Electronic Supplementary Material (ESI) for Soft Matter

Supplementary Information

Induced Dye Leakage by PAMAM G6 Does Not Imply Dendrimer Entry into Vesicle Lumen

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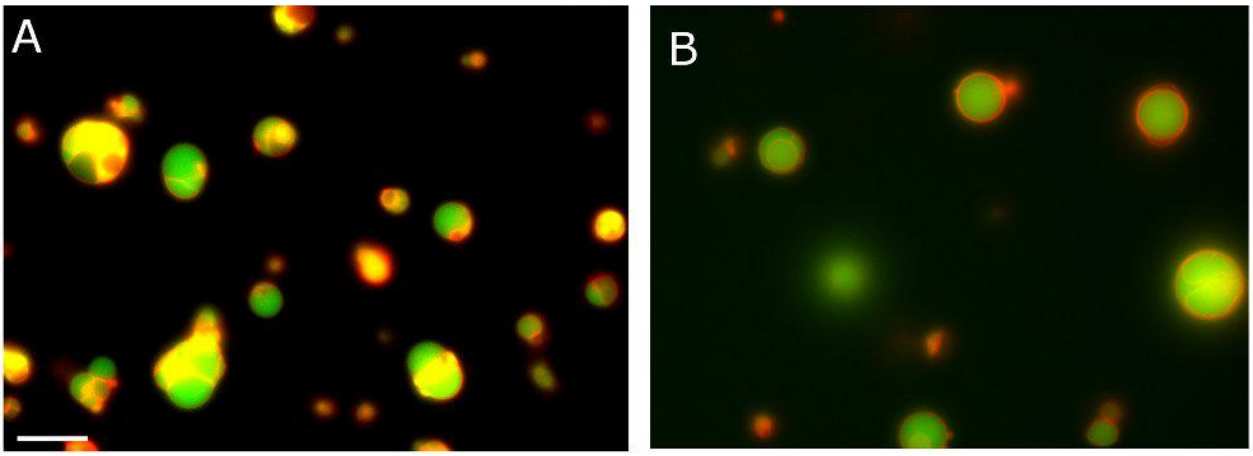

Figure SI1. Addition of buffer alone to giant unilamellar vesicles (GUVs) creates no leakage. As a control for dendrimer addition, the same volume of PBS buffer was added to GUVs composed of A) POPC and B) 20:80 mol% POPC/DPPC. DiD-C18 (red) and Alexa 488 (green) were used to visualize the membranes and the vesicle aqueous lumen, respectively. Even after 1 h of incubation at ambient conditions no leakage of Alexa 488 was observed, demonstrating that the GUVs stay intact during the applied mechanical stress that occurs when the dendrimer solution is added.

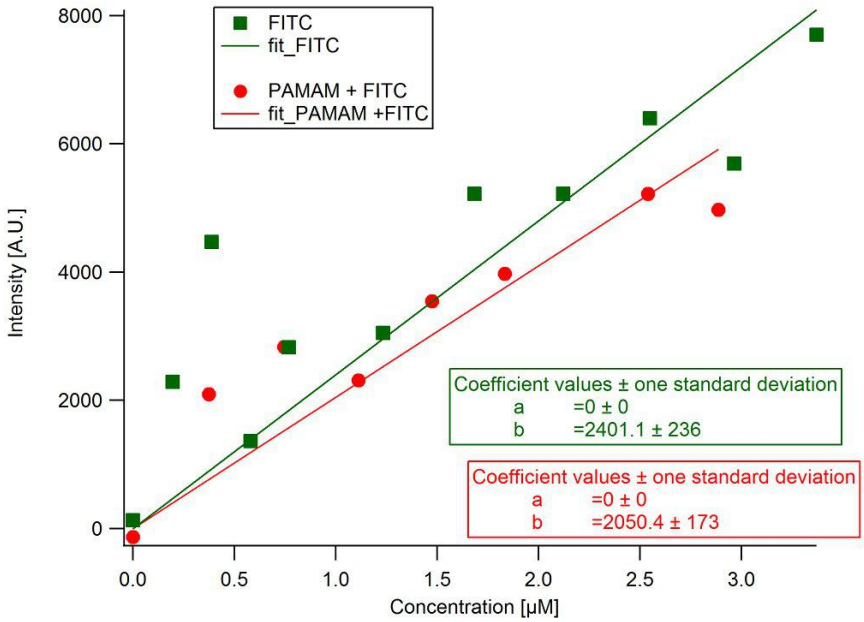

Figure SI2. Spectrofluorescence measurements of FITC and FITC-labeled dendrimers used to determine the degree of PAMAM dendrimers labeling. Red circles show emission intensity from FITC in PBS and green squares show intensity from FITC-conjugated to dendrimers in PBS. Filled and dashed line show linear fits experimental data from FITC and FITC-dendrimer complexes, respectively. Spectrofluorimetry was performed with an excitation wavelength of 460 nm and emission wavelength of 520 nm using a Fluoromax-4 spectroflourometer (Horiba, Edison New Jersey, USA). Low labeling ratio around 1 fluorophore per dendrimer was desired for minimization of potential effects of the fluorophore. We achieved a slightly lower labeling ratio with 0.85 FITC molecules per dendrimer, which proved sufficient to visualize the dendrimer by fluorescence microscopy.

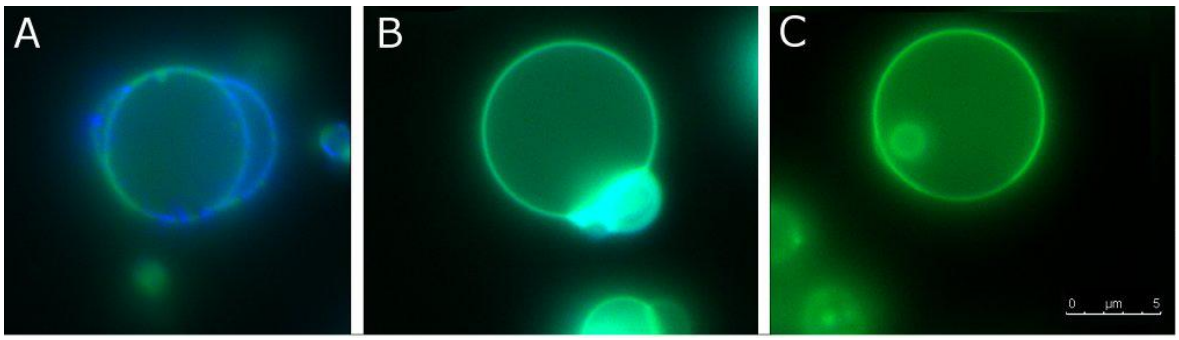

Figure SI3. Visualization of phase coexistence in GUVs using two fluorophores DiD C-18 and DiI c-12. A) GUVs composed of POPC/DPPC at a molar ratio of 20:80 show phase coexistence as visualized by differential labeling with the two lipid dyes. By contrast, vesicles composed of pure B) POPC or C) DPPC show no phase separation as both lipid dyes are homogenously distributed in the membranes. Scale bar, 5 µm. The experiment is in good agreement of previous studies showing phase coexistence in 20:80 mol% POPC/DPPC.

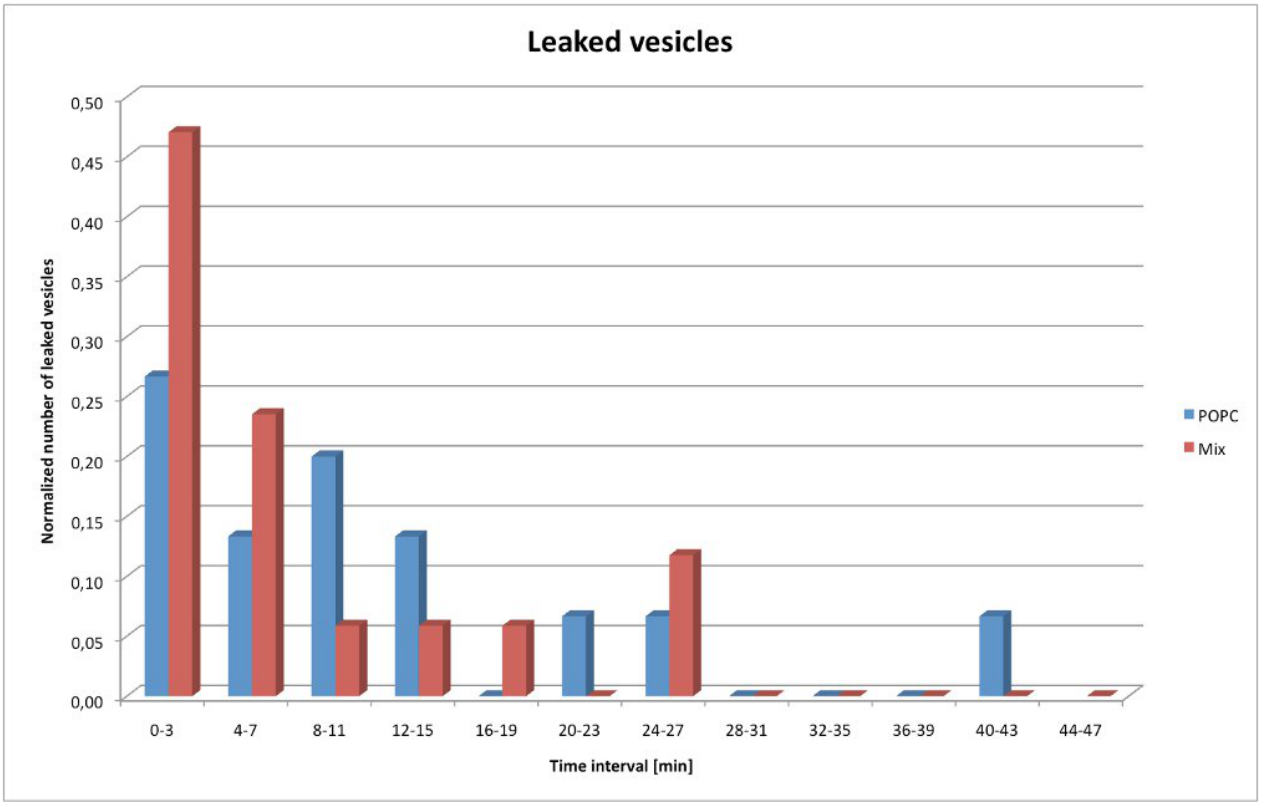

Figure SI4. Histogram showing normalized number of leaked vesicles in time intervals of 3 minutes. Alexa 488-loaded GUVs were prepared from pure POPC or POPC/DPPC mixture (20:80 molar ratio). Upon addition of 10 µM dendrimer solution, leakage was monitored by fluorescence microscopy. For each vesicle composition, data were collected from 3 separate experiments and a total number of 15 and 17 leakage events were observed for POPC and 80 mol% DPPC vesicles, respectively. The graph indicates that leakage time is shorter for DPPC containing vesicles than for the pure POPC vesicles. During minutes 0-7, almost 50% more DPPC vesicles leak as compared to pure POPC vesicles. During minutes 8-15, 50% more of the POPC vesicles leak. Although the data seam consistent it is important to note that only a small number of leakage events has been observed per experiment, due to the relative large size of the GUVs compared to the image area. Therefore since the presented results are based on relatively few data points the quantitative significance for the results are small and should merely be considered as mere indications.

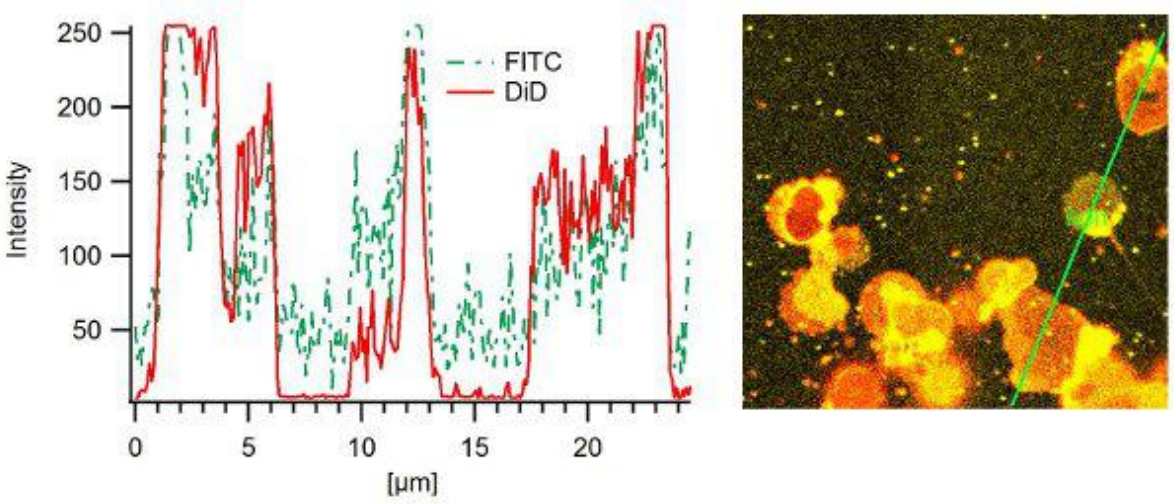

Figure SI5. Intensity profile over collapsed vesicle composed of POPC/POPG (75:25, molar ratio) as a result of dendrimer interaction. FITC-labeled PAMAM G6 dendrimers (10 µM) were added to the sample. A) Intensity profile for FITC labeled dendrimer (dashed line) and lipid dye DiD (solid line) corresponding to the marked green line in the fluorescence image (B). The signals from FITC-labeled dendrimers and the membrane dye co-localized, indicating that the bilayer stacks formed after vesicle collapse contain dendrimer in close contact with the bilayer. Most likely the highly cationic dendrimer can act as a linkage between the membranes forming a lamellar type structure as previously seen in SAXS measurements.[1]

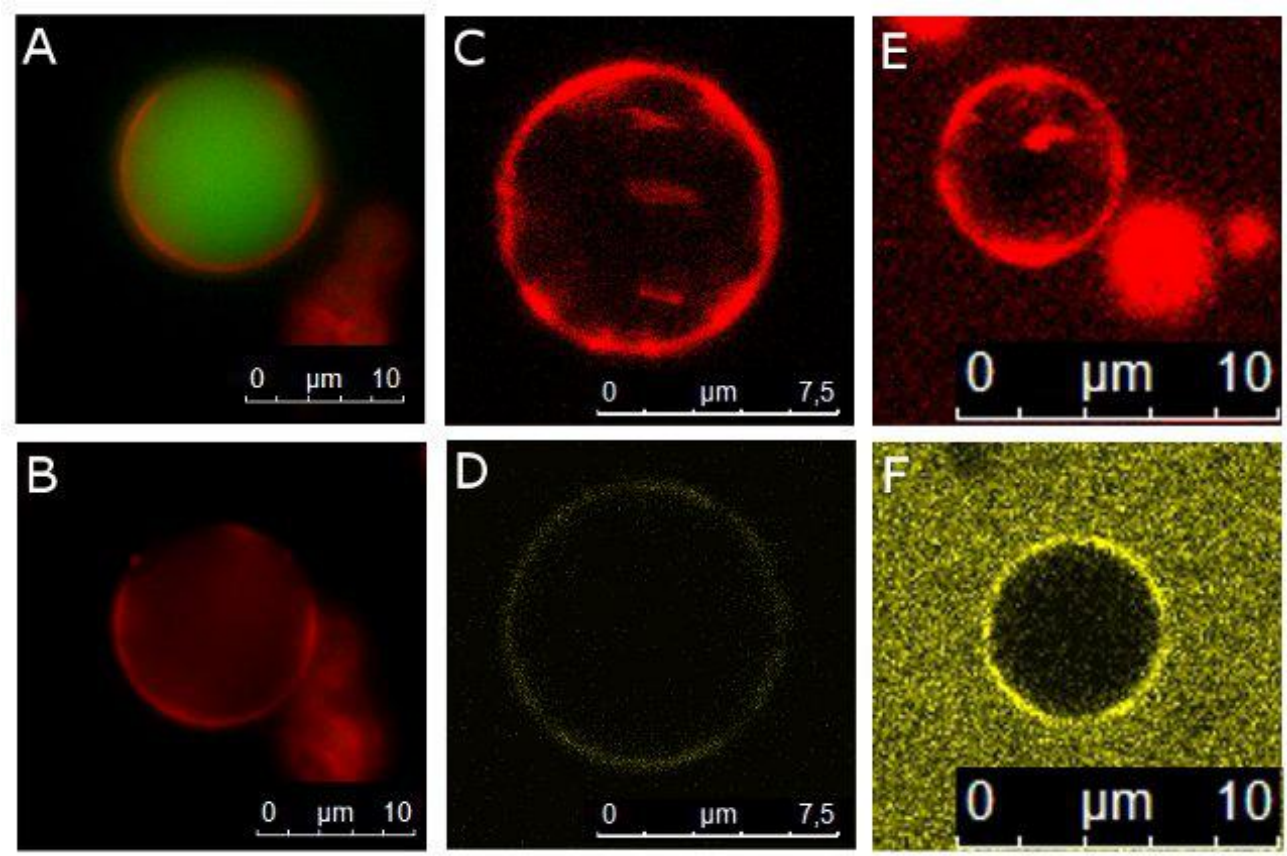

Figure SI6. Dendrimers Interaction with GUVs composed of DPPG/DPPC (25/75, molar ratio). Lipid dye DiI-C18 (red) was used to visualize the membrane in A-C and E. In A-B, Alexa 488 (green) was used to visualize vesicle lumen before and after addition of 1 µM PAMAM G6. In C-D and E-F, 1 µM and 10 µM FITC-labeled PAMAM G6 (yellow) was added to respective vesicle. In GUVs composed of DPPG/DPPC, 1 µM PAMAM G6 dendrimers bind to the membrane (C-D) and induces leakage of the soluble dye (A-B). However, no significant dendrimer translocation into the vesicle lumen was observed even upon addition of 10 µM FITC-labeled dendrimers (E-F). The vesicles show phase separation indicating the local concentration of DPPG might be higher than 25 mol%.

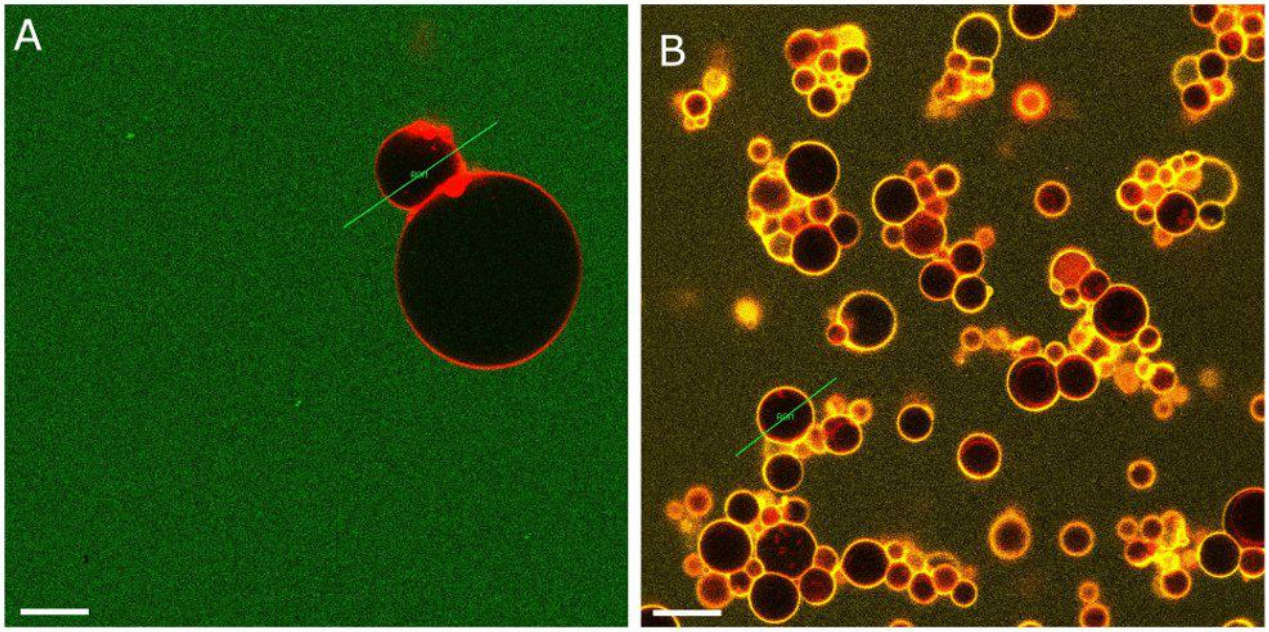

Figure SI7. Images used to generate the intensity line profiles in Figure 2 A and B, respectively. A) GUV composed of POPC after addition of 10 µM FITC-labeled PAMAM G6 (green). B) GUV composed of 25 mol% POPG after addition of 10 µM FITC-labeled PAMAM G6 (yellow). Lipid dye DiD (red) was used to visualize the membranes. Scale bar, 10 µm.

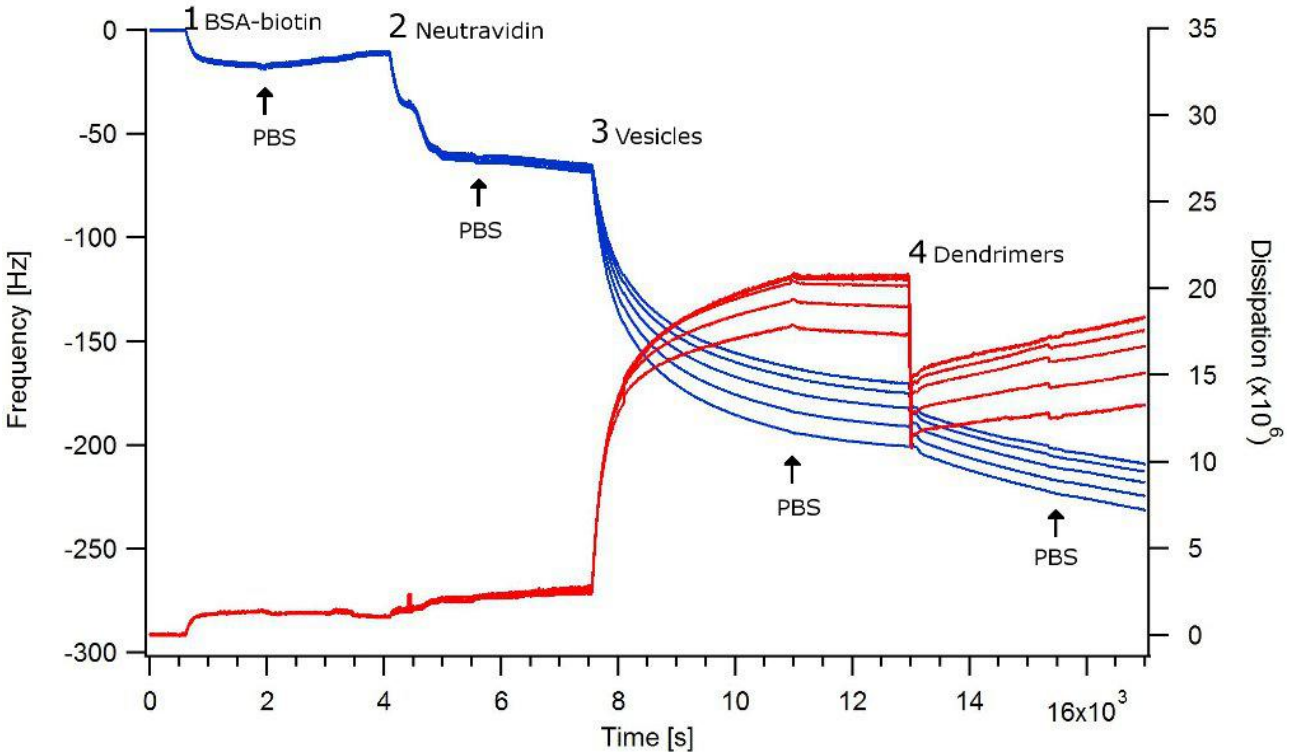

Figure SI8. Analysis of PAMAM dendrimers interaction with small unilamellar vesicles (SUV) composed of POPG/POPC (25/75, molar ratio) by QCM-D. Change in Frequency (blue) and Dissipation (red) is shown in as function of time. 1) Addition of 1 g/l BSA-biotin:BSA (1:10) followed by 2) 0.025 g/l neutravidin, 3) SUV containing 25 mol% POPG and 4) addition of 1 µM PAMAM G6 dendrimer. Between each step the system was rinsed with PBS buffer to remove unbound molecules or vesicles from the liquid cell, as indicated by the arrows. The 5^{th}, 7^{th}, 9^{th} 11^{th} and 13^{th} overtones are shown in the graph. Addition of viscous structures like the vesicles (3) having large wet mass induces a large increase in dissipation and decrease in frequency. When the dendrimers were added an initial decrease in dissipation is seen indicating the adsorbed layer becomes more rigid.

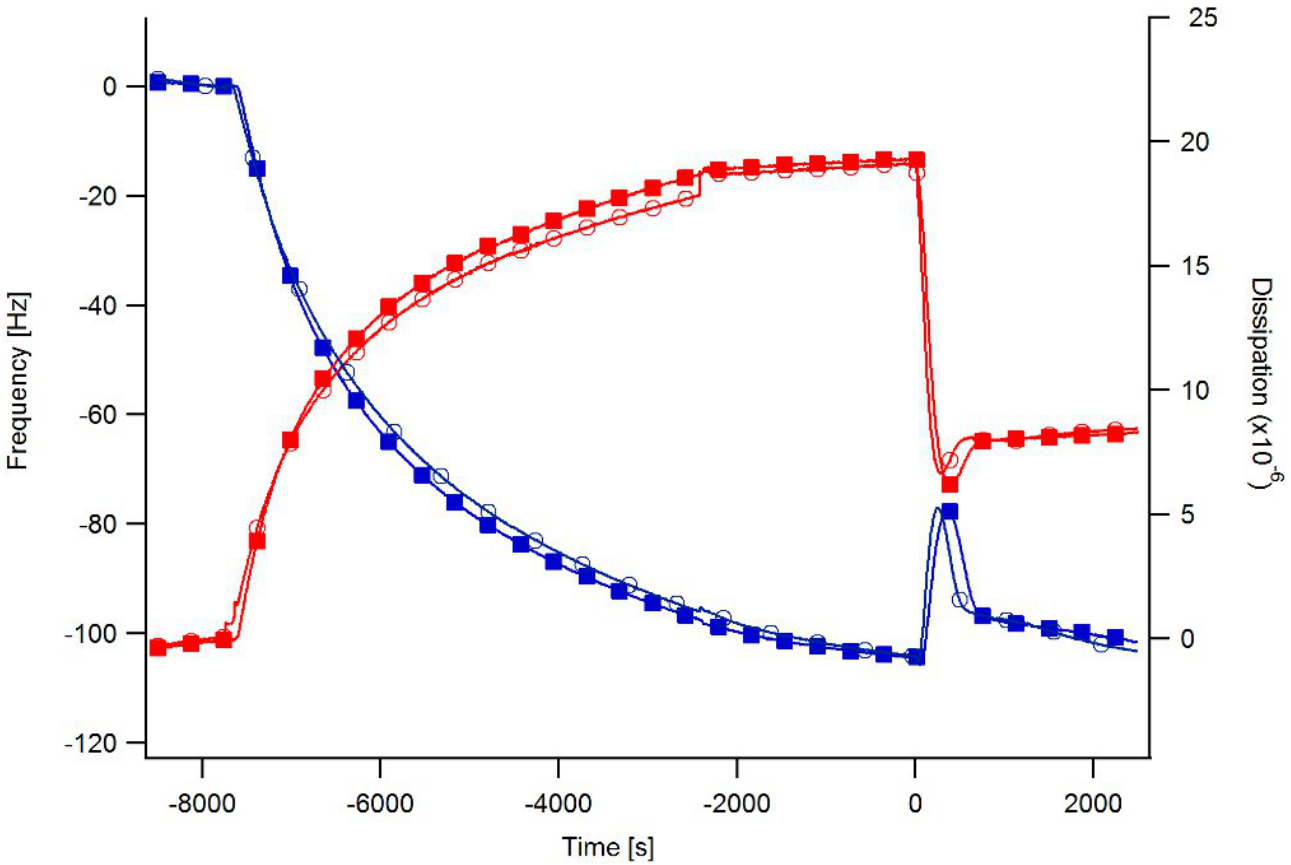

Figure SI9. Analysis of FITC-PAMAM/PAMAM dendrimers interaction with SUVs composed of POPG/POPC (25/75, molar ratio) by QCM-D. Change in Frequency (blue) and Dissipation (read) for the 7th overtone is shown. At time -7750 s a vesicle solution was added to induce vesicle tethering on the surface followed by removal of unbound vesicles with buffer rinsing at t = -2700 s. At t = 0 s, 1 µM PAMAM dendrimer (open circles) and 0.1 µM FITC-labeled PAMAM dendrimer (filled squares) solutions were added. There was no significant difference between the responses of FITC-labeled and non-labeled dendrimer, hence we expect that the fluorophore has no effect on dendrimer interaction with the vesicle membrane.

References:

1. A. Akesson, K. M. Bendtsen, M. A. Beherens, J. S. Pedersen, V. Alfredsson and M. C. Gomez, *Phys Chem Chem Phys*, 2010, **12**, 12267-12272.