Supporting information

EXPERIMENTAL Procedures

1. Synthesis of monomers and polymers

General : All reagent used in the synthesis were obtained from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification if not specified. Ultrapure water was obtained using PURLAB Prima 7 (Elga). The final products were purified by dialysis in ultrapure water using Spectrapore1000WCO membranes (Spectra/por® Dialysis Membranes, Interchim). The products were then lyophilized (Telstar Cryodos, Froilabo). ^{1}H and ^{13}C spectra were recorded on a Bruker 300 MHz NMR spectrometer. ^{1}H NMR shifts were assigned by 2D COSY experiments. The ^{1}H NMR data are reported as followed : chemical shift (ppm), multiplicity (bs : broad singlet, bm : broad multiplet, s : singlet , d : doublet, t : triplet, m : multiplet) and the peak integration. The deuterated solvents (D_2O, DMSO-d_6, Trimethylsilyl-3-propionic acid-d_4) were purchased from Eurisotop. The mass spectra were obtained using an "Api 2000 SCIEX" ESI-MS.

Linear PEI (1) from poly(2-ethyl-2-oxazoline): 10g of poly(2-ethyl-2-oxazoline) (0.02 mole ; molar mass 50,000 $g.mol^{-1}$) were dissolved in 115 mL of freshly distilled water. 75 mL of hydrochloric acid (37 %) were added dropwise and the mixture was refluxed for 48 hours under gentle stirring. The solution was then cooled at room temperature and sodium hydroxide pellets were slowly added so as to bring the pH in the 12-14 range in order to precipitate the lPEI. The polymer was then collected by filtration through a sintered glass. The precipitate was washed with distilled water up to neutrality of the rinsing solvent and lyophilized up to complete removal of water. lPEI was then recovered as white powder (4.232g, 96.3%). ^{1}H NMR (D_2O): δ =3.50 (s, 4H, -NH-C$\underline{H_2}$-C$\underline{H_2}$-). ^{13}C (D_2O): δ = 48.97 (s, 2C, -NH-$\underline{C}H_2$-$\underline{C}H_2$-)

Synthesis of N-2-(3(3H-imidazol-4-yl) propanoic acid) acrylamide (N-acryloyl-L-histidine) (2): 3.1g of L-histidine (20 mmol) were dissolved in 10 mL of ultrapure water containing 1g of sodium hydroxide (25 mmol). 1.8g of acryloyl chloride (19.8 mmol) was added dropwise. During addition, the reaction mixture was kept at a temperature below 5°C during the overall addition. The reactive solution was stirred at room temperature for 18 hours and then acidified to pH=2 with 0.1N hydrochloric acid. The unreacted L-histidine was

precipitated in acetone (400 mL), and the N-acryloyl-L-histidine was recovered after solvent removal and lyophilisation. The resulted white powder was dissolved in acidic water (3 mL; pH = 2) and then precipitated in acetone (50 mL). The expected white precipitate was recovered by filtration through sintered glass and then dried under vacuum at room temperature. The yield is 35.8% (1.8g). ^{1}H NMR (DMSO-d_6): δ = 8.5 (s, 1H, imidazol); 7.2 (s, 1H, imidazol); 6.31 (m, 1H, $CH_2CHCONH$); 6.04 (d, 1H, $CH_2CHCONH$); 5.58 (d, 1H, $CH_2CHCONH$); 4.6 (m, 1H, CHCOOH); 3.10 (m, 2H, CCH_2CH). ^{13}C (D_2O): δ = 167.78 (s, 1C, CHCOOH); 162.76 (s, 1C, NCH_2CH_2CONH); 127.93 (s, 1C, imidazol); 123.55 (s, 1C, $CH_2CHCONH$); 123.26 (s, 1C, imidazol)); 123.05 (s, 1C, $CH_2CHCONH$); 111.72 (s, 1C, imidazol); 46.39 (s, 1C, CHCOOH); 30.9 (s, 1C, CCH_2CHN); 0 (s, TSP : ref). ESI-MS m/z = 210.20 uma for [$M+H^+$].

Modification of lPEI by histidinylation (3): To a solution of lPEI (3.7g, 86 mmol of ethylenimine units) in 73.5 mL of ultrapure water (pH=7), N-2-(3(3H-imidazol-4-yl) propanoic acid) acrylamide (6.9g, 33 mmol) was added and stirred at 100°C under N_2 atmosphere for 24 h. After reaction completion, the solution was allowed to cool down at room temperature. The product was dialyzed against ultrapure water for 24 h. Then, the product was lyophilized up to complete removal of water. His-lPEI was then recovered as a pale yellow powder at a 25% yield (grafting ratio: 24% of the ethylenimine units). ^{1}H NMR spectroscopy (D_2O) δ =8.5 (s, 1H, imidazol); 7.2 (s, 1H, imidazol); 4.32 (bs, 1H, CHCOOH); 3.2 (bs, 4H, ($NHCH_2CH_2$), ethylenimine units); 3.61 (bs, 2H, CCH_2CHN); 2.8 (bm, 4H, ($NRCH_2CH_2$), histidinylated ethylenimine units); 2.57 (bs, 2H, NCH2CH_2CONH); 2.35 (bs, 2H, NCH_2CH_2CONH). ^{13}C (D_2O): δ= 179.33 (s, 1C, CHCOOH); 177.98 (s, 1C, NCH_2CH_2CONH); 135.92 (s, 1C, imidazol); 132.56 (s, 1C, imidazol); 119.22 (s, 1C, imidazol); 57.15 (s, 1C, CHCOOH); 51.92 (s, 1C, ($NRCH_2CH_2NH$), histidinylated ethylenimine units); 47.70 (bs, 2C, ($NHCH_2CH_2$), ethylenimine units); 46.16 (s, 1C, ($NRCH_2CH_2NH$), histidinylated ethylenimine units); 50.63 (s, 1C, NCH_2CH_2CONH); 35.23 (s, 1C, -C-CH_2-CH-N); 28.89 (bs, 1C, NCH_2CH_2CONH); 0 (s, TSP : ref).

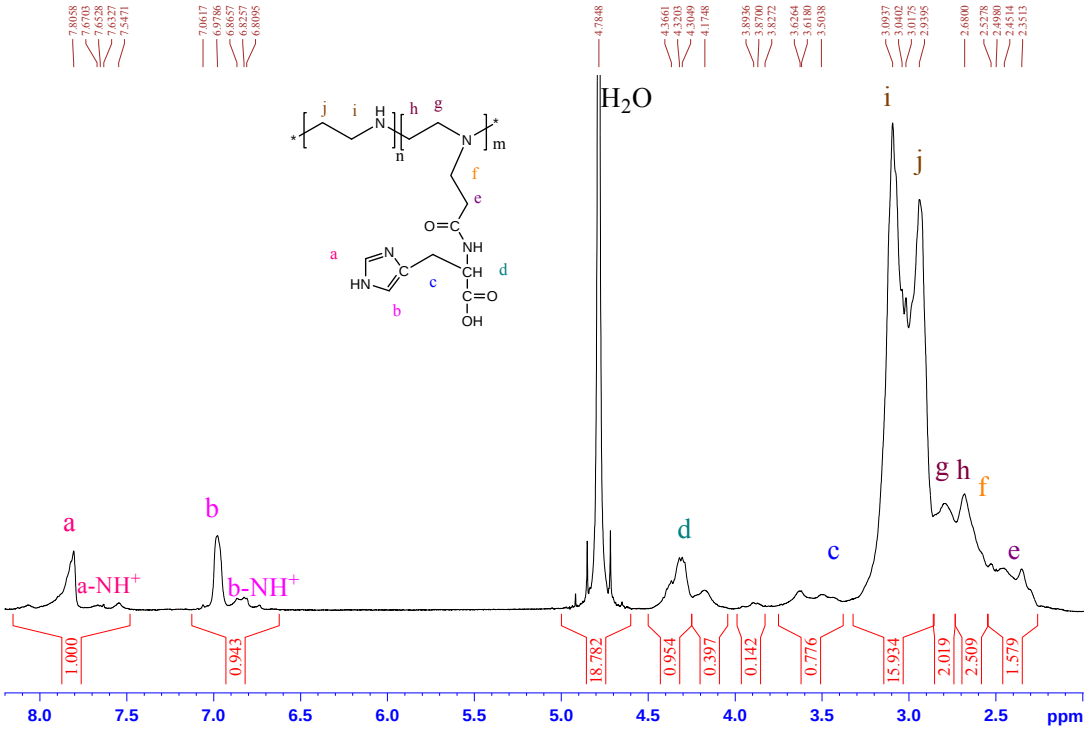

Figure 1. [1] H-NMR spectrum of His_{24}-lPEI in D_2O at 25°C.

Acid-Base Titration: The buffering capacity of His-lPEI polymers was determined by acid-base titration. 25 mM of ethylenimine moieties were dissolved in 6 mL of ultrapure water and then 3 mL of 0.1 N HCl were added to the solution in order to adjust the pH close to 2. Aliquots (20 µL) of 0.05 M NaOH were added in the His-lPEI solution, and pH was measured after each addition with a pH-metre WTW (inoLab 720) and an electrode pH-electrode SenTix 41. lPEI 22KDa was titrated in the same way as control. Buffering capacity was calculated by the reciprocal slope of titration plot in the pH range 5.1 to 7.4 (with units of mL of 0.05 N NaOH/ pH unit or simply mL).

2. His-lPEI/DNA complex characterization

Gel Electrophoresis Shift Assay: The ability of His-lPEI to complex pDNA was examined by agarose gel electrophoresis with pTG11033 (a plasmid DNA of 9514 bp from Trangène S.A., Strasbourg, France) encoding the firefly luciferase gene under the control of the human cytomegalovirus (CMV) promoter. Agarose gel (0.6%, w/v) containing ethidium bromide (1 µg/mL) was prepared in TBE buffer (89 mM Tris (tris(hydroxymethyl)aminomethane), 89 mM boric acid, 2 mM EDTA, pH=8.3). Each sample was dissolved in Hepes buffer pH=7.4. pTG11033 (1µL, 1µg/µL) was mixed with an equal volume of polymer at w/w ratios 0 - 8 and

incubated for 30 min at room temperature before the addition of loading buffer (1µL of blue Juice, Invitrogen, Carlsbad, CA). An aliquot (20 µL) of each sample was subjected to gel electrophoresis at 90 mV. pDNA complexation is evidenced by the complete lack of pDNA migration.

Particle Size and ζ potential Measurements: The size of His-lPEI/pDNA polyplexes pTG11033 was measured by dynamic light scattering with a Zetazizer 3000 (Malvern Instruments, Orsay, France). The system was calibrated with 204 nm latex nanosphere size standards (Duke Scientific Corps Palo Alto, CA). Polyplexes with pDNA/ His-lPEI w/w of 1:6 were formed by incubating His-lPEI (60µL, 1µg/µL in 10 mM Hepes buffer, pH=7.4) with pDNA (10 µL, 1µg/µL in 140 µL 10 mM Hepes buffer, pH=7.4). After 30 min at room temperature, the solution was diluted by adding 1.2 mL of 10 mM Hepes buffer, pH 7.4. The particle size was determined at 25°C. All measurements were performed in triplicate. The ζ potential determined by electrophoretic mobility with ZetaSizer 3000 were 8.9 mV, 6.6 mV, 8.4 mV, 1.3 mV, 3.1 mV, 2.9 mV and -13.6 mV for His_2-lPEI, $His_{7.4}$-lPEI, His_{11}-lPEI, His_{16}-lPEI, His_{22}-lPEI, His_{24}-lPEI and His_{32}-lPEI,respectively. The system was calibrated with DTS 1050 standard (Malvern).

Cells and Cell Culture

Human epithelial ovarian carcinoma cells (HeLa) (CCL2; ATCC, Rockville MD, USA) were maintained by regular passage in minimum essential medium (MEM; Gibco, Invitrogen SARL, Cergy Pontoise, France) supplemented with 1% of non-essential amino acid solution (Gibco), 10% heat-inactivated fetal bovine serum (FBS), 2mM L-glutamine and 100 U/ml penicillin and 50 U/ml streptomycin (Gibco). Human Embryo Kidney HEK293–T7 cells (kindly given by Drs. L. Huang and M. Brisson, University of Pittsburgh, Pittsburgh, PA) were maintained by regular passage in DMEM supplemented with 10% heat-inactivated FBS,

2mM L-glutamine and 100 U/ml penicillin and 50 U/ml streptomycin, 1% sodium pyruvate (Life Technologies), 100 U/ml penicillin and 50 U/ml streptomycin, and 400 µg/mL geneticin. The mouse skeletal muscle (C2C12; CRL1772, ATCC, Rockville MD, USA) cells line was maintained by regular passage in DMEM supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin and 50 U/mL streptomycin. Cells were harvested once a week by treatment with phosphate-buffered saline (PBS) containing 1 µg/ml trypsin and 5 mM ethylenediamine tetraacetic acid (EDTA). The immortalized human tracheal epithelial cell line (ΣCFTE29o- cells) was from a CF patient homozygous for the ΔF508 mutation and was kindly given by Dr D. C. Gruenert (University of Vermount, Birlington, VT, USA). Five hundred thousand cells were plated on tissue culture plasticware (75 cm2*)* coated with fibronectin (0.01 mg/ml), collagen (0.03 mg/ml), and bovine serum albumin (BSA) (0.1 mg/ml) contained in MEM and grown in 20 ml MEM supplemented with 10% non-heat-inactivated FBS, 2 mM Lglutamax and antibiotics (100 units/ml penicillin and 100 µg/ml streptomycin). The culture medium was changed every 2 days. On day 8, cells were harvested by treatment with trypsin at 37°C for 10min. Cells were incubated at 37°C in a humidified 5% CO_2 atmosphere. Cells were mycoplasma-free as evidenced by MycoAlert® Mycoplasma Detection Kit (Lonza, Levallois Perret, France).

Transfection efficiency of polyplexes: The gene transfer efficiency of Histidine modified lPEI-polyplexes was evaluated by transfection of cells using pTG11033 encoding the luciferase gene under the promoter of the cytomegalovirus CMV. The cells in 24 wells plate were transfected for four hours at 37°C with 0.5 mL of polyplexes (i.e. 2.5 µg pDNA per well) in culture medium containing 10% serum. Then, the medium was removed and replaced with fresh complete culture medium. The luciferase activity (RLU) was measured upon 48 hours culture and was expressed as RLU/mg of protein. The number of transfected cells was assessed by flow cytometry upon transfection with polyplexes made with a plasmid DNA encoding the green fluorescent protein (EGFP) under the CMV promoter. Forty-eight hours after transfection, cells were harvested by treatment with trypsin, washed in complete

medium, and the pellet was suspended in sheath fluid. The number of transfected cells and the cell-associated fluorescence were measured by flow cytometry (FACSort, Becton Dickinson; λex=488 nm; λem=520 nm).

Cytotoxicity assay: The cell viability was evaluated by the colorometric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MTT (50 µL of a 5 mg/mL stock solution in phosphate buffered saline) was added to the cell culture 48 h after either polyplexes transfection or incubation with pDNA free polymers. After 4 h incubation at 37 °C, MTT converted into an insoluble dye in living cells was then solubilised with acidic isopropanol. The absorbance was measured at 570 nm and expressed as a percentage of the absorbance measured for untreated cells cultured under the same conditions used for treated cells. The cytotoxicity of Hist-lPEI was measured as a function of the % of grafted histidine residues. As shown in Figure 2, the highest the grafted histidine ratio, the lowest is the toxicity.

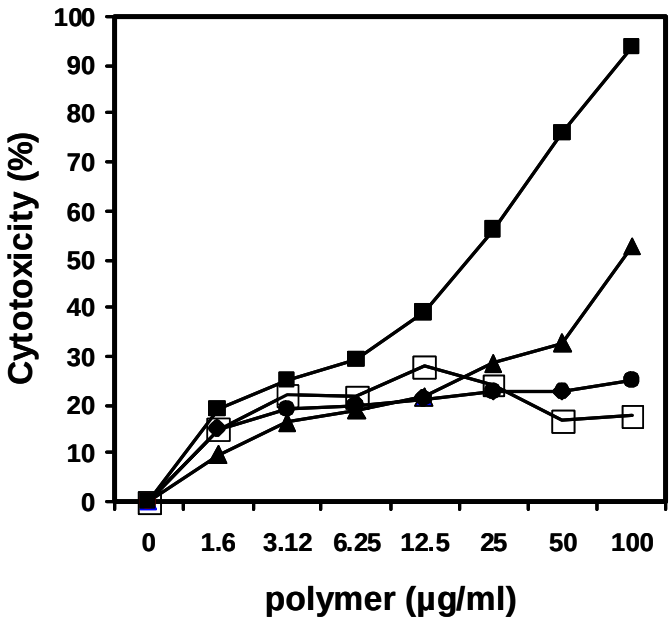

Figure 2. Cytotoxicity on HeLa cells of ■ lPEI, ▲ $His_{7.4}$-lPEI, ● His_{16}-lPEI, □ His_{25}-lPEI.