The Effect and Role of Carbon Atoms in Poly(beta-amino ester)s for DNA Binding and Gene Delivery

Corey J. Bishop, Tiia-Maaria Ketola, Stephany Y. Tzeng, Joel C. Sunshine, Arto Urtti, Helge Lemmetyinen, Elina Vuorimaa-Laukkanen, Marjo Yliperttula, Jordan J. Green

Experimental Procedure

I. Materials (*Reagents, assays, cells and instruments*)

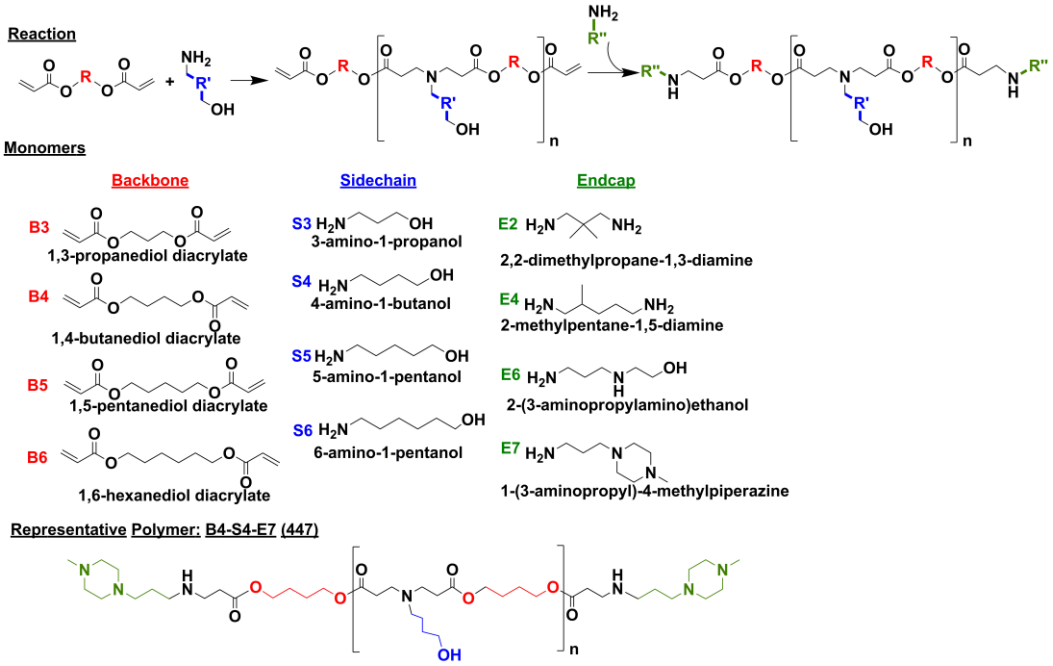
The polymers were synthesized from commercially available monomers: 1,3-propanediol diacrylate (B3) (Monomer-Polymer and Dajac Laboratories Inc.), 1,4-butanediol diacrylate (B4) (Alfa Aesar), 1,5-pentanediol diacrylate (B5) (Monomer-Polymer and Dajac Laboratories Inc.), 1,6-hexanediol diacrylate (B6) (Alfa Aesar), 3-amino-1-propanol (S3), 4-amino-1-butanol (S4) (Alfa Aesar), 5-amino-1-pentanol (S5) (Alfa Aesar), 6-amino-1-hexanol (S6) (Sigma Aldrich), 2,2-dimethyl-1,3-propanediamine (E2) (Sigma Aldrich), 2-methyl-1,5-diaminopentane (E4) (TCI America), 2-(3-aminopropylamino)ethanol (E6) (Sigma Aldrich), 1-(3-aminopropyl)-4-methylpiperazine (E7) (Alfa Aesar). Other reagents include the following and were used as received: peptide $(KK)_2KGGC$ (Biomatik), tetrahydrofuran (THF) (Sigma Aldrich), dimethyl sulfoxide (DMSO), (Sigma Aldrich), ethidium bromide (ETB; Sigma Aldrich), Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA), OptiMEM I (Invitrogen), plasmid enhanced green fluorescent protein (pEGFP-N1) DNA (Clontech), amplified and purified by Aldevron (Fargo, ND). The breast cancer cell line (MDA-MB-231; ATCC) is of human origin and was cultured using DMEM high glucose 1x media and supplemented with 10% heat inactivated fetal bovine serum (FBS) and 100 U/mL of penicillin and 100 μg/mL of streptomycin (Invitrogen). The glioblastoma multiforme (GBM) cell line (GBM319) was derived from brain tumor stem cells from a 79-year old patient, was cultured as previously described in DMEM:Ham's F12 (1:1) (Invitrogen) supplemented with 10% heat inactivated FBS and 1x Antibiotic-Antimycotic (Invitrogen).[1] All cells were cultured in a humid 37°C and 5% CO_2 atmosphere. Propidium iodide (PI) (Invitrogen), 25 mM sodium acetate buffer (NaAc, pH=5.2) (Sigma Aldrich), CellTiter® Aqueous One Solution Cell Proliferation Assay (Promega), Gel Permeation Chromatography (GPC) (Waters®, Breeze 2 software), a Bruker nuclear magnetic resonance (NMR) spectrometer, UV-Vis Spectrometer (Synergy2, BioTek®, Gen5 software), and a BD Accuri™ C6 flow cytometer equipped with HyperCyt® (Intellicyt Corp.) for high-throughput were used following manufacturer instructions. A Visi-Blue™ Transilluminator was used for imaging agarose gels. The single photon counting instrumentation consisted of a PicoQuant GmBH, PicoHarp 300 controller and a PDL 800-B driver.

II. Methods

Polymer Synthesis and Fractionation

Diacrylate monomers that form the polymer backbones (B3, B4, B5, B6) and amine monomers that form the polymer side chains (S3, S4, S5, S6) were mixed neat using 1.05:1, 1.2:1, or 1.4:1 mole ratios and endcapped as previously described with slight modification (E2, E4, E6, E7) (Scheme 2).[2] Briefly, the base polymer (diacrylate and side chain) reactions were carried out for 24 hours at 90°C, solvated in THF and endcapped for 1 hr using a 0.5 M amine monomer solution. Subsequently, the polymers were purified in anhydrous diethyl ether and vacuum dried for at least 24 hours and then fractionated by gel permeation chromatography (Waters Corp.,

Milford MA) using THF Styragel columns (3 7.8 x 300 mm in series). Two minute time fractions were collected at a 1 mL/min flow rate and again ether purified and vacuum dried for 48 hours. The polymers were then solvated in anhydrous DMSO to 100 mg/mL and stored at -20°C in small aliquots to minimize freeze-thaw cycles. GPC was used to assess molecular weight of the fractionated polymers. Synthetic PBAE polymers are referred to by the order of their constituent monomers: backbone acrylate monomer, side chain amine monomer, and end group amine monomer. For example, B4-S4-E7 is 447 as an abbreviation (Scheme 2).



Scheme 2. Reaction of PBAE synthesis; backbone (B3-6), sidechain (s3-6) and various endcap (E2, E4, E6, E7) monomers used in the PBAE library. A representative polymer (447) is shown.

Nuclear Magnetic Resonance

Representative acrylate-terminated base polymers and amine-terminated end-capped polymers were analyzed via ^{1}H NMR. Polymers designated as "ether-purified" were synthesized in THF (or, in the case of 44 base polymer, dissolved in THF without reaction) and then precipitated into diethyl ether as described. After 48 hr drying under vacuum, polymers were dissolved in deuterated chloroform ($CDCl_3$) with 0.03% v/v tetramethylsilane (TMS) at 10-20 mg/mL Other 44 base polymers were not purified after neat synthesis and were similarly dissolved in $CDCl_3$ with TMS. All spectra were obtained with Bruker instruments (400 MHz, Topspin 2.0 or 2.1 software) and analyzed with NMR Processor v.12 (ACD Labs, Toronto, Canada).[2]

Fluorescence Measurements

Plasmid DNA encoding enhanced green fluorescent protein (pEGFP) at 0.0975 mg/mL (300 µM of phosphate concentration) was added to ETB (20 µM) in a 15:1 mole ratio in 250 µL of 25 mM sodium acetate (NaAc, pH 5.2). The resulting intercalated DNA-ETB complex was a homogeneous pink color. Subsequently, 250 µL of each polymer was added to the resulting solution in polymer weight to DNA weight ratio (w/w) ranging from 1.2 to 47 w/w (N/P ratios ranging from 1 to 40) and was immediately mixed thoroughly. The polyplexes were allowed to stabilize for 10 minutes before beginning fluorescence measurements. The time-resolved fluorescence was measured by a time-correlated single photon counting (TCSPC) system (PicoQuant GmBH) consisting of a PicoHarp 300 controller and a PDL 800-B driver. The samples were excited with the pulsed diode laser head

LDH-P-C-485 at 483 nm with 130 ps time resolution. The signals were detected with a microchannel plate photomultiplier tube (Hamamatsu R2809U). To diminish the influence of the scattered excitation, a cut-off filter was used in front of the monitoring monochromator. To study the decay associated spectra (DAS), the decays were collected with a constant accumulation time in the 560–670 nm wavelength range with 10 nm increments. The decays were simultaneously fitted to the sum of two exponents in the equation (1):

$$I(t,\lambda) = a_1(\lambda)e^{-t/\tau_1} + a_2(\lambda)e^{-t/\tau_2} \quad (1)$$

where τ_i is the global lifetime and $a_i(\lambda)$ is the local amplitude at a particular wavelength. The factors $a_i(\lambda)$ represent the DAS (Figure S2), which in the case of a mixture of different non-interacting fluorescing species corresponds to the individual spectra of the species (ETB bound to DNA and ETB free in solution). The photomultiplier tube becomes increasingly less sensitive at higher wavelengths which was taken into account. The spectral areas (A_i) of the components can be calculated by integrating the pre-exponential factors over the measured wavelength range as indicated in the following equation:

$$A_i = \int a_i(\lambda)d\lambda \quad (2)$$

The short-living component, corresponding to free ETB in the bulk solution, has a lower fluorescence quantum yield than the long-living component corresponding to ETB bound to DNA. The relative fluorescence quantum yield of the short-living component, ϕ_{rel} = 0.112 (equation 3), was calculated from the steady state absorption (UV-VIS spectrophotometer Shimadzu UV-3600) and fluorescence (Fluorolog Yobin Yvon-SPEX, l_{ex} = 483 nm) spectra according to the following equation:

$$\phi_{rel} = \frac{\phi_{ETB}}{\phi_{DNA-ETB}} = \frac{I_{ETB}A_{DNA-ETB}}{I_{DNA-ETB}A_{ETB}} \quad (3)$$

where ϕ_{ETB} is the quantum yield of ETB free in solution, $\phi_{DNA\text{-}ETB}$ is the quantum yield of the DNA-ETB complex, I_i is the area of the fluorescence spectra with an excitation wavelength of 483 nm and A_i is the absorbance at wavelength of 483 nm. The corrected spectral area (A_i^c) for the short living component is obtained by dividing A_1 by ϕ_{rel}. As polymer (P) is added to the DNA-ETB complex, the polymer binds DNA and the ETB is freed into solution as follows:

$$\text{DNA-ETB} + \text{P} \rightleftharpoons \text{DNA-P} + \text{ETB} \quad (4)$$

The proportion of the short-living decay component of the total area of the DAS spectra, *B*, is the proportion or ratio of free ETB and is directly proportional to the amount of formed polyplexes (or the fraction of DNA bound to polymer). Thus, the bound fraction of DNA, B, can be assessed by monitoring the ratio of free ETB and can be calculated from the spectral areas of the components as follows:

$$B = \frac{A_1^c}{A_1^c + A_2} \quad (5)$$

The bound fraction of DNA as a function of amine concentration was assessed and the maximum was determined. All data points up to the maximum bound fraction were used to determine the binding constants. Of note, the initial concentration of ETB in the system is chosen such that without polymer there is no free ETB.

Binding Constant Calculation

The Hill plot equation for multivalent ligands binding to multi-subunit substrates was used to estimate the cooperativity and binding constants for the polyplex formation[3-6]:

$$\ln \frac{A_1^c}{A_2} = \alpha \ln [P] + \alpha \ln K \quad (6)$$

K^{α} is the overall binding constant for the reaction DNA + nP ⇌ DNA-P_n, K is the binding constant for the binding of one functional amine group according to the reaction DNA-P_{x-1} + P ⇌ DNA-P_x (X = 1, 2, …, n) and the slope of the Hill plot, α, is the experimental Hill´s coefficient (α = 1 for non-cooperative systems, α < 1 for negative cooperativity and α > 1 for positive cooperativity). The error in K is calculated from the standard error of the y-value in the linearly fitted Hill plots.

Particle Diameter and Zeta Potential

Particle diameter was determined by nanoparticle tracking analysis (NTA) using a NanoSight NS500 (Amesbury, UK, 532 nm laser), and zeta potential was determined using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK, detection angle 173°, 633 nm laser) in triplicate. Polymer/DNA nanoparticles were made at a 60 w/w ratio in 25 mM sodium acetate buffer (pH = 5.2) at a DNA concentration of 0.005 mg/ml and diluted into 1x PBS, pH 7.4. Particles were diluted 100-fold into PBS before NTA measurement. Particles were diluted 5-fold into PBS when using the Zetasizer; average electrophoretic mobilities were measured at 25°C, and zeta potentials (ZP) were analyzed using the Smoluchowski model. Additional experiments of representative polyplexes were conducted at concentrations comparable to delivery conditions at various pHs (5 and 7.4) and various ionic strengths (150, 75, 38, 19 mM) using dynamic light scattering (Malvern Instruments, UK).

Transfection and Cytotoxicity (Relative Metabolic Activity)

MDA-MB-231 and GBM319 cells were seeded in 96-well plates at 15,000 cells per well and allowed to adhere overnight at 37°C and 5% CO_2. Polymers and DNA were diluted in 25 mM NaAc and mixed in a 1:1 v/v ratio at 30, 60, and 90 w/w. Particles were allowed to self-assemble for 10 minutes prior to *in vitro* delivery. Subsequently, 20 µL of particle solution was delivered to each well already containing 100 µL of media (10% or 70% serum) for a DNA dosage of 600 ng/well (5 µg/mL) in quadruplicate. Naked DNA at the same final concentration in 25 mM sodium acetate and an untreated group were used as negative controls. Lipofectamine 2000 was used as a positive control to deliver 100 and 200 ng of DNA per well using a 2.5:1 v/w ratio (Lipofectamine reagent:DNA) in quadruplicates (following manufacturer recommendations). After 4 hours of incubation, the wells were aspirated and replenished with fresh media. To assess relative metabolic activity as an indication of toxicity at 24 hours post-delivery, each of the wells were aspirated and incubated with 110 µL of a 10:1 mixture of culture media to CellTiter 96® Aqueous One Solution in quadruplicate according to the manufacturer's instructions. The absorbance at 490 nm was measured using the Synergy2 UV-Vis spectrometer.

Flow Cytometry

The transfection efficacy was assessed using flow cytometry at 48 hours post-delivery. The 96-well plates were aspirated, washed with PBS, and trypsinized. After quenching with 2% FBS (in PBS) with propidium iodide (PI) at 1:200 v/v, the contents were transferred to a round-bottom 96-well plate and centrifuged at 800 RPM for 5 minutes. After centrifuging, all but 30 µL of buffer was removed, and each cell pellet was triturated before loading on the Hypercyt high-throughput reader. FlowJo (v. 7.6) was used for gating and further analysis. Singlets were identified using FSC-H vs SSC-H; dying cells were identified with PI (a DNA intercalator which fluoresces with a

compromised cell membrane) using FSC-H vs FL3-H; FL1-H vs FL3-H was used to identify the GFP-positive population.

Geometric and arithmetic fluorescence means of the flow cytometer's FL1-A channel can be an indicator of the relative amount of EGFP present on a per cell basis. Normalized fluorescence means of the FL1-A channel were calculated by dividing the viable singlet population's FL1-A mean fluorescence by the untreated conditions' mean fluorescence.

Heparin Competition Release Assay

Gel electrophoresis was accomplished using 1% agarose gels containing 1 μg/mL of ETB in a 1x TAE buffer. The gels were loaded with 15 μL of polyplexes at 60 w/w (pEGFP-N1 of 0.01 mg/mL). The polyplexes were allowed to stabilize for 10 minutes. Just prior to the loading the polyplexes were added to glycerol (30% v/v). The gels were run for 1 hour using 100 volts and imaged using a Visi-Blue™ Transilluminator. Four representative polymers ranging from the weakest to the strongest binding constants were used for the release assay (44, 447 Low M_w, 446, 447 High M_w).

Statistics

All binding constants are reported as previously described; transfection and toxicity plots show the mean and standard error of the mean. All other physical characterizations and data plotted show the mean and standard deviation. One-way ANOVA tests were used with Tukey post-hoc analyses to assess significance between multiple groups. Differences were considered significant with p-values < 0.05 (* < 0.05, ** < 0.01, *** < 0.001).

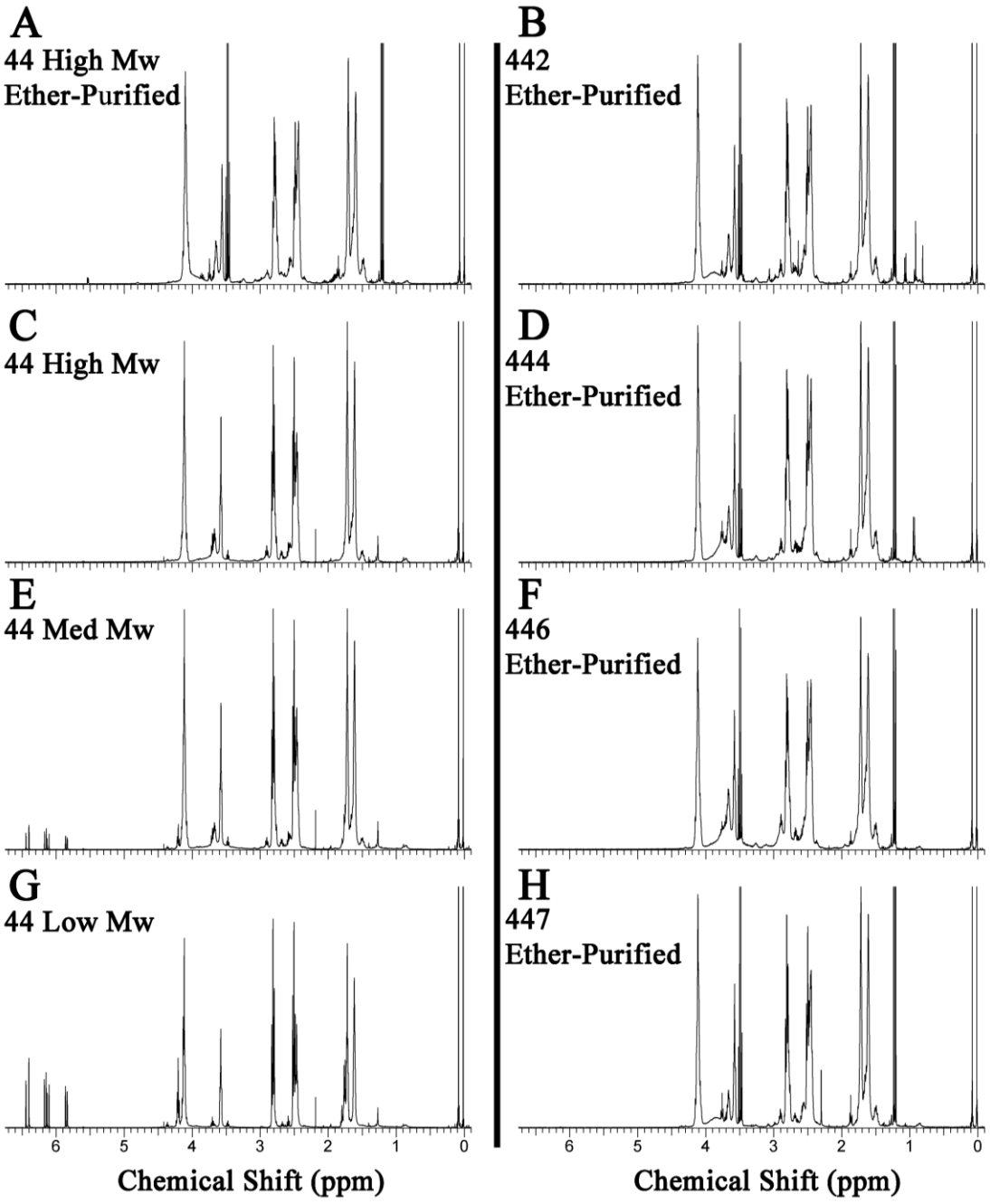


Figure S1. ^{1}H NMR spectra of polymers 44 High (A, C), Med (E), Low M_w (G), 442 (B), 444 (D), 446 (F), and 447 (H). These spectra are consistent with NMR analyses published previously (Sunshine, Akanda, et al.) along with spectra of the other polymers used in this study.[2] (See below for further peak analyses.)

Nuclear Magnetic Resonance Spectra

Some of the spectra above include the following sharp peaks corresponding to the solvent in which the polymer was synthesized (tetrahydrofuran, THF) or diethyl ether, used to precipitate the polymer:

THF: 1.85 ppm
Diethyl ether: 3.45-3.55 ppm (q, $CH_3C\boldsymbol{H_2}OC\boldsymbol{H_2}CH_3$)

Diethyl ether: 3.15-3.25 ppm (t, C***H***$_3$CH$_2$OCH$_2$C***H***$_3$)

Solvent peaks were not considered during analysis. Shown in the spectra below:

<u>44 (B4-S4) (all molecular weights)</u>
1.45-1.6 (m, NCH$_2$C***H***$_2$CH$_2$CH$_2$OH and NCH$_2$CH$_2$C***H***$_2$CH$_2$OH)
1.6-1.75 (t, COOCH$_2$C***H***$_2$C***H***$_2$CH$_2$OOC)
2.35-2.6 (t, COOC***H***$_2$CH$_2$NCH$_2$C***H***$_2$OOC and t, NC***H***$_2$CH$_2$CH$_2$CH$_2$OH)
2.7-2.85 (t, COOCH$_2$C***H***$_2$NC***H***$_2$CH$_2$OOC)
3.55-3.7 (t, NCH$_2$CH$_2$CH$_2$C***H***$_2$OH)
4.0-4.2 (t, COOC***H***$_2$CH$_2$CH$_2$C***H***$_2$OOC)
5.8-5.9 (d, CH$_2$OOCCH=C***H***H)
6.1-6.2 (dd, CH$_2$OOCC***H***=CHH)
6.35-6.5 (d, CH$_2$OOCCH=CH***H***)

<u>442 (B4-S4-E2)</u>
0.9-0.95 (s, NHCH$_2$C(CH$_3$)$_2$CH$_2$NH$_2$)
1.45-1.6 (m, NCH$_2$C***H***$_2$CH$_2$CH$_2$OH and NCH$_2$CH$_2$C***H***$_2$CH$_2$OH)
1.6-1.75 (t, COOCH$_2$C***H***$_2$C***H***$_2$CH$_2$OOC)
2.35-2.6 (t, COOC***H***$_2$CH$_2$NCH$_2$C***H***$_2$OOC and t, NC***H***$_2$CH$_2$CH$_2$CH$_2$OH and
t, NHC***H***$_2$C(CH$_3$)$_2$C***H***$_2$NH$_2$)
2.7-2.85 (t, COOCH$_2$C***H***$_2$NC***H***$_2$CH$_2$OOC)
3.55-3.7 (t, NCH$_2$CH$_2$CH$_2$C***H***$_2$OH)
4.0-4.2 (t, COOC***H***$_2$CH$_2$CH$_2$C***H***$_2$OOC)

<u>444 (B4-S4-E4)</u>
0.9-1.0 (m, NCH$_2$CH$_2$C***H***$_2$CH(C***H***$_3$)CH$_2$N)
1.45-1.6 (m, NCH$_2$C***H***$_2$CH$_2$CH$_2$OH and NCH$_2$CH$_2$C***H***$_2$CH$_2$OH and NCH$_2$C***H***$_2$CH$_2$C***H***(CH$_3$)CH$_2$N)
1.6-1.75 (t, COOCH$_2$C***H***$_2$C***H***$_2$CH$_2$OOC)
2.35-2.5 (t, COOC***H***$_2$CH$_2$NCH$_2$C***H***$_2$OOC and t, NC***H***$_2$CH$_2$CH$_2$CH$_2$OH and
m, NC***H***$_2$CH$_2$CH$_2$CH(CH$_3$)C***H***$_2$N)
2.7-2.85 (t, COOCH$_2$C***H***$_2$NC***H***$_2$CH$_2$OOC)
3.55-3.7 (t, NCH$_2$CH$_2$CH$_2$C***H***$_2$OH)
4.0-4.2 (t, COOC***H***$_2$CH$_2$CH$_2$C***H***$_2$OOC)

<u>446 (B4-S4-E6)</u>

1.45-1.6 (m, NCH$_2$C***H***$_2$CH$_2$CH$_2$OH and NCH$_2$CH$_2$C***H***$_2$CH$_2$OH)
1.6-1.75 (t, COOCH$_2$C***H***$_2$C***H***$_2$CH$_2$OOC and quin, NCH$_2$C***H***$_2$CH$_2$NHCH$_2$CH$_2$OH)
2.35-2.6 (t, COOC***H***$_2$CH$_2$NCH$_2$C***H***$_2$OOC and t, NC***H***$_2$CH$_2$CH$_2$CH$_2$OH and
m, NC***H***$_2$CH$_2$C***H***$_2$NHC***H***$_2$CH$_2$OH)
2.7-2.85 (t, COOCH$_2$C***H***$_2$NC***H***$_2$CH$_2$OOC)
3.55-3.7 (t, NCH$_2$CH$_2$CH$_2$C***H***$_2$OH and t, NCH$_2$CH$_2$CH$_2$NHCH$_2$C***H***$_2$OH)
4.0-4.2 (t, COOC***H***$_2$CH$_2$CH$_2$C***H***$_2$OOC)

447 (B4-S4-E7)

1.45-1.6 (m, $NCH_2C\boldsymbol{H_2}CH_2CH_2OH$ and $NCH_2CH_2C\boldsymbol{H_2}CH_2OH$ and
t, $NCH_2C\boldsymbol{H_2}CH_2N<(CH_2CH_2)_2>NCH_3$)

1.6-1.75 (t, $COOCH_2C\boldsymbol{H_2}C\boldsymbol{H_2}CH_2OOC$)

2.3 (s, $NCH_2CH_2CH_2N<(CH_2CH_2)_2>NC\boldsymbol{H_3}$)

2.35-2.6 (t, $COOC\boldsymbol{H_2}CH_2NCH_2C\boldsymbol{H_2}OOC$ and t, $NC\boldsymbol{H_2}CH_2CH_2CH_2OH$ and m, $NC\boldsymbol{H_2}CH_2C\boldsymbol{H_2}N<(C\boldsymbol{H_2}C\boldsymbol{H_2})_2>NCH_3$)

2.7-2.85 (t, $COOCH_2C\boldsymbol{H_2}NC\boldsymbol{H_2}CH_2OOC$)

3.55-3.7 (t, $NCH_2CH_2CH_2C\boldsymbol{H_2}OH$)

4.0-4.2 (t, $COOC\boldsymbol{H_2}CH_2CH_2C\boldsymbol{H_2}OOC$)

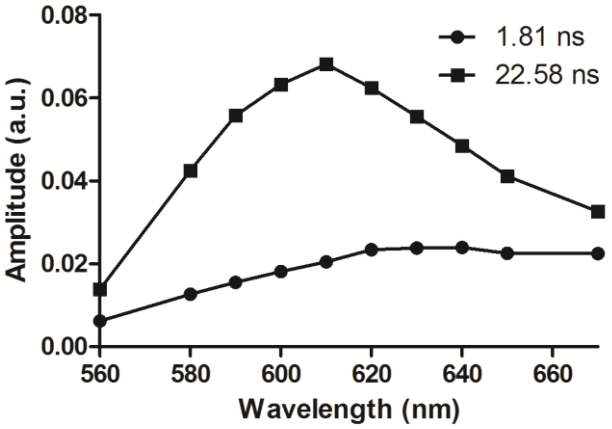


Figure S2. Decay-associated spectra. The fluorescence lifetimes of ethidium bromide bound to DNA and free in the solution are 22.58 and 1.81 ns, respectively, in this particular case.

Table S1. List of PBAE polymers and their number average molecular weights (M_n), weight average molecular weights (M_w), polydispersity indices (PDI), degree of polymerizations (DP), Hill coefficients (α), binding constants (*K*), diameters (nm), and zeta potentials (ZP; mV).

Varying	Polymer	M_n (kDa)	M_w (kDa)	PDI	DP	α	*K* (M^{-1})	Diameter (nm)	ZP (mV)
Molecular Weight	447 Low M_w	7.9	10.3	1.3	27	0.40	$4.2 \pm 0.1 \times 10^3$	180	14
	447 Med M_w	10.4	14.7	1.4	35	0.22	$5.8 \pm 0.3 \times 10^4$	135	6
	447 High M_w	32.0	91.6	2.9	110	0.23	$1.23 \pm 0.03 \times 10^5$	171	14
Backbone	346	7.5	11.2	1.5	27	0.24	$4.8 \pm 0.2 \times 10^5$	122	14
	446	8.3	11.8	1.4	28	0.38	$7.97 \pm 0.09 \times 10^3$	130	18
	546	7.0	9.1	1.3	23	0.22	$1.03 \pm 0.04 \times 10^4$	178	15
	646	8.1	10.0	1.2	24	1.16	$1.19 \pm 0.04 \times 10^3$	230	15
Sidechain	437	8.1	10.3	1.3	29	0.28	$1.15 \pm 0.01 \times 10^5$	170	9
	447 Med M_w	10.4	14.7	1.4	35	0.22	$5.8 \pm 0.3 \times 10^4$	134	6
	457	10.3	13.1	1.3	33	0.41	$3.5 \pm 0.1 Ex10^3$	160	9
	467	10.3	12.5	1.2	31	0.39	$4.7 \pm 0.3 \times 10^3$	165	8
Endcap	44	9.3	11.6	1.2	32	0.62	526 ± 9	180	11
	442	7.5	10.4	1.4	25	0.42	$3.5 \pm 0.2 \times 10^3$	200	12
	444	7.4	10.3	1.4	25	0.50	$3.4 \pm 0.1 \times 10^3$	190	13
	446	8.3	11.8	1.4	28	0.38	$7.97 \pm 0.09 \times 10^3$	130	18
	447 Low M_w	7.9	10.3	1.3	27	0.40	$4.2 \pm 0.1 \times 10^3$	180	14

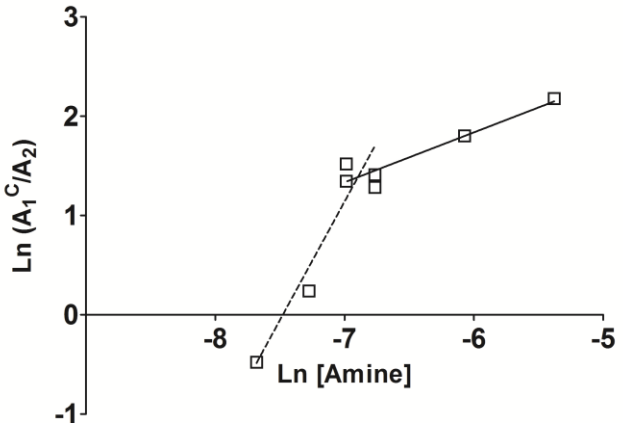


Figure S3. Hill plot of peptide $(KK)_2KGGC$.

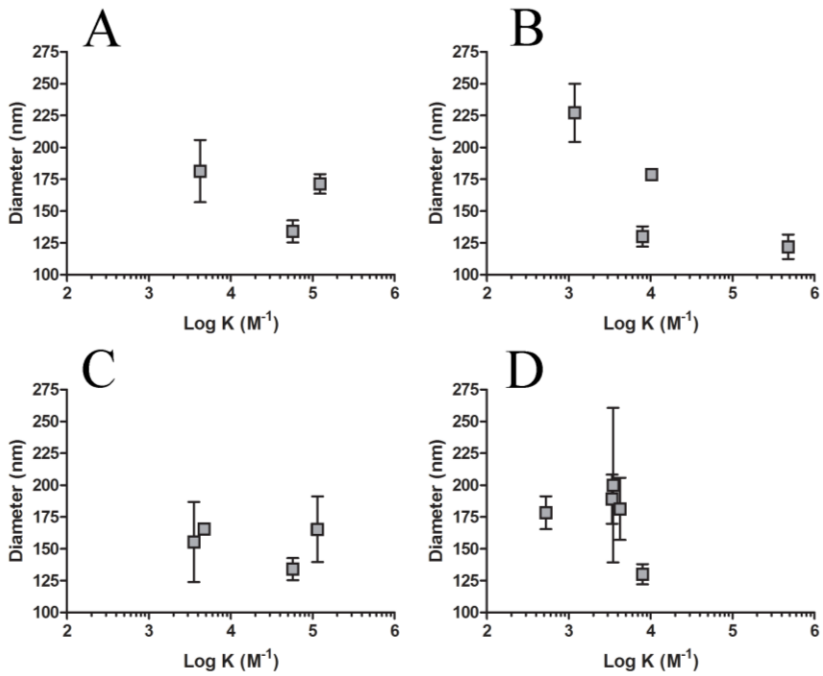


Figure S4. The relationship between polyplex diameter and the binding constant (M^{-1}) of each of the series comparing M_w (A), backbone (B), sidechain (C), and endcaps (D).

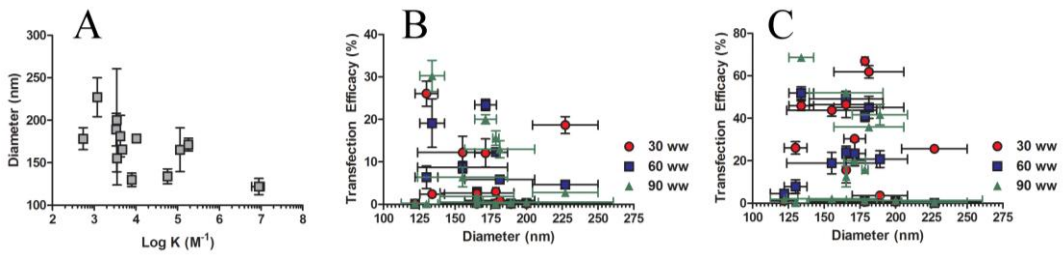


Figure S5. All diameters versus binding constants (A); dependence of transfection efficacy on polyplex diameters in MDA-MB-231 (B) and GBM319 cells (C).

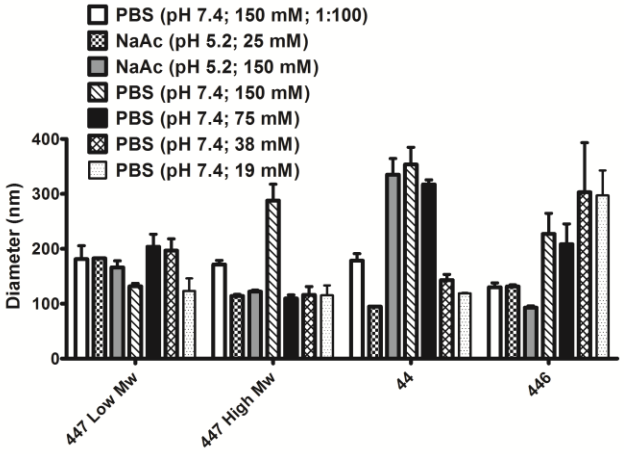


Figure S6. Diameter of four representative polymers at various pHs and ionic strengths. (White group was via NTA; remainder was via DLS.)

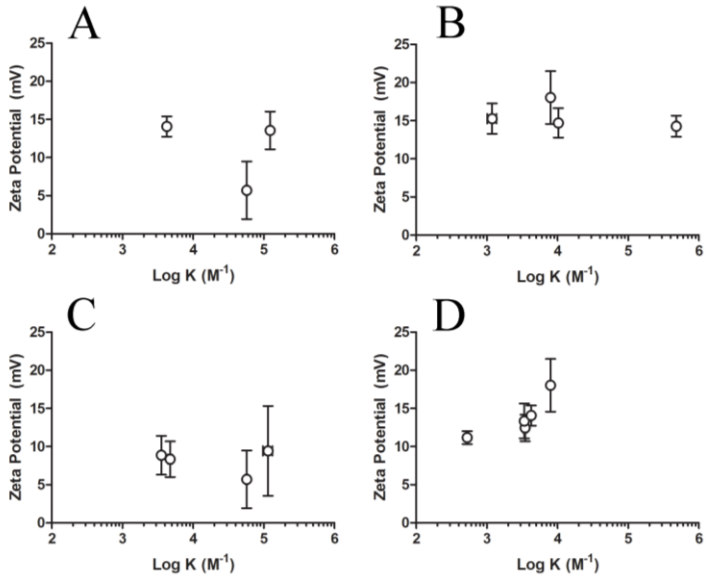


Figure S7. The relationship between zeta potential and the binding constant (M^{-1}) of each of the series comparing M_w (A), backbone (B), sidechain (C), and endcaps (E).

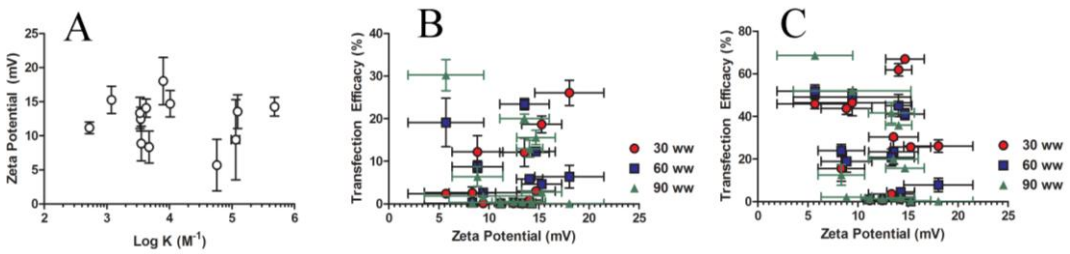


Figure S8. All ZP values irrespective of series versus binding constants (A); dependence of transfection efficacy on ZP in MDA-MB-231 (B) and GBM319 cells (C).

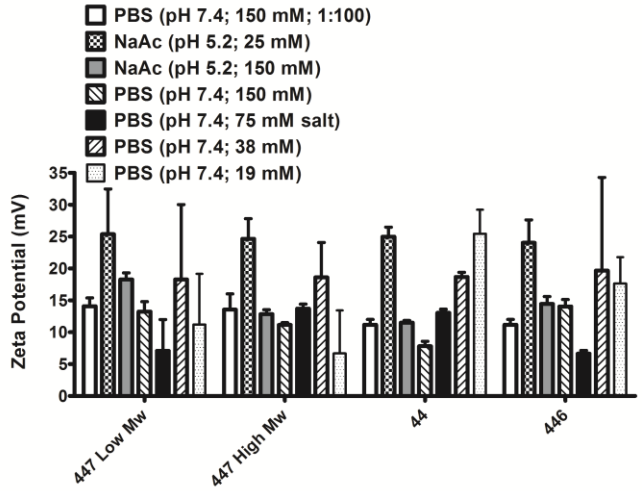


Figure S9. ZP of four representative polymers at various pHs and ionic strengths.

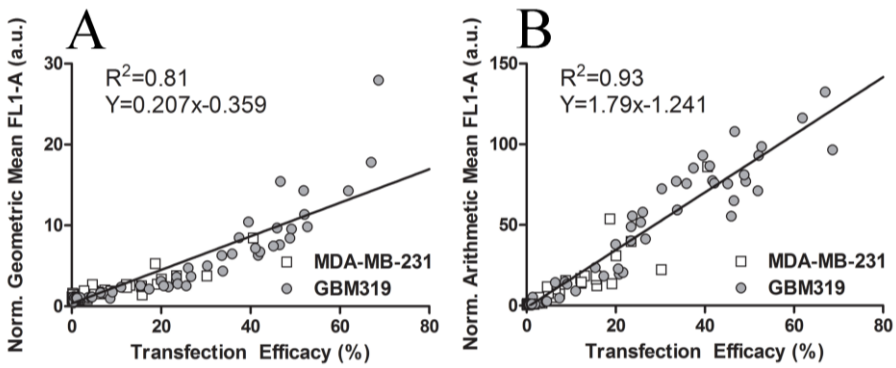


Figure S10. Normalized geometric (A) and arithmetic (B) means versus transfection efficacy in the MDA-MB-231 and GBM319 cell lines.

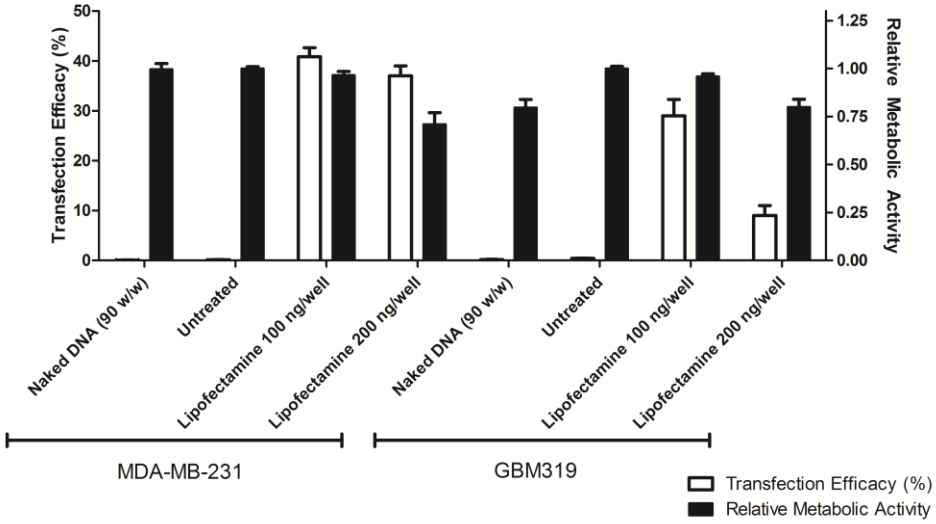


Figure S11. Positive (Lipofectamine 2000 at 100 and 200 ng/well) and negative controls (naked DNA and untreated) for transfection and relative metabolic activity in MDA-MB-231 and GBM319 cells.

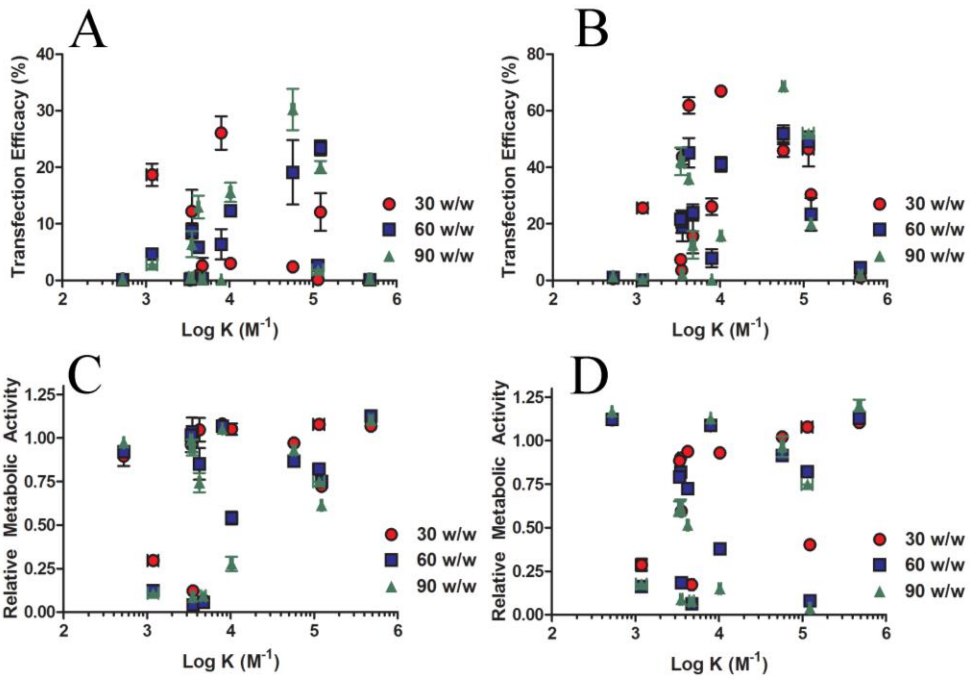


Figure S12. All binding constants for each of the series of comparison against transfection efficacy in MDA-MB-231 cells (A) and GBM319 cells (B), as well as cytotoxicity in MDA-MB-231 cells (C) and GBM319 cells (D).

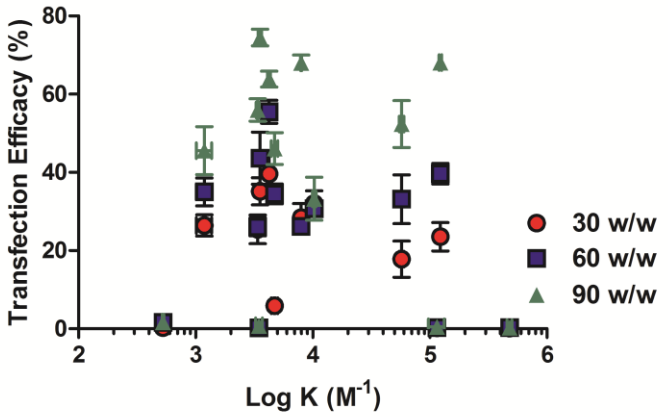


Figure S13. Binding constants compared to transfection efficacy using 70% serum in the GBM319 cell line.

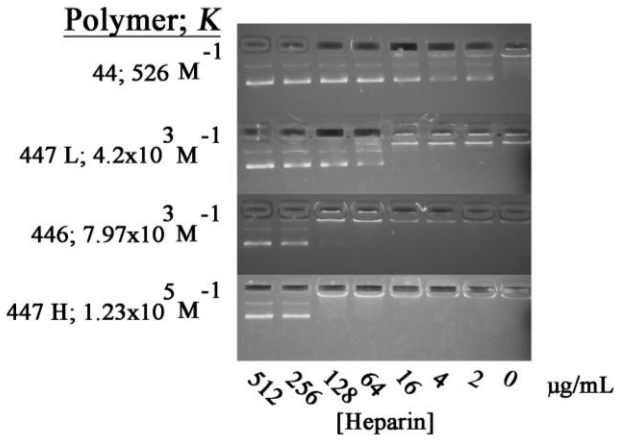


Figure S14. Heparin (ranging from 0 to 512 µg/mL) competition release assay of four representative polymers using gel electrophoresis; binding constants range from 526 (weakest *K* measured) to 1.23x10^5 M^{-1} (strongest *K* measured).

REFERENCES

1. Tzeng, S. Y.; Guerrero-Cazares, H.; Martinez, E. E.; Sunshine, J. C.; Quinones-Hinojosa, A.; Green, J. J., *Biomaterials* **2011,** *32*, 5402.
2. Sunshine, J. C.; Akanda, M. I.; Li, D.; Kozielski, K. L.; Green, J. J., *Biomacromolecules* **2011,** *12*, 3592.
3. Nanduri, V.; Sorokulova, I. B.; Samoylov, A. M.; Simonian, A. L.; Petrenko, V. A.; Vodyanoy, V., *Biosens. Bioelectron.* **2007,** *22*, 986.
4. Michel, D., *Biophys. Chem.* **2007,** *129*, 284.
5. Gelamo, E. L.; Tabak, M., *Spectrochim. Acta A* **2000,** *56*, 2255.
6. Gelamo, E. L.; Silva, C. H. T. P.; Imasato, H.; Tabak, M., *BBA-Protein Struct. M* **2002,** *1594*, 84.