Supporting Information

Use of Primary and Secondary Polyvinylamines for Efficient Gene Transfection

Mathilde Dréan,[a,b] Antoine Debuigne,[b]CristineGoncalves,[c]Christine Jérôme[b], Patrick Midoux,[c]Jutta Rieger,[a] Philippe Guégan[a]

a Sorbonne Universités, UPMC Univ Paris 06, CNRS, Institut Parisien de Chimie Moléculaire, Equipe Chimie des Polymères, 4 Place Jussieu, F-75005 Paris, France.

b Center for Education and Research on Macromolecules (CERM), Department of Chemistry, University of Liege (ULg), Sart-Tilman, Allée de la Chimie 3, Bat. B6a, B-4000 Liège, Belgium.

c Centre de Biophysique Moléculaire, UPR4301 CNRS, Rue Charles Sadron, 45071 Orléans Cedex 2, France

Table S1. Determination of the hydrolysis level of the poly(*N*-vinylamines) (PVAm) and poly(*N*-methylvinylamines) (PMVAm) by elemental analysis.

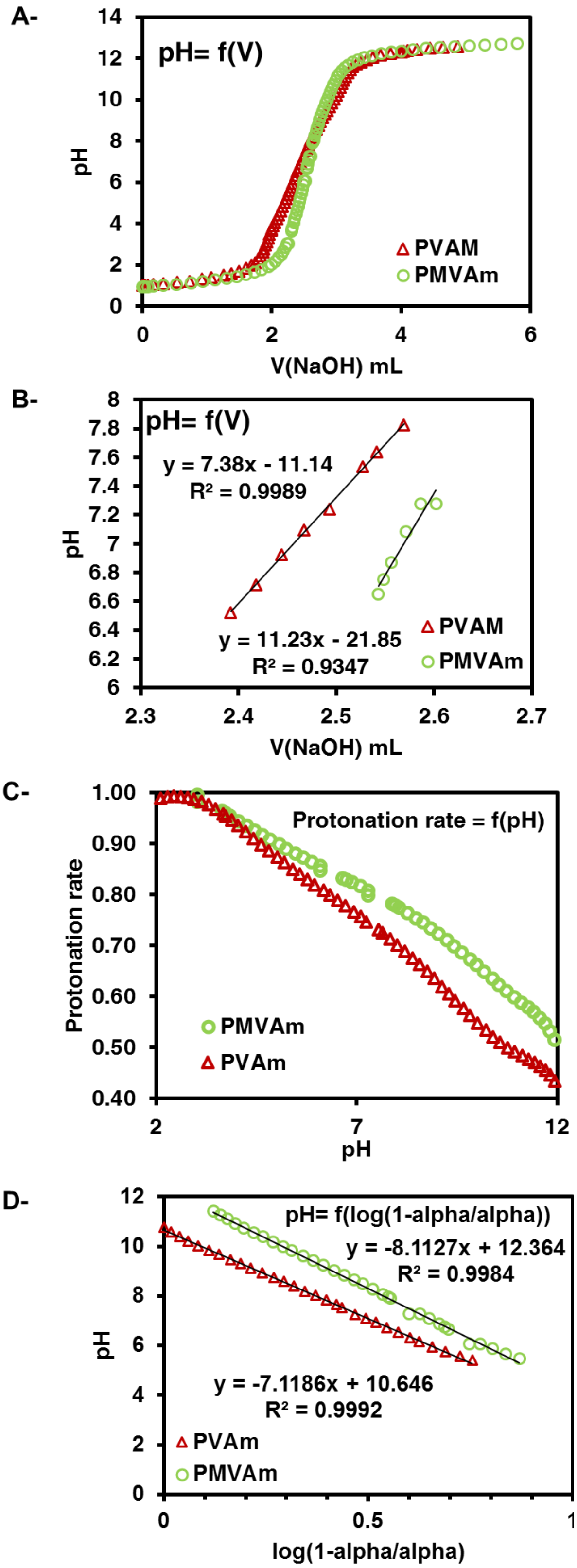
Entry	Polymer	%C[e]	%N[e]	C/N_{exp} [e]	$C/N_{theor.\ full\ hydrolysis}$ [f]	% of hydrolysis[g]
1	PN700-Fs[a]	32.115	17.650	2.904	1.716	94
2	PN150-Cs[a]	n.d.	n.d.	n.d.	n.d.	n.d.
3	PN255-Cs[a]	33.105	17.675	1.873	1.716	91[h]
4	PN660-Cs[a]	32.595	18.085	1.802	1.716	94[h]
5	PN940-Cs[a]	32.080	17.630	1.820	1.716	95[h]
6	PN1510-Cs[a]	32.485	17.930	1.812	1.716	94[h]
7	PN50-R[b]	32.420	17.920	1.809	1.716	99[i]
8	PN170-R[b]	32.840	17.875	1.837	1.716	98[i]
9	PN200-R[b]	31.825	18.150	1.753	1.716	≥ 99[i]
10	PM100-Fh[c]	41.620	14.105	2.951	2.573	78[j]
11	PM140-Fh [c]	38.945	13.410	2.904	2.573	81[j]
12	PM165-Fh [c]	41.700	14.975	2.785	2.573	88[j]
14	PM285-Fh [c]	46.165	15.060	3.065	2.573	71[j]
15	PM110-Ch[c]	43.630	15.135	2.883	2.573	82[j]
16	PM265-Ch[c]	43.895	15.680	2.799	2.573	87[j]
17	PM310-Ch[c]	42.400	15.700	2.701	2.573	93[j]
18	PM680-Ch[c]	43.610	16.010	2.724	2.573	91[j]
19	PM155-Fm23[d]	52.480	13.450	3.902	2.573	23 [j]
20	PM155-Fm 37[d]	50.800	13.935	3.645	2.573	37 [j]
21	PM155-Fm 44[d]	48.570	13.740	3.535	2.573	44 [j]
22	PM155-Fm 54[d]	46.665	13.940	3.348	2.573	54 [j]
23	PM155-Fm 64[d]	44.345	13.895	3.191	2.573	64 [j]
24	PM155-Fm 76[d]	43.760	14.670	2.983	2.573	76 [j]
25	PM155-Fm 94[d]	42.005	15.695	2.676	2.573	94 [j]

[a] Hydrolysis conditions:HCl 2N at 120°C for 14h.[b]Hydrazinolysis conditions:[NVPi]/[hydrazine]= 1/24, in 1.4-dioxane/MeOH 1/2 at 65°C for one night.[c] Hydrolysis conditions:HCl 6N at 120°C for 64h.[d]Hydrolysis conditions:HCl3 N at 100°C[e] Determined by elementary analysis.[f] Calculated for full hydrolysis of the amides moieties. [g]NVA hydrolysis level = 100 × (1-$f_{NVA\ residual}$) where $f_{NVA\ residual}$ is the molar fraction of the residual non-hydrolyzed NVA units, and NMVA hydrolysis level = 100 × (1-$f_{NMVA\ residual}$) where $f_{NMVA\ residual}$ is the molar fraction of the residual non-hydrolyzed NMVA units.[h-j]$f_{NVA\ residual}$, $f_{NMVA\ residual}$ and $f_{NVPi\ residual}$ are determined based on formulas h-j (see below) established by taking into account the molar fraction of each comonomer in the copolymer precursor (F_{NVA}^0, F_{NMVA}^0 and F_{NVPi}^0) and the respective numbers of carbon and nitrogen atoms in the hydrolyzed and non-hydrolyzed monomer units. MM_c and MM_N are the molar mass of C and N, respectively.n.d. = not determined.

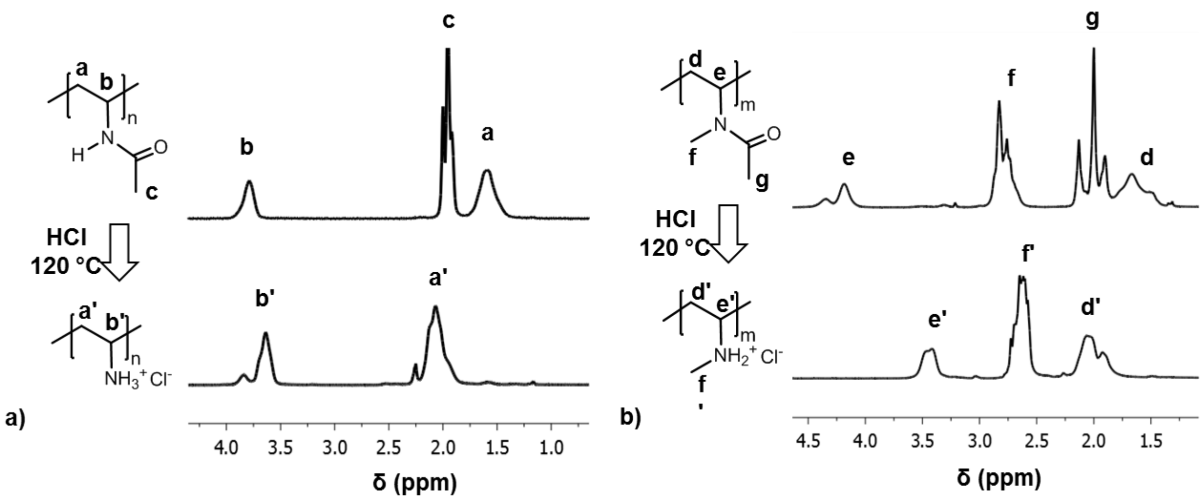
$$^{h}F_{NVA\ residual} = \frac{[MM_N \times \frac{C}{N}] - [2 \times MM_C]}{2 \times MM_c}$$

$$^{i}F_{residual\ \ NVPi} = \frac{\left[MM_N \times \frac{C}{N}\right] - [2 \times MM_C]}{8 \times MM_C}$$

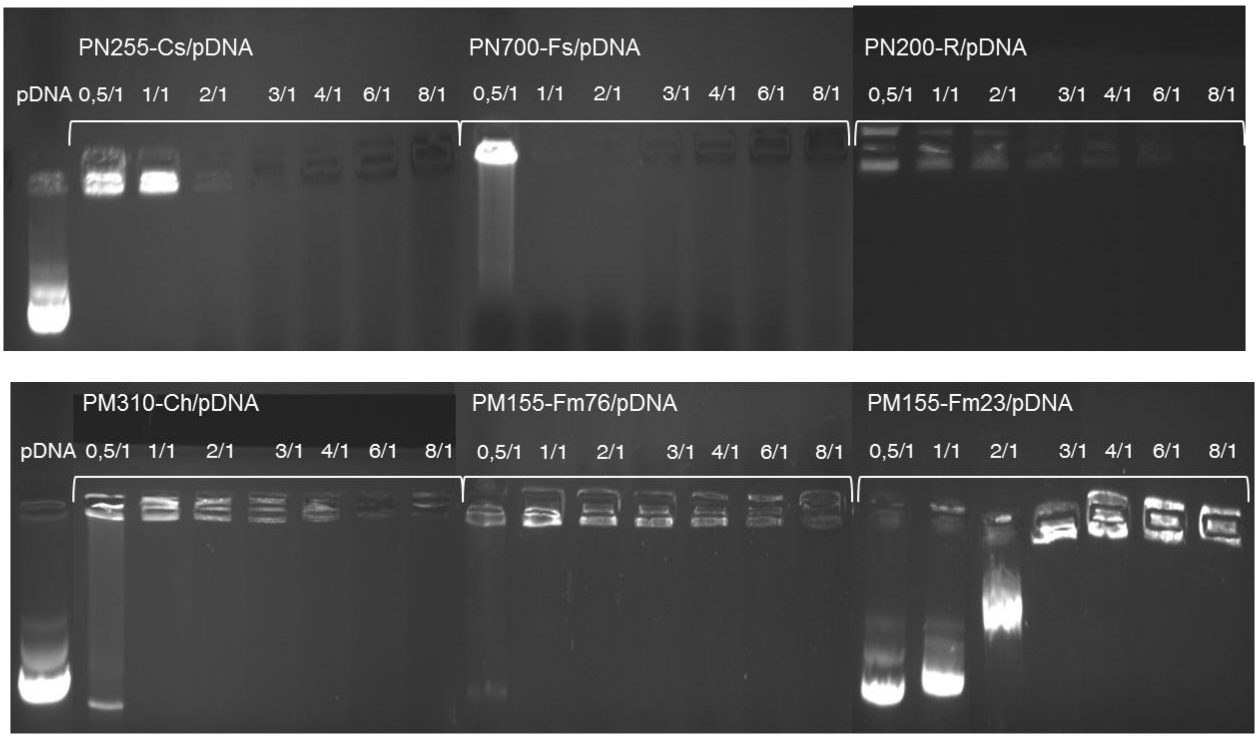
$$^{j}F_{NMVA\ residual} = \frac{\left[MM_N \times \frac{C}{N}\right] - [3 \times MM_C]}{2 \times MM_c}$$



<u>Figure S1</u>. (A) Titration curves of aqueous solutions of PN255-Cs (PVAm) and PM140-Fh(PMVAm) (50 mg/ml in 10 mL of HCl 1M) with 0.5N NaOH, (B) linear regression of the titration curves of aqueous solutions of PN255-Cs and PM140-Fhin order to determine their buffer capacities between $6.5 < pH < 7.5$, (C) the resulting protonation curves versus the pH and (D) determination of the pKa.



<u>Figure S2.</u>[1]H NMR analyses of **a**) PNVA ($M_{\text{nSEC-MALLS}}$ = 56300 g/mol, $Đ$ = 1.18) and **b**) PNMVA ($M_{\text{nSEC-MALLS}}$ = 30800 g/mol, $Đ$ = 1.12) samples before and after acid hydrolysis (6 N HCl/120°C). Spectra were recorded at 298K in D_2O.



<u>Figure S3.</u>Agarose gel electrophoresis retardation assays of polyplexes made with different polyvinylamines (PN) (top) and poly(*N*-methylvinylamines) (PM) (bottom). Polyplexes were formed with various polymer/pDNA weight ratios (WR).

Table S2.Characteristics of polyvinylamine (PVAm) synthesized by RAFT polymerization of *N*-vinylphthalimide and successive hydrazinolysis.

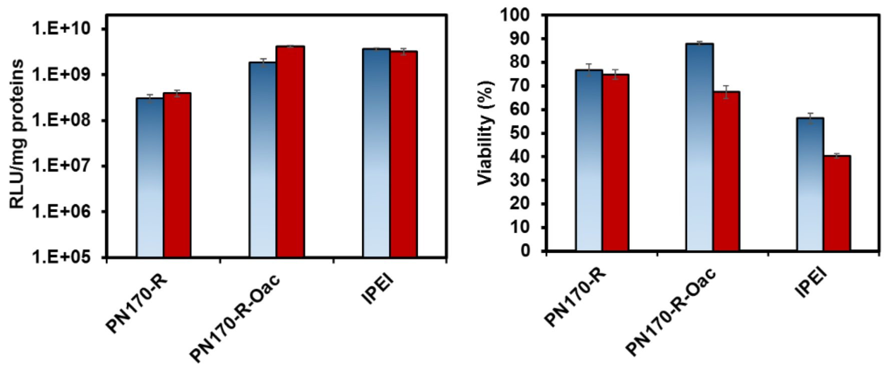
Entry	Name	PNVPi[a]					PVAm [b]	
		$M_{n,th}$[c]	M_n^{LS} (kg/mol)[d]	DP_n[e]	$Đ$[f]	Conv. (%)	% of hydrazinolysis[g]	M_n (kg/mol)[h]
1	PN50-R	5	8	46	1.43	> 99	99	2.0
2	PN170-R	21	29	167	1.52	81	98	7.2
3	PN200-R	23	35	200	1.61	59	99	8.6

[a] Conditions for PN50-R, PN170-R and PN200-R are respectively: [NVPi]/[AIBN]/[CTA]= 25/0.25/1, 150/0.25/1 and 227/0.25/1, for 12 h, 24 h and 72 h. [b] Conditions of the hydrazinolysis: [PNVPi]/[hydrazine]= 1/24, in 1.4-dioxane/MeOH 1/2 at 65°C for one night. [c]$M_{n,th}$= DP_n^{th} x conversion x $MM_{monomer}$ [d] M_n^{LS}determinedby SEC in DMF equipped with a MALLS detector, dn/dc_{PNVPi}= 0.131. [e] Calculated using the following formula: DP_n= $M_n/MM_{monomer}$. [f] Determined by SEC in DMF using a PMMA calibration. [g]Determined by elemental analysis (SI Table S1 for crude EA analysis and calculations).[h]Number-average molar mass calculated by the molar mass of the precursor.

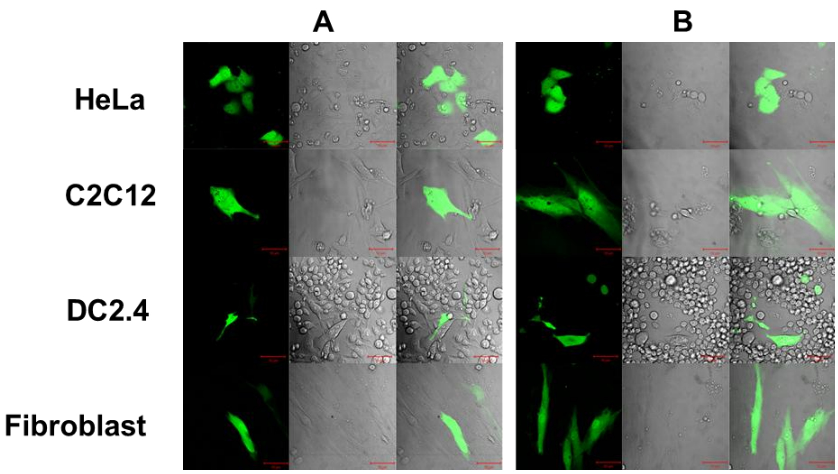
Table S3. Characteristics of pDNA complexes made with PVAm polymers prepared via RAFT polymerization

Entry	Polyplexes	polymer/pDNA WR[a]	N/P[b]	D_h[c] (nm)	ζ[d] (mV)
1	PN50-R-plex	1	7	3490	+27
		3	22	150	+37
2	PN170-R-plex	1	7	460	+40
		3	21	140	+44
3	PN200-R-plex	1	7	90	+23
		3	22	130	+40
2’	PN170-R-OAc[e]	1	2	185	+27
		3	5	140	+33

[a] WR = polymer/pDNA weight ratio. [b] amine/phosphate molar ratio calculated as described in experimental part. [c]Hydrodynamic diameters D_h of the polyplexes at 298K in HEPES 10 mM, pH 7.4.[d] ζ potential of polyplexes at 298K in HEPES 10 mM, pH 7.4. [e]Obtained by acetylation of PN170-R (degree of acetylation = 50%).



<u>Figure S4.</u> (A) Transfection efficiency and (B) cell viability of HeLa cells. Transfection was performed with PVAm (made by RAFT) before (PN170-R) and after 50 % acetylation (PN170-R-OAc) polyplexes at two polymer/pDNA ratios (ratio 1 (blue) and ratio 2 (red): lower and higher amount of polymer, Table S3). The luciferase activity was measured 48h after the transfection and expressed as RLU/mg of protein. The cell viability was evaluated by MTT assay 48h after transfection and expressed as percentagerelative to untreated cells.



<u>Figure S5.</u>HeLa, C2C12, DC2.4 cells and fibroblasts were transfected with PM140-Fhpolyplexes at N/P = 3 (A) and 6 (B) containing pDNA encoding EGFP. EGFP-positive cells were analyzed by fluorescent confocal microscopy. Fluorescence images (left), phase contrast images (middle) and their merge (right).