Electronic Supplementary Material (ESI) for Soft Matter
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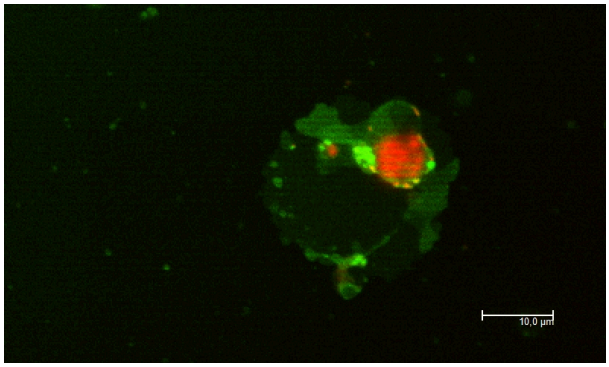
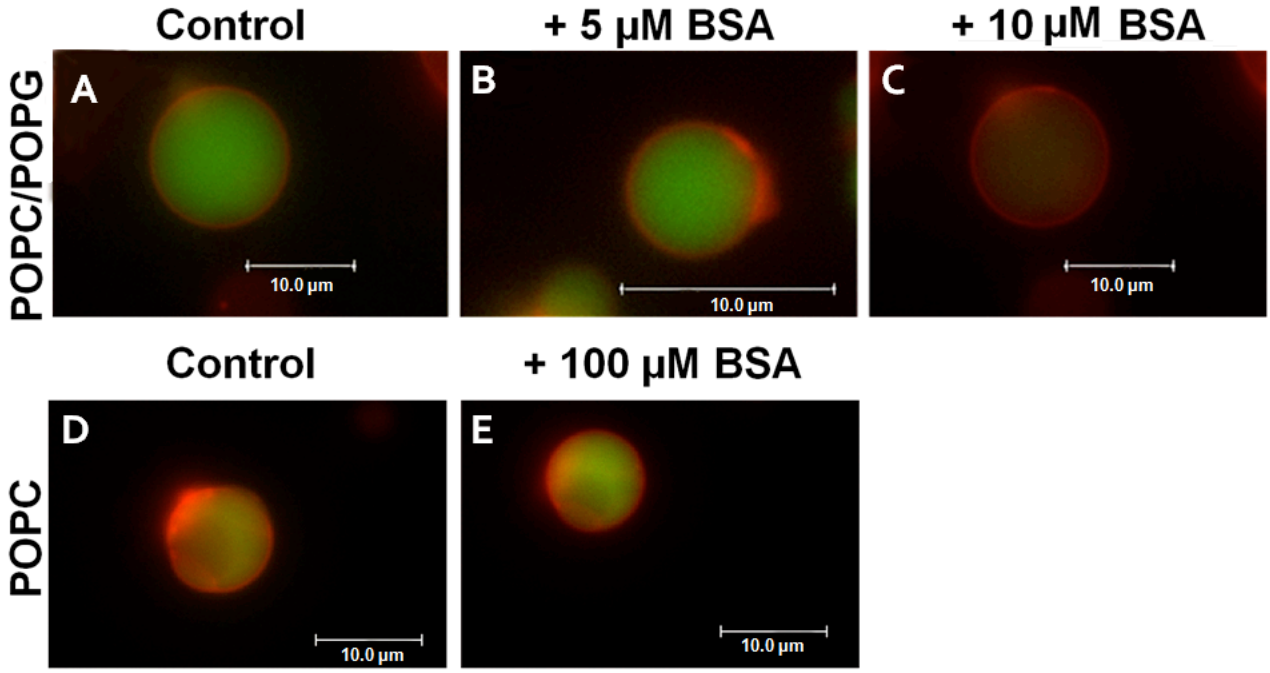
Dendrimer Impact on Vesicles can be Tuned Based on the Lipid Bilayer Charge and the Presence of Albumin.

Francesca Ruggeri[a],Anna Åkesson[a], Pierre-Yves Chapuis[a], Catherine Anna Skrzynski Nielsen[a], Marco P. Monopoli[a], Kenneth A. Dawson[a], Thomas Günther Pomorski[a] and Marité Cárdenas[a].

Supporting Information

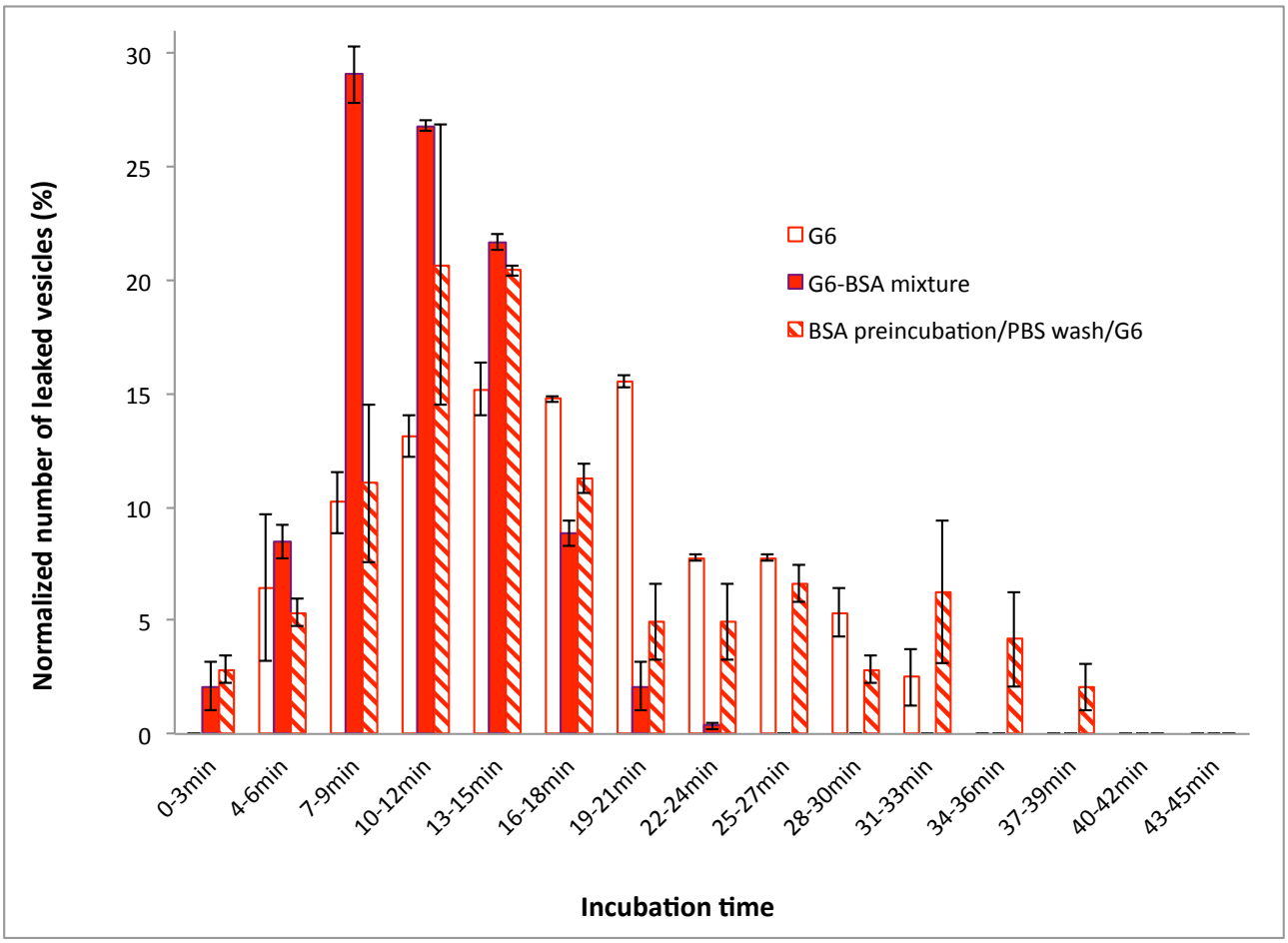


Supporting Information Figure S1. Typical image of the microscope slide surface showing vesicle collapse (red channel) upon the action of FITC labeled PAMAM G6 (green channel) and BSA on negatively charged vesicles. For details on mechanism of vesicle collapse see our earlier work on Soft Matter, 2012, 8, 8972-8980.



Supplementary Figure S2. Effect of BSA on leakage and morphology of giant unilamellar vesicles (GUVs). DiD-C18 (red) and Alexa 488 (green) were used to visualize by fluorescence microscopy the membrane and the vesicle aqueous lumen, respectively. Addition of PBS served as control and did not affect integrity of GUVs composed of POPC/POPG at a molar ratio of 75:25 (A) or pure POPC (D). Likewise, neither the integrity nor the structure of POPC/POPG vesicles was affected by the presence of 5 µM BSA addition (B). However, we noted that BSA started to affect the vesicles at BSA concentrations of

10 μM or above (C): some of the vesicles leaked but the overall three-dimensional structure of the GUVs remained preserved. For POPC vesicles, no leakage or shape changes were observed for BSA concentrations up to 100 μM (E).



Supporting Information Figure S3. Leakage analysis of Alexa 488-loaded POPC/POPG GUVs in the presence of PAMAM G6 with and without BSA at identical conditions as those for Figure 2. In this case, the vesicles were formed by electroswelling instead of the hydration method. The figure also includes data for GUV pre-incubated with BSA and subjected to 3 volume exchanges against PBS prior to addition of PAMAM G6. Data represent the mean of 2 independent experiments (with at least 30 vesicles analyzed at each experiment). Error bars show standard deviations. The lipid concentration was kept constant at ~14 μM although in this case the absolute lipid concentration is hard to control due to the method for electroswelling. Nevertheless, the trends are similar to those found for GUV made by the hydration method (Figure 2) and thus we can conclude that there is no significant effect on the method of vesicle formation on how dendrimers interact with them. For GUV preparation by electroswelling, the commercially available Vesicle Prep Pro® system was used at a 0.5 g/L lipid concentration using an alternating voltage (3 V) with a frequency of 5 Hz for 120 min.

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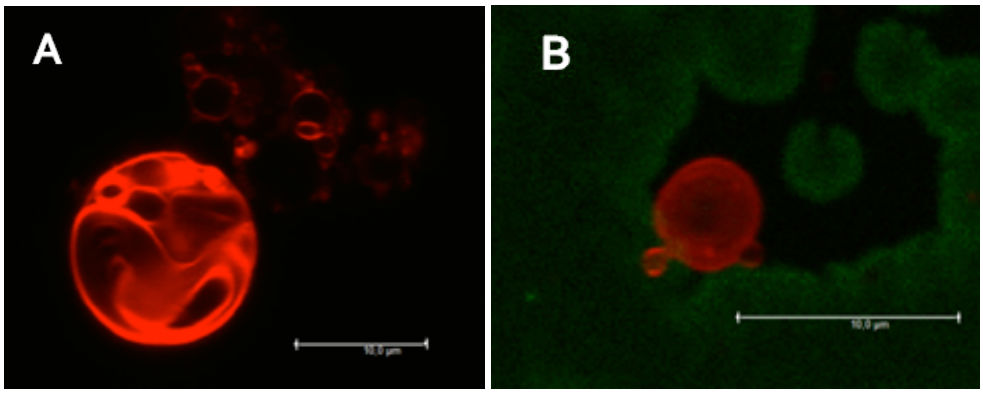
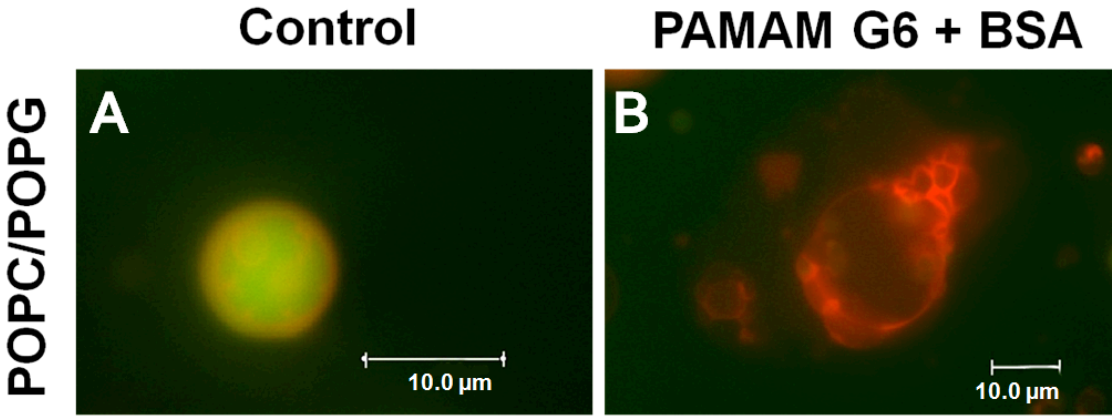


Supporting Information Figure S4. Confocal fluorescence microscopy images for DiD-C18 (red) labeled POPC vesicles before (A) and after addition of FITC (green) -PAMAM G6 and BSA (B) at conditions similar to those used in Figure 3 (0.5 μM G6 and 5 μM BSA). At these low concentrations, neither dendrimers nor dendrimers-BSA complexes induce any significant effect on the vesicle structure or dendrimers accumulation at the vesicle surface (the image focuses on the microscope slide surface). Instead dendrimers interacted preferentially with the BSA treated surface (the focus on the right image is done on the surface to show the preferential interaction with the surface. From this it is clear that PAMAM G6 dendrimer have a higher affinity for BSA than POPC vesicles.



Supplementary Figure S5. The synergistic effect of PAMAM G6 and BSA towards negatively charged GUVs is still present at physiological ionic strength. GUVs were prepared from POPC/POPG mixtures (75:25, molar ratio). DiD-C18 (red), and Alexa 488 (green) were used to visualize by fluorescence microscopy the membrane vesicle and aqueous lumen, respectively. (A) Vesicles after 1 h in PBS containing 150 mM NaCl. (B) Vesicles after 1 h in the presence of 0.5 μM PAMAM G6 pre-incubated with 5 μM BSA (1:10,molar ratio); content leakage and inter-vesicle aggregation occurred. Quantitative analysis revealed that 99% of vesicles leaked during the 1 h incubation in excellent agreement with the value obtained in 100 mM NaCl (Figure 2). Taken together, these data corroborate that the reported effects are valid even at higher ionic strengths.

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List of Tables

Table S1. Physical-chemical properties of the chemicals used in this work.

Type of molecule	Name	MW (Da):	Net charge pH 7.4
Lipids	POPC	760	Zero
	POPG	771	Negative
	POPS	784	Negative
	DOPE-Biotin	1105	Zero
Dendrimer			
	PAMAM (polyamidoamine) generation 6	58046 (7 nm diameter)	Positive (256 amino surface groups)
Protein			
	Bovine Serum Albumin	66500 (4 nm diameter)[1]	Negative (pI = 4.7)

* Zeta potential for protein and dendrimers solution were measured using a zetasizer Nano Z. Three samples were measured giving a zeta potential for BSA and G6 dendrimers solutions of -11.4 ± 0.9 mV and 16.9 ± 0.5 mV, respectively.

Table S2. Luminescence properties of the dyes used in this work.

Dye:	Excitation (nm): Confocal/wide field	Emission(nm): Confocal/wide field	Location Confocal/wide field
DIDC18(5)	633/590-650	640-700 /665-735	Lipid bilayer
Alexa 488	499/490-510	519/520-550	Vesicles lumen
Atto 550	555	577	Vesicles lumen
FITC	494/490-510	518/490-510	Dendrimers

References:

(1) Seng Ang, W.; Elimelech, M. *Journal of Membrane Science Journal of Membrane Science* **2007**, *296*, 83.