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Supporting information

DyNAvectors: Dynamic constitutional vectors for adaptive DNA transfection

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Materials: Squalene (98%), 1,3,5-benzenetrialdehyde, poly-(ethyleneglycol)-bis(3-aminopropyl) terminated (Mn~1500 g/mol^{-1}) branched polyethylenimine (Mn~0.8kDa and 2kDa, 50 wt. % in H_2O) and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. Plasmid pEYFP was purchased from Clontech Laboratories Inc. (Mountain View, CA, USA), and the Plasmid Midi Kit from Qiagen (Düsseldorf, Germany). Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), penicillin G, neomycin, streptomycin were purchased from Gibco BRL (Gaithersburg, MD, USA), and the cell culture plates from Corning (New York, NY, USA).

Methods: ***NMR spectra*** have been recorded on a Bruker Avance III 400 instrument operated at 400.1 and 100.6 MHz for ^{1}H and ^{13}C nuclei, respectively, at room temperature. Chemical shifts were reported in ppm, and referred to 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid sodium salt (TSP) as internal standard. ***XPS analyzes*** were performed with an AXIS NOVA instrument (Kratos Analytical, Manchester, United Kingdom) using monochromatic AlKα radiation (1486.6 eV), with 20 mA current and 15 kV voltage (300W). The working pressure in the analysis chamber was typically $5x10^{-9}$ Torr. The high resolution spectra were collected using a pass energy of 20 eV and a step size of 0.1 eV. All the binding energies were referenced to the C1s peak at 285.0 eV to compensate for the surface charging effects. The composition and chemical state were investigated on the basis of the areas and binding energies of C 1s, and N 1s photoelectron peaks. Data were analyzed using the Vision Processing software (Vision2 software, Version 2.2.10).***TEM images*** were obtained on a HT7700 Hitachi Transmission Electron Microscope. The samples were prepared by placing a drop of polyplexes aqueous suspension on a carbon-coated copper grid and by allowing the solvent to evaporate at room temperature. After drying, the samples were examined in high resolution mode, under an operating potential of 100 kV. ***Elemental analysis*** was performed using a scanning electron microscope (SEM) (Quanta 200-FEI) equipped with an energy-dispersive X-ray spectroscopy (EDX). EDX system allows quantitative and qualitative compositional analysis.

Synthesis of PEGylated Sq (SQ-PEG). PEG(NH_2)$_2$ (1170 mg, 0.78 mmol, 1.1 eq) was dissolved in 10 ml of acetonitrile and added to a stirring solution of 1,1',2-Tris-nor-squalene aldehyde. [24] (275 mg, 0.716 nmol, 1 eq) in 10 ml of acetonitrile. The resulted solution was stirred for 24 h at room temperature. The product was formed in quantitative yield and used with no further purification. The solution with conc. 36.6 mM was kept in acetonitrile for further experiments.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ=7.63 (1H, t, J=4.8, C*H*=N), 5.23-5.00 (5H, m, C*H*=C), 3.74-3.34 (140 H, m, CH_2-CH_2-O), 2.95(2H, t, J=6.4, CH_2-NH_2), 2.35-2.27 (2H, m, CH_2) 2.23-2.15 (2H, m, CH_2), 2.23-1.98 (16H, m, CH_2-CH_2), 1.91-1.80 (3H, m, CH_3), 1.67 (3H, m, =C(CH_3)-CH_3), 1.59 (12H, m, =C(CH_3)). ^{13}C NMR (400 MHz, $CDCl_3$) δ=161.54 (*C*=N), 134.13 (CH3-*C*=C), 131.21 (*C*(CH_3)$_2$), 124.39 (CH_2-*C*=CH), 124,25 (CH_2-*C*=C), 72.59 (O*C*H_2-CH_2),), 70.56 (O-*C*H_2-*C*H_2-O), 70.31 (O-*C*H_2), 70.12 (O-*C*H_2), 69.44 (O-*C*H_2), 61.67(N-*C*H_2), 39.71 (*C*H_2-CH_2), 39.57 (*C*H_2-NH_2), 33.03 (CH_2), 28.56 (CH_2), 28.28 (CH_2), 26.75(CH_2), 26.65 (CH_2), 25.71 (CH_2), 17.69 (CH_3),16.06 (CH_3), 16.01 (CH_3).

Preparation of MU6: SQ-PEG (115 mg, 62 nmol), 1,3,5-Benzenetrialdehyde **3** (10 mg, 62 nmol) and H_2N-PEG-NH_2 **2** (92 mg, 62 nmol) were dissolved in 2 ml acetonitrile. Reaction mixture was stirred for 24 h at room temperature. Solvent was evaporated and residue was taken in water (3 ml) and PEI800 (74 mg, 93 nmol) in 300 µl water was added to the mixture and stirred for another 48 h at room temperature. The P1-P13 series was prepared in the same manner with appropriate amounts of reagents. IR (*v*): 2887.34, 1647.16, 1583.51, 1467.78, 1379.06, 1359.77, 1344.34, 1280.69, 1242.12, 1149.54, 1112.89, 1060.81, 962.42, 842.87 cm^{-1}.Elemental analysis (EDX) calculated for $C_{229}O_{63}N_{32}$: (At%) C, 70.7, N, 9.8, O,19.5; found (At%) C, 77.66, N, 6.75, O, 15.60.

Preparation of samples for TEM analysis: Mixtures of 1μg pEYFP in 20μL solution water and the calculated amount of **MU6** were prepared to get the desired N/P ratio. After one hour incubation time, 10 μL of each resulted suspension were deposited on greed and air dried, at room temperature.

Preparation of polyplexes P1-P13 loading solutions for gel electrophoresis: The N/P ratio was expressed as **MU**/pEYFP ratio, which determines the ratio of nitrogens (N) determinate by the presence of PEI to phosphates (P) in the nucleic acid. 15 μl of loading solution contain 0.5 mg of pEYFP, appropriate amount of **MU** for specific N/P ratio, 5 μl succrose (25%in MQ Water) in DNase free 1x TAE buffer solution (pH=7.4). After incubation for 30 minutes, solutions were loaded in a 1 % agarose gels, and ran at 90 V, for 120 minutes, in TAE buffer solution (40 mM Tris–HCl, 1%, acetic acid, 1 mM EDTA). The gel was stained with ethidium bromide and visualized with UV bio imaging system.

Preparation of plasmid DNA: The pCS2+NLS-eGFP plasmid, encoding a nuclear localized enhanced green fluorescent protein, was propagated in E.Coli DH5α, extracted and purified with EZNA ENDO-FREE PLASMID MINI II kit (Omega Bio-Tek, Inc.).

Cell cultures: In vitro transfection efficiency of polyplexes was evaluated in HeLa cells. HeLa cells (from CLS-Cell-Lines-Services-GmbH, Germany) were grown in tissue culture flasks with alpha-MEM medium (Lonza) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% Penicillin-Streptomycin-Amphotericin B mixture (10K/10K/25 μg in 100 ml, Lonza). Medium was changed with fresh one every 3 or 4 days. Once cells reached confluency, they were detached with 1x Trypsin-Versene-EDTA mixture (Lonza), washed with phosphate buffered saline (PBS, Invitrogen), centrifuged at 200 x g for 3 minutes and subcultured into new tissue culture flasks.

Flow cytometry and microscopy: HeLa cells were harvested by trypsinization, counted using a Countess Automated Cell Counter (Invitrogen), and, 24 hours before transfection, were seeded in 24 well tissue culture plates at a density of $4x10^4$ cells/well in 500 μL complete medium/well. For each sample/well

were used 1.5 µg of pDNA and the corresponding quantity of polymer for desired N/P ratio. Polymer and pDNA were mixed in a total volume of 20 µL/sample (volume was adjusted with PBS), vortexed briefly and allowed to rest at room temperature. After one hour, transfection mixture was added to the cells without removing the existent medium and the very next day 500 µL of total alpha-MEM medium were added to each sample. After 48 hours from transfection, plates were inspected using an inverted microscope Leica DMI 3000 and images were acquired with fluorescence GFP filter. Immeadiatelly after microscopy, cells were detached with trypsin, washed with PBS, centrifuged at 300 x g for 5 minutes and resuspended in 400 µL/sample FACSFlow solution (BD Biosciences). The cell suspensions were analyzed for transfection efficiency and cytotoxicity by flow cytometry (Navios, Beckman Coulter) using Kaluza v1.3 software (Beckman Coulter). For cytotoxicity, half volume from each sample was incubated with 1 µL propidium iodide 50 µL/mL (Sigma Aldrich) . GFP positive cells were counted with FITC channel and dead cells under FL-3 channel. At least 3 replicates were included for each sample.

Table 1S. Products resulted by different ratios combinations between **1 – 4**. **IU**-intermediate unit **5** and **6**, **MU**-multifunctional unit **7** and **8**, P-polyplex **10.**

Sample	Molar ratio				Polyplex/pEYFP, N/P ratio for complete complexation
	1	SQ-PEG, **2**	$PEG(NH_2)_2$, **3**	bPEI**, 4**	
	IU (**5** and **6**)				
	MU (**7** and **8**)				
	P (**10**)				
1	1	1	0	1	10
2	1	1	0,5	0,5	-
3	1	1	1	1	5
4	1	0,5	1	0,5	-
5	1	0,5	1	1	5
6	1	1	1	1,5	5
7	1	1	0,5	1,5	5
8	1	1	0	1,5	5

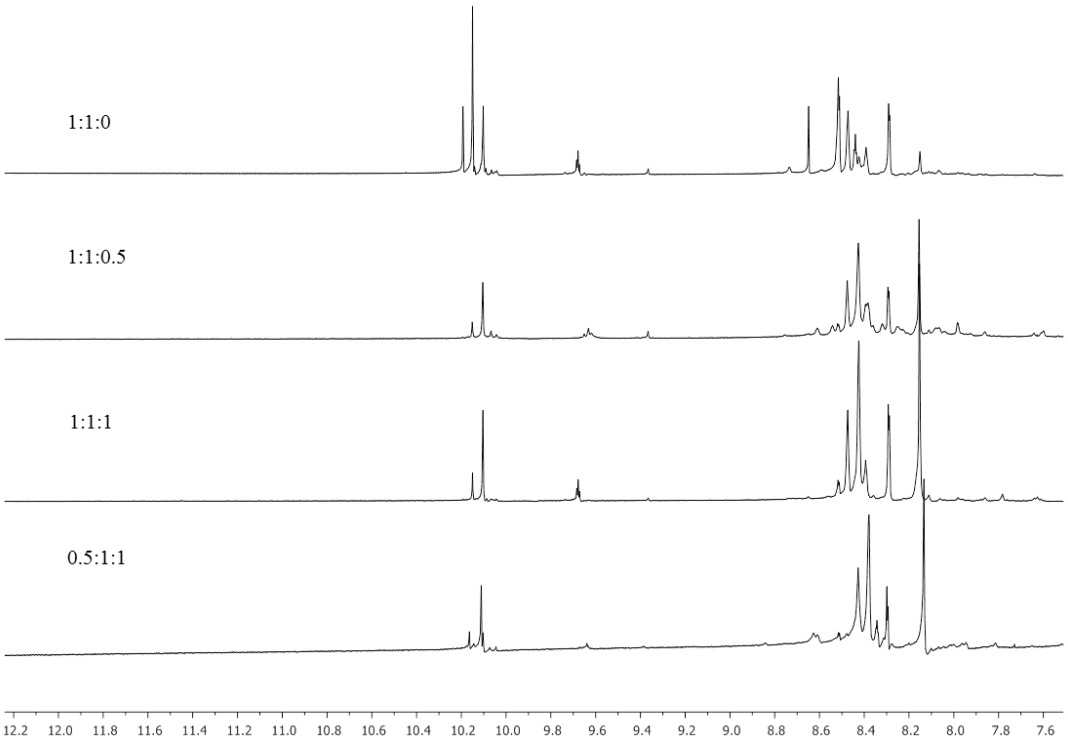

Figure 1S. Aromatic region of the ^{1}HNMR spectra for **IU**1 –**IU**4 in CD_3CN.

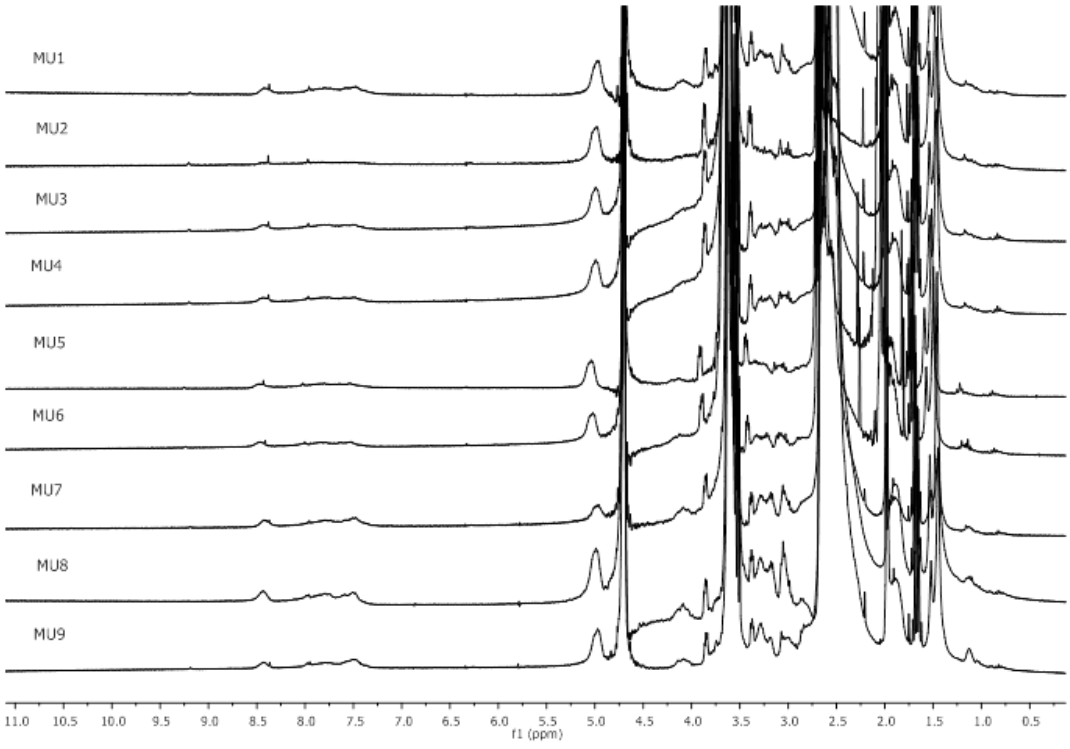

Figure 2S. ^{1}HNMR spectra in D_2O of **MU1** – **MU8** in D_2O.

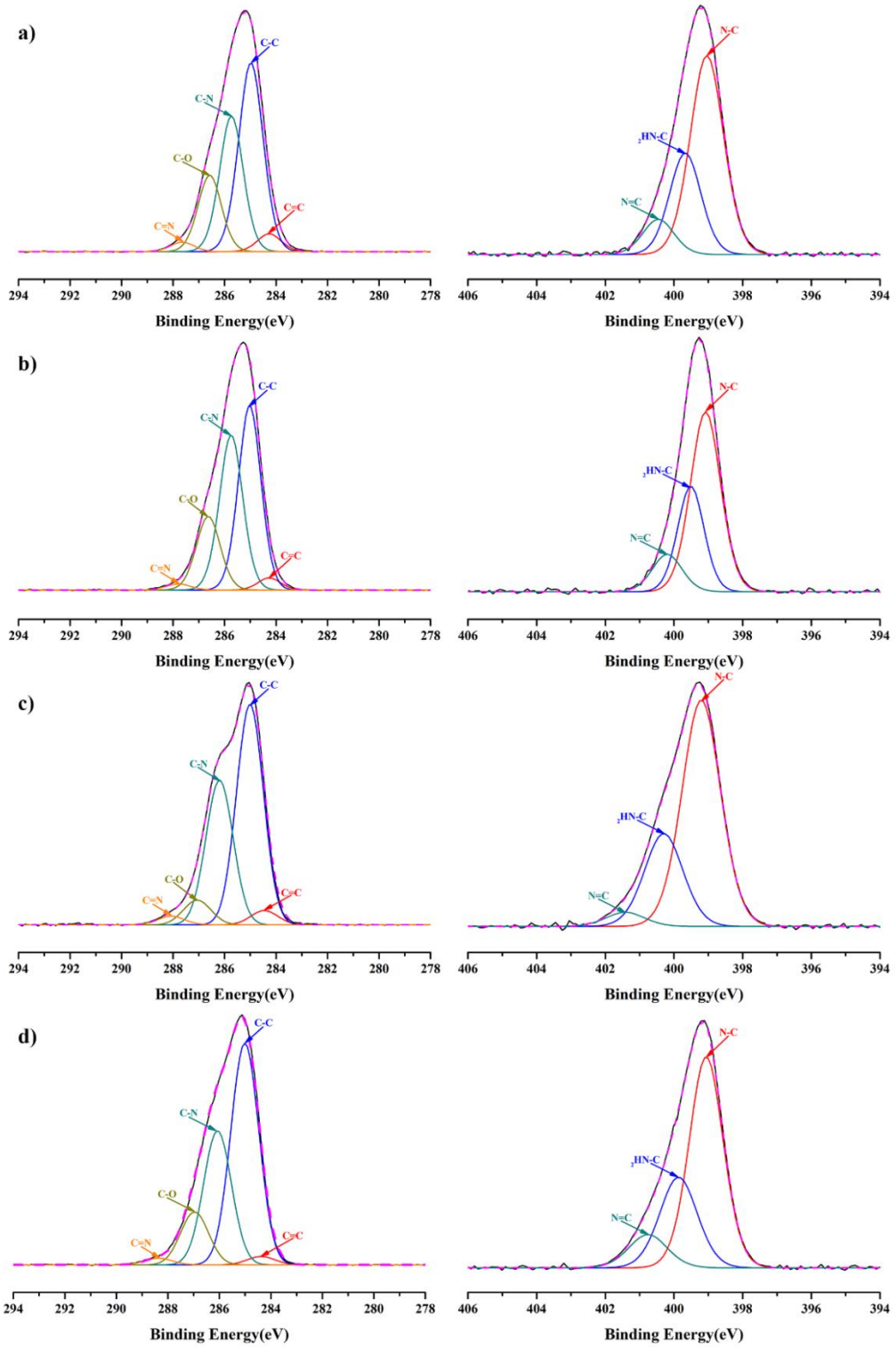

Figure 3S. Detailed C 1s and N 1s XPS spectra of **MU1** (a, a'), **MU2** (b, b'), **MU8** (c,c'), **MU6** (d, d'): experimental data (black lines) and fitting results(colored lines).

The formation of imine -N=C- bonds are also confirmed by X-ray photoelectron spectroscopy (XPS) (Figure 3S). The C_{1s} spectra are reminiscent with the presence of five peaks corresponding to: a) 284.2-284.4 eV assigned to C=C bond, b) 285.0 eV assigned to C-C and C-H bonds; c) 285.7-286.2 eV assigned to C-N bond; d) 286.6-287.1 eV attributed to single bond C-O and e) 287.6-288.3 eV assigned to C=N bonds. The corresponding N_{1s} XPS spectra (Figure 3S) are revealing three main components: a) 399.1-399.2 eV, characteristic to N-C bond, b) 399.8-400.2 eV assigned to $–C-NH_2$ bond and c) a high binding energy component at 400.6-401 eV related to N=C bonds.

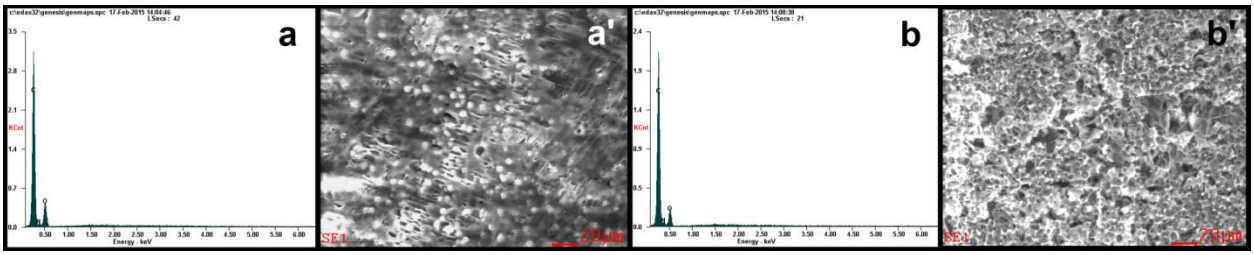

Figure 4S. EDX spectra and SEM images for lyophilized **MU6** (a) and **MU8** (b).

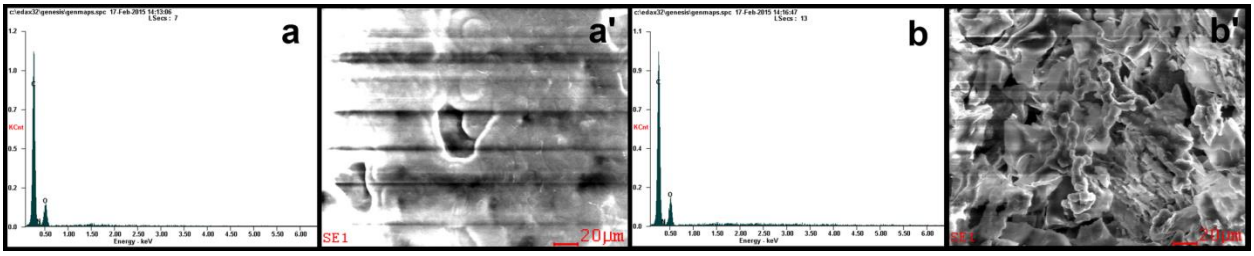

Figure 5S. EDX spectra and SEM images for lyophilized **MU1** (a) and **MU3** (b).

Table S2. Elemental composition (Wt%=mass % and At%= atomic %) found from EDX data and theoretical calculated composition for **MU6, MU8, MU1, MU3**.

	found		*calculated*	
MU6	*Wt%*	*At%*	*Wt%*	*At%*
C	73.05	77.66	65.35	70.7
N	07.40	06.75	10.61	9.8
O	19.54	15.60	24.02	19.5
	found		*calculated*	
MU8	*Wt%*	*At%*	*Wt%*	*At%*
C	74.07	78.23	67.49	72.2
N	10.71	09.70	14.69	13.5
O	15.21	12.06	17.81	14.3
	found		*calculated*	
MU1	*Wt%*	*At%*	*Wt%*	*At%*
CK	73.60	78.11	68.10	73.0
NK	07.49	06.81	11.55	10.6
OK	18.92	15.08	20.33	16.4
	found		*calculated*	
MU3	*Wt%*	*At%*	*Wt%*	*At%*
CK	73.13	77.86	65.56	71.1
NK	05.82	05.31	8.21	7.6
OK	21.05	16.83	26.22	21.3

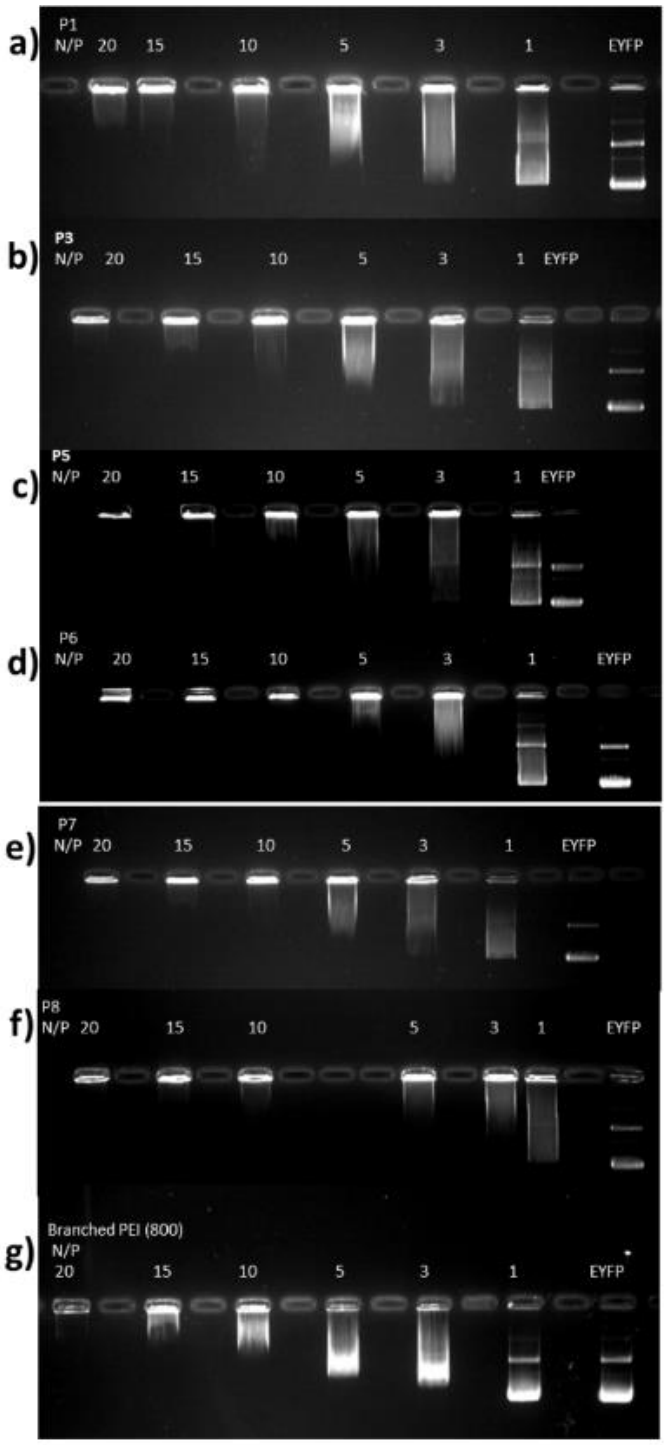

Figure 6S. Agarose gel electrophoresis assays for compounds a)-f) **P5-P8** at N/P ratio of 1, 3, 5, 10, 15, 20, g) PEI800 at N/P ratio of 1, 3, 5, 10, 15, 20 as reference. Agarose gel (1% w/v) in TAE (Tris-Acetate-EDTA) buffer at pH 7.4, the amount of dsDNA and pEYFP was kept constant in all the experiments and used as reference in line EYFP.

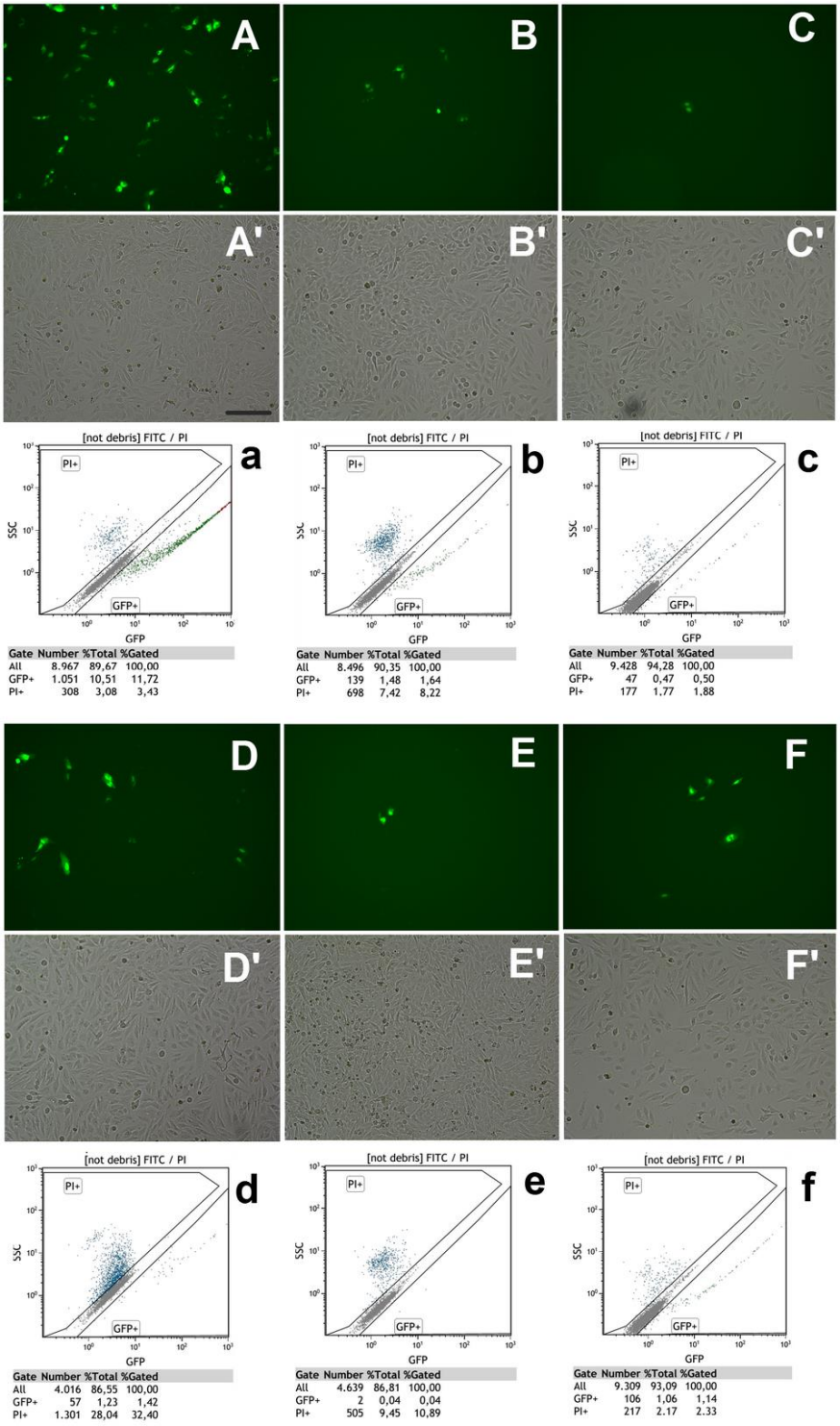

Figure 7S. Illustration of transfection efficiency of polyplexes. Fluorescence microscopy and flow cytometry were used to evaluate the capability of vectors to transfect HeLa cells. Fluorescence images for N/P 50 (A, B, C) and N/P 200 (D, E, F), modulation contrast for N/P 50 (A', B', C') and N/P 200 (D', E', F') and flow cytometry data for N/P 50 (a, b, c) and N/P 200 (d, e, f) for **P6**, **P8** and PEI 800 respectively.

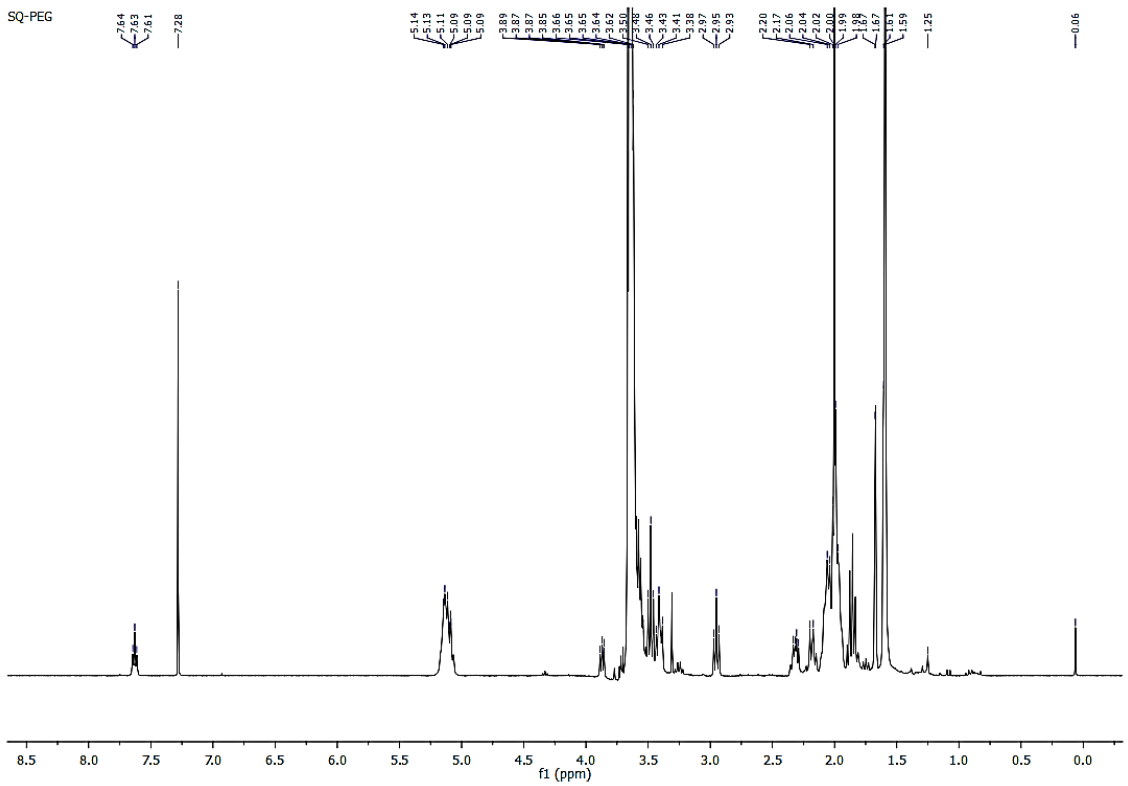

Figure 8S. ^{1}H NMR spectrum of SQ-PEG in $CDCL_3$.

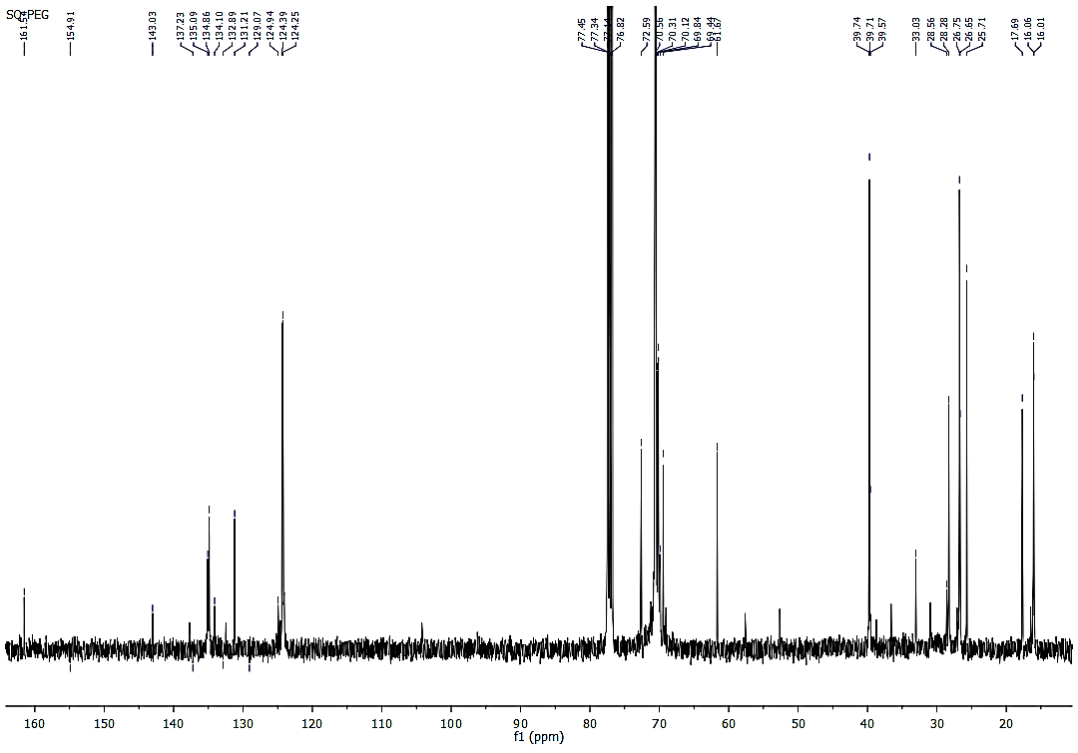

Figure 9S. ^{13}C NMR spectrum of SQ-PEG in $CDCL_3$