Azlactone-Functionalized Polymers as Reactive Templates for Parallel Polymer Synthesis: Synthesis and Screening of a Small Library of Cationic Polymers in the Context of DNA Delivery

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Supplementary Information

Experimental Section

General Considerations. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Bruker AC+ 250 (250.133 MHz) and Bruker AC+ 300 (300.135 MHz) spectrometers. Chemical shift values are given in ppm and are referenced with respect to residual protons from solvent. Gel permeation chromatography (GPC) was performed using a GPCmax-VE2001 Solvent/Sample module (Viscotek Corp., Houston, TX) and two PlusPore Organic GPC Columns (Polymer Laboratories, Amherst, MA) in series. For the characterization of poly(2-vinyl-4,4-dimethylazlactone) (PVDMA), THF was used as the eluent at a flow rate of 1.0 mL/min. For the characterization of polymers **P1**-**P12**, THF with 0.1 M triethylamine (TEA) was used as the eluent at a flow rate of 1.0 mL/min. Data were collected using the refractive index detector of a Viscotek TDA 302 triple detector array and processed using the OminiSEC 4.5 software package. Molecular weights and polydispersities are reported relative to monodisperse polystyrene standards. Attenuated total reflectance infrared spectroscopy data were collected on a Bruker TENSOR 27 FTIR instrument (Billerica, MA) outfitted with an ATR transmission cell from Pike Technologies (Madison, WI). Fluorescence microscopy images used to evaluate the expression of enhanced green fluorescent protein in transfection experiments were recorded

using an Olympus IX70 microscope and were analyzed using the Metavue version 4.6 software package (Universal Imaging Corporation). Data were stored in single channel, 12-bit TIF format. Additional image processing was limited to false coloring and scaling. Absorbance and luminescence measurements used to characterize cell viability and metabolism, luciferase expression, and total cell protein were made using a PerkinElmer EnVision multilabel plate reader (MTT Assays: Ex: 550 nm; Luciferase: Em: 700 nm cutoff; BCA: Ex: 560 nm).

Materials. Primary amine-functionalized compounds **1-12** were purchased from Aldrich Chemical Company (Milwaukee, WI), Acros Organics (Morris Plains, NJ), and Alfa Aesar Organics (Ward Hill, MA). 2,2'-Azobisisobutyronitrile (AIBN) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Linear poly(ethyleneimine) (LPEI) was synthesized by hydrolysis of the side chains of poly(2-ethyloxazoline) (MW = 50,000; obtained from Polysciences, Inc., Warrington, PA) and purified prior to use in analogy to procedures previously described.[1,2] Branched poly(ethyleneimine) (BPEI) was purchased from Aldrich Chemical Co. (MW = 25,000, Milwaukee, WI). 2-Vinyl-4,4-dimethylazlactone (VDMA) was a generous gift from Dr. Steven M. Heilmann (3M Corporation, Minneapolis, MN). Anhydrous THF was purchased from Acros Organics (Morris Plains, NJ). Plasmid DNA constructs containing the firefly luciferase reporter gene (pCMV-Luc) and enhanced green fluorescence protein reporter gene (pEGFP-N1) were purchased from Elim Biopharmaceuticals, Inc. (San Francisco, CA). Monkey kidney fibroblasts (COS-7 cells) used in transfection assays were purchased from American Type Culture Collection (Manassas, VA) and grown at 37°C, 5% CO_2 in Dulbecco's modified Eagle's medium, 90%; fetal bovine serum, 10%; penicillin, 100 units/ml; streptomycin, 100 μg/ml. Luciferase detection kits used in transfection assays were purchased from Promega

Corp. (Madison, WI). BCA protein assay kits used in total cell protein assays were purchased from Pierce Biotechnology, Inc. (Rockford, IL). All other materials and solvent were used as received without any further purification unless otherwise noted.

Polymerization of 2-Vinyl-4,4-dimethylazlactone. 2-Vinyl-4,4-dimethylazlactone (VDMA) was purified by passage through inhibitor removal resin followed by passage through a plug of silica gel prior to polymerization. The initiator 2,2'-azobisisobutyronitrile (AIBN, 14.1 mg, 0.086 mmol) was weighed into a 25 mL round-bottomed flask equipped with a stir bar. Ethyl acetate (12 mL) was added and the solution was purged with N_2 for 10 minutes. VDMA (3.36 g, 24.2 mmol) was added to the flask via syringe. The N_2 purge was continued for an additional 5 minutes. The solution was stirred under N_2 at 60 °C for 24 hours. After 24 hours, the viscous reaction mixture was cooled to room temperature. Approximately 5 mL of CH_2Cl_2 were added to the flask and the polymer was precipitated into hexanes to produce a white solid. The polymer was redissolved in CH_2Cl_2 and precipitated twice more in hexanes to obtain a white solid in 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.37 (br s, (-C***H*****3**)$_2$), 1.62-2.1 (br m, -C***H*****2**CH-), 2.69 (br s, -CH$_2$C***H***-). FT-IR (ATR, cm^{-1}): 2980-2900 (C-H), 1820 (lactone C=O), 1672 (C=N). M_n = 73,873; PDI = 2.65. For experiments designed to synthesize lower molecular weight PVDMA (see text) by atom-transfer radical polymerization (ATRP): Copper(I) bromide (18.8 mg, 13.1 mmol) was added to a 10 mL round-bottomed flask equipped with a stir bar. The flask was capped with a septum and purged with N_2 for 10 mins. VDMA (3.063 g, 22 mmol) was added to the flask through a syringe. Anhydrous toluene (3 mL) and PMDETA (54.5 μL, 44.9 mg, 0.26 mmol) were added to the flask via syringe and the solution was purged with N_2 for an additional 5 mins at 50 ºC. The initiator ethyl-2-bromoisobutyrate (63.4 μL, 84.3 mg, 0.43

mmol) was added to the flask. The reaction was stirred under N_2 at 50 ºC for 21.5 hrs. After cooling to room temperature, the viscous reaction mixture was diluted with CH_2Cl_2 and passed through a silica gel column to remove the copper complex. The polymer was then precipitated into hexanes twice to obtain an off-white solid in 38% yield. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.37 (br s, (-C*H*$_3$)$_2$), 1.62-2.1 (br m, -C*H*$_2$CH-), 2.69 (br s, -CH$_2$C*H*-). FT-IR (ATR, cm^{-1}): 2980-2900 (C-H), 1820 (lactone C=O), 1672 (C=N). M_n = 5,840, PDI = 1.11.

Synthesis of Polymers P1–P12. In a typical experiment, poly(2-vinyl-4,4-dimethylazlactone) (PVDMA) (150 mg, 1.08 mmol with respect to the molecular weight of the polymer repeat unit) was dissolved in 5 mL anhydrous THF in 12 different reaction vials, and 1.5 equivalent of each different primary amine-functionalized side chain (compounds **1**-**12**, see main text) (1.62 mmol) was added to each vial. The resulting solutions were stirred at 50 °C for 24 hours. The reaction solutions were concentrated under vacuum and precipitated into an acetone/hexane solution (40 ml, 1:2 v/v). Precipitated polymer was collected by decanting the supernatant, and the polymer was precipitated twice more using the above procedure. The final products were dried under vacuum. Exhaustive functionalization of PVDMA in each case was confirmed by the absence of the carbonyl peak of the azlactone functionality of PVDMA (at 1820 cm^{-1}) in each product polymer, as determined using attenuated total reflectance infrared spectroscopy. The structure of each of the polymers (referred to hereafter as polymers **P1**-**P12**, see structures in main text and in additional pages below) were confirmed by ^{1}H nuclear magnetic resonance (NMR). ^{1}H NMR data for each polymer are as follows: Polymer **P1**: ^{1}H NMR (300.135 MHz, D_2O, δ): 1.48 (br s, 8H), 2.21 (br m, 9H), 3.30 (br s, 2H). Polymer **P2**: ^{1}H NMR (300.135 MHz, D_2O, δ): 1.51 (br m, 10H), 2.18 (br m, 9H), 3.19 (br s, 2H). Polymer **P3**: ^{1}H NMR (300.135 MHz, $CDCl_3$, δ): 0.99 (br

s, 14H), 1.47 (br m, 6H), 2.55 (br s, 3H), 3.00 (br m, 4H), 6.71 (br s, 2H). Polymer **P4**: ^{1}H NMR (300.135 MHz, D_2O, δ): 1.03 (s, 8H), 1.51 (br s, 6H), 2.59 (br s, 7H), 3.31 (br s, 2H). Polymer **P5**: ^{1}H NMR (300.135 MHz, D_2O, δ): 0.98 (s, 8H), 1.51 (br m, 8H), 2.52 (br s, 7H), 3.19 (br s, 2H). Polymer **P6**: ^{1}H NMR (300.135 MHz, $CDCl_3$, δ): 0.99 (br m, 9H), 1.44 (br s, 12H), 2.50 (br m, 7H), 3.84 (br s, 1H), 5.84 (br s, 2H). Polymer **P7**: ^{1}H NMR (300.135 MHz, $CDCl_3$, δ): 1.43 (br m, 12 H), 2.50 (br s, 7H), 3.32 (br s, 2H), 6.72 (br s, 2H). Polymer **P8**: ^{1}H NMR (300.135 MHz, $CDCl_3$, δ): 1.37 (br m, 14 H), 2.21 (br s, 7H), 3.21 (br s, 2H), 6.70 (br s, 2H). Polymer **P9**: ^{1}H NMR (300.135 MHz, $CDCl_3$, δ): 1.56 (br m, 16 H), 2.37 (br s, 7H), 3.26 (br s, 2H), 7.63 (br s, 2H). Polymer **P10**: ^{1}H NMR (300.135 MHz, $CDCl_3$, δ): 1.59 (br m, 19 H), 2.21 (br m, 5H), 3.14 (br m, 3H), 7.53 (br s, 2H). Polymer **P11**: ^{1}H NMR (300.135 MHz, $CDCl_3$, δ): 1.47 (br m, 8H), 2.46 (br s, 7H), 3.29 (br s, 2H), 3.68 (s, 4H), 6.64 (br s, 2H). Polymer **P12**: ^{1}H NMR (300.135 MHz, $CDCl_3$, δ): 1.67 (br m, 10H), 2.45 (br s, 7H), 3.29 (br s, 2H), 3.69 (s, 4H), 7.36 (br s, 2H). The molecular weights and polydispersities of these polymers are presented in Table S1, below.

Table S1: Molecular Weights and Polydispersities of Polymers **P1-P12**.[a]

Polymer	M_n	PDI
P1	33,500	1.78
P2	43,013	1.61
P3	35,960	2.06
P4	37,633	1.57
P5	39,675	1.66
P6	43,114	1.81
P7	31,044	1.52
P8	30,396	1.42
P9	32,520	1.60
P10	31,019	1.53
P11	58,139	3.45
P12	59,411	3.74

[a]As determined by GPC, relative to polystyrene standards.

Preparation of Samples for Agarose Gel Retardation Assays and Cell Transfection Experiments. DNA/polymer complexes (polyplexes) were formed by adding 50 μL of a plasmid DNA solution (2 μg/50 μL in water) to a gently vortexing solution of polymer (50 μL in either 20 mM HEPES, pH = 7.2 or 20 mM acetate buffer, pH = 5.0). In every case, the concentration of polymer in this volume of buffer was adjusted to yield a desired DNA/polymer weight ratio (e.g., 1:1, 1:2, 1:3, etc.; see main text). Formations of polyplexes (30 μL) prepared at desired DNA/polymer ratios were mixed with a loading buffer and analyzed on a 1% agarose gel (HEPES, 20 mM, pH = 7.2, 108 V, 50 min). DNA bands were visualized by ethidium bromide staining.

Gerneral Protocols for Cell Transfection Experiments and Characterization of Transgene Expression. COS-7 cells were grown in clear or opaque 96-well culture plates (for experiments

using pEGFP-N1 and pCMV-Luc, respectively) at initial seeding densities of 15,000 cells/well in 200 μL of growth medium (90% Dulbecco's modified Eagle's medium, 10% fetal bovine serum, penicillin 100 units/mL, streptomycin 100 μg/mL). After plating, all cells were incubated at 37 °C for 24 hours and transfection experiments were conducted at approximately 80% confluence.

For experiments conducted using pEGFP-N1, formulations of polyplexes (30 μL) prepared at desired pEGFP-N1/polymer ratios (as described above, and further in the main text) were added to cells after an initial replacement of serum-containing media (DMEM + 10% FBS) with 200 μL of OptiMEM media. Cells were incubated for four hours, and media was aspirated from all samples and replaced with 200 μL of DMEM. Culture plates were incubated for 48 hours at 37 °C, at which point cell morphology and gene expression were visualized using phase contrast and fluorescence microscopy.

For experiments conducted using pCMV-Luc, formulations of polyplexes (30 μL) prepared at desired pCMV-Luc/polymer ratios (as described above, and further in the main text) were added to cells after an initial replacement of serum-containing media (DMEM + 10% FBS) with 200 μL of OptiMEM media. Cells were incubated for four hours, and media was aspirated from all samples and replaced with 200 μL of DMEM. Culture plates were incubated for 48 hours at 37 °C, at which point luciferase expression and total cell protein were determined with commercially available luciferase assay kits and BCA protein assay kits, respectively, using the manufacturers' specified protocols and a PerkinElmer EnVision multilabel plate reader. Luminescence was expressed in relative light units and was normalized to total cell protein.

For experiments conducted to evaluate the influence of polymer structure on cell viability and metabolism, formulations of polyplexes (30 μL) prepared at desired pEGFP-N1/polymer ratios (as described above) were added to cells after an initial replacement of serum-containing

media (DMEM + 10% FBS) with 200 μL of OptiMEM media. Cells were incubated for four hours, and media was aspirated from all samples and replaced with 200 μL of DMEM. Culture plates were incubated for 48 hours at 37 °C, at which point 25 μL of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in phosphate buffered saline (PBS, pH = 7.4, 137 mM NaCl) was added to each well of the culture plates. Cells were further incubated for four hours, and PBS solution was aspirated from all samples and replaced with 200 μL of DMSO to dissolve MTT crystals. Absorbance at 550 nm was measured after cells were incubated for an additional 10 minutes.

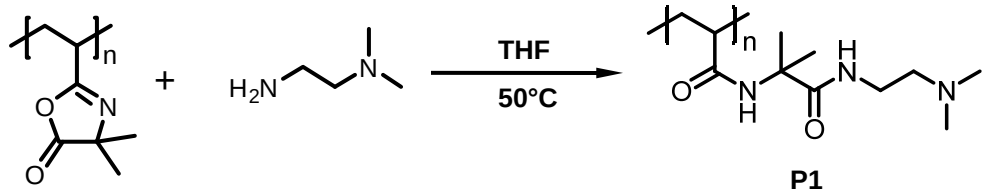
References

(1) Liu, X. H.; Yang, J. W.; Miller, A. D.; Nack, E. A.; Lynn, D. M. *Macromolecules* 2005, *38*, 7907-7914.

(2) Brissault, B.; Kichler, A.; Guis, C.; Leborgne, C.; Danos, O.; Cheradame, H. *Bioconjugate Chemistry* 2003, *14*, 581-587.

Supplementary Data for Experiments Using Polymer P1

A)



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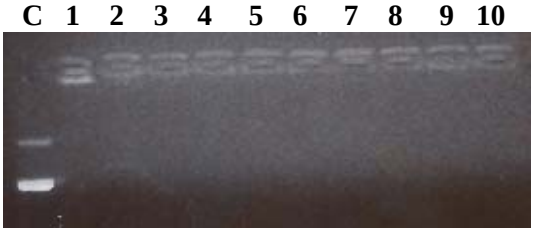

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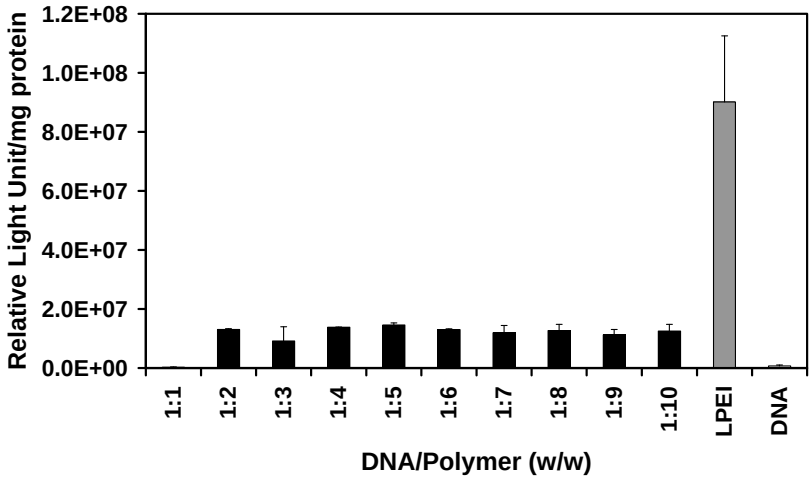
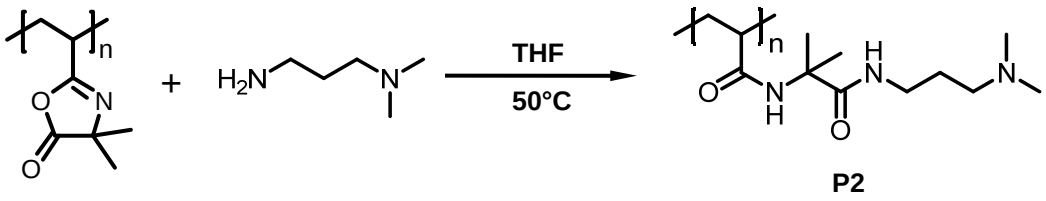


A) Reaction scheme showing synthesis of polymer **P1**. B) Results of agarose gel electrophoresis experiments to characterize the self-assembly of polymer **P1** with plasmid DNA encoding EGFP (pEGFP-N1). The leftmost lane labeled "C" corresponds to a DNA control (no polymer). Additional lanes labeled 1-10 correspond to DNA/polymer weight ratios ranging from 1:1 to 1:10, respectively. DNA bands were visualized by ethidium bromide staining. C) Results of an initial screening experiment showing levels of transgene expression as a function of DNA/polymer weight ratios for COS-7 cells treated with polyplexes formed using plasmid DNA (pCMV-Luc) encoding firefly luciferase. Light units are arbitrary and normalized to total cell protein. Experiments were performed in triplicate. Experiments performed in the absence of polymer (DNA only) and polyplexes formed using LPEI at a DNA/polymer weight ratio of 1:2 are shown for comparison. See Experimental Section for additional details of all experiments and assays.

Supplementary Data for Experiments Using Polymer P2

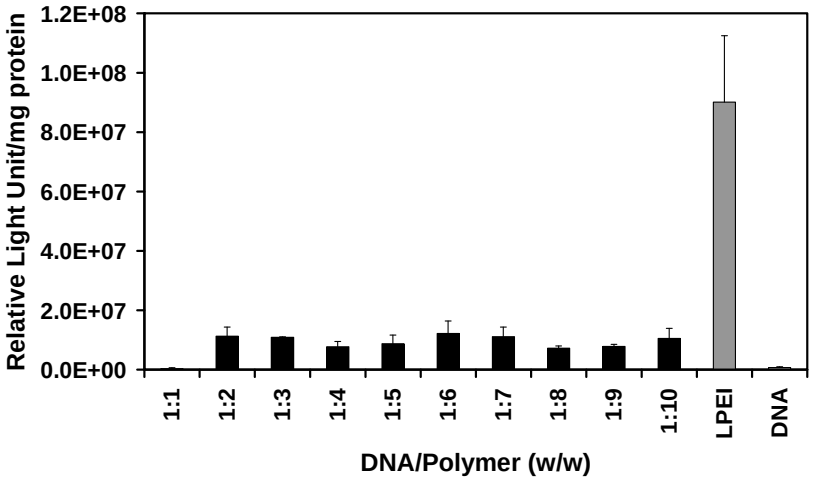
A)



P2

B)

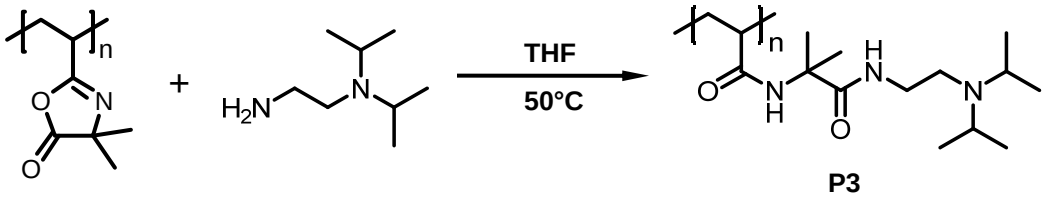
C)



A) Reaction scheme showing synthesis of polymer **P2**. B) Results of agarose gel electrophoresis experiments to characterize the self-assembly of polymer **P2** with plasmid DNA encoding EGFP (pEGFP-N1). The leftmost lane labeled "C" corresponds to a DNA control (no polymer). Additional lanes labeled 1-10 correspond to DNA/polymer weight ratios ranging from 1:1 to 1:10, respectively. DNA bands were visualized by ethidium bromide staining. C) Results of an initial screening experiment showing levels of transgene expression as a function of DNA/polymer weight ratios for COS-7 cells treated with polyplexes formed using plasmid DNA (pCMV-Luc) encoding firefly luciferase. Light units are arbitrary and normalized to total cell protein. Experiments were performed in triplicate. Experiments performed in the absence of polymer (DNA only) and polyplexes formed using LPEI at a DNA/polymer weight ratio of 1:2 are shown for comparison. See Experimental Section for additional details of all experiments and assays.

Supplementary Data for Experiments Using Polymer P3

A)



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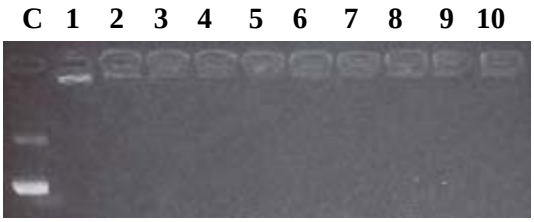

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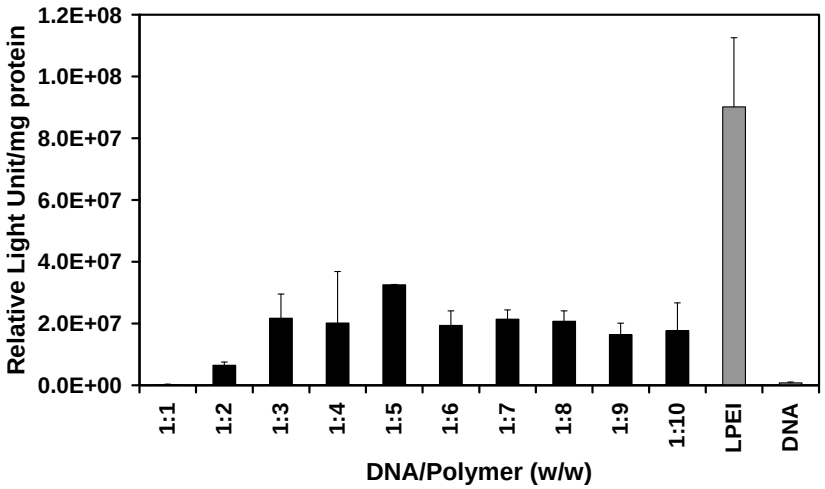
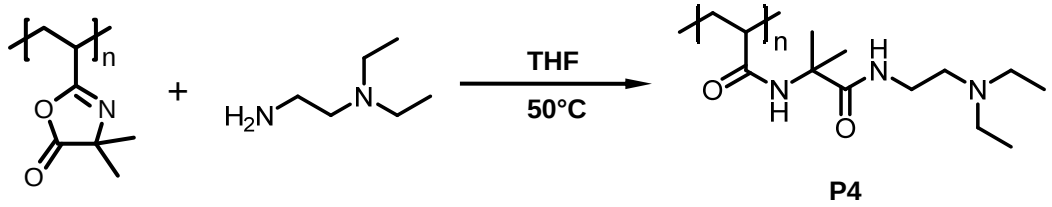


A) Reaction scheme showing synthesis of polymer **P3**. B) Results of agarose gel electrophoresis experiments to characterize the self-assembly of polymer **P3** with plasmid DNA encoding EGFP (pEGFP-N1). The leftmost lane labeled "C" corresponds to a DNA control (no polymer). Additional lanes labeled 1-10 correspond to DNA/polymer weight ratios ranging from 1:1 to 1:10, respectively. DNA bands were visualized by ethidium bromide staining. C) Results of an initial screening experiment showing levels of transgene expression as a function of DNA/polymer weight ratios for COS-7 cells treated with polyplexes formed using plasmid DNA (pCMV-Luc) encoding firefly luciferase. Light units are arbitrary and normalized to total cell protein. Experiments were performed in triplicate. Experiments performed in the absence of polymer (DNA only) and polyplexes formed using LPEI at a DNA/polymer weight ratio of 1:2 are shown for comparison. See Experimental Section for additional details of all experiments and assays.

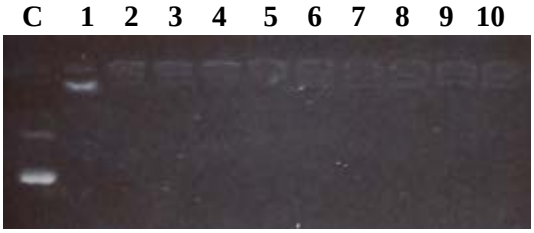
Supplementary Data for Experiments Using Polymer P4

A)



P4

B)



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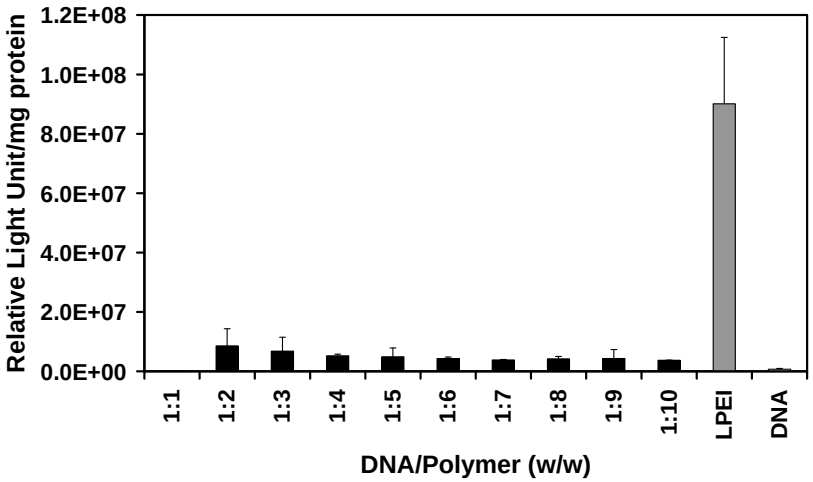
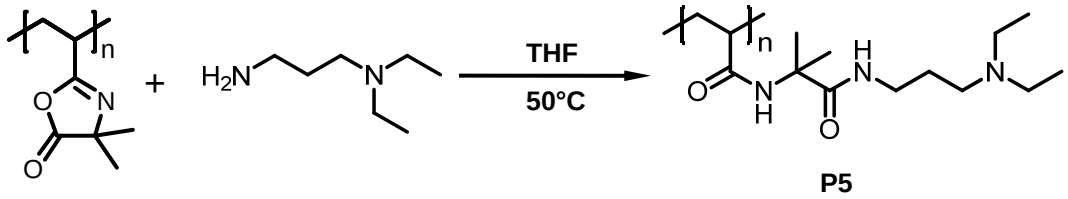


A) Reaction scheme showing synthesis of polymer **P4**. B) Results of agarose gel electrophoresis experiments to characterize the self-assembly of polymer **P4** with plasmid DNA encoding EGFP (pEGFP-N1). The leftmost lane labeled "C" corresponds to a DNA control (no polymer). Additional lanes labeled 1-10 correspond to DNA/polymer weight ratios ranging from 1:1 to 1:10, respectively. DNA bands were visualized by ethidium bromide staining. C) Results of an initial screening experiment showing levels of transgene expression as a function of DNA/polymer weight ratios for COS-7 cells treated with polyplexes formed using plasmid DNA (pCMV-Luc) encoding firefly luciferase. Light units are arbitrary and normalized to total cell protein. Experiments were performed in triplicate. Experiments performed in the absence of polymer (DNA only) and polyplexes formed using LPEI at a DNA/polymer weight ratio of 1:2 are shown for comparison. See Experimental Section for additional details of all experiments and assays.

Supplementary Data for Experiments Using Polymer P5

A)



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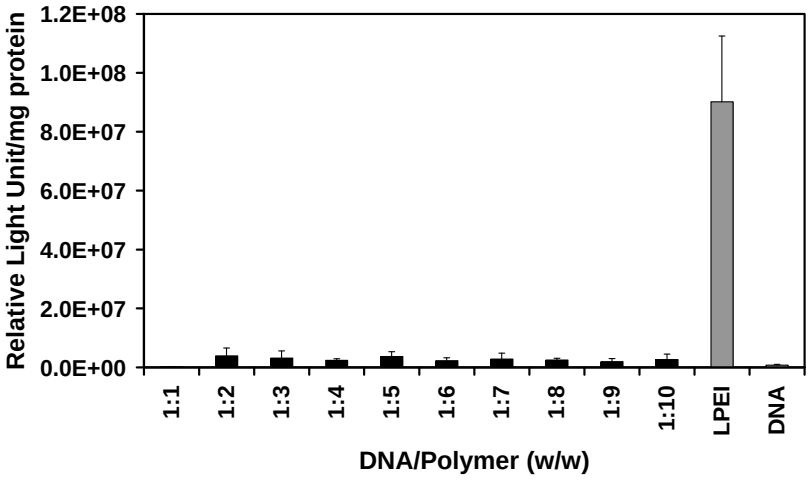
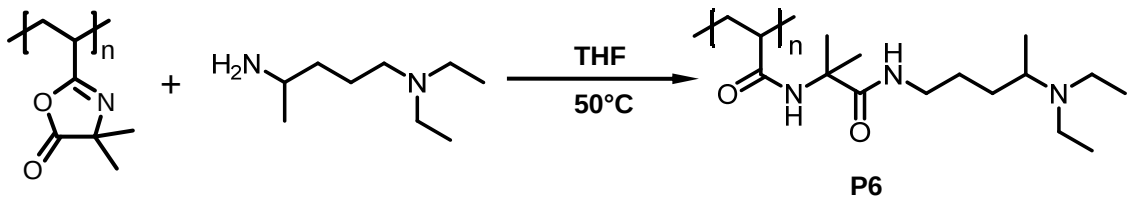


A) Reaction scheme showing synthesis of polymer **P5**. B) Results of agarose gel electrophoresis experiments to characterize the self-assembly of polymer **P5** with plasmid DNA encoding EGFP (pEGFP-N1). The leftmost lane labeled "C" corresponds to a DNA control (no polymer). Additional lanes labeled 1-10 correspond to DNA/polymer weight ratios ranging from 1:1 to 1:10, respectively. DNA bands were visualized by ethidium bromide staining. C) Results of an initial screening experiment showing levels of transgene expression as a function of DNA/polymer weight ratios for COS-7 cells treated with polyplexes formed using plasmid DNA (pCMV-Luc) encoding firefly luciferase. Light units are arbitrary and normalized to total cell protein. Experiments were performed in triplicate. Experiments performed in the absence of polymer (DNA only) and polyplexes formed using LPEI at a DNA/polymer weight ratio of 1:2 are shown for comparison. See Experimental Section for additional details of all experiments and assays.

Supplementary Data for Experiments Using Polymer P6

A)



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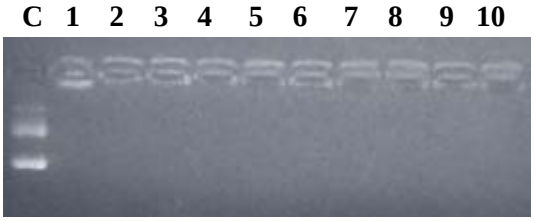

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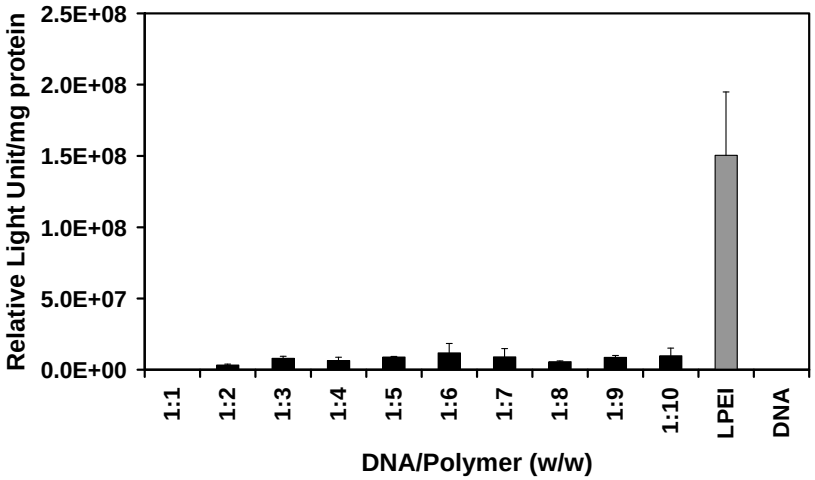
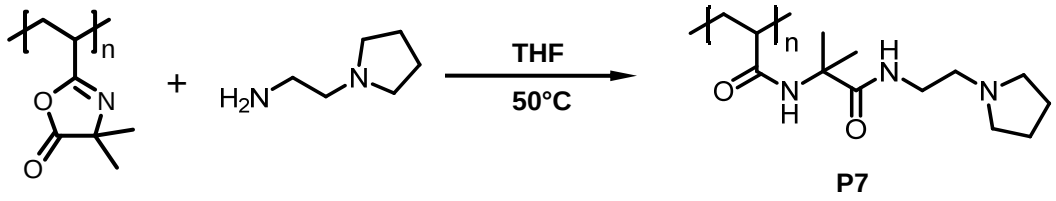


A) Reaction scheme showing synthesis of polymer **P6**. B) Results of agarose gel electrophoresis experiments to characterize the self-assembly of polymer **P6** with plasmid DNA encoding EGFP (pEGFP-N1). The leftmost lane labeled "C" corresponds to a DNA control (no polymer). Additional lanes labeled 1-10 correspond to DNA/polymer weight ratios ranging from 1:1 to 1:10, respectively. DNA bands were visualized by ethidium bromide staining. C) Results of an initial screening experiment showing levels of transgene expression as a function of DNA/polymer weight ratios for COS-7 cells treated with polyplexes formed using plasmid DNA (pCMV-Luc) encoding firefly luciferase. Light units are arbitrary and normalized to total cell protein. Experiments were performed in triplicate. Experiments performed in the absence of polymer (DNA only) and polyplexes formed using LPEI at a DNA/polymer weight ratio of 1:2 are shown for comparison. See Experimental Section for additional details of all experiments and assays.

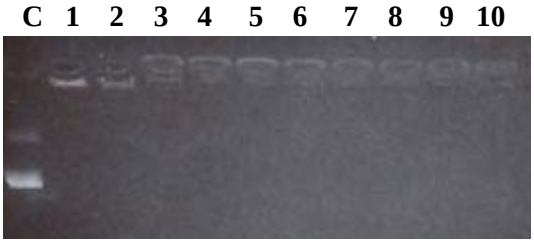
Supplementary Data for Experiments Using Polymer P7

A)

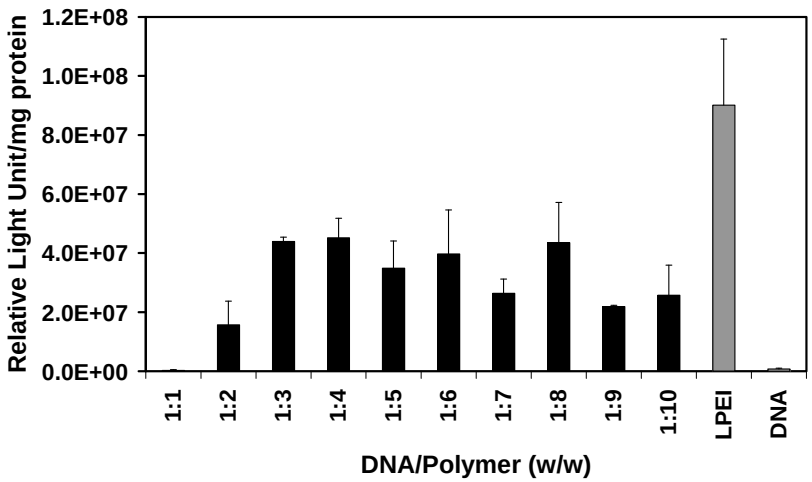


P7

B)



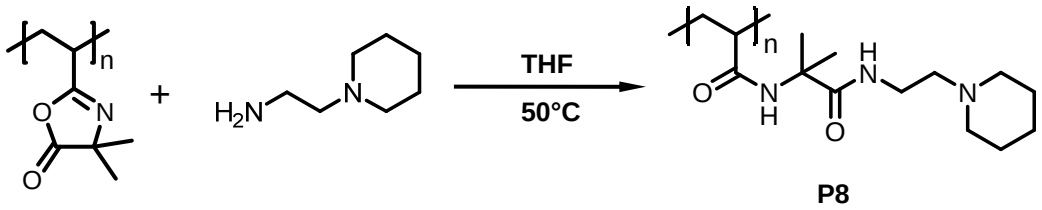
C)



A) Reaction scheme showing synthesis of polymer **P7**. B) Results of agarose gel electrophoresis experiments to characterize the self-assembly of polymer **P7** with plasmid DNA encoding EGFP (pEGFP-N1). The leftmost lane labeled "C" corresponds to a DNA control (no polymer). Additional lanes labeled 1-10 correspond to DNA/polymer weight ratios ranging from 1:1 to 1:10, respectively. DNA bands were visualized by ethidium bromide staining. C) Results of an initial screening experiment showing levels of transgene expression as a function of DNA/polymer weight ratios for COS-7 cells treated with polyplexes formed using plasmid DNA (pCMV-Luc) encoding firefly luciferase. Light units are arbitrary and normalized to total cell protein. Experiments were performed in triplicate. Experiments performed in the absence of polymer (DNA only) and polyplexes formed using LPEI at a DNA/polymer weight ratio of 1:2 are shown for comparison. See Experimental Section for additional details of all experiments and assays.

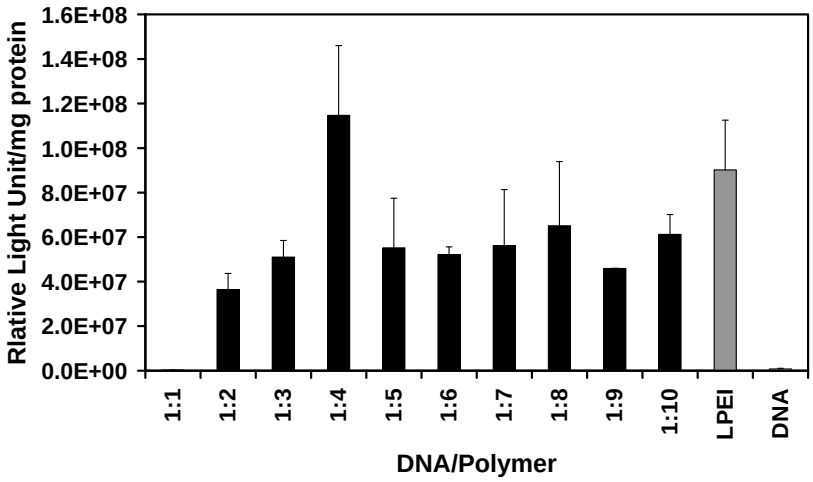
Supplementary Data for Experiments Using Polymer P8

A)



B)

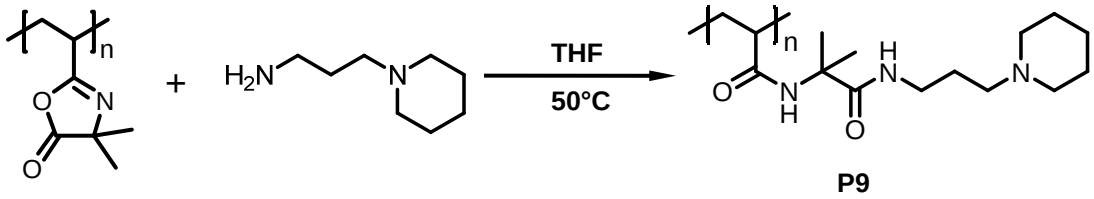
C)



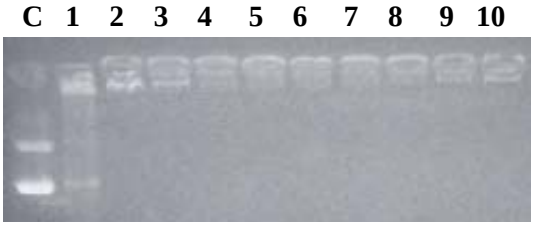
A) Reaction scheme showing synthesis of polymer **P8**. B) Results of agarose gel electrophoresis experiments to characterize the self-assembly of polymer **P8** with plasmid DNA encoding EGFP (pEGFP-N1). The leftmost lane labeled "C" corresponds to a DNA control (no polymer). Additional lanes labeled 1-10 correspond to DNA/polymer weight ratios ranging from 1:1 to 1:10, respectively. DNA bands were visualized by ethidium bromide staining. C) Results of an initial screening experiment showing levels of transgene expression as a function of DNA/polymer weight ratios for COS-7 cells treated with polyplexes formed using plasmid DNA (pCMV-Luc) encoding firefly luciferase. Light units are arbitrary and normalized to total cell protein. Experiments were performed in triplicate. Experiments performed in the absence of polymer (DNA only) and polyplexes formed using LPEI at a DNA/polymer weight ratio of 1:2 are shown for comparison. See Experimental Section for additional details of all experiments and assays.

Supplementary Data for Experiments Using Polymer P9

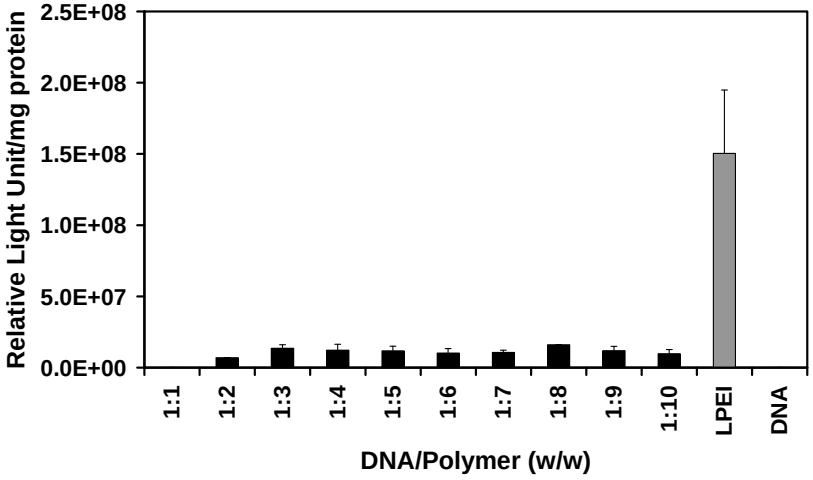
A)



B)



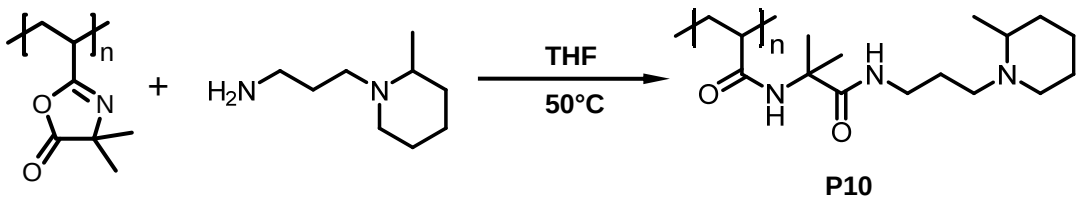
C)



A) Reaction scheme showing synthesis of polymer **P9**. B) Results of agarose gel electrophoresis experiments to characterize the self-assembly of polymer **P9** with plasmid DNA encoding EGFP (pEGFP-N1). The leftmost lane labeled "C" corresponds to a DNA control (no polymer). Additional lanes labeled 1-10 correspond to DNA/polymer weight ratios ranging from 1:1 to 1:10, respectively. DNA bands were visualized by ethidium bromide staining. C) Results of an initial screening experiment showing levels of transgene expression as a function of DNA/polymer weight ratios for COS-7 cells treated with polyplexes formed using plasmid DNA (pCMV-Luc) encoding firefly luciferase. Light units are arbitrary and normalized to total cell protein. Experiments were performed in triplicate. Experiments performed in the absence of polymer (DNA only) and polyplexes formed using LPEI at a DNA/polymer weight ratio of 1:2 are shown for comparison. See Experimental Section for additional details of all experiments and assays.

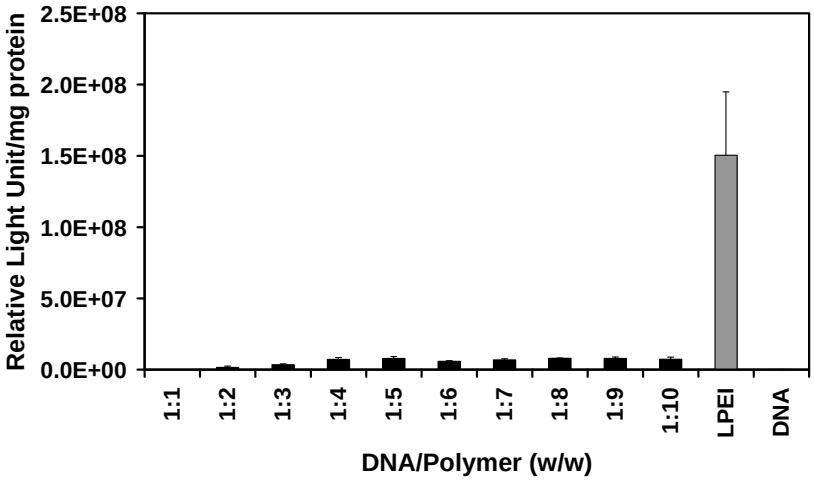
Supplementary Data for Experiments Using Polymer P10

A)



B)

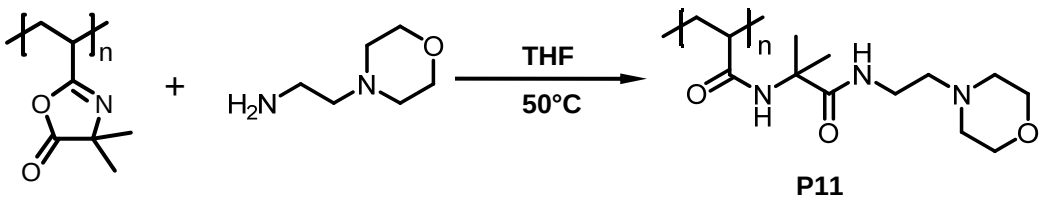
C)



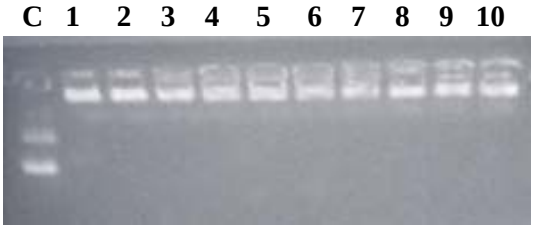
A) Reaction scheme showing synthesis of polymer **P10**. B) Results of agarose gel electrophoresis experiments to characterize the self-assembly of polymer **P10** with plasmid DNA encoding EGFP (pEGFP-N1). The leftmost lane labeled "C" corresponds to a DNA control (no polymer). Additional lanes labeled 1-10 correspond to DNA/polymer weight ratios ranging from 1:1 to 1:10, respectively. DNA bands were visualized by ethidium bromide staining. C) Results of an initial screening experiment showing levels of transgene expression as a function of DNA/polymer weight ratios for COS-7 cells treated with polyplexes formed using plasmid DNA (pCMV-Luc) encoding firefly luciferase. Light units are arbitrary and normalized to total cell protein. Experiments were performed in triplicate. Experiments performed in the absence of polymer (DNA only) and polyplexes formed using LPEI at a DNA/polymer weight ratio of 1:2 are shown for comparison. See Experimental Section for additional details of all experiments and assays.

Supplementary Data for Experiments Using Polymer P11

A)



B)



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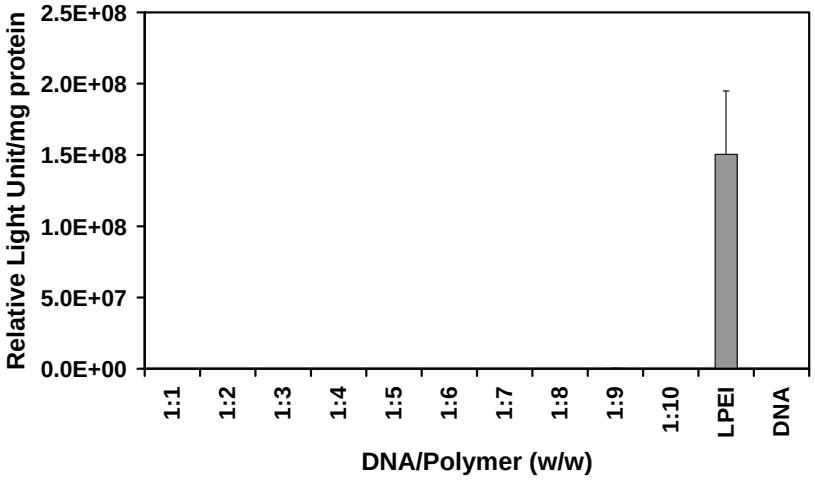
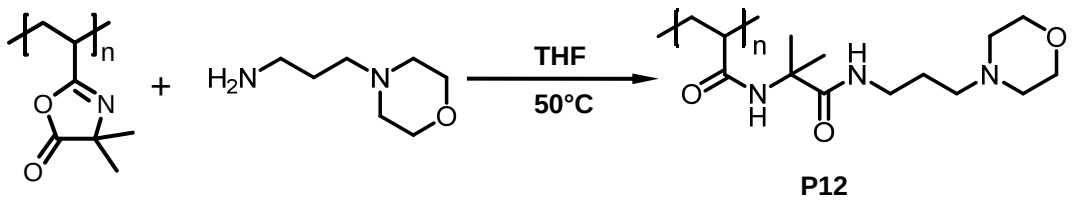


A) Reaction scheme showing synthesis of polymer **P11**. B) Results of agarose gel electrophoresis experiments to characterize the self-assembly of polymer **P11** with plasmid DNA encoding EGFP (pEGFP-N1). The leftmost lane labeled "C" corresponds to a DNA control (no polymer). Additional lanes labeled 1-10 correspond to DNA/polymer weight ratios ranging from 1:1 to 1:10, respectively. DNA bands were visualized by ethidium bromide staining. C) Results of an initial screening experiment showing levels of transgene expression as a function of DNA/polymer weight ratios for COS-7 cells treated with polyplexes formed using plasmid DNA (pCMV-Luc) encoding firefly luciferase. Light units are arbitrary and normalized to total cell protein. Experiments were performed in triplicate. Experiments performed in the absence of polymer (DNA only) and polyplexes formed using LPEI at a DNA/polymer weight ratio of 1:2 are shown for comparison. See Experimental Section for additional details of all experiments and assays.

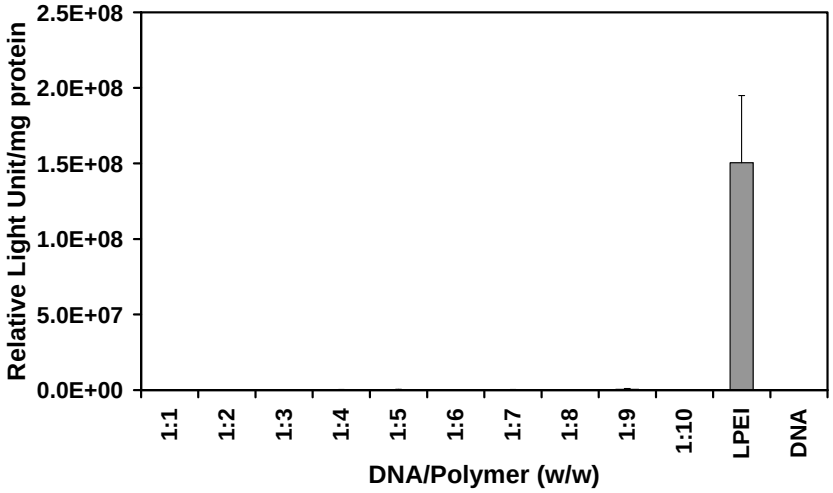
Supplementary Data for Experiments Using Polymer P12

A)



B)

C)



A) Reaction scheme showing synthesis of polymer **P12**. B) Results of agarose gel electrophoresis experiments to characterize the self-assembly of polymer **P12** with plasmid DNA encoding EGFP (pEGFP-N1). The leftmost lane labeled "C" corresponds to a DNA control (no polymer). Additional lanes labeled 1-10 correspond to DNA/polymer weight ratios ranging from 1:1 to 1:10, respectively. DNA bands were visualized by ethidium bromide staining. C) Results of an initial screening experiment showing levels of transgene expression as a function of DNA/polymer weight ratios for COS-7 cells treated with polyplexes formed using plasmid DNA (pCMV-Luc) encoding firefly luciferase. Light units are arbitrary and normalized to total cell protein. Experiments were performed in triplicate. Experiments performed in the absence of polymer (DNA only) and polyplexes formed using LPEI at a DNA/polymer weight ratio of 1:2 are shown for comparison. See Experimental Section for additional details of all experiments and assays.

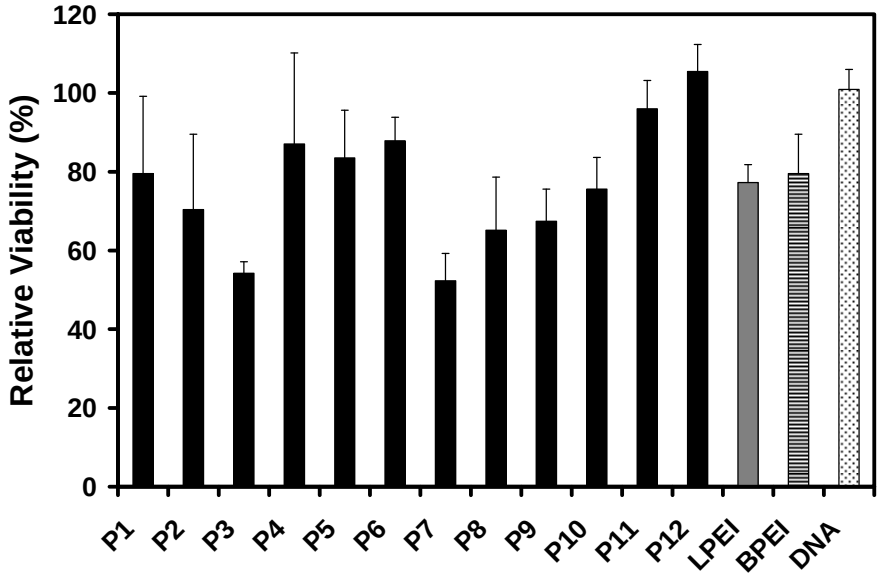


Figure S1: Characterization of the influence of polyplexes on the metabolism and viability of COS-7 cells, as determined using an MTT assay. Experiments were conducted using polyplexes formed using plasmid DNA and polymers **P1**-**P12**. Polyplexes used in each case were formed at the DNA/polymer ratios (w/w) determined to mediate the highest levels of transfection for each polymer (see text, and additional data in Supporting Information above). Experiments were performed in triplicate. Experiments performed in the absence of polymer (DNA only) and using polyplexes formed using LPEI and BPEI at DNA/polymer weight ratios of 1:2 are shown for comparison. See Materials and Methods for additional details related to the formation of polyplexes and cell culture and cytotoxicity experiments.

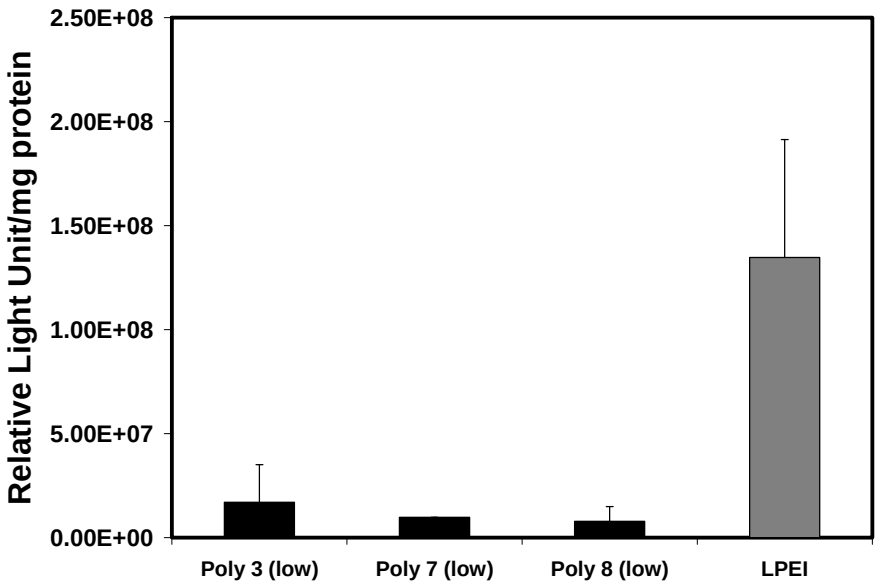


Figure S2: Levels of transgene expression as a function of polymer structure for COS-7 cells treated with polyplexes formed using polymers **P3**, **P7**, and **P8** synthesized from low molecular weight PVDMA (M_n = 5,800; PDI = 1.11) with plasmid DNA encoding firefly luciferase. Light units are arbitrary and normalized to total cell protein. Experiments were performed in triplicate. Experiments performed with polyplexes formed using LPEI at DNA/polymer weight ratios of 1:2 are shown for comparison.