

Supporting Information

Surface diffusion of dendronized polymers correlates with their transfection potential

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Methods

Instrumental characterisation methods.

¹H NMR spectra were measured using Varian 400 MHz and Bruker 500 MHz spectrometers, using CD₃OD as the solvent for copolymers **3a-d**, azido- polymers **4a-d**, dendronized polymers **6a-d** and fluorinated dendronized polymers **7a-d**. Chemical shifts were references to the solvent peak, $\delta = 3.31$ ppm for CD₃OD. Gel permeation chromatography (GPC) was used to determine the molecular weight and polydispersity index of polymers (Waters Styragel HR 4 DMF 4.6 ×300 mm column, 5 μ m). Agilent Technologies 1100 Series GPC and Agilent GPC software were used for measurements and data analysis respectively. Measurements were taken using DMF as the eluent at a flow rate of 0.3 mL/min at 50 °C, and calibrated against poly(methyl methacrylate) standard. Elemental analysis was conducted at the Campbell Microanalytical Laboratory, University of Otago.

ATRP of copolymer.

In a typical reaction, inhibitors for HEMA **1** and GMA **2** were removed *via* a basic alumina column, and dissolved separated in methanol (MeOH) at a ratio of 1:3 (monomer : MeOH). Monomer solutions were degassed *via* ‘freeze-pump-thaw’ methods and backfilled with nitrogen gas. CuBr (100 mg, 0.70 mmol) was added to the reaction flask followed by 2,2'-bipyridine (bpy, 392 mg, 2.5 mmol) before addition of monomer solutions at various ratios (Table S1). 2-(4-Morpholino)ethyl 2-bromoisobutyrate initiator (ME-Br, 210 μ L, 1 mmol) was added and the reaction proceeded at 80 °C under standard Schlenk conditions. After 2 h, reaction was opened to air and MeOH (15 mL) was added. The product was collected under reduced pressure and redissolved in minimal MeOH, and purified by repeated precipitation in excess diethyl ether. Solid product was dried under vacuum. Final copolymers **3** were collected and composition was determined by ¹H NMR as previously described.¹ Composition, molecular weight and PDI of polymers are reported in Table S1.

Table S1. Monomer feed ratios for ATRP reactions, and molecular weight and PDI measurements of resulting copolymers as measured by GPC.

Polymer	Feed ratios		GMA mol% (¹ H integration)	Mw (kDa)	PDI
	GMA/MeOH	HEMA/MeOH			
3a	0.8 mL (1.5 mmol)	15.2 mL (31.2 mmol)	3.7	13.6	1.25
3b	1.9 mL (3.6 mmol)	14.1 mL (29.0 mmol)	8.9	15.7	1.21
3c	4 mL (7.5 mmol)	12 mL (24.7 mmol)	16.3	21.5	1.30
3d	6.4 mL (12 mmol)	9.6 mL (19.7 mmol)	27.9	17.5	1.30

Incorporation of PAMAM dendrons.

Copolymers **3** were reacted to afford azido-functionalised copolymers **4** by reaction with excess sodium azide (NaN₃) in the presence of excess ammonium chloride (NH₄Cl), as

previously described. Amounts were scaled accordingly for each polymer species. Pure product **4** was obtained by repeated precipitation in ether and dried under vacuum.

4.5 generation (4.5G) propargyl PAMAM dendrons were synthesized using methods described by Lee *et al.*² and Lin *et al.*³

Azido-functionalised polymers **4** and 4.5G dendrons were then reacted *via* azide-alkyne copper catalyzed click reaction, and the dendron generation finalized to 5G by reaction with ethylene diamine to give dendronized polymers **6**. Products were purified by dialysis against MilliQ water (4 × 4 L, 10,000 MWCO).

Partial fluorination of dendronized polymer surface, and PAMAM dendrimer surface, was achieved by methods adapted from Wang *et al.*⁴

Elemental analysis of final fluorinated samples can be found in Table S2 below.

Table S2. Experimental elemental analysis of fifth generation dendron-functionalised copolymers **6a-d** and **7a-d**.

Polymer	% C	% H	% N	% F
6a	50.86; 50.63	8.22; 8.25	15.09; 15.02	-
	40.60; 40.54	7.90; 7.95	15.54; 15.44	-
6b	45.14; 45.84 (50.36)*	7.99; 8.21	17.18; 17.48 (19.14)*	-
	46.31; 46.51	8.02; 8.16	17.29; 17.22	-
6c	46.71; 46.43 (50.24)*	7.27; 7.62	17.13; 17.06 (20.77)*	-
	6d	50.23; 49.94	9.00; 8.82	22.05; 21.91
PAMAM	48.57; 48.56	8.88; 9.06	22.16; 22.33	-
7a	40.91; 40.80	5.86; 5.84	10.04; 9.93	20.31; 20.05
7b	38.41; 38.45	5.50; 5.02	10.93; 10.80	23.95; 23.81
7c	39.74; 39.79	5.43; 5.28	12.60; 12.60	23.10; 23.14
7d	39.03; 38.94	5.40; 5.29	11.55; 11.41	27.49; 27.67
PAMAM-F	35.34; 35.26	4.08; 4.02	10.39; 10.43	31.17; 31.45

*Corrected for counter ion and water content – sample was hygroscopic; measurements were conducted twice in duplicate with repeated drying, before being normalized to average carbon percentage previously reported.

References

- (1) Kretzmann, J. A.; Ho, D.; Evans, C. W.; Plani-Lam, J. H. C.; Garcia-Bloj, B.; Mohamed, A. E.; O'Mara, M. L.; Ford, E.; Tan, D. E. K.; Lister, R.; et al. Synthetically Controlling Dendrimer Flexibility Improves Delivery of Large Plasmid DNA. *Chem. Sci.* **2017**, *8* (4), 2923–2930. <https://doi.org/10.1039/C7SC00097A>.
- (2) Lee, J. W.; Kim, B.-K.; Kim, H. J.; Han, S. C.; Shin, W. S.; Jin, S.-H. Convergent Synthesis of Symmetrical and Unsymmetrical PAMAM Dendrimers. *Macromolecules* **2006**, *39* (6), 2418–2422. <https://doi.org/10.1021/ma052526f>.
- (3) Lin, Y.-J.; Tsai, B.-K.; Tu, C.-J.; Jeng, J.; Chu, C.-C. Synthesis of β -Cyclodextrin and Poly(amido Amine) Dendron Click Cluster and Its Synergistic Binding Property. *Tetrahedron* **2013**, *69* (7), 1801–1807. <https://doi.org/10.1016/j.tet.2012.12.073>.

- (4) Wang, M.; Liu, H.; Li, L.; Cheng, Y. A Fluorinated Dendrimer Achieves Excellent Gene Transfection Efficacy at Extremely Low Nitrogen to Phosphorus Ratios. *Nat. Commun.* **2014**, *5*, 3053. <https://doi.org/10.1038/ncomms4053>.

Supplemental Data

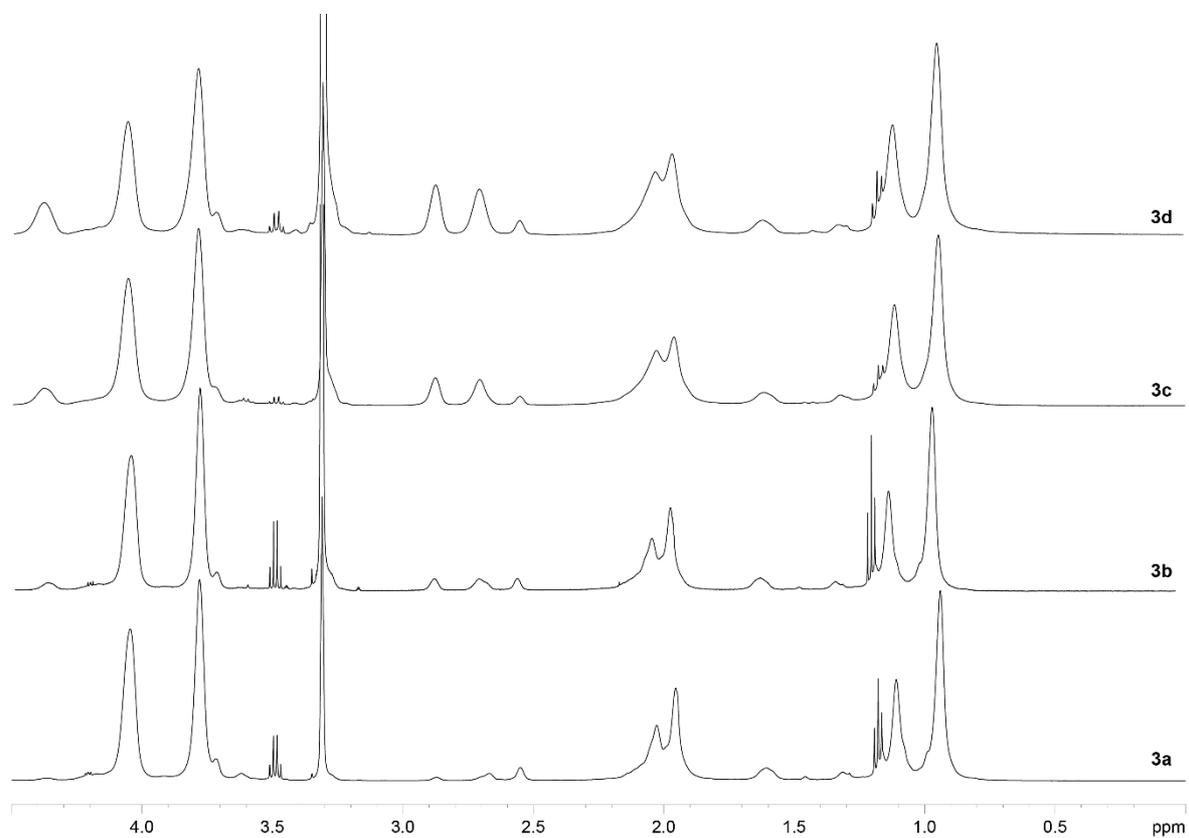


Figure S1. ¹H NMR spectra of copolymer backbones. Spectra were taken in MeOD at 500 MHz. Peaks corresponding to the epoxide moiety on glycidyl methacrylate (δ 2.7, 2.9 ppm) were used to calculate GMA incorporation for copolymer composition, against the backbone methylene bridge (δ 1.9 – 2.1, b).

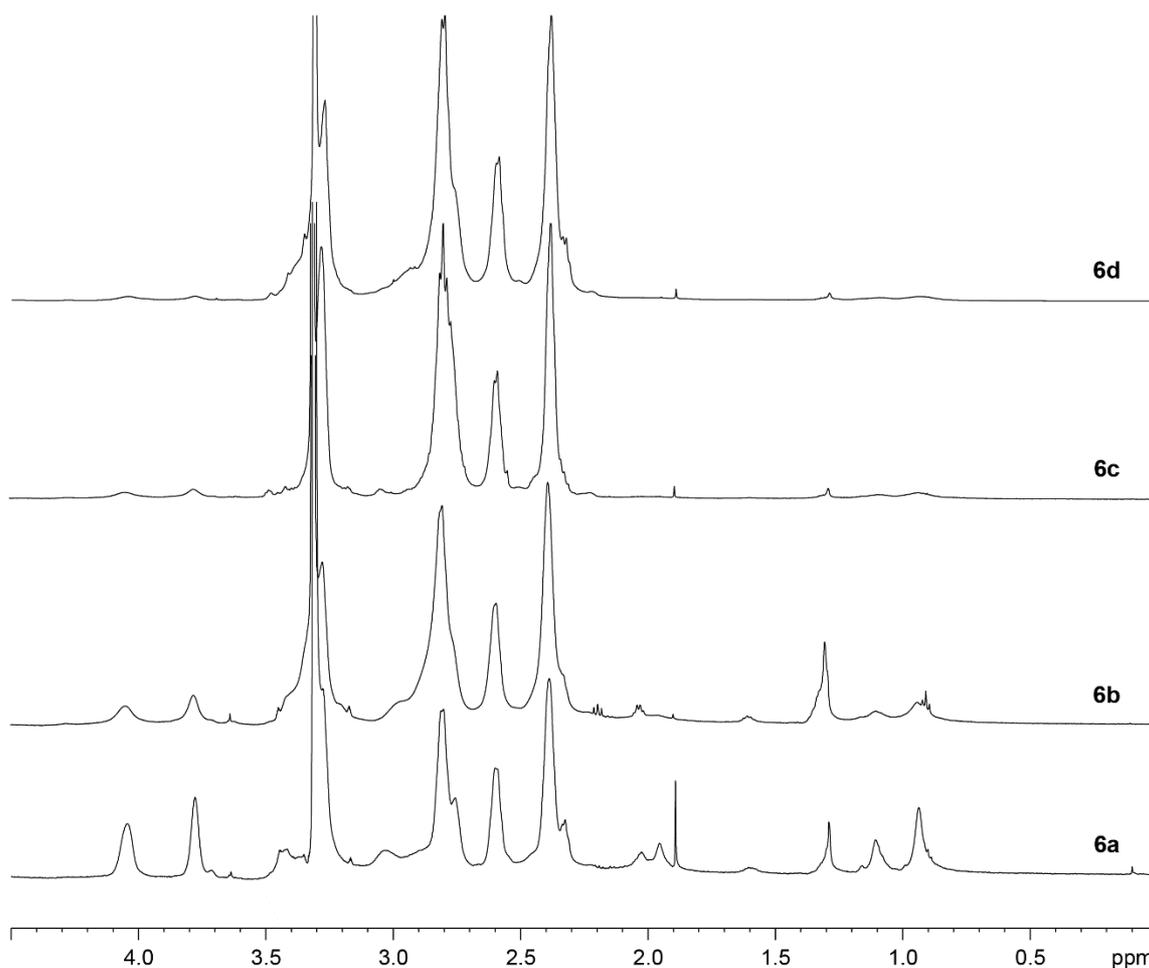


Figure S2. ^1H NMR spectra of polymers with 5th generation PAMAM dendrons attached. ^1H NMR spectra were taken in MeOD at 500 MHz. Peaks corresponding to polymeric backbone are suppressed as dendron substitution increases.

Table S3. Mean, standard deviation and percentage population for “Population 2”, fitted with a normal distribution. Mean and standard deviation are given in nm.

Polymer	Population 2		
	Mean	Std Dev	%
7a	1.20	0.36	35.7
7b	3.78	0.83	39.0
7c	2.51	0.49	47.0
7d	1.32	0.41	42.8
PAMAM	-	-	-

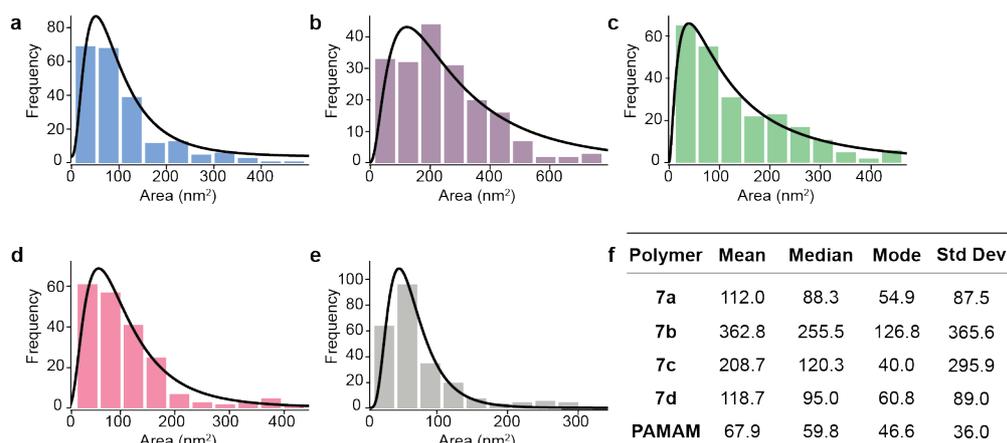


Figure S3. Area distributions of polymer samples. Area was measured by thresholding intensity using ImageJ. Area distributions for polymers **7a–d** and PAMAM are given in (a–e) respectively. Distributions were fitted with a log-normal fit, statistical summary of each of the distributions is given in (f).

Table S4. Full statistical breakdown for multiple comparison analysis from Fig 3f. Statistical analysis was performed using one-way ANOVA to perform multiple comparisons (** $p \leq 0.01$, **** $p \leq 0.0001$).

	7a	7b	7c	7d	PAMAM
7a	-	**	****	****	****
7b	-	-	**	****	****
7c	-	-	-	****	****
7d	-	-	-	-	ns
PAMAM	-	-	-	-	-

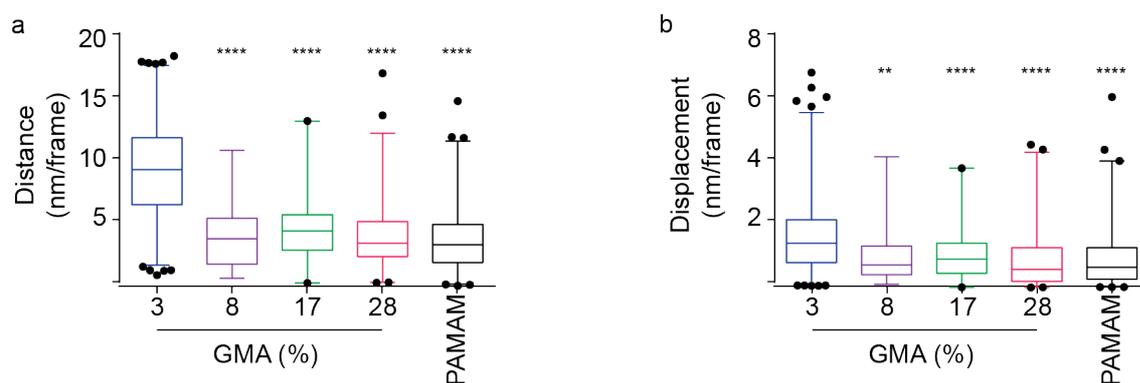


Figure S4. Distance and displacement of polymers. Total distance travelled (a) and final displacement (b) given for each of the polymer samples **7a–d** and PAMAM. Box plots show centre line as median, box limits as upper and lower quartiles, and whiskers representing 1st – 99th percentile range. Statistical analysis was performed using one-way ANOVA to perform multiple comparisons (** $p \leq 0.01$, **** $p \leq 0.0001$).

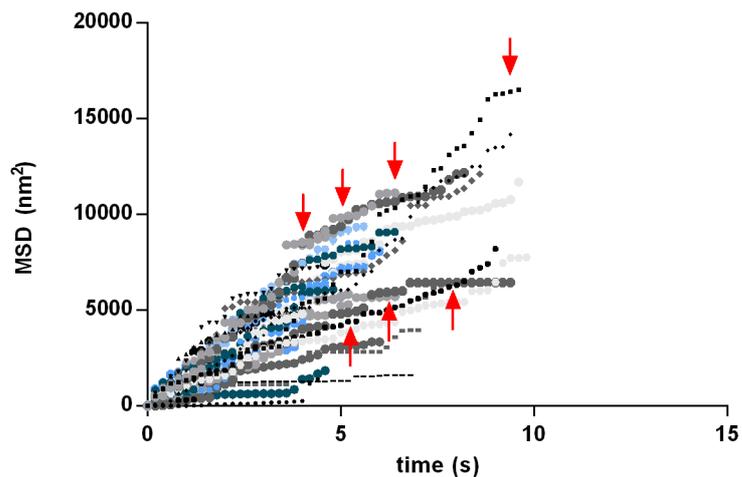


Figure S5. Mean square diffusion (MSD) vs time for polymer 7a. Representative MSD vs. time plot, with described plateaus highlighted with red arrows. As these plateaus may be due to times of significantly stronger polymer-surface binding, diffusion coefficients were instead represented and calculated from a distribution to further understand these events.

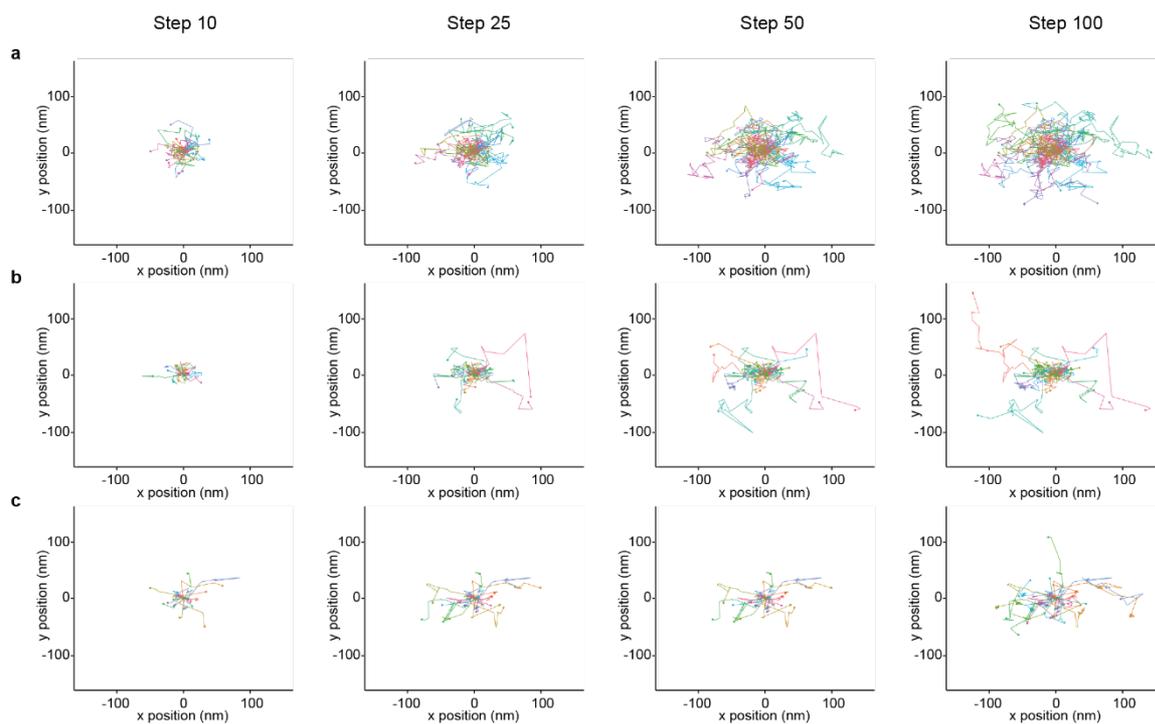


Figure S6. Representative mapping of polymer movement. Movement was mapped over time for polymers **7a**, **7c** and **7d**, as demonstrated in (a–c) respectively. Random movement is observed, without bias in either x or y direction, confirming that there is insignificant tip-sample interaction. Polymers also demonstrate undergoing random jumps.

Table S5. Mean, median and mode diffusion coefficients (given in $\text{nm}^2 \text{s}^{-1}$) for distributions given in Fig 4, fitted with a two component log-normal distribution. The relative percentage of each sample represented is also given.

Polymer	Population 1				Population 2			
	Mean	Median	Mode	%	Mean	Median	Mode	%
7a	589	453	268	55.7	224	59.0	4.10	44.3
7b	127	38.0	3.42	84.3	1239	1064	785	15.7
7c	168	45.2	3.25	100				0
7d	35.0	10.2	0.871	78.3	1224	1007	681	21.7
PAMAM	109	14.9	0.282	100				0

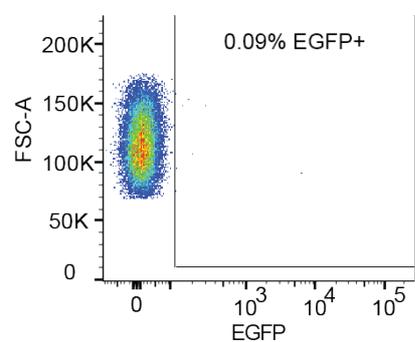


Figure S7. Flow cytometry micrograph for negative control. Untransfected MCF-7 cells were used for gating EGFP fluorescence.