate

GFP Antisense
CGGUGGUGCAGAUGAACUUCAGGGUCA

Six-stranded RNA cube functionalized with six DS RNAs against RhoA:

Strand A

GGCAACUUUGAUCCCUCGGUUUAGCGCCGGCCUUUUCUCCCACACUUUCACGU
UCCUGCUUCAUUUUGGCUAACUCCCGCCUU

Strand B

GGGAAAUUUCGUGGUAGGUUUUGUUGCCCGUGUUUCUACGAUUACUUUGGUCU
UCCUGCUUCAUUUUGGCUAACUCCCGCCUU

Strand C

GGACAUUUUCGAGACAGCAUUUUUUCCCGACCUUUGCGGAUUGUAUUUUAGGU
UCCUGCUUCAUUUUGGCUAACUCCCGCCUU

Strand D

GGCGCUUUUGACCUUCUGCUUUAUGUCCCCUAUUUCUUAAUGACUUUUGGCCU
UCCUGCUUCAUUUUGGCUAACUCCCGCCUU

Strand E

GGGAGAUUUAGUCAUUAAGUUUUACAAUCCGCUUUGUAAUCGUAGUUUGUGUU
UCCUGCUUCAUUUUGGCUAACUCCCGCCUU

Strand F

GGGAUCUUUACCUACCACGUUUUGCUGUCUCGUUUGCAGAAGGUCUUUCCGAU
UCCUGCUUCAUUUUGGCUAACUCCCGCCUU

Six-stranded RNA ring functionalized with six DS RNAs against RhoA:

Strand A

GGGAAUCCGUCCACUGGAUUCCCGUCACAGAGCCUGCCUGUGACUUCCUGCUU
CAUUUUGGCUAACUCCCGCCUU

Strand B

GGGAAUCCGCAGGCUGGAUUCCCGUCACAGAGAACGCCUGUGACUUCCUGCUU
CAUUUUGGCUAACUCCCGCCUU

Strand C

GGGAAUCCGCGUUCUGGAUUCCCGUCACAGACGUCUCCUGUGACUUCCUGCUU
CAUUUUGGCUAACUCCCGCCUU

Strand D

GGGAAUCCGAGACGUGGAUUCCCGUCACAGUCGUGGUCUGUGACUUCCUGCUU
CAUUUUGGCUAACUCCCGCCUU

Strand E

GGGAAUCCACCACGAGGAUUCCCGUCACAGAACCAUCCUGUGACUUCCUGCUUC
AUUUUGGCUAACUCCCGCCUU

Strand F

GGGAAUCCGAUGGUUGGAUUCCCGUCACAGAGUGGACCUGUGACUUCCUGCUU
CAUUUUGGCUAACUCCCGCCUU

RNA fiber functionalized with DS RNAs against RhoA:

Strand A

GGGAAUCCAAGGAGGCAGGAUUCCCGUCACAGAAGGAGGCACUGUGAC

Strand B

GGGAACGUAAGCCUCCAACGUUCCCGGAUGCUAAGCCUCCAAGCAUCCUUCCU
GCUUCAUUUUGGCUAACUCCCGCCUU

RhoA Sense
pGGCGGGAGUUAGCCAAAAUGAAGCAGG

RhoA Antisense
CCUGCUUCAUUUUGGCUAACUCCCGCCUU

GFP Sense with Alexa 488
pACCCUGAAGUUCAUCUGCACCACCG-Alexa488

DNA-Sense-Al488
GGAGACCGTGACCGGTGGTGCAGATGAACTTCAGGGTCATT-Alexa488

DNA-Anti-Sense-Iowa Black
Iowa Black Quencher-TGACCCTGAAGTTCATCTGCACCACCGGTCACGGTCTCC

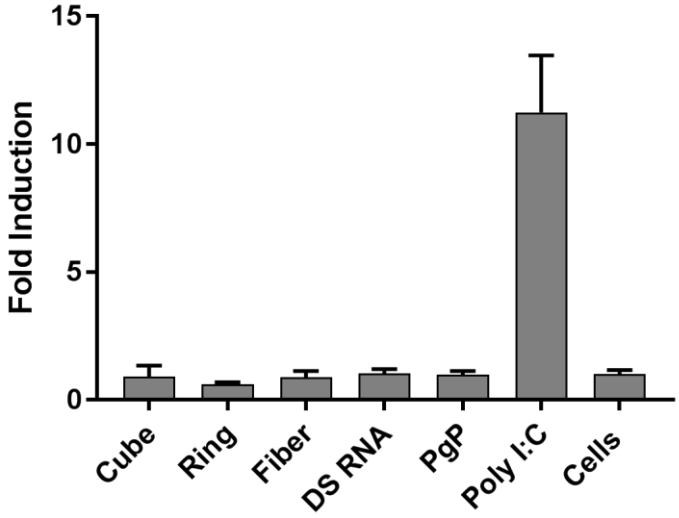
DNA-Anti-Sense
TGACCCTGAAGTTCATCTGCACCACCGGTCACGGTCTCC

SUPPORTING TABLES

	Abs at 540nm			
	sample 1	sample 2	sample 3	% hemolysis
Triton X-100	0.63	0.621	0.684	100±6.68%
PBS	0.136	0.142	0.136	0.59±0.68%
PgP 6 µg (Equivilent to 1 µg NANP)	0.109	0.107	0.102	0.1±0.6%
PgP 12 µg (Equivilent to 2 µg NANP)	0.068	0.065	0.07	-2.48±0.15%
PgP/rings(GFP) 1µg	0.095	0.096	0.088	-8.24±0.85%
PgP/rings(GFP) 2µg	0.127	0.115	0.114	-3.20±1.42%
PgP/cubes(GFP) 1µg	0.119	0.094	0.104	-5.70±2.47%
PgP/cubes(GFP) 2µg	0.14	0.143	0.141	1.24±0.30%
PgP/fibers (GFP) 1µg	0.097	0.125	0.092	-5.95±3.49%
PgP/fibers(GFP) 2µg	0.128	0.134	0.145	0.13±1.69%
PgP/DS RNA(GFP) 1µg	0.116	0.106	0.11	-4.77±0.99%
PgP/DS RNA(GFP) 2µg	0.139	0.148	0.141	1.50±0.93%

Supplementary Table S1. Hemocompatibility of various PgP/NANP polyplexes at N/P ratio of 30/1 on rat erythrocytes *in vitro. % hemolysis= (A_{sample}-A_{PBS}) / ($A_{Tritonx-100}$-A_{PBS}) X 100*

SUPPORTING FIGURES



Supplementary Figure S1: hTLR3 stimulation as measured by HEK-Blue™ hTLR3 cells. Positive control Poly I:C stimulates a response whereas none of the PgP/NANPs trigger hTLR3 activation. In all experiments, cubes (PgP/cubes(GFP)), rings (PgP/rings(GFP)), fibers (PgP/fibers(GFP)), and individual DS RNAs(GFP) are compared.