Supporting Information

An acid-labile block copolymer of PDMAEMA and PEG as potential carrier for intelligent gene delivery systems

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Materials and Methods.

3-Amino-1,2-propanediol (Jinan Richeschem Chemicals), trifluroacetate (Jinan Meihua Chemicals), trimethylorthoformate and 2-bromo-2-methylpropanoyl bromide (BMPB, Acros), poly(ethylene glycol) monomethyl ether with molecular weight of 5000 (mPEG5k, Fluka), isopropyl alcohol (HPLC grade, Tianjin Shield Company), copper (I) bromide (CuBr) (Aldrich) were used as received. 2-(dimethylamino)ethyl methacrylate (DMAEMA, Fluka) was distilled under reduced pressure in nitrogen atmosphere prior to use. All other reagents and solvents were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. Pyridine was dried with anhydrous KOH and distilled under reduced pressure. Triethylamine (Et_3N) was treated with anhydrous KOH and sodium, followed by distillation under nitrogen atmosphere. Dichloromethane, chloroform and toluene was washed with concentrated sulfuric acid, saturated sodium bicarbonate, distilled water successively and dried over anhydrous $CaCl_2$. Dichloromethane and chloroform were distilled from CaH_2. Toluene was refluxed and distilled in the presence sodium/benzophenone. Tetrahydrofuran (THF) was distilled from CaH_2 before use. 2, 2'-Bipyridine (Bpy, Beijing Shiying Chemicals) and N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA, Aldrich) were used as received. Tris(2-dimethylaminoethy1)amine (Me_6TREN) was synthesized as reported.[1] Peptide MT20,

the hydrophobic portion of melittin tail (GIGAVLKVL TTGLPALISWI), was synthesized by the standard Fmoc procedure.

^{1}H-NMR spectra were recorded on Varian Mercury 300 MHz or Bruker 400 MHz NMR spectrometer using D_2O or *d*-chloroform as solvent and tetramethylsilane as an internal standard. Size-exclusion chromatography (SEC) analysis of polymer solutions (concentration: 10 mg/mL) was performed on an equipment consisting of a Waters 515 HPLC pump, a 2410 refractive index detector and three Waters Styragel columns (HT2, HT3, and HT4). The columns were thermostatted at 35^{o}C and THF was used as eluent at a flow rate of 1.0 mL/min. A series of narrowly dispersed polystyrenes were used as standards and a Millennium 32 software was employed to calculate the molecular weight and polydispersity index of polymers.

Synthesis

2,2,2-trifluoro-N-(2,3-dihydroxypropyl) acetamide (P1). A solution of 3-amino-1,2-propanediol (16.6 g, 0.18 mol) in THF (125 mL) was treated dropwise with ethyl trifluroacetate (25 mL, 0.21 mol) at 0 ^{o}C over 1.5 h. After an additional reaction of 2 h at room temperature, the solution was concentrated at reduced pressure. The residue was diluted with ethyl acetate (150 mL), washed successively with 0.5 M $NaHSO_4$ and saturated brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Then the solution was precipitated into cold $CHCl_3$, separated by decantation to give thick syrup at ambient temperature, yield 57%. ^{1}H NMR (300 MHz, D_2O, δ ppm): 3.2-3.4(m, 2H, NH-C*H*$_2$-), 3.4-3.6(m, 2H, -C*H*$_2$-OH), 3.8(m, 1H, OH-C*H*-).

2,2,2-trifluoro-N-(2-methoxy-1,3-dioxolan-4-yl)methyl acetamide (P2). A solution of P1 (19.4 g, 104 mmol) in CH_2Cl_2 (50 mL) was treated with p-TSA·H_2O (0.2 g, 1.1 mmol) and trimethylorthoformate (50 mL). The mixture was reacted for 5 h at room temperature, followed by addition of 200 mL CH_2Cl_2. The organic solution was washed successively with saturated $NaHCO_3$, NaCl solutions twice and dried over anhydrous K_2CO_3. After filtration, the organic solution was concentrated and precipitated in cold petroleum ether to afford a white solid after dried in vacuum, yield 43%. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.3-3.4 (d, 3H, O-C*H*$_3$, cis and trans), 3.4-3.6 (m, 2H, NH-C*H*$_2$-), 3.7-4.2 (2H, m, CH-C*H*$_2$-O), 4.5 (m, 1H,

NH-CH_2-C*H*-), 5.8 (1*H*,d,O_3-C*H*, cis and trans), 7.0-7.7(b, 1H, CO-N*H*-).

2,2,2-trifluoro-N-(2-(ω-methoxy)PEGyl-1,3-dioxolan-4-yl)methyl acetamide (P3). A mixture of P2 (1.01 g, 4.4 mmol), mPEG-5K (5.5 g, 1.1 mmol), anhydrous PTSA (6.9 mg , 0.038 mmol) , pyridine (42 mg, 0.53 mmol) and toluene (20 mL) was refluxed under nitrogen cover for 8 h, cooled to ambient temperature and treated with chloroform (100 mL). The organic solution was worked up similar to compound P2. Crude product was precipitated in cold ether to give white powder (P3), yield 80%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.4(s, 3H,O-C*H*$_3$), 3.5-3.9 (OC*H*$_2$C*H*$_2$O), 4.0-4.2 (m, 2H, -CH-C*H*$_2$-O), 4.5 (m, 1H, NH-CH_2-C*H*-), 5.9 (d, 1*H*, O_3-C*H*, cis and trans), 7.1, 8.0 (b, 1H, CO-N*H*-).

4-aminomethyl-2-(ω-methoxy)PEGyl-1,3-dioxolane (P4). A solution of compound P3 (2.0 g, 0.4 mmol) in THF (30 mL) was treated dropwise with 4% aqueous NaOH (30 mL) at 0 ^{o}C. After an additional reaction for 6 h at room temperature, the solution was washed with ether. The aqueous solution was concentrated and the residue was extracted with $CHCl_3$. The organic solution was dried over anhydrous K_2CO_3, flittered and concentrated. Then the solution was precipitated into ether twice to yield white powder products (P4), yield 84%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.4(s, 3H, O-C*H*$_3$), 3.5-3.9 (440H, OC*H*$_2$C*H*$_2$O), 4.0-4.4 (m, 3H, NH_2-CH_2-C*H*-C*H*$_2$-O), 5.8-5.9(d, 1H, O_3-C*H*, cis and trans)

2-bromo-N-(2-(ω-methoxy)PEGyl-1,3-dioxolan-4-yl)methyl -2-methylpropanamide (P5). A solution of P4 (1.5 g, 0.3 mmol) and triethylamine (95 mg, 0.96 mmol) in 25 mL of CH_2Cl_2 was cooled to 0-5 ^{o}C in an ice bath. To this solution, 2-bromo-2-methylpropanoyl bromide (0.14 g, 0.6 mmol) dissolved in 5 mL of CH_2Cl_2 was added dropwise over 0.5 h. After an additional reaction of 4 h, the reaction mixture was filtered. The filtrate was treated by similar procedure as for P3 to afford white powder (P5), Yield 80%. ^{1}H NMR (400M, $CDCl_3$, δ ppm): 1.8 (s, 6H, (C*H*$_3$)$_2$CBr), 3.4(s, 3H, O-C*H*$_3$), 3.5-3.9 (OC*H*$_2$C*H*$_2$O), 4.0-4.2(m, 2H, -CH-C*H*$_2$-O), 4.4-4.5 (m, 1H, NH-CH_2-C*H*-), 5.8-5.9 (d, 1H,O_3-C*H*, cis and trans), 7.0, 7.3 (b, 1H, CO-N*H*-).

ω-methoxyPEGyl 2-bromo-2-methylpropanate (P6). P6 was prepared by the procedure as described in literature.[2]

Polymerizations

Atom transfer radical polymerization of DMAEMA. The block copolymers were prepared by ATRP method. Macromolecular initiator **P5** or **P6**, monomer DMAEMA, ligand and solvent were charged into schlenk tube. After three freeze-pump-thaw cycles, CuBr was charged under N_2 atmosphere and the tube was sealed under vacuum. The polymerization was performed under vacuum for certain hours and terminated by exposure to air. After dilution with $CHCl_3$, the solution was dried with anhydrous K_2CO_3. Copper salt in the polymerization mixture was removed through a basic Al_2O_3 column using chloroform as eluent. After partial removal of the organic solvent at reduced pressure, the concentrated residue was added dropwise into hexane to precipitate out the crude polymer. Trace of the intact macromolecular initiator in the crude product was removed by repetitive precipitation in ether. In a typical polymerization procedure, as for sample C, the weight ratio of initiator (P5): monomer: CuBr: Me_6TREN was 0.5 g: 1.2 g: 15 mg: 28 μL (molar ratio = 1:80:1:1). The polymerization was carried out in isopropyl alcohol/H_2O (2.5 mL/1mL) at 25 ^oC for 2 h. Sample B was obtained in a similar way. The chain length of PDMAEMA in the block copolymers was determined from their ^1H NMR spectra by comparing the peak integration of –N $(CH_3)_2$ protons (2.1- 2.5 ppm) on the DMAEMA repeating units with mPEG5k repeating units -$(CH_2CH_2O)_{110}$ protons (3.5-3.9 ppm). (Figure S7 and S8). Furthermore, altering the charge ratio of initiator to monomer and ligand can easily mediate copolymer products with various molecular weights (Table S1). As for ligands, Me_6TREN is considered as one of most powerful ligands with Cu(I) halide in ATRP, [3] allowing rapid polymerization of acrylates at room temperatures. But the polydispersities were high due to slow deactivation. Thus, Me_6TREN was used to synthesize the copolymer with relatively short cationic chain. To prepare copolymers of higher molecular weight, the mild ligands Bpy and PMDETA were used. Moreover, there are residual PEG macroinitiator in the crude products based on SEC analysis. During the fractional purification in ether as mentioned above, the copolymers with relative small molecular weight were removed together with PEG macroinitiator. Thus the final products obtained show higher PDMAEMA contents than in charge ratios.

Conventional radical polymerization of DMAEMA. In a polymerization tube, 1.0 g of DMAEMA and 5 mg of AIBN were dissolved in 4.0 mL of toluene. The solution was purged with

nitrogen for 15min and frozen, and the tube was sealed under vacuum. The polymerization was carried out at 60 ^{o}C for 24 h. The purified polymer was obtained by precipitation in hexane twice.

Table S1 Characterization of a series of acid-labile block copolymers

Entry	I:M:CuBr:L[1]	Mn[2]	PDI[2]	Mn[3]
PEG_{110}-*a*-$PDMAEMA_{140}$ (I)	1:80:1:1	21000	1.55	27000
PEG_{110}-*a*-$PDMAEMA_{290}$ (II)	1:95:1:2	42000	1.38	51000
PEG_{110}-*a*-$PDMAEMA_{410}$ (III)	1:180:1.5:1.5	50000	1.42	70000

1. I for initiator P5, M for monomer DMAEMA, L for ligand Me_6TREN (I), Bpy (II) and PMDETA (III).
2. obtained from GPC analysis.
3. obtained from ^{1}H NMR analysis

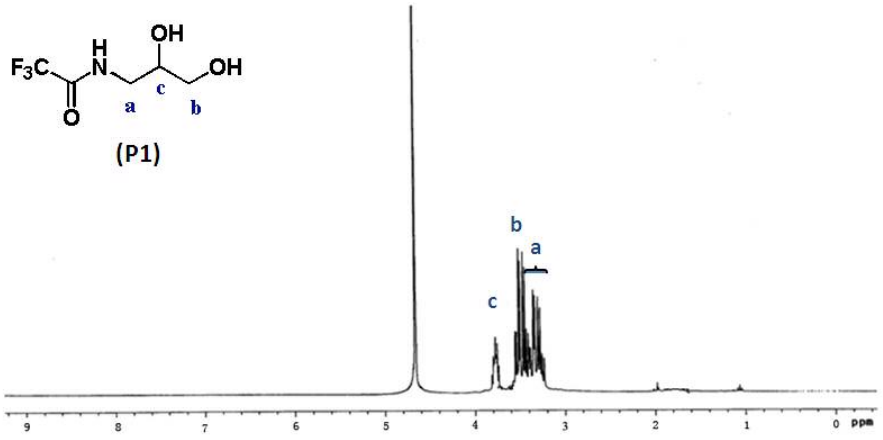

Figure S1 ^{1}H NMR spectrum of 2,2,2-trifluoro-N-(2,3-dihydroxypropyl)acetamide (P1) in D_2O

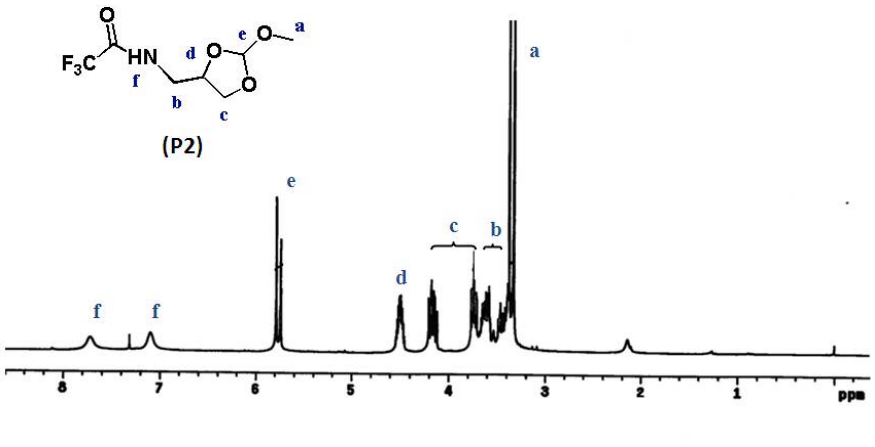

Figure S2 ^{1}H NMR spectrum of 2,2,2-trifluoro-N-((2-methoxy-1,3-dioxolan-4-yl)methyl) acetamide (P2) in $CDCl_3$

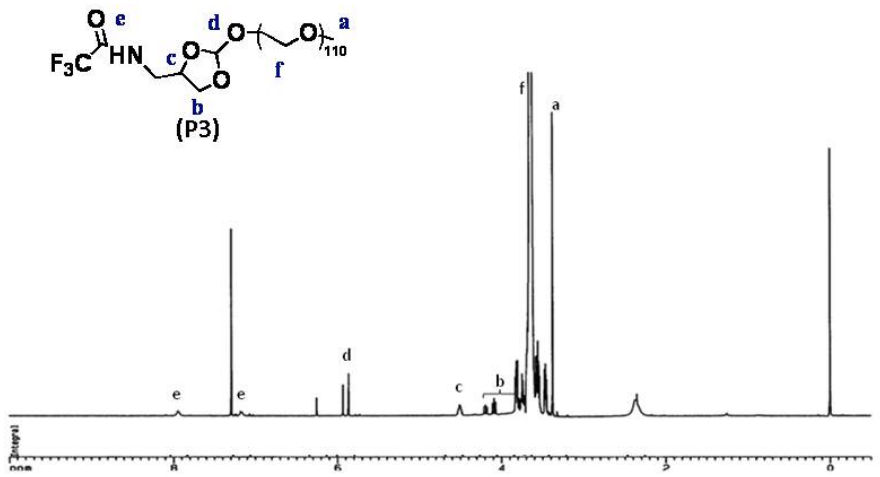

Figure S3 [1]H NMR spectrum of 2,2,2-trifluoro-N-(2-(ω-methoxy)PEGyl-1,3-dioxolan-4-yl)methyl acetamide (P3) in $CDCl_3$

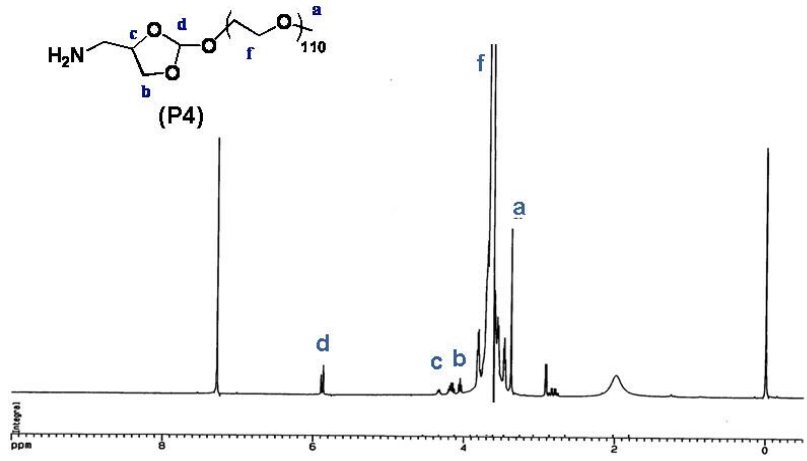

Figure S4. 1H NMR spectrum of 4-aminomethyl-2-(-methoxy)PEGyl-1,3-dioxolane (P4) in CDCl3

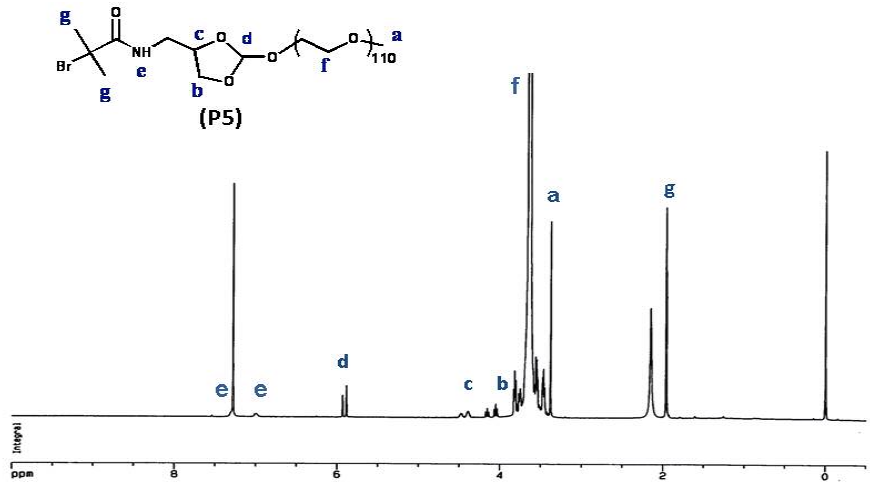

Figure S5. [1]H NMR spectrum of 2-bromo-N-(2-(ω-methoxy)PEGyl-1,3-dioxolan-4-yl)methyl -2-methylpropanamide (P5) in $CDCl_3$

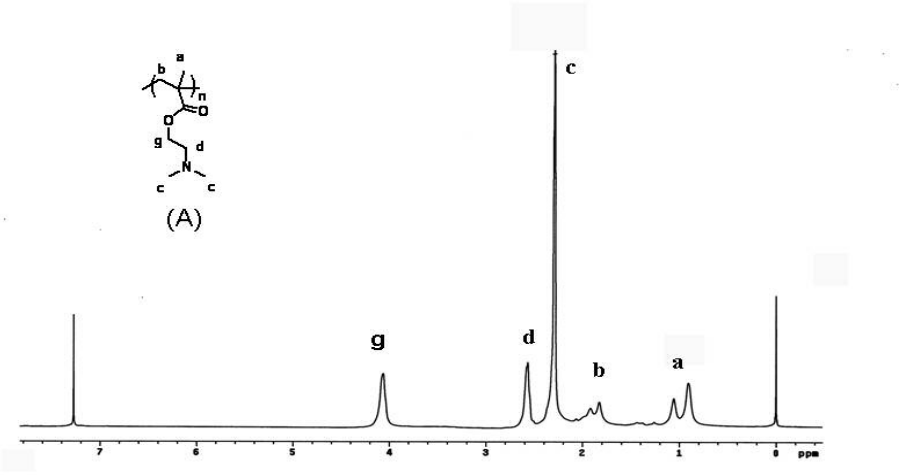

Figure S6. ^{1}H NMR spectrum of homopolymer PDMAEMA (A) in $CDCl_3$

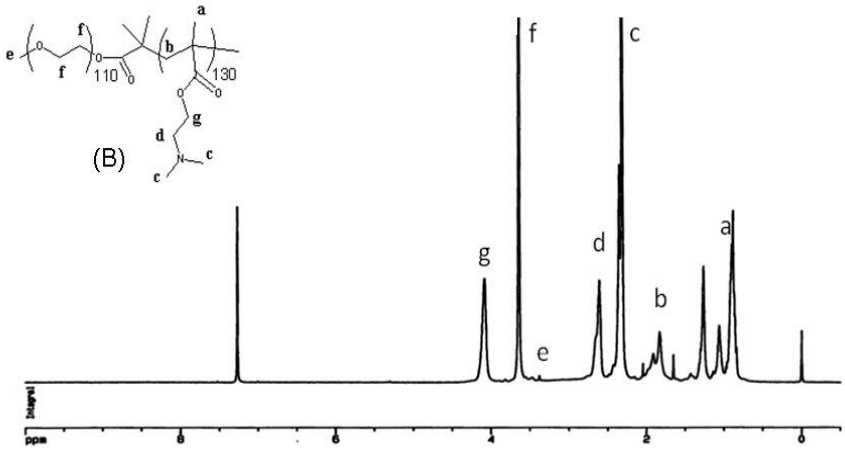

Figure S7. ^{1}H NMR spectrum of stable block copolymer PEG-*b*-PDMAEMA (B) in $CDCl_3$

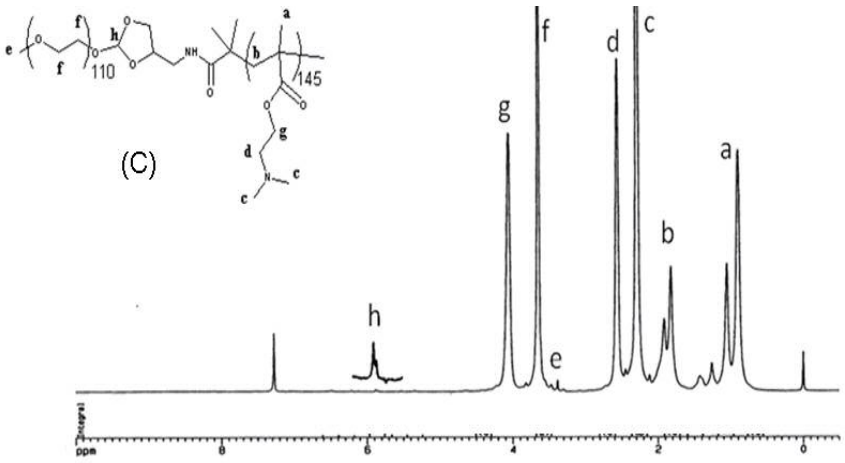

Figure S8. ^{1}H NMR spectrum of acid-labile block copolymer PEG-*a*-PDMAEMA (C) in $CDCl_3$

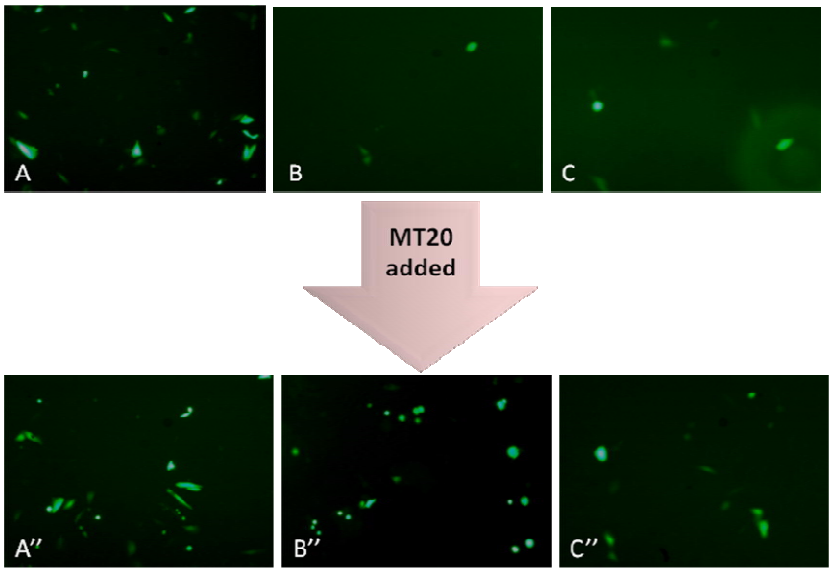

Figure S9. In vitro EGFP gene transfection of various polyplexes on 293T cells at the optimized N/P ratio (N/P=12). A, B, and C denote the intact polymers PDMAEMA, PEG-*b*-PDMAEMA, and PEG-*a*-PDMAEMA, respectively. A'', B'' and C'' denote the samples for PDMAEMA, PEG-*b*-PDMAEMA, PEG-*a*-PDMAEMA in the presence of MT20, respectively.

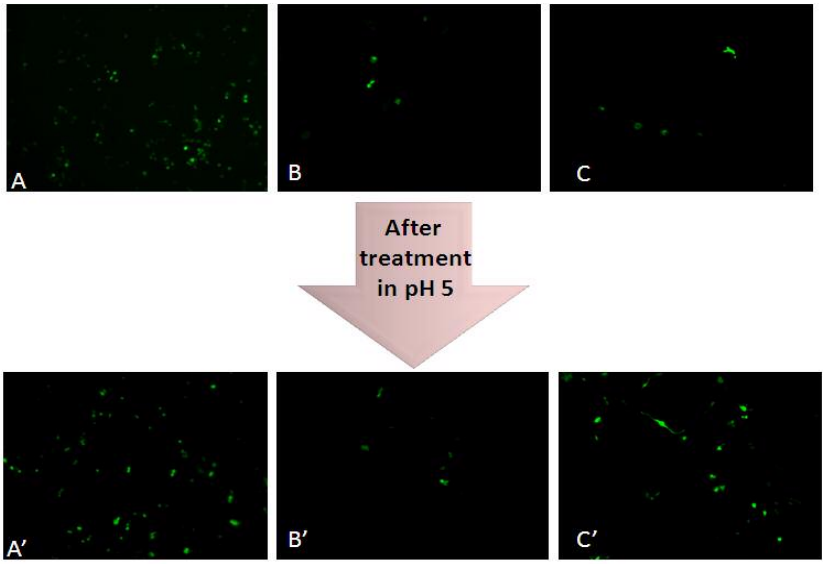

Figure S10. In vitro EGFP gene transfection of various polyplexes on 293T cells at the optimized N/P ratio (N/P=12). A, B, C denote the intact polymers PDMAEMA, PEG-*b*-PDMAEMA, and PEG-*a*-PDMAEMA, respectively. A', B', and C' denote the acid-treated samples for PDMAEMA, PEG-*b*-PDMAEMA, and PEG-*a*-PDMAEMA, respectively.

Reference:

1. Ciampolini, M.; Nardi, N. *Inorg. Chem.* **1966**, *5*, 41-44.
2. Jiang, X.; Luo, S.; Armes, S. P.; Shi, W.; Liu, S. *Macromolecules* **2006**, *39,* 5987-5994.
3. Teodorescu, M.; Matyjaszewski, K. *Macromol. Rapid Commun.* **2000**, *21*, 190–194.