Supplementary Information

Flexible cyclic siloxane core enhances the transfection efficiency of polyethylenimine-based non-viral gene vectors

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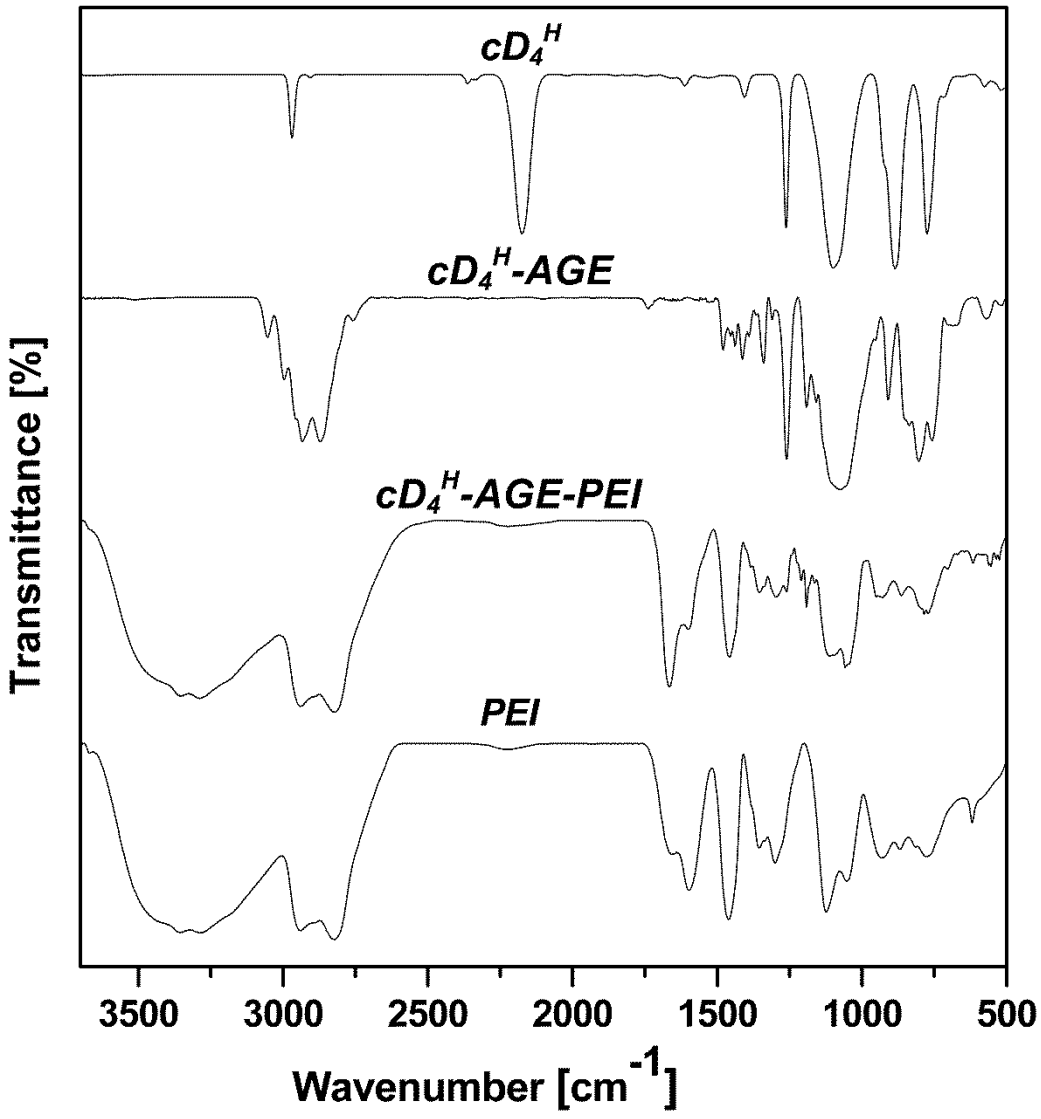


Figure SD-1. *FTIR spectra of the cD_4^H–AGE–PEI conjugate and its precursors.*

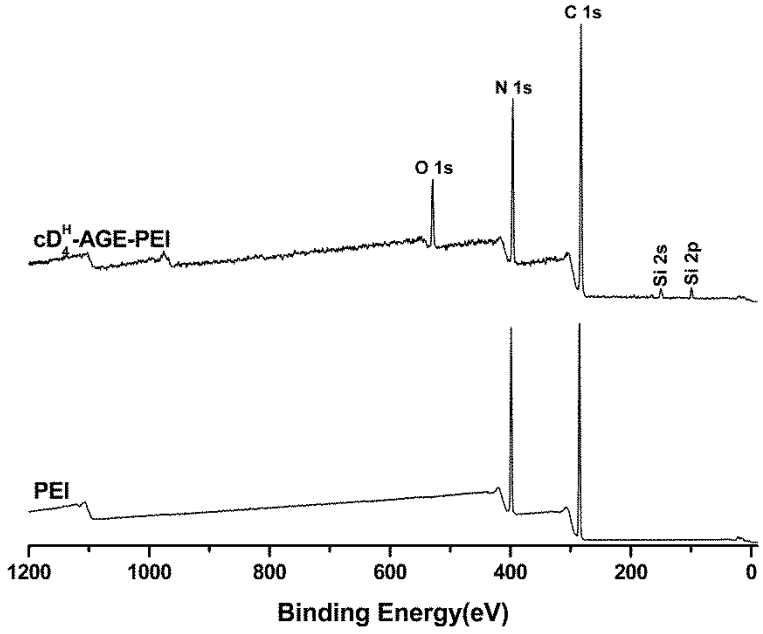


Figure SD-2. *XPS wide scan of cD_4^H–AGE–PEI and PEI.*

Table SD-1.*PEI and cD_4^H–AGE–PEI high resolution spectra. C 1s and N 1s assignments.*

Assignment / Sample	C 1s								N 1s					
	C-Si		C-C/C-H		C-N		C-O		N-C		HN-C		H_2N-C	
	eV*	%**	eV*	%**	eV*	%**	eV*	%**	eV*	%**	eV*	%**	eV*	%**
PEI	-	-	285.0	73.9	285.4	26.1	-	-	398.4	75.4	398.8	12.2	399	12.4
cD_4^H–AGE–PEI	284.1	9.5	285.0	59.8	285.7	28.0	286.8	2.7	398.4	76.6	398.8	16.4	399.5	7.0

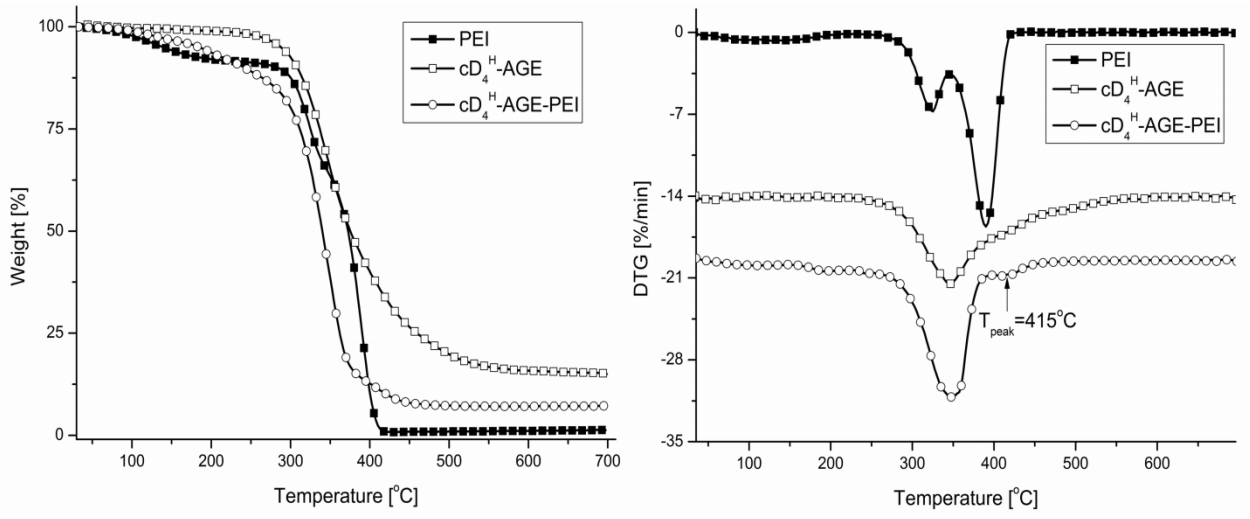


Figure SD-3. *The TGA and DTG curves of PEI, cD_4^H–AGE precursor, and cD_4^H–AGE–PEI conjugate.*

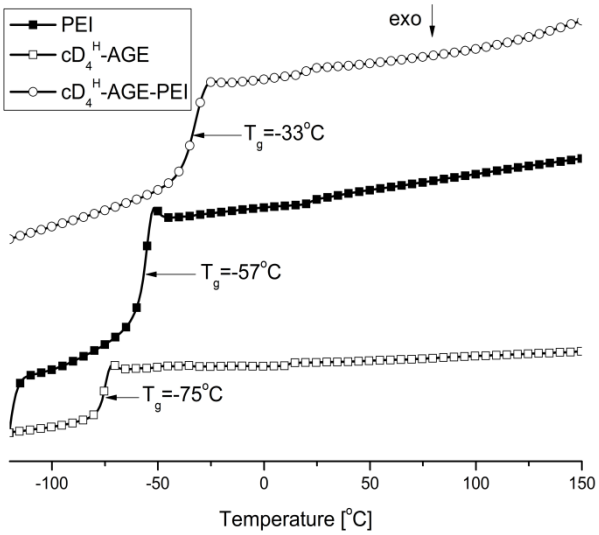


Figure SD-4. *Second DSC heating curves of the precursors, and of cD_4^H–AGE–PEI conjugate.*

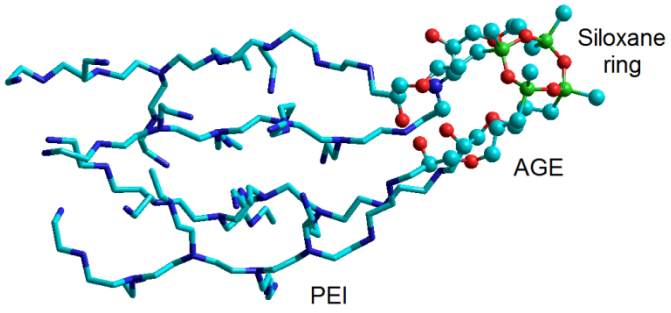


Figure SD-5. *Optimized molecular structure of cD_4^H–AGE–PEI conjugate in ground state.*

Table SD-2. *Summary of the results of molecular dynamics simulation regarding the interaction between DNA and cD_4^H–AGE–PEI conjugate, in explicit water environment, at 298 K.*

Simulation time, t (ns)	**0.00**	**2.00**	**4.00**	**6.00**	**8.00**	**10.00**
Potential Energy, kcal/mol	-136843	-315107	-314887	-314857	-314820	-314903
Intermolecular Distance, Å	30.50	14.32	11.37	11.27	11.39	11.69
Number of atoms in contact	0	387	520	611	565	500
Number of hydrogen bonds	0	1	9	8	10	9
Energy of H-bonds, kcal/mol	0.00	5.84	49.80	40.02	48.17	41.53
R_g (DNA), Å	13.77	13.67	13.41	13.53	13.55	13.46
R_g (cD_4^H–AGE–PEI), Å	10.42	11.22	11.20	10.96	11.12	11.14
RMSD (DNA), Å	0.00	1.87	2.26	2.28	2.11	2.02
RMSD (cD_4^H–AGE–PEI), Å	0.00	7.85	7.47	7.64	7.88	8.09

Mass spectrometry analysis.

The cD4H–AGE–PEI sample was dissolved in methanol and diluted with water/methanol mixture, to obtain a concentration of 100 μg/mL. The ESI-MS spectrum of hyperbranched 2 kDa PEI sample in 9:1 v/v methanol-water solution is shown in Figure SD-7. The distribution of singly protonated oligomers observed stretches from the 2-mer ion at m/z 104.07, to the 46-mer ion at m/z 1998.63. Doubly charged ions series with 43/2 m/z units are also observed in the mass spectrum, being attributed to $[H_2N–(CH_2CH_2NH)_n–H + 2H]^{2+}$ species. Hyperbranching cannot be detected by ESI-MS, because it does not affect the overall composition of the polymer or its repeat unit.

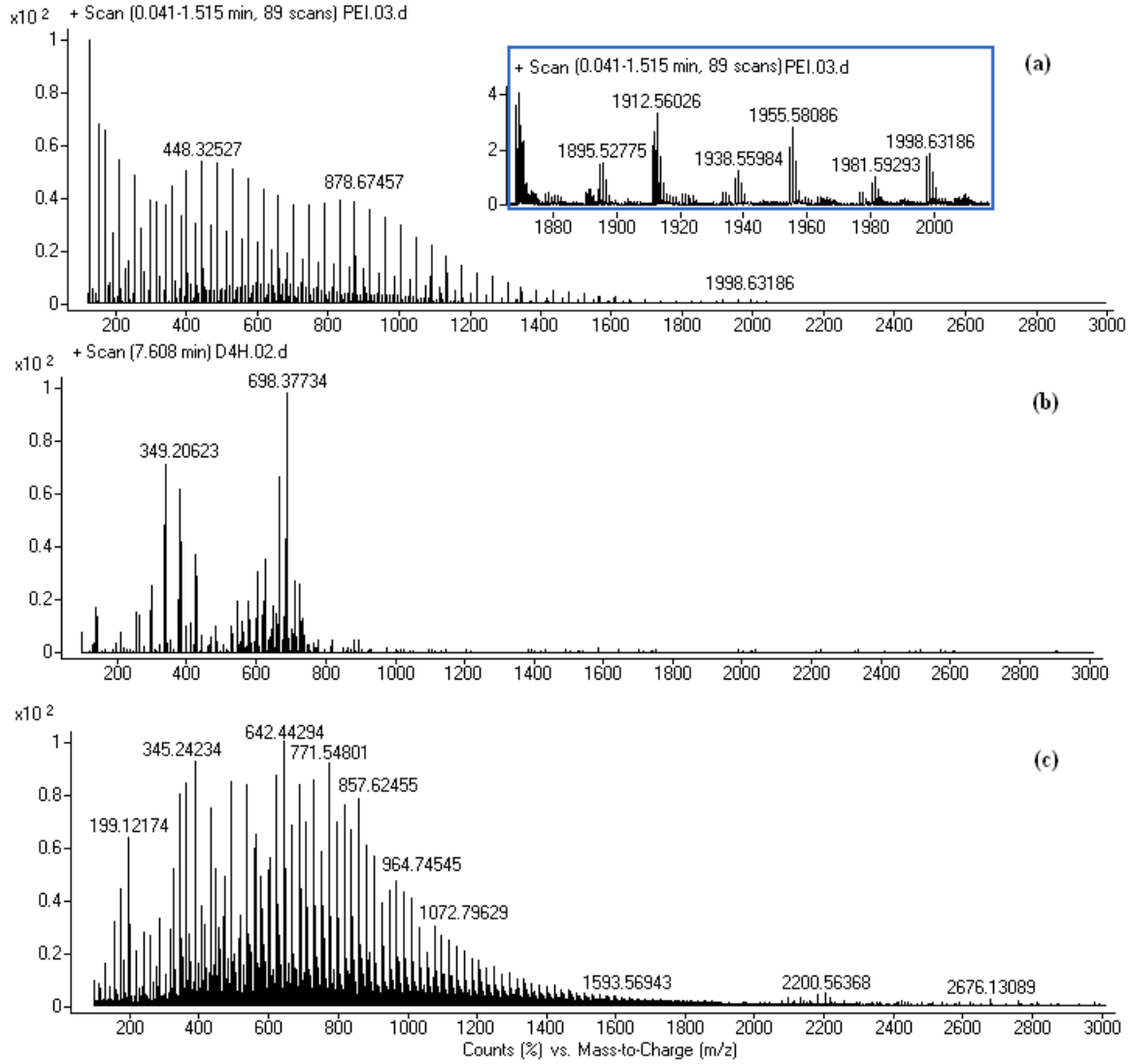


Figure SD-6. *Electrospray mass spectra of (a) PEI 2000, (b) cD_4^H–AGE precursor, and (c) cD_4^H–AGE–PEI conjugate, all dissolved in 9:1 v/v methanol-water solution.*

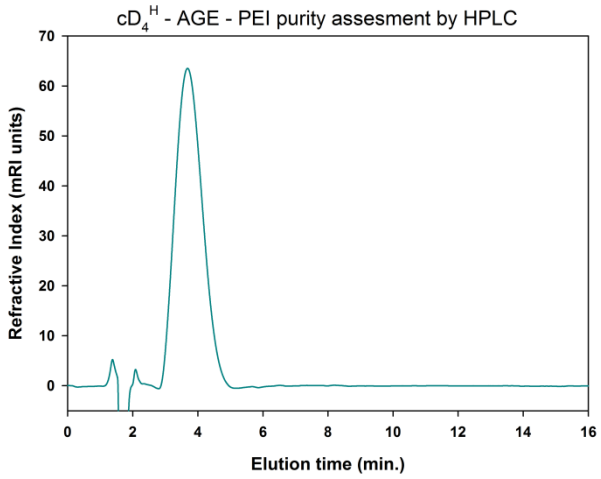
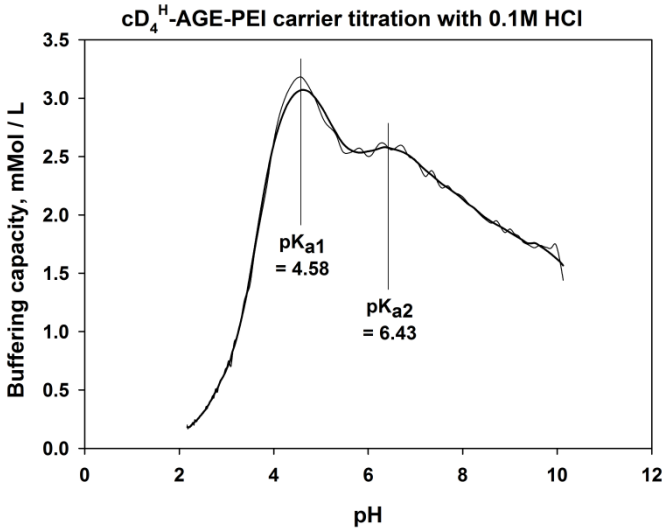


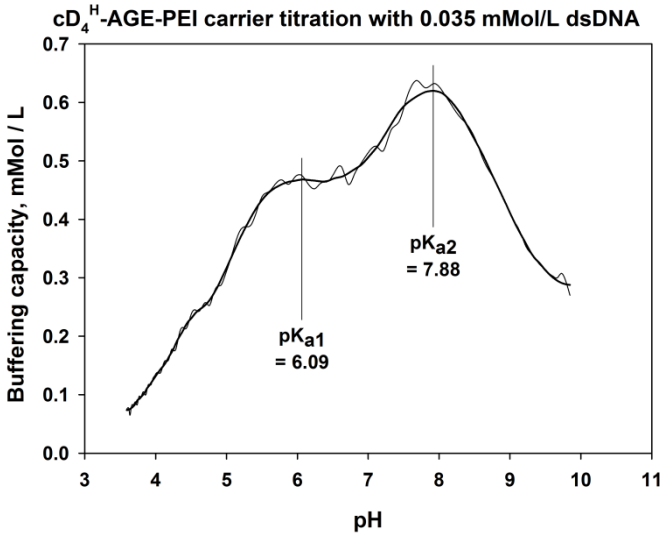
Figure SD-7. *HPLC chromatogram of cD_4^H–AGE–PEI conjugate, dissolved in 9:1 v/v methanol-water solution.*

The full scan ESI-MS spectrum of cD_4^H–AGE sample is shown in Figure SD-6.b. The most abundant peak, observed at m/z 698.38, corresponds to the single charge protonated ion of the precursor species, $[M + H]^+$, and the peak at m/z 349.21 corresponds to their double charge protonated ion $[M + H]^{2+}$ (where M denotes the molecular weight of cD_4^H–AGE precursor).

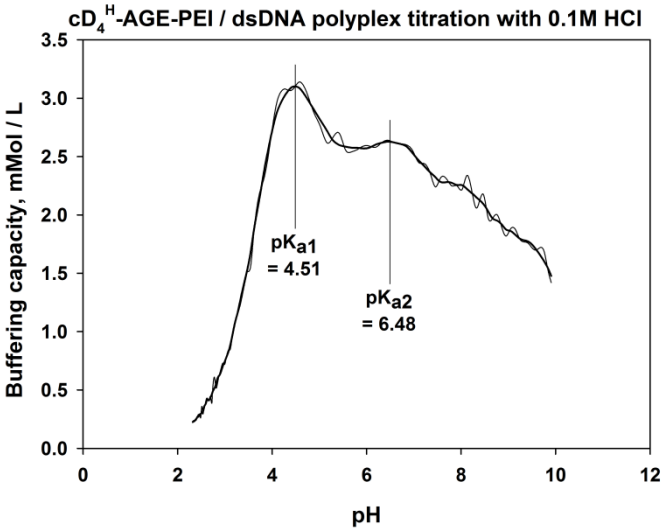
The HPLC analyses were carried out using a Perkin Elmer HPLC system with a Flexar Quaternary LC Pump, a Flexar LC Autosampler, a Flexar UV-VIS and Refractive Index LC Detectors. A Zorbax SB C18 (5µm, 150 mm x 4.6mm) column was used. Temperature was kept at 35°C. The mobile phase was a mixture of 10:90 v/v water and methanol HPLC grade. The flow was 1 mL/min, and the injection volume was of 20 µL.



(a)



(b)



(c)

Figure SD-8. *The values of pKa constants determined by potentiometric titration, and calculated based on the maxima of the buffering capacity of the investigated species.*
(1 mMol/L = 1 nMol/μL = 1 pMol/nL)
The curves were smoothed using the Savitzky–Golay algorithm, with a 13 points window.

(a) Buffering capacity of the naked carrier, when titrated with HCl.

(b) Buffering capacity of the naked carrier, when titrated with dsDNA.

(c) Buffering capacity of the loaded carrier (as polyplex entities), when titrated with HCl.