Electronic Supplementary Material (ESI) for Soft Matter.

New Microscopic Insight Into Generation Dependent Membrane Penetration and Reorganization by Dendrimers

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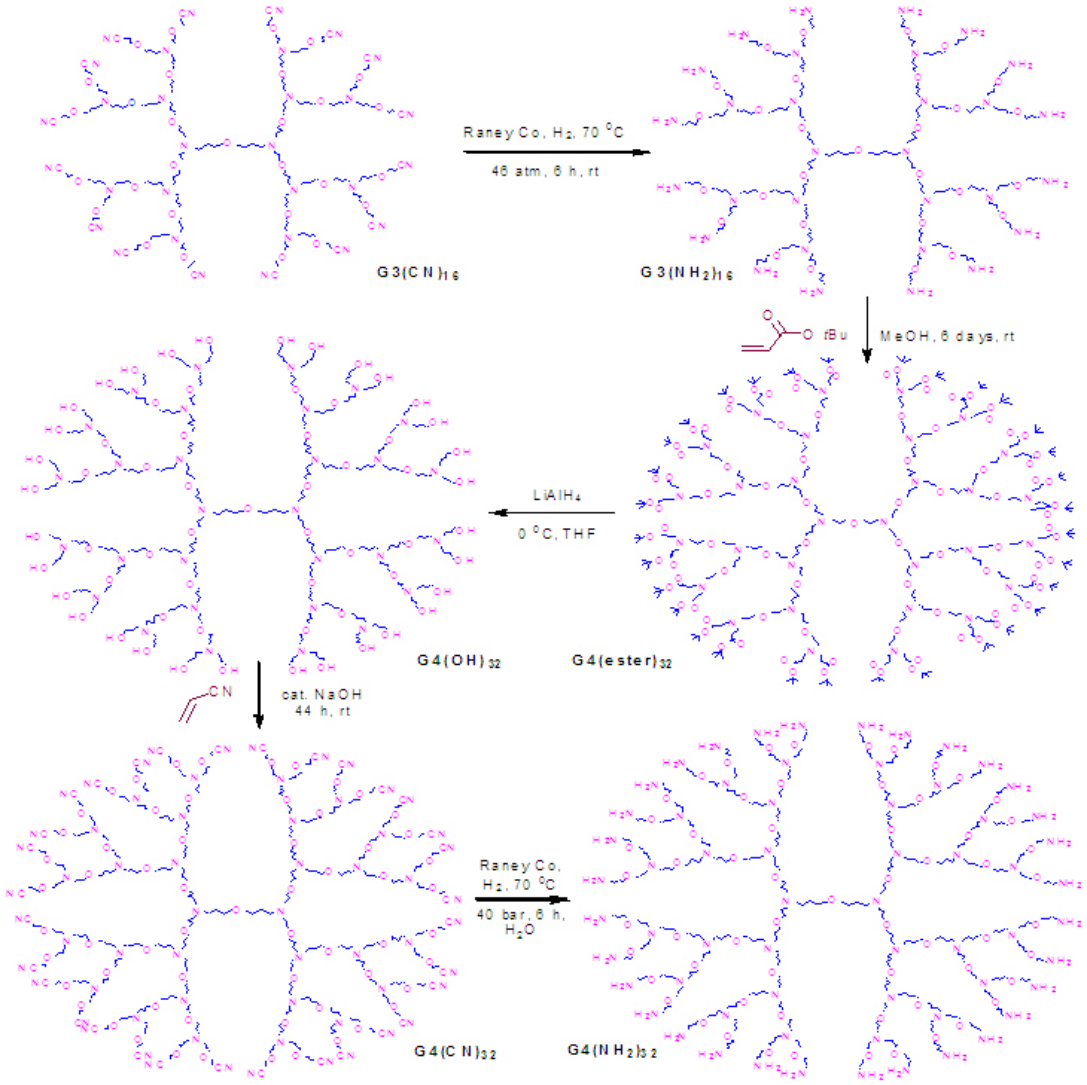

FIG. 1: Schematic of the synthesis procedure of G3 and G4 Amine terminated oxygen Core PETIM dendrimers.

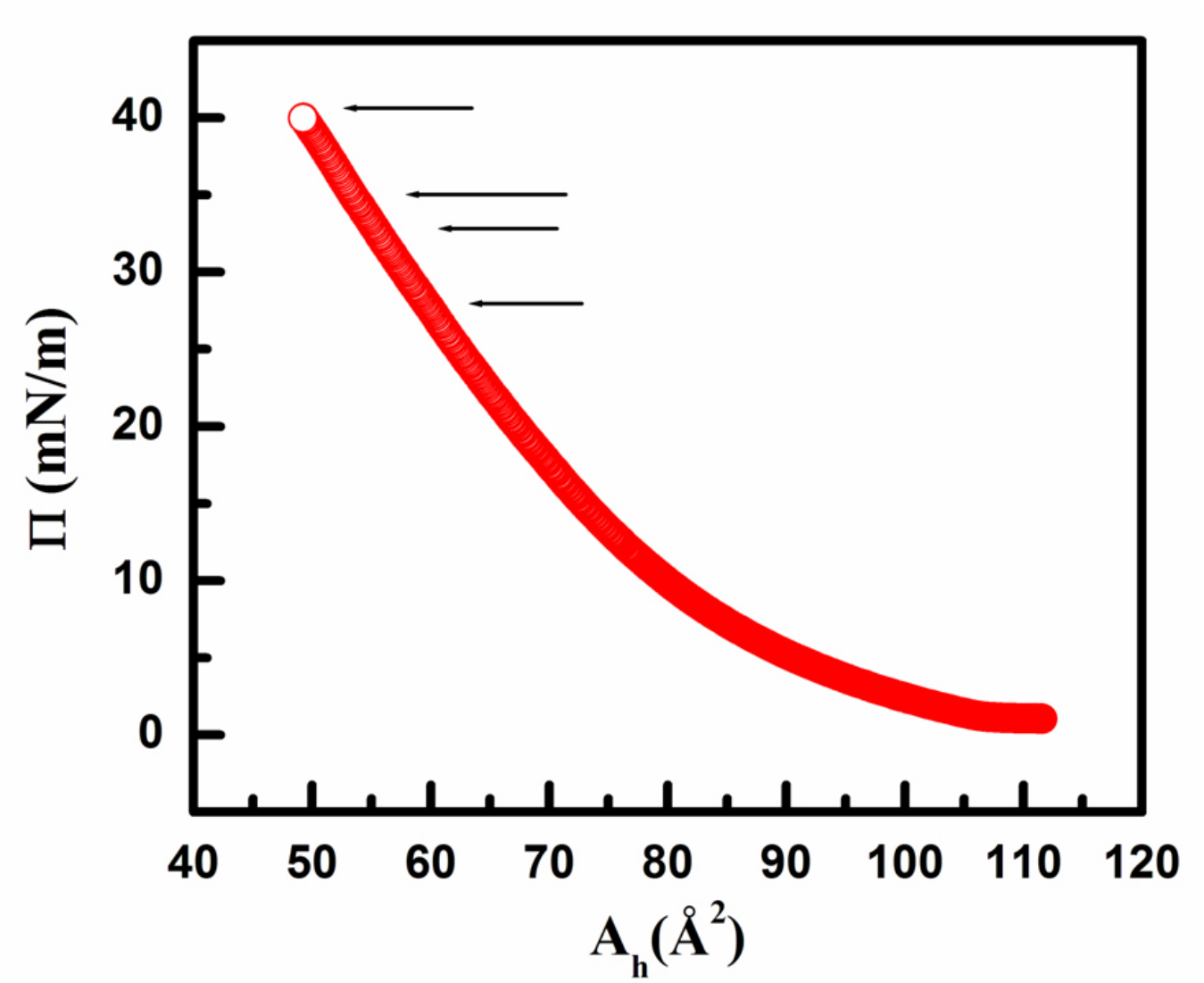

FIG. 2: Typical Π Vs A_h isotherm of DMPC monolayer at the air/water interface collected at temperature 27^0C. The horizontal arrows indicate the positions on the isotherm where DMPC SLBs were transferred.

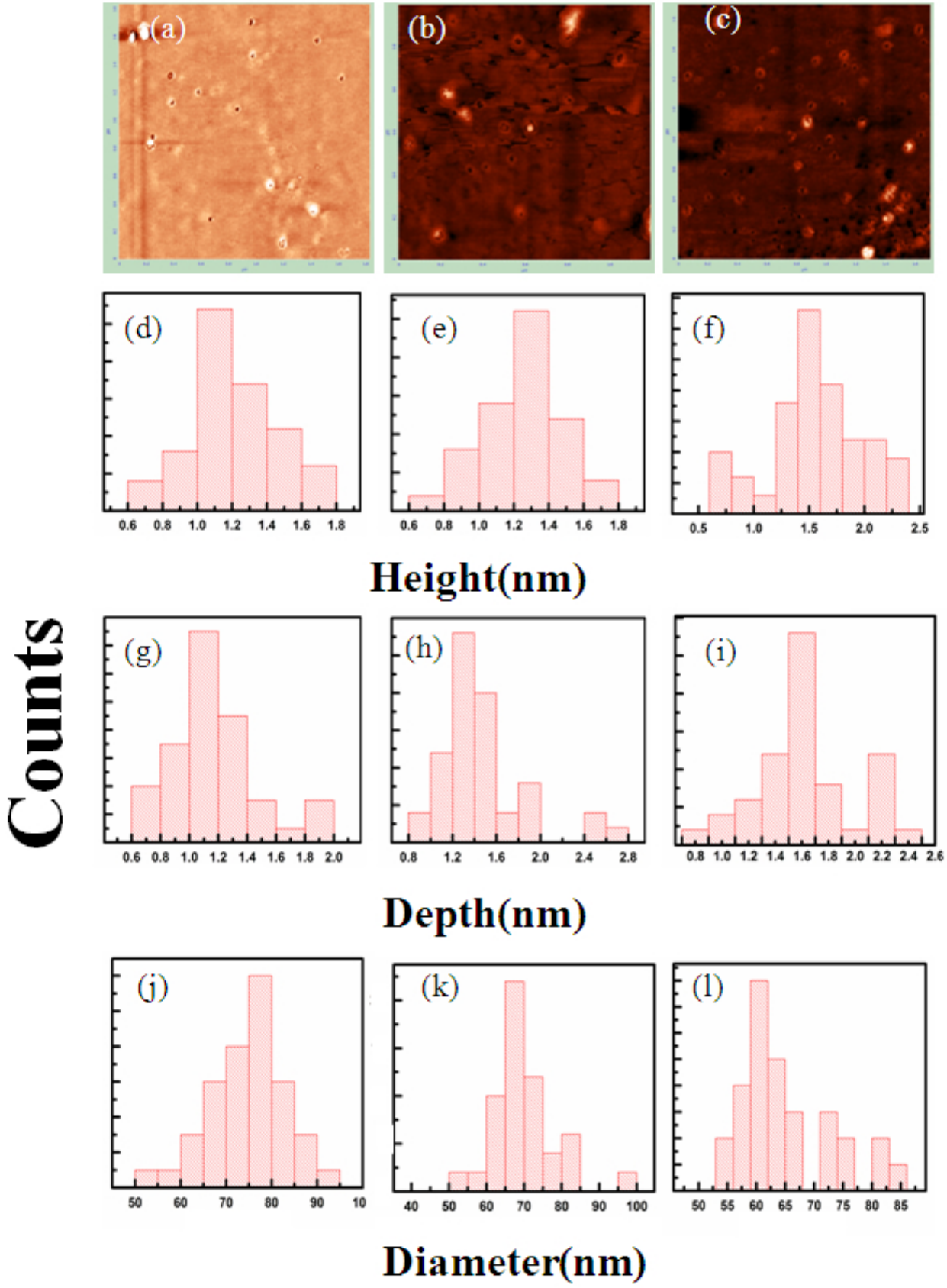

FIG. 3: AFM images of DMPC SLB deposited at A_h=56Å^2 and incubated with (a) 0.7 μM. (b)0.43 μM. (c) 0.14 μM. Height distributions of the pores formed by dendrimer incubated DMPC SLBs are shown for concentration (d) 0.7 μM. (e)0.43 μM. (f) 0.14 μM. Depth distributions of pores formed by dendrimers incubated DMPC SLB are shown for concentration (g) 0.7 μM. (h) 0.43 μM. (i) 0.14 μM. Diameter distributions of pores formed by dendrimers incubated DMPC SLB are shown for concentration (j) 0.7 μM. (k) 0.43 μM. (l) 0.14 μM .

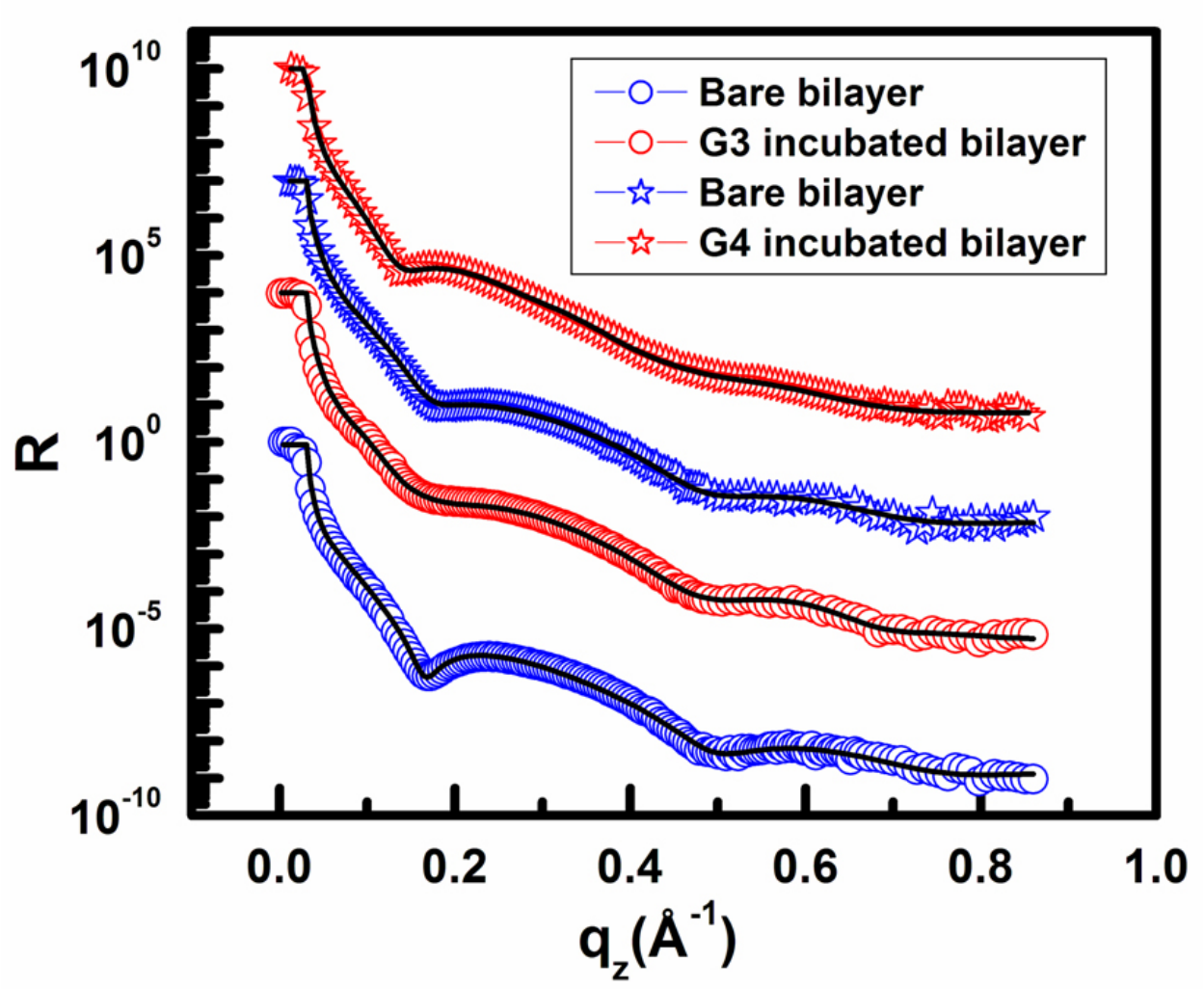

FIG. 4: X-ray reflectivity, R Vs q_z for DMPC SLBs before and after incubation with G3 & G4 PETIM dendrimers corresponding to the Fresnel normalized data in Fig. 4(b) in main manuscript. bare SLB (◯), SLB incubated with G3 dendrimers (◯), bare SLB (☆), SLB incubated with G4 dendrimers (☆). Solid line represents the corresponding best fit with the proposed model shown in Fig. 4 (c)-(d) in the main manuscript.

SYNTHESIS OF OXYGEN-CORE AMINE TERMINATED G3 AND G4 PETIM DENDRIMERS.

The synthesis was performed as reported previously[1] by following the synthetic sequence shown in fig. 1.

General methods:

All chemicals were purchased from commercial sources and were used without further purification. Solvents were dried and distilled according to literature procedure. Analytical TLC was performed on commercial Merck plates coated with alumina GF254 (0.25 mm). Alumina (neutral) was used for column chromatography. ^{1}H and ^{13}C NMR spectra were recorded on a 400 and 100 MHz spectrometer, respectively, with residual solvent signal as the internal standard. The following abbreviations are used to explain the multiplicities: s, singlet; d, doublet; t, triplet; m, multiplet.

G3(NH$_2$)$_{16}$: The third generation amine terminated PETIM dendrimer was synthesized following previously reported procedures[1]. Briefly, G3(CN)$_{16}$ (0.70 g, 21.26 mmol) was transferred to a hydrogenation reactor vessel in H_2O and Raney Co as a catalyst was added. The reaction mixture was hydrogenated (H_2, 46 atm) at 70^{0}C for 6 h. The reaction mixture was cooled, filtered and the filtrate was concentrated in vacuo to afford the corresponding amine-functionalized dendrimer. FT-IR (neat) ν (cm^{-1}) 3419.9, 2943.4, 2858,8, 2805.0, 1373.9, 1152.0, 1112.1; ^{1}H NMR (400 MHz, D_2O)δ 1.4 (bs, 12 H), 2.2 (t, J = 8 Hz, 8 H), 2.41 (bs, 4 H), 3.18-3.21 (m, 12 H); ^{13}C NMR (100 MHz, D_2O)δ 23.3, 25.4, 29.6, 29.9, 37.7, 48.9, 49.9, 68.2, 68.8, 68.9 .

G4(ester)$_{32}$: A solution of G3(NH$_2$)$_{16}$ (0.70 g, 20.8 mmol) in MeOH was added drop-wise, to a solution of tert-butyl acrylate (1.1 mL, 7.29 mmol) in MeOH with continuous stirring. After completion of the addition, the reaction was stirred at room temperature for 72 h, with further additions of 0.5 mL of tert-butyl acrylate after 24 and 48 h. The excess tert-butyl acrylate and MeOH were removed under in vacuo and the product extracted into hexane. The hexane portion was concentrated in vacuo to afford G4(ester)$_{32}$ in 97% yield, as a colorless viscous liquid. FT-IR (neat)ν (cm^{-1}) 2975.9, 2932.8, 2855.0, 2814.3, 1731.4, 1367.7, 1255.8, 1157.9, 1117.8. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 288 H), 1.64-1.68 (m, 116 H), 2.32 (t, J = 8 Hz, 64 H), 2.43 (t, J = 8 Hz, 116 H), 2.69 (t, J = 7.2 Hz, 64 H), 3.37 (bs, 116 H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.1, 30.9, 33.7, 49.4, 68.9, 80.2, 172.0.

G4(OH)$_{32}$: A solution of the G4(ester)$_{32}$ dendrimer (1.2 g, 160 mmol) in THF was added drop-wise to a suspension of $LiAlH_4$ (0.22 g, 5.63 mmol) in THF over a period of 15 min. at 0^{0}C, and the reaction mixture was stirred for 4 h at room temperature, then cooled to 0^{0}C, quenched with ice-cold water, filtered and the filtrate concentrated in vacuo. The inorganic material was precipitated using MeOH, filtered and the filtrate concentrated. The resultant residue was subsequently dissolved in $CHCl_3$, filtered and the filtrate concentrated in vacuo. Yield: 92 %. FT-IR (neat) ν(cm^{-1}) 3369.1, 2942.2, 2867.5, 1650.6,1220.6, 1111.6, 1055.4. ^{1}H NMR (400 MHz, $CDCl_3$) 1.69-1.77 (m, 180 H), 2.46-2.52 (m, 116 H), 2.60 (t, J = 8 Hz, 64 H), 3.41 (t, J = 8 Hz, 116 H), 3.70 (app. s, 64 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.9, 27.2, 28.7, 50.8, 50.9, 52.8, 62.4, 62.8, 68.8, 69.1, 69.2 .

G4(CN)$_{32}$: Acrylonitrile (140 μL, 2 mmol) and aq. NaOH (40%) (5 μL, 570 mmol) were added to the alcohol-functionalized dendrimer (0.30 g, 570 mmol) at room temperature and the mixture was allowed to stir for 20 h. Excess acrylonitrile (70 μL) and aq. NaOH (40%) (2.5 μL) was added further and the reaction mixture left to stir for another 24 h. The reaction mixture was diluted with $CHCl_3$ and water and the product was extracted. The organic layer was then dried using Na_2SO_4 and concentrated in vacuo. The crude reaction mixture was purified by column chromatography (neutral Al_2O_3), to afford G4(CN)$_{32}$ as a pale yellow gum. Yield: 87%. FT-IR (neat) ν(cm^{-1}) 2938.1, 2809.1, 2868.9, 2250.8, 1653.9, 1648.2, 1465.3, 1458.1, 1368.9, 1152.2, 1111.7; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.70-1.73 (m, 180 H), 2.48-2.51 (m, 180 H), 2.62 (t, J = 6.24 Hz, 64 H), 3.41 (s, 116 H), 3.52 (t, J = 6.16 Hz, 64 H), 3.68 (t, J = 5.64 Hz, 64 H); ^{13}C NMR (100 MHz, $CDCl_3$) d 18.8, 27.3, 27.4, 50.4, 50.7, 65.2, 69.0, 69.1, 69.3, 118.0 .

G4(NH$_2$)$_{32}$: G4(CN)$_{32}$ (30 mg, 0.0043 mmol) was transferred to a hydrogenation reactor vessel in H_2O and Raney Co as a catalyst was added. The reaction mixture was hydrogenated (H_2, 50 atm) at 70^{0}C for 6 h. The reaction mixture was cooled, filtered and the filtrate was concentrated in vacuo to afford the corresponding amine-functionalized dendrimer in a quantitative yield. FT-IR (neat) ν(cm^{-1}) 3341.0, 2929.5, 2859.4, 1590.1, 1478.8, 1350.2, 1113.1, 1017.2; ^{1}H NMR (400 MHz, D_2O) 1.54-1.57 (m, 180 H); 2.38 (t, J = 7.6 Hz, 160 H,), 2.53, (t, J =

7.08 Hz, 180 H,) ^{13}C NMR (100 MHz, D_2O) δ 25.3, 30.1, 37.6, 48.7, 49.9, 68.2, 68.7, 68.9 .

SUPPORTED LIPID BILAYER PREPARATION

The supported lipid bilayers were deposited on silicon (100) by Langmuir-Blodgett method [2–10]. All the films were transferred at higher pressure $\approx$ 40 mNm^{-1} . A typical isotherm shown in Fig. 2. After deposition the samples are kept in a box under DI water to keep the hydration layer which prevents further degradation in samples. The dendrimer in DI water solution was used to incubate the samples. Incubation was done in a home made teflon cell in-situ during the measurement at various dendrimer densities. The sample was kept horizontally inside the dendrimer solution for a fixed time($\sim$ 30 minute) at 37°C. After incubation the sample was washed multiple times with DI water to remove unbound dendrimers.

AFM IMAGING

Here detailed analysis of AFM images described in main manuscript (Fig. 1.& 2.) as well as additional AFM data is presented. Figure. 3 shows the concentration dependent self assembled pattern formation and membrane penetration by G4 PETIM dendrimers on SLBs deposited at A_h=56Å^2. The corresponding diameter, height above and depth below, the mean SLB surface, respectively are also shown. This is indicative of the extent of penetration of the G4 PETIM dendrimers with their incubation concentration.

X RAY REFLECTIVITY MEASUREMENTS

X ray reflectivity data on the bare and dendrimer incubated SLBs are shown in Fig. 4. The reflectivity data was fitted using standard reflectivity software (IGOR with motofit package) .The model used in the fitting is basically a multiple layer model with specific head and tail part of the lipid molecules alongwith the hydration layer at both the film-substrate and film-air interface. The fitting parameters (both thickness as well as electron density(ρ) values) are tabulated in the table I in main manuscript. From the table I of main text it is clear that after incubation the ρ value for top leaflet(both head and tail) decreases after G4 incubation whereas for G3 it remains almost same. The reduction in ρ values can be attributed to the penetration of dendrimers inside the lipid layer.

GID DATA ANALYSIS

The individual peaks in the GID data can be attributed to diffraction from different crystal planes. From the position of each peak we can estimate different parameters from the interplanar spacing value. Ideally for a perfect in plane hexagonal lattice symmetry one can expect a single peak in Q_{xy}- Q_z plane. In practice, two peaks instead of one are representative of a distorted hexagonal lattice. Thus for a distorted hexagon, γ is the second parameter (along with a or b) which has to be estimated and it should be always $\prec$120°. In our measurement we have found two peak whose miller indices are [(1,0)+(0,1)] and (1,-1). From the interplanar spacing value we have estimated the value of a (=b) and γ using the Eqn. 1 of the main manuscript and is tabulated in table I. For a distorted 2D hexagon we have estimated the area (A) as

$$A = \frac{3\sqrt{3}a^2}{2} \tag{1}$$

The no of occupancy(N) of atoms in a 2D inplane hexagonal lattice is 3/2. Thus the area/head group(A_h) can be defined as.

$$A_h = \frac{A}{N} \tag{2}$$

The values of A_h for both bare bilayer and incubated bilayer with G3 and G4 dendrimer is shown in the table I.

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TABLE I: Parameters for SLB extracted using a distorted hexagonal lattice model.

		In Plane					out of Plane
		a, b(Å)	$d_{(10)}$, $d_{(1\bar{1})}$(Å)	$\gamma(^\circ)$	L_{xy}(Å)	A_h(Å^2)	L_z(Å)
G3	Before	5.26±0.03	4.55, 4.21	114.49	205.2±5.82	47.92±0.40	28.99±0.09
	After	5.45±0.03	4.72, 4.36	114.45	184.1±3.9	51.44±0.45	27.06±0.05
G4	Before	5.30±0.02	4.58, 4.30	115.48	215.4±5.0	48.65±0.38	30.34±0.06
	After	5.65±0.02	4.86, 4.52	114.47	148.6±2.0	55.28±0.43	27.25±0.06

MD SIMULATIONS

Supplementary video 1 and video 2 showing the full atomistic simulations of G3 and G4 DMPC SLB-dendrimer systems, respectively, have been attached as separate files. The mass density and thickness (from the FWHM of density profile) of DMPC bilayer are measured in both cases G3 and G4 and compared with the free standing DMPC bilayer is tabulated in Table I in main text. It is observed that dendrimers are thinning the bilayer and order of thinning is more in case of G4 compared to G3 and indicating the G4 dendrimers are more uidizing the bilayer compared to G3 dendrimers.

TABLE II: Simulation details

system	Number of Lipids	Number of Waters	Number of Dendrimer atoms	Total Number of atoms
DMPC	512	25600		137216
DMPC+G3	512	28651	(5×613) 3065	149434
DMPC+G4	512	38702	(5×1285) 6325	182947

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