SUPPORTING INFORMATION

Investigating the properties of L-lysine dendrimers through physico-chemical characterisation techniques and atomistic molecular dynamics simulations

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Supporting Methods

MD Simulations

Three-dimensional model templates for the L-lysine dendrimers up to generation 6 were generated using dendrimer builder as implemented in the Materials Studio software (BIOVIA, Dassault Systèmes, BIOVIA Materials Studio, Release 2017 (17.1.0.48), San Diego: Dassault Systèmes, 2020). Models were built for boc-protected L-lysine and L-Lysine (ammonium trifluoroacetate) dendrimer systems. For the L-lyisne (ammonium trifluoroacetate) system a chloride anion was modelled rather than trifluoroacetate anion. Two protonation states were considered for the L-Lysine salt systems, corresponding to a high pH system (pH > 12) where all the amine groups are in the unprotonated state and a low pH system (pH <3) where all amine groups are protonated.

The initially generated structures were subsequently solvated and optimized. The boc-protected L-lysine systems were solvated in methanol and the L-Lysine(ammonium chloride) systems were solvated in water (simple point charge (SPC) water model). In the next step an annealing scheme was employed to generate improved starting structures for MD analysis. A temperature ramp of 300–700 K over 2 ns using the NPT ensemble mode (with a Langevin thermostat method) before the annealed systems were allowed to relax for 5ns at 300 K was employed to obtain the initial equilibrated configurations for the systems studied.

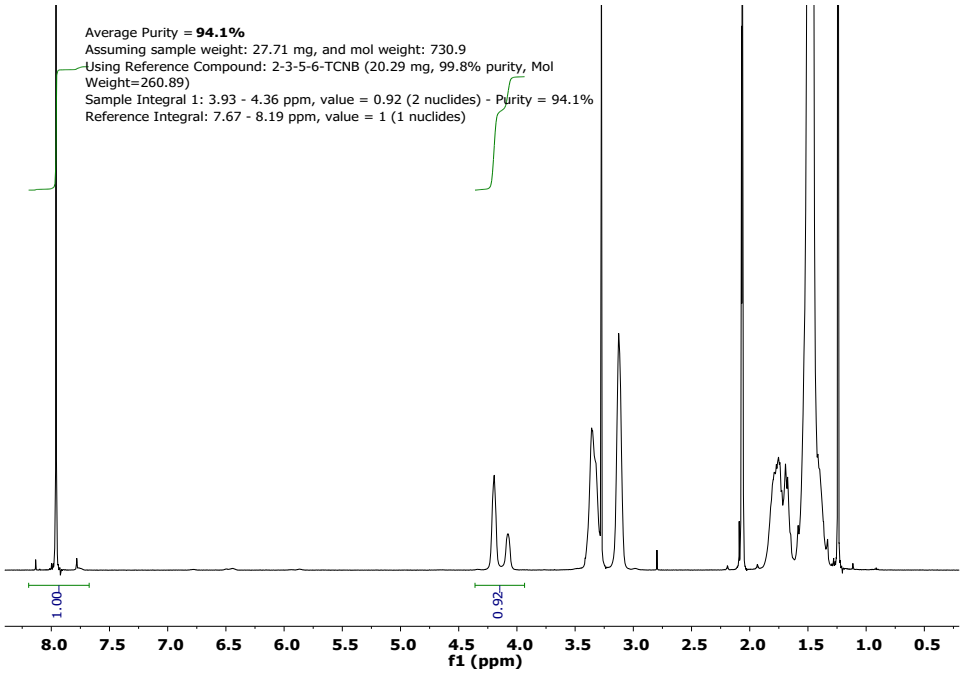
The optimizations and simulated annealing runs were performed with the Desmond molecular dynamics system (Schrödinger Release 2020-1: Desmond Molecular Dynamics System, D. E. Shaw Research, New York, NY, 2020. Maestro-Desmond Interoperability Tools, Schrödinger, New York, NY, 2020.) and the OPLS3 force-field.[1]

On completion of the setup protocol production MD simulations were carried out for each of the systems. An initial 5 step relaxation protocol is performed on each system studied, 1) a NVT ensemble with Brownian dynamics for 100 ps at 10 K and all non-hydrogen solute atoms restrained, 2) a NVT ensemble using a Langevin thermostat for 12 ps at 10 K and all non-hydrogen solute atoms restrained, 3) a NPT ensemble using a Langevin thermostat and Langevin barostat for 12 ps at 10 K and 1.01325 bar

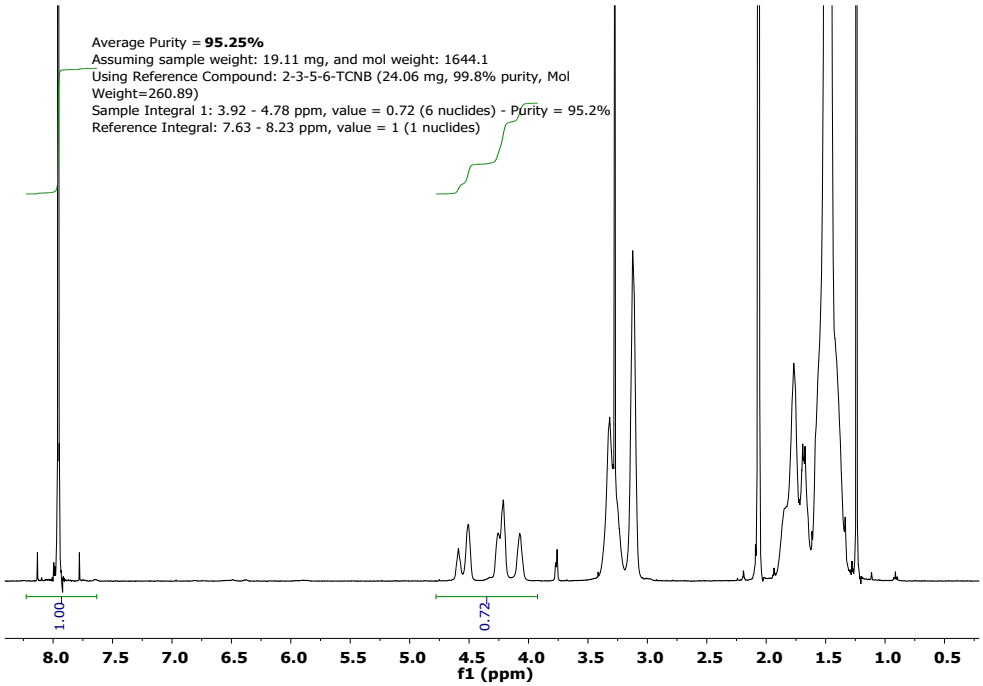
and all non-hydrogen solute atoms restrained, 4) a NPT ensemble using a Langevin thermostat and Langevin barostat for 12 ps at 300 K and 1.01325 bar and all non-hydrogen solute atoms restrained and 5) a NPT ensemble using a Langevin thermostat and Langevin barostat for 24 ps at 300 K and 1.01325 bar. Following the initial relaxation protocol a 50 ns NPT (300K, 1.01325 bar) MD simulation was carried out using a time step of 2 femtoseconds, the temperature was regulated with the Nose-Hoover chain thermostat with a relaxation time of 1.0 ps and the pressure was regulated to 1 bar with the Martyna–Tobias–Klein barostat and a relaxation time of 2.0 ps. The RESPA integrator was used to integrate equations of motions with a 2.0 fs time step for bonded and near interactions and a 6.0 fs time step for far interactions. A cutoff of 9Å was applied to non-bonded interactions. The smooth particle mesh Ewald method was used to treat long-range electrostatics with a tolerance of 10^{-9}. Trajectory snapshots were recorded with a 2 ps interval. All simulations were performed with the DESMOND simulation package utilising the OPLS3 force-field.

The simulation trajectories were used to calculate the radius of gyration and solvent accessible surface areas of each of the dendrimer systems reported. The final 40 Ns of the simulation trajectories were used to calculate the reported properties. The radius of gyration (Rg) is plotted as a function of the simulation time for each generation of dendrimer studied in S.I. Figure 11. In addition from the simulation trajectories the hydrodynamic radius (Rh) has been calculated using the HYDROPRO (v.10) program[2] for the PLL-boc and the PLL dendrimer at high pH. As input every trajectory was saved every 8 ps over the final 40 ns of the production run. The resulting structures were used as input to the HYDROPRO program, and from the calculated translational diffusion coefficients the hydrodynamic radius was computed using the Stokes-Einstein relationship.

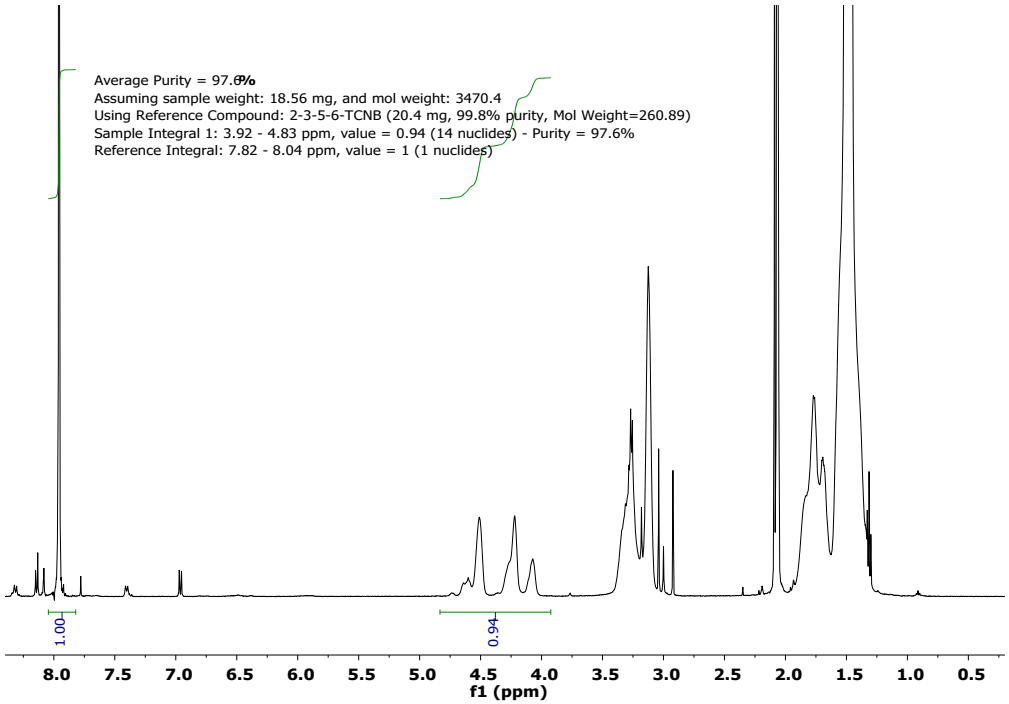
Supporting Data



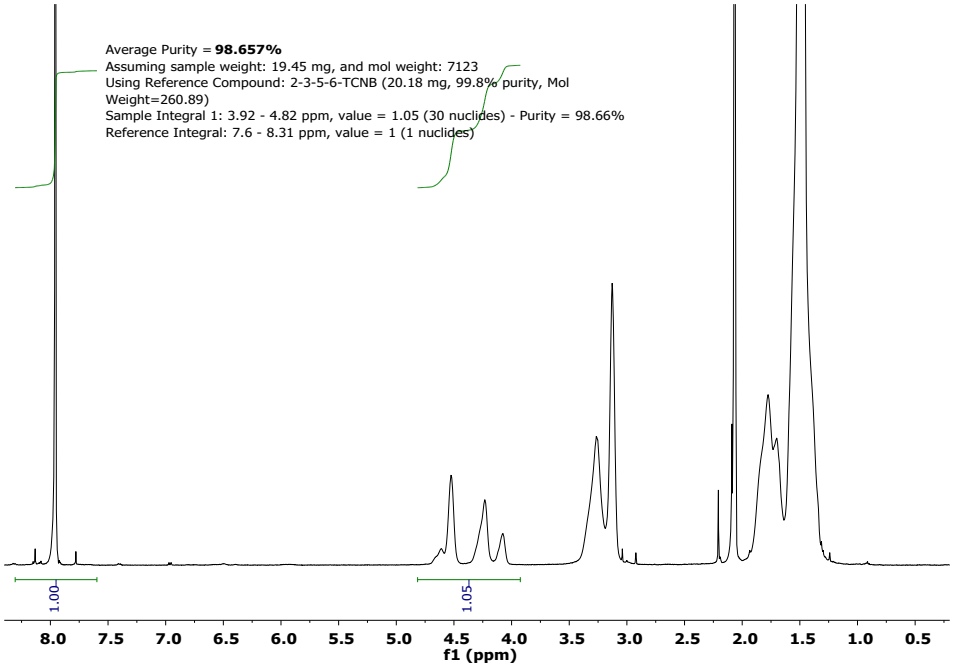
S.I. Figure 1. ^{1}HNMR (500 MHz, d4-acetic acid) for Generation 1 boc-protected lysine dendrimer. Purity determined using the MestReNova v.9.0 in built purity script.



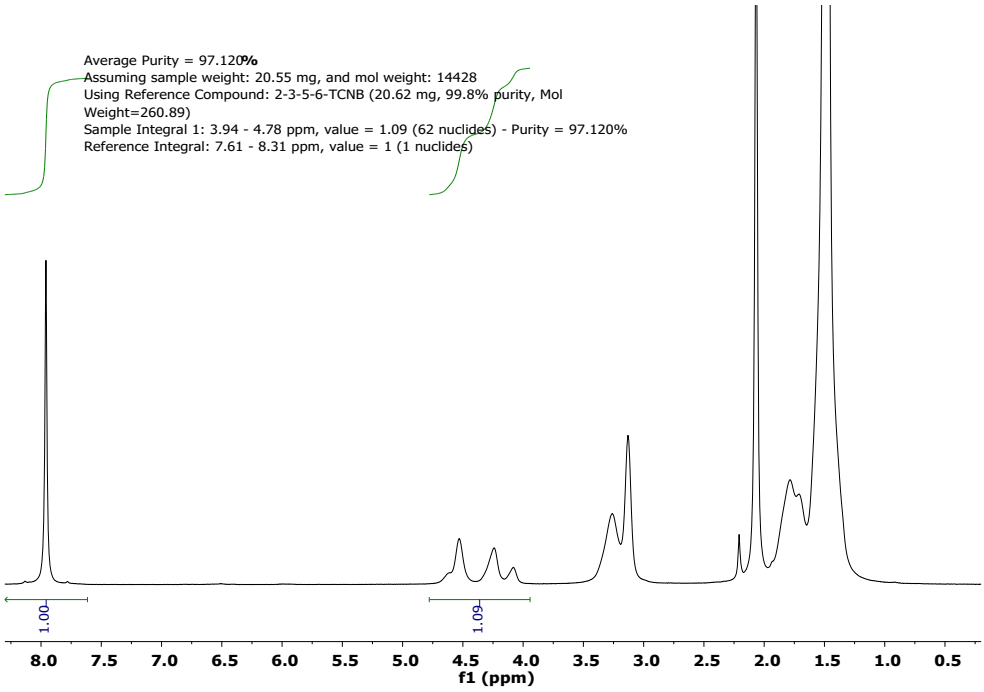
S.I. Figure 2. ^{1}HNMR (500 MHz, d4-acetic acid) for Generation 2 boc-protected lysine dendrimer. Purity determined using the MestReNova v.9.0 in built purity script.



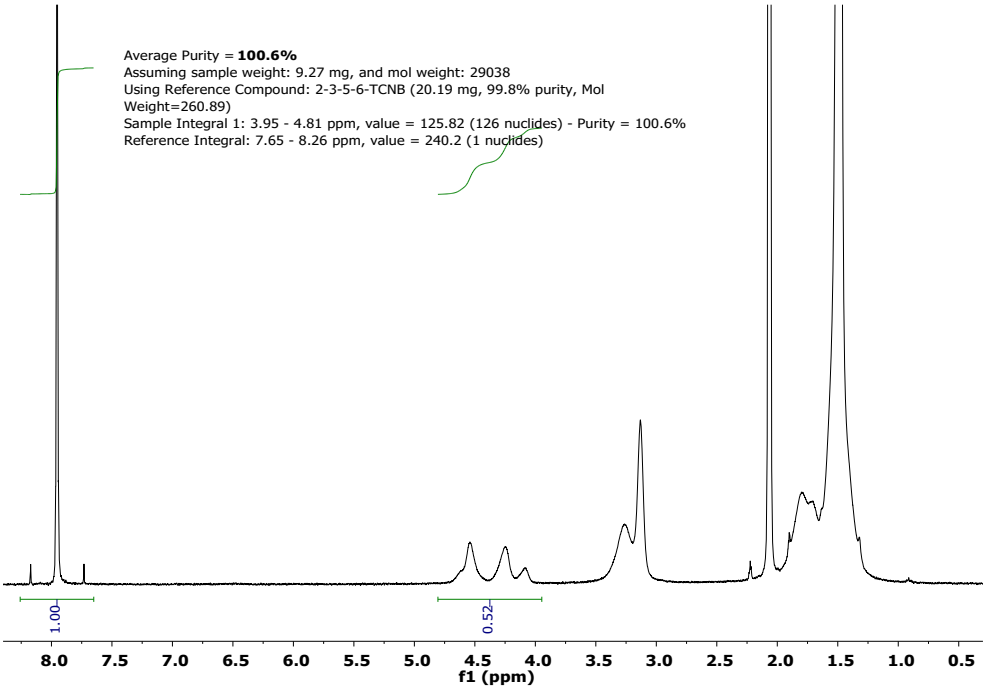
S.I. Figure 3. ^{1}HNMR (500 MHz, d4-acetic acid) for Generation 3 boc-protected lysine dendrimer. Purity determined using the MestReNova v.9.0 in built purity script.



S.I. Figure 4. ^{1}HNMR (500 MHz, d4-acetic acid) for Generation 4 boc-protected lysine dendrimer. Purity determined using the MestReNova v.9.0 in built purity script.



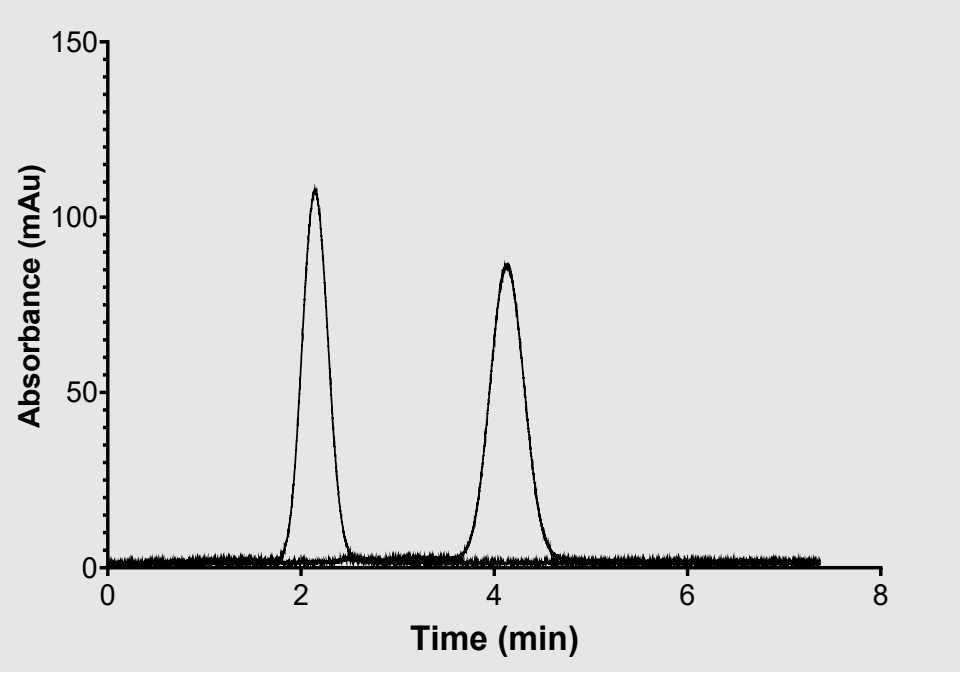
S.I. Figure 5. ^{1}HNMR (500 MHz, d4-acetic acid) for Generation 5 boc-protected lysine dendrimer. Purity determined using the MestReNova v.9.0 in built purity script.



S.I. Figure 6. ^{1}HNMR (500 MHz, d4-acetic acid) for Generation 5 boc-protected lysine dendrimer. Purity determined using the MestReNova v.9.0 in built purity script.

SI Table 1. Expected molar masses versus the masses found by MALDI-TOF-MS for both boc-protected and deprotected dendrimers, generation 1-6.

Description	Expected Molar mass (g/mol)	$[M + Na]^+$ m/z	Description	Expected Molar mass (g/mol)	$[M + Na]^+$ m/z
G1-PLL[Boc]$_4$	730.9	753.2	**G1-PLL[NH$_2$.TFA]$_4$**	330.5	-
G2-PLL[Boc]$_8$	1644.1	1665.5	**G2-PLL[NH$_2$.TFA]$_8$**	843.2	865.4
G3-PLL[Boc]$_{16}$	3470.5	3490.0	**G3-PLL[NH$_2$.TFA]$_{16}$**	1868.6	1889.8
G4-PLL[Boc]$_{32}$	7123.2	7146.5	**G4-PLL[NH$_2$.TFA]$_{32}$**	3919.4	3946.9
G5-PLL[Boc]$_{64}$	14428.6	14440.5	**G5-PLL[NH$_2$.TFA]$_{64}$**	8021.0	8043.6
G6-PLL[Boc]$_{128}$	29039.4	29064.8	**G6-PLL[NH$_2$.TFA]$_{128}$**	16224.2	16241.9



SI Figure 7. Taylor Dispersion Analysis data (using two-window detection at 214nm) for generation 6 boc-protected PLL dendrimer run in methanol.

SI Table 2. Comparison of hydrodynamic radii determined from MD-SEC and Taylor Dispersion Analysis for the boc-protected and deprotected PLL dendrimers

	boc-protected		**PLL-ammonium salts**	
Description	R_H (nm) (Taylor Dispersion Analysis)	R_H (nm) (MD-SEC)	R_H (nm) (Taylor Dispersion Analysis)	R_H (nm) (MD-SEC)
G1	0.57	0.81	0.36	0.61
G2	1.10	1.13	0.80	0.90
G3	1.47	1.47	0.95	1.24
G4	1.79	1.89	1.35	1.66
G5	2.48	2.42	1.92	2.16
G6	3.14	3.04	2.47	2.76

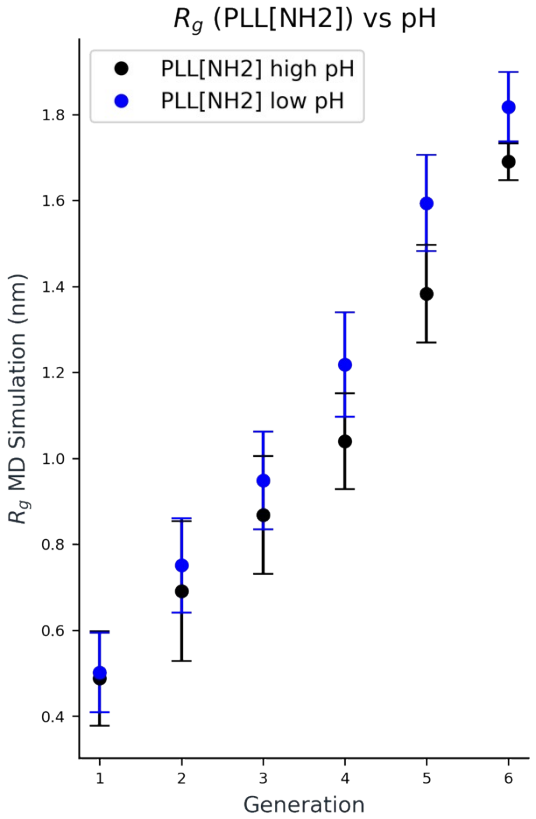
SI Table 3. Properties of PLL-boc protected dendrimer systems studied with molecular dynamics in methanol.

Generation	no. atoms	MW	pH	no. Cl^-	no. methanol	Charge	R_g(nm)	R_h (nm)
G1	117	730.9	neutral	0	531	0	0.72	0.92
G2	261	1644.1	neutral	0	781	0	0.91	1.18
G3	549	3470.5	neutral	0	1432	0	1.13	1.52
G4	1125	7123.2	neutral	0	2155	0	1.42	1.93
G5	2277	14428.6	neutral	0	3409	0	1.79	2.51
G6	4581	29039.4	neutral	0	5348	0	2.19	2.97

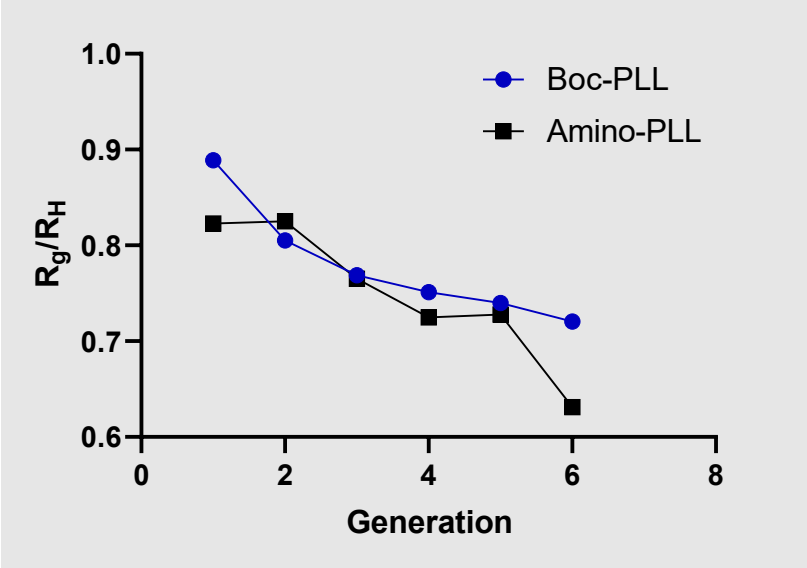
SI Table 4. Properties of PLL dendrimer systems studied with molecular dynamics in aqueous.

Generation	no .atoms	MW	pH	no. Cl^-	no. water	Charge	R_g(nm)	R_h (nm)
G1	61	334.5	low	4	861	+4	0.50	0.68
G2	149	851.2	low	8	1578	+8	0.75	0.94
G3	325	1884.7	low	16	3007	+16	0.95	1.29
G4	677	3951.6	low	32	4168	+32	1.22	1.63
G5	1381	8085.5	low	64	6474	+64	1.59	2.12
G6	2917	16353.2	low	128	12376	+128	1.82	2.58
G1	57	330.5	high	0	892	0	0.49	-
G2	141	843.2	high	0	1631	0	0.69	-
G3	309	1868.6	high	0	3110	0	0.87	-

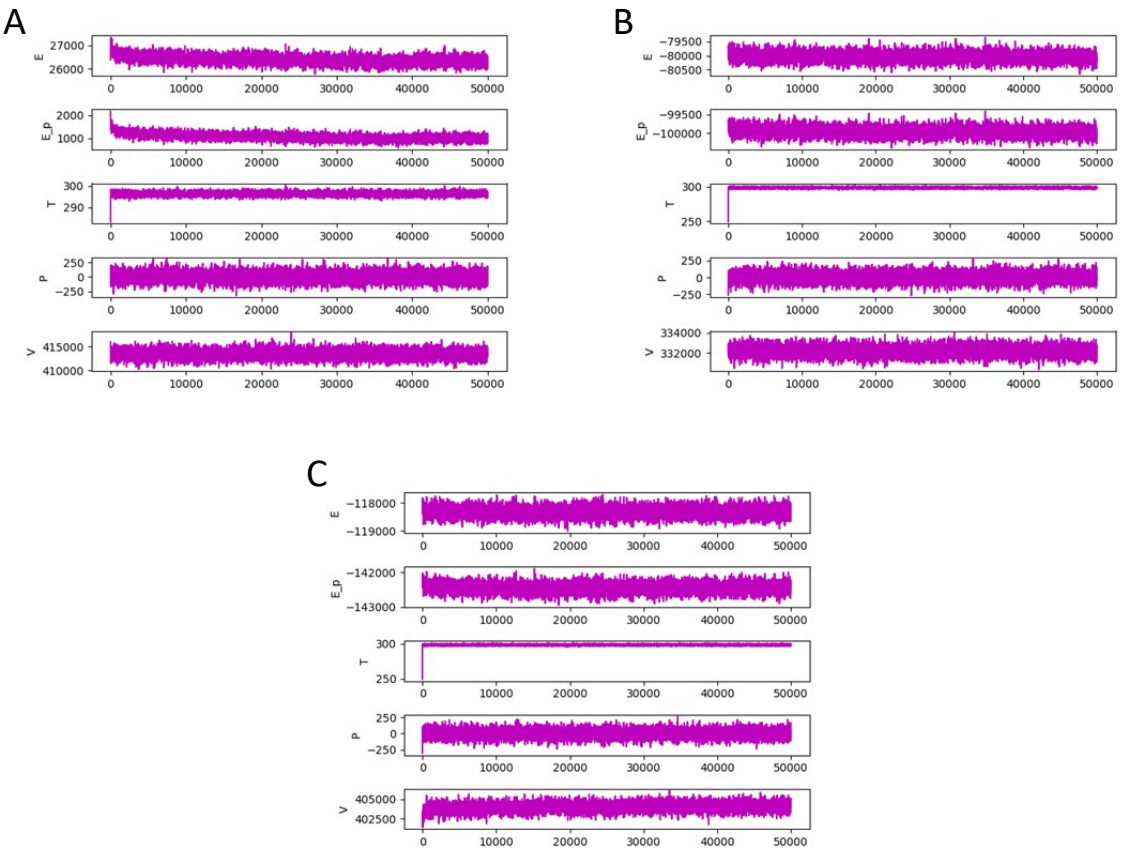
G4	645	3919.4	high	0	4322	0	1.04	-
G5	1317	8021.0	high	0	6856	0	1.38	-
G6	2917	16224.2	high	0	10122	0	1.69	-



SI Figure 8. Variation of simulated radius of gyration for amino lysine dendrimers generations under low and high pH conditions. Error bars are the 95% confidence limits for R_g calculated from the standard deviation of the simulated R_g distribution.

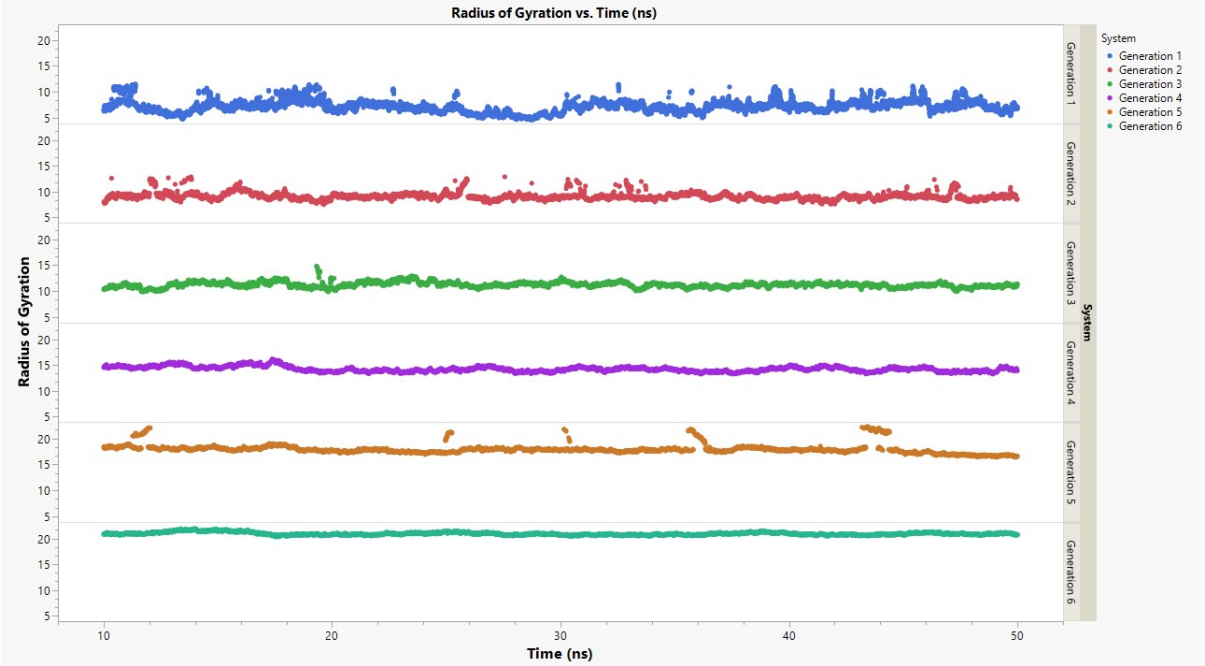


SI Figure 9. R_g/R_H plot for boc- and amino-PLL dendrimers (low pH) from experimental R_H and simulated R_g

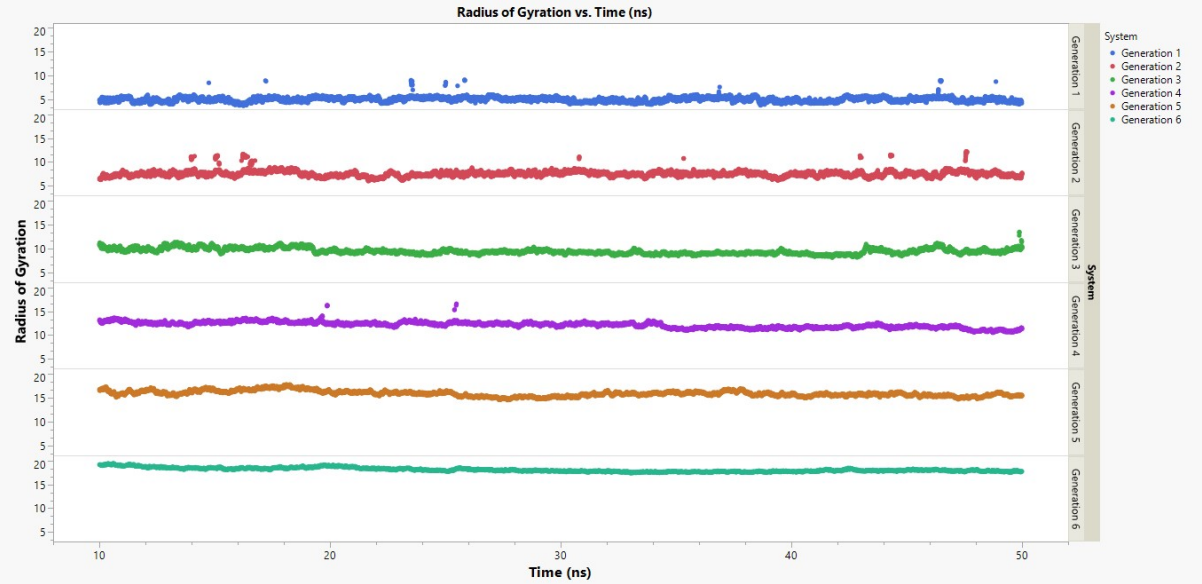


SI Figure 10. Simulation analysis for the generation 6 l-lysine dendrimers studied, boc-protected L-lysine (A), L-Lysine neutral (B) and L-lysine fully charged (C). The properties analysed over the 50 ns simulations are E the total energy (kcalmol^{-1}), E_p the potential energy (kcalmol^{-1}), T the temperature (K), P the pressure (bar) and V the volume (Å^3).

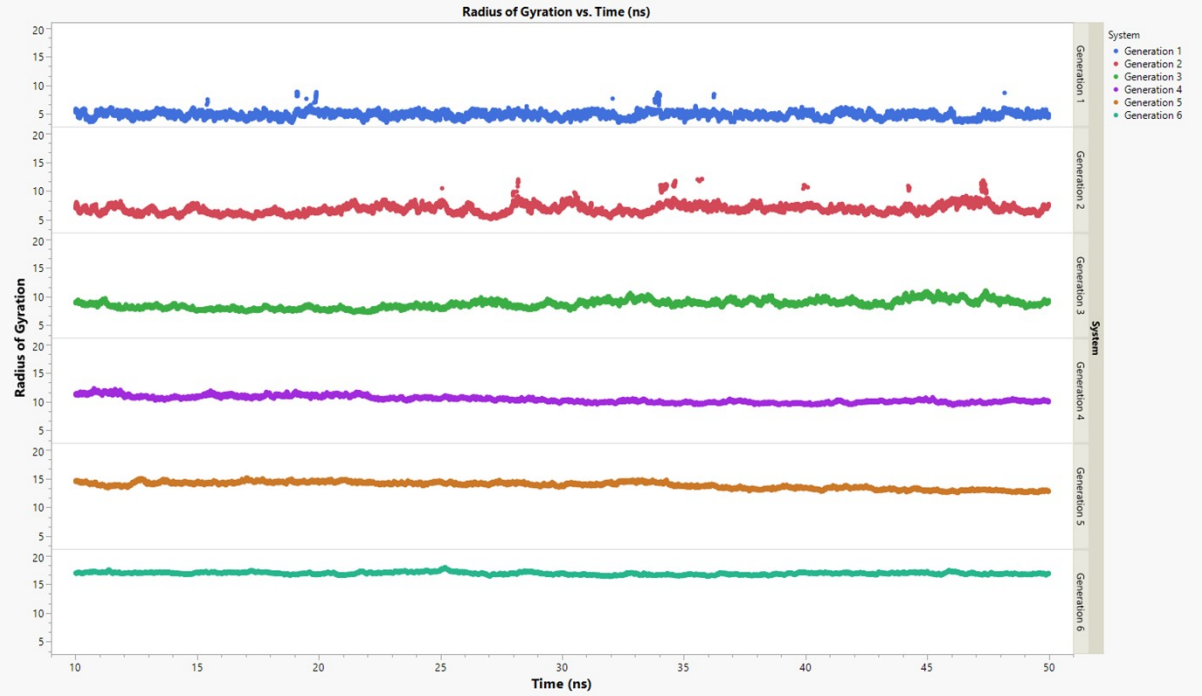
(a)



(b)



(C)



S.I. Figure 11. The radius of gyration (Rg (Å)) as a function of simulation time for (a) the neutral boc-protected L-lysine dendrimer in methanol, (b) L-Lysine dendrimer in water under low pH conditions and (c) the L-Lysine dendrimer in water under high pH conditions (c).

1. E. Harder, W. Damm, J. Maple, C. Wu, M. Reboul, J. Y. Xiang, L. Wang, D. Lupyan, M. K. Dahlgren, J. L. Knight, J. W. Kaus, D. S. Cerutti, G. Krilov, W. L. Jorgensen, R. Abel and R. A. Friesner, *Journal of Chemical Theory and Computation*, 2016, **12**, 281-296.
2. A. Ortega, D. Amorós and J. García de la Torre, *Biophysical Journal*, 2011, **101**, 892-898.