Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supplementary Information

Leaky membrane fusion: an ambivalent effect induced by antimicrobial polycations

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Additional cryo-TEM images

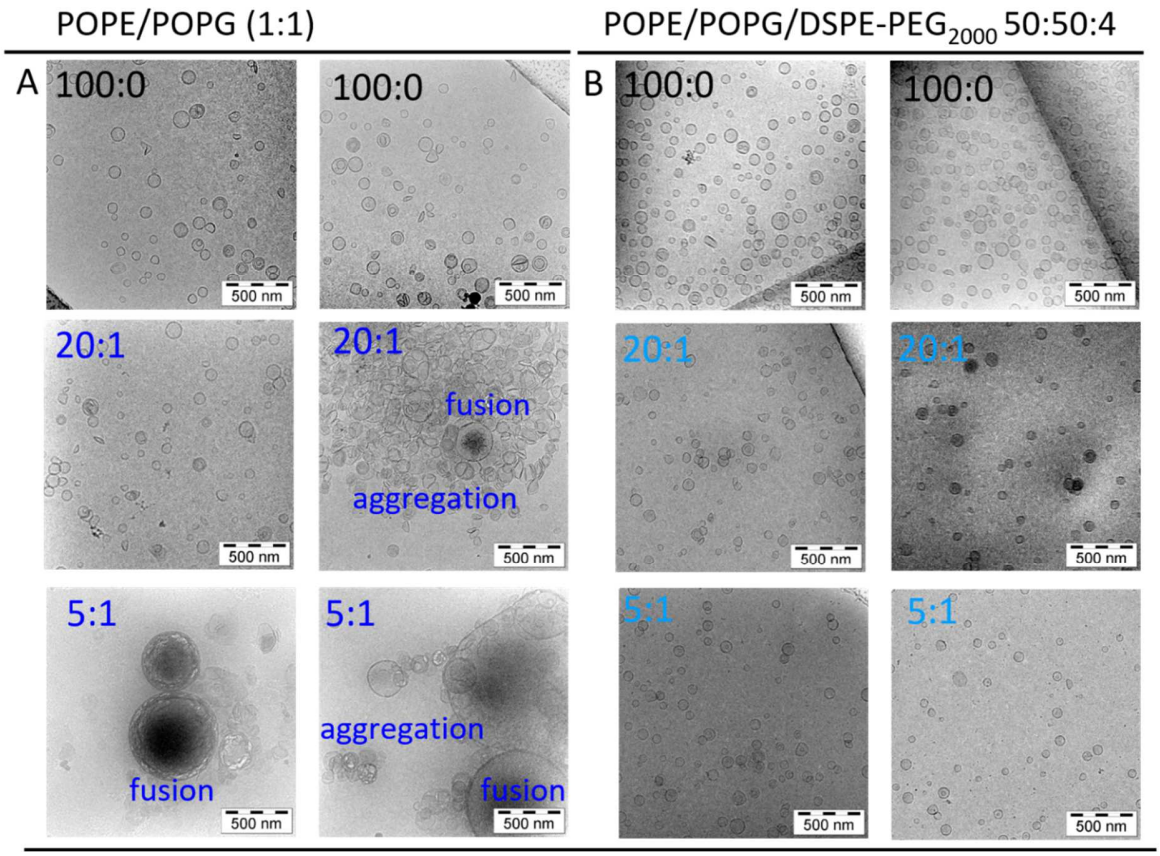

Figure S 1 Two additional cryo-TEM images of each condition. Samples initially contained extruded LUVs composed of POPE/POPG (1:1) (A) or POPE/POPG/DSPE-PEG$_{2000}$ (50:50:4) (B) in the absence (black) and presence (dark blue or light blue) of poly-NM. The lipid/poly-NM subunits molar ratio is indicated. 8-10 mM extruded LUVs were incubated 1 day before cryo-TEM with or without poly-NM in MOPS buffer (25 mM MOPS; 130 mM NaCl; pH 7.0).

No influence of the PEG-chains on poly-NM binding

To check for direct interactions of the PEG-chains with poly-NM, a solution of 0.12 mM $mPEG_{2000}$ (Sigma-Aldrich, St. Louis, MO, USA) was titrated into a solution of 0.15 mM poly-NM. The heats of injection resemble typical heat of dilution. There is no apparent interaction of poly-NM with the PEG chains ($mPEG_{2000}$).

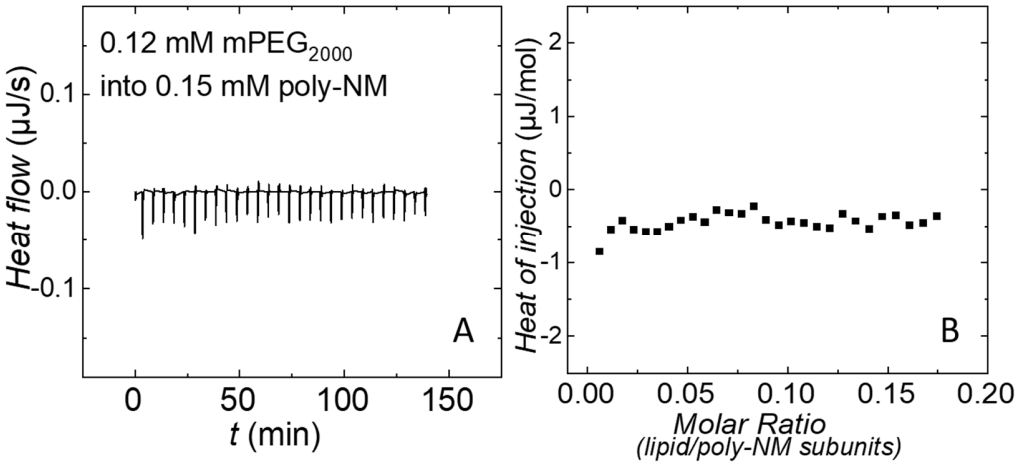

Figure S 2 ITC data (raw data in A and integrated data in B) of the injection of soluble $mPEG_{2000}$ into a solution of poly-NM. 0.12 mM $mPEG_{2000}$ (corresponding to 4 mol % in 3 mM PEG-vesicles) was injected into 0.15 mM poly-NM. Note that the injection volume is 10 µL here (only 5 µL in Figure 3). (25 mM MOPS; 130 mM NaCl; pH 7.0; 25 °C).

Potential effects of turbidity/ light scattering: ANTS-DPX leakage compared to calcein leakage

The leakage of calcein detected via fluorescence-lifetime (Figure 4) was confirmed independently using steady-state fluorescence of the vesicles containing ANTS and DPX examined in thesigmal contents mixing assay. After the contents mixing experiment, additional DPX was added to the buffer to quench the fluorescence of ANTS that has leaked out of the vesicles. Both leakage assays yield comparable L_{total} as a function of poly-NM concentration. There is no indication of the leakage results being affected by turbidity.

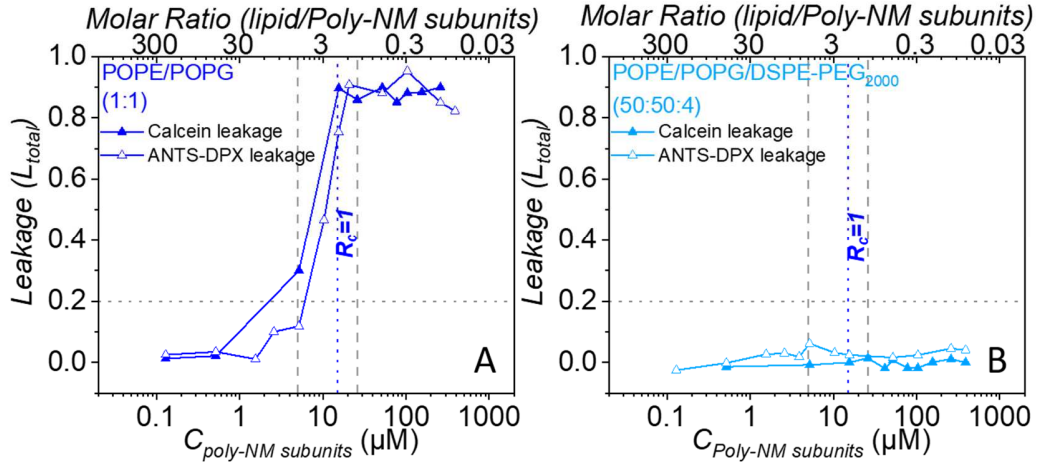

Figure S 3 Comparison of leakage of calcein (filled triangle) or ANTS-DPX (open triangle) induced by poly-NM in 30 µM LUVs composed of POPE/POPG (1:1) (A) and POPE/POPG/DSPE-PEG_{2000} (50:50:4) (B). LUVs (100-130 nm diameter) were incubated with or without poly-NM for 24-26 h (25 mM MOPS; 130 mM NaCl; pH 7.0; 25 °C). Calcein leakage data of POPE/POPG (1:1) are reproduced from Ref. Shi et al. 2021 with permission from the PCCP Owner Societies.

ANTS/DPX leakage Method: 20 µL 450 mM DPX solution was added to the samples after the content mixing assay (*i.e.* after 24 h incubation time). Leakage efficiency in the presence of poly-NM based on ANTS/DPX was calculated as:

$$ANTS/DPX\ leakage\ efficiency = \frac{I_{S0} - I_S}{I_{S0} - I_{S100}}$$

Where I_S is the fluorescence intensity of ANTS at 525 nm which was recorded 2 h after the addition of external DPX in the presence of poly-NM. The subscripts 0 and 100 correspond to the negative control (in the absence of poly-NM) and positive control of leakage (in the presence of 1 v/v % TX100), respectively.

Full time course of leakage of POPE/POPG vesicles induced by poly-NM in the absence and presence of DSPE-PEG$_{2000}$ in vesicles and soluble mPEG$_{2000}$ in solution

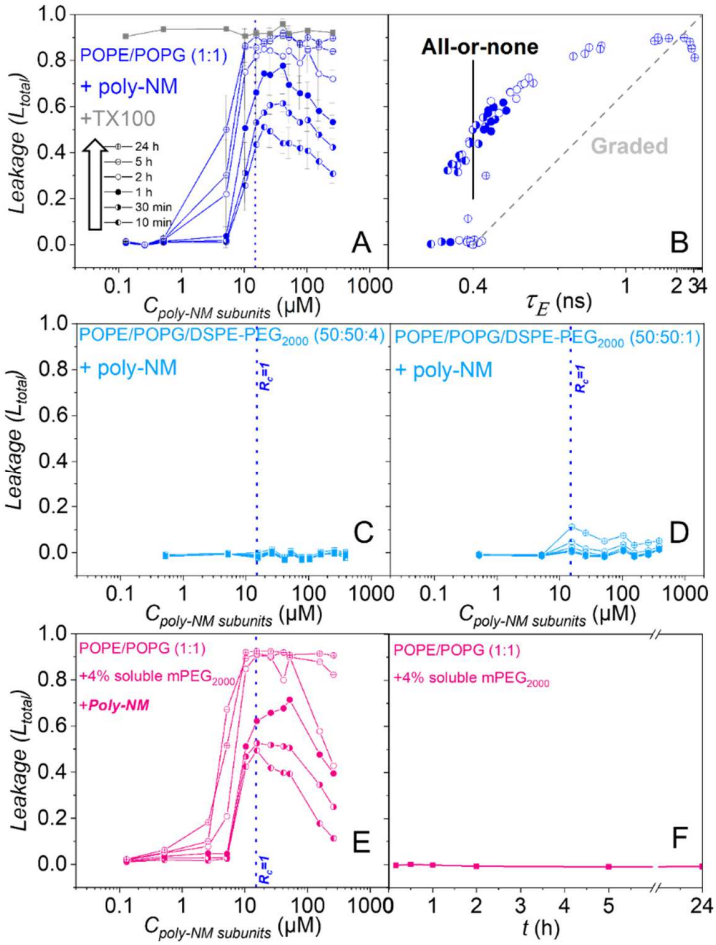

Figure S 4 Comparison of poly-NM induced leakage of 30 µM POPE/POPG (1:1) vesicles in the absence (A and B, in dark blue) and presence of DSPE-PEG$_{2000}$ in vesicles (C: 1% and D: 4%, in light blue) and 1.2 µM soluble mPEG$_{2000}$ (equivalent to 4% with respect to the lipid concentration, E and F, in pink). A: The mean values of four independent repetitions is depicted with a standard deviation. B: Total leakage as a function of calcein fluorescence lifetime. The theoretical behaviors for all-or-none and graded leakage are indicated. Data are taken from Shi et al. 2021 with permission from the PCCP Owner Societies. C and D: Total leakage as a function of poly-NM subunits concentration in the presence of 4 % (C) and 1% (D) DSPE-PEG$_{2000}$ in vesicles. E: Total leakage as a function of poly-NM subunits concentration in the presence of 4 % soluble mPEG$_{2000}$ with respect to lipid concentration (1.2 µM). LUVs are preincubated with soluble mPEG$_{2000}$ for 10 min before the addition of poly-NM. F: Leakage of POPE/POPG vesicles in the presence of 1.2 µM soluble mPEG$_{2000}$ (equivalent to 4% with respect to the lipid concentration) without the addition of poly-NM until 24 h. Different incubation times are indicated and all legends as in A. (25 mM MOPS; 130 mM NaCl; pH 7.0; 25 °C)

For comparison, the full details of leakage induced by poly-NM in POPE/POPG vesicles are reproduced from Shi et al. 2021 with permission from the PCCP Owner Societies. There is no significant leakage (< 5 %) of POPE/POPG vesicles in the presence of 1% and 4 % DSPE-PEG_{2000} in vesicles until 5 h (Figure S 4 C, D). Moreover, the poly-NM-induced leakage behavior seems to be unchanged in the absence and presence of 4 % soluble $mPEG_{2000}$ (Figure S 4 E, F).

Lipid Mixing Assays

Comparison of total lipid mixing using two different references

We use two types of reference values for detecting lipid mixing: 1. Micelle reference: direct addition of TX100 to solubilize the FRET-lipid labelled LUVs into micelles (a common reference for entirely suppressed FRET) (left axis in Figure S 5). 2. Maximal dilution reference: LUVs prepared separately containing only 1/5 of FRET labels (each 0.1% of NBD and Rho) for an estimated value better reflecting the actual extent of lipid mixing (right axis in Figure S 5). The maximal dilution reference indicates an absolute lipid mixing efficiency of up to 80%.

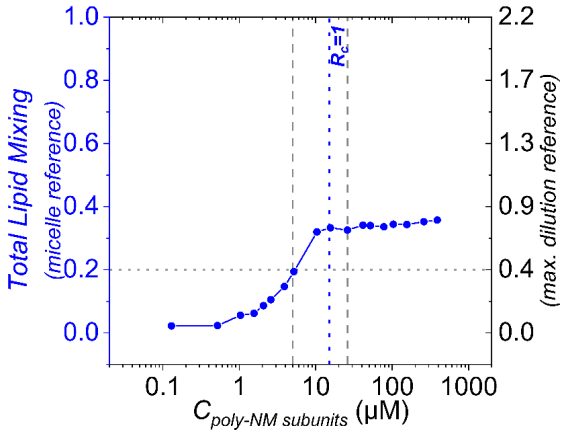

Figure S 5 Total lipid mixing of 30 μM extruded LUVs composed of POPE/POPG (1:1) induced by poly-NM by using micelle reference (by addition of 1 v% TX100) and maximal dilution reference (1 h incubation, 25 mM MOPS; 130 mM NaCl; pH 7.0; 25 °C).

Comparison of lipid mixing induced by poly-NM and Ca^{2+}

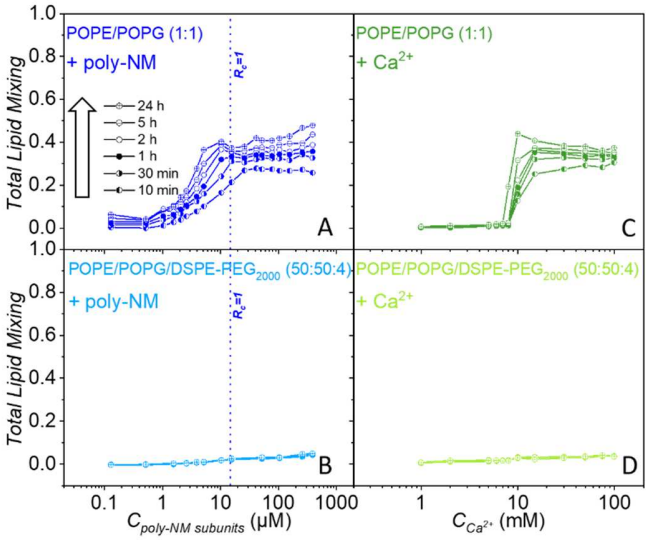

Figure S 6 Total lipid mixing of 30 µM extruded LUVs composed of POPE/POPG (1:1) (A, C) and POPE/POPG/DSPE-PEG_{2000} (50:50:4) (B, D) induced by poly-NM (dark and light blue) and Ca^{2+} (dark and light green). Various incubation times are indicated (25 mM MOPS; 130 mM NaCl; pH 7.0; 25 °C).

The time course of total lipid mixing induced by poly-NM and Ca^{2+} is similar, indicating a comparable mechanism of lipid mixing in both cases.

Comparison of uninterrupted kinetics of total lipid mixing induced by poly-NM and Ca2+

We performed uninterrupted kinetics of total lipid mixing induced by 5 µM poly-NM and 10 mM Ca^{2+}. We use the same settings and analysis for the measurement that only differed in the additional stirring magnet at a low stirring speed. The uninterrupted kinetics of total lipid mixing are in good agreement with the timescales of lipid mixing (only measured at individual time points).

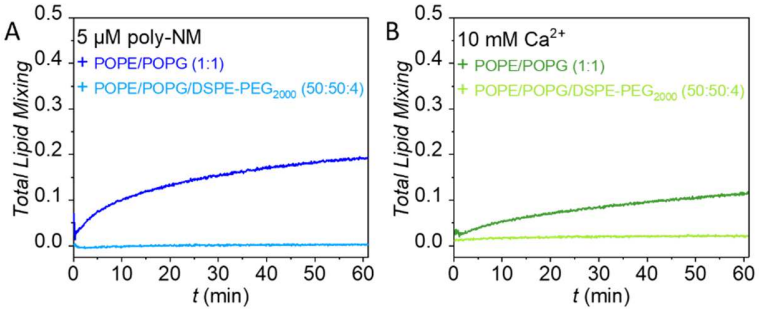

Figure S 7 Uninterrupted kinetics of total lipid mixing induced by 5 µM poly-NM (A) or 10 mM Ca^{2+} (B) in 30 µM LUVs composed of POPE/POPG (1:1) or POPE/POPG/DSPE-PEG_{2000} (50:50:4) (25 mM MOPS; 130 mM NaCl; pH 7.0, 25 °C).

Content Mixing Assay

Comparison of content mixing induced by poly-NM with and without correction of light scattering

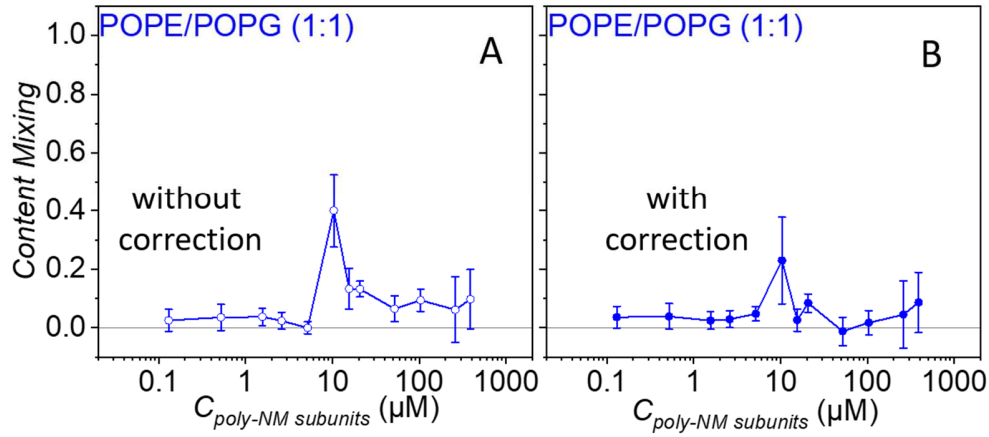

Figure S 8 Poly-NM induced content mixing of 30 μM LUVs (100-130 nm) composed of POPE/POPG (1:1) without (A) and with (B) correction of light scattering. The mean value and standard deviation of three independent measurements are shown. LUVs are incubated with or without poly-NM for 1 h (25 mM MOPS; 130 mM NaCl; pH 7.0; 25 °C).

The time course of content mixing induced by poly-NM

A change in content mixing over longer incubation times (until 24h) was not observed.

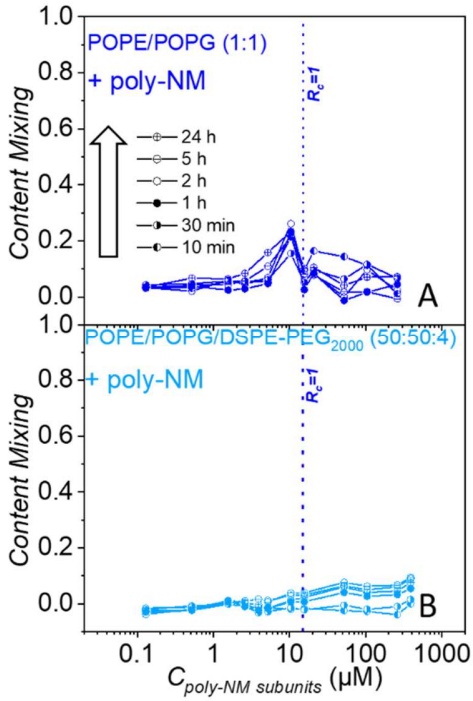

Figure S 9 Time course of content mixing of 30 μM extruded LUVs composed of POPE/POPG (1:1) (A) and POPE/POPG/DSPE-PEG$_{2000}$ (50:50:4) (B) induced by poly-NM (dark and light blue). various incubation times are indicated (25 mM MOPS; 130 mM NaCl; pH 7.0; 25 °C).

Particle sizes in the leakage experiment with additional vesicles

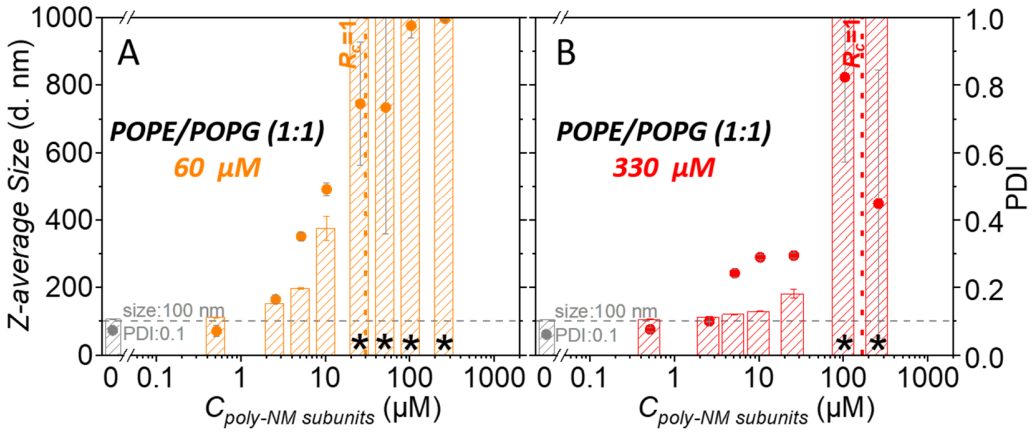

Figure S 10 Dynamic light scattering: Particle size (z-average diameter, bar graph) and polydispersity indices (PDI, dot diagram) of extruded LUVs composed of POPE/POPG (1:1) (A: 60 µM, orange; B: 330 µM, red) 24h after addition of poly-NM. Data in gray represent LUVs in the absence of poly-NM. The dotted lines represent the theoretical charge saturation ratio (R_c = 1) of POPE/POPG LUVs at different lipid concentrations. Particle sizes larger than 1000 nm are marked by an asterisk. (25 mM MOPS; 130 mM NaCl; pH 7.0; 25 °C)

No significant leakage, lipid mixing, and content mixing of POPC LUVs induced by poly-NM

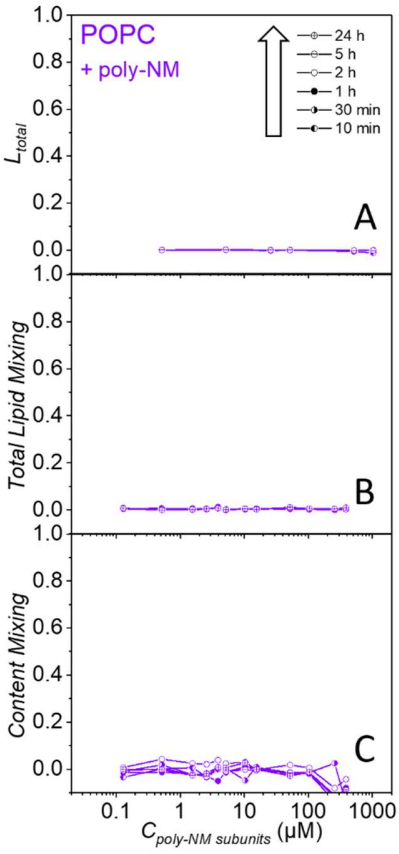

Figure S 11 Poly-NM induced no significant leakage (A), lipid mixing (B) and content mixing (C) of POPC LUVs until 24 h. LUVs (100-130 nm) were incubated with and without poly-NM in MOPS buffer (25 mM MOPS, 130 mM NaCl, pH 7.0) at 25 °C. Incubation times are indicated. Leakage results for 1 h are reproduced from Ref. Shi et al. 2021 with permission from the PCCP Owner Societies.

We checked for possible leakage and fusion of POPC LUVs induced by poly-NM. There is no significant leakage, lipid mixing, and content mixing until 24h, which might be explained by the weak or missing interaction of highly charged and negligibly hydrophobic poly-NM with zwitterionic POPC vesicles (Shi et al. 2021).

Overview of all effects examined at various lipid and polycation concentrations

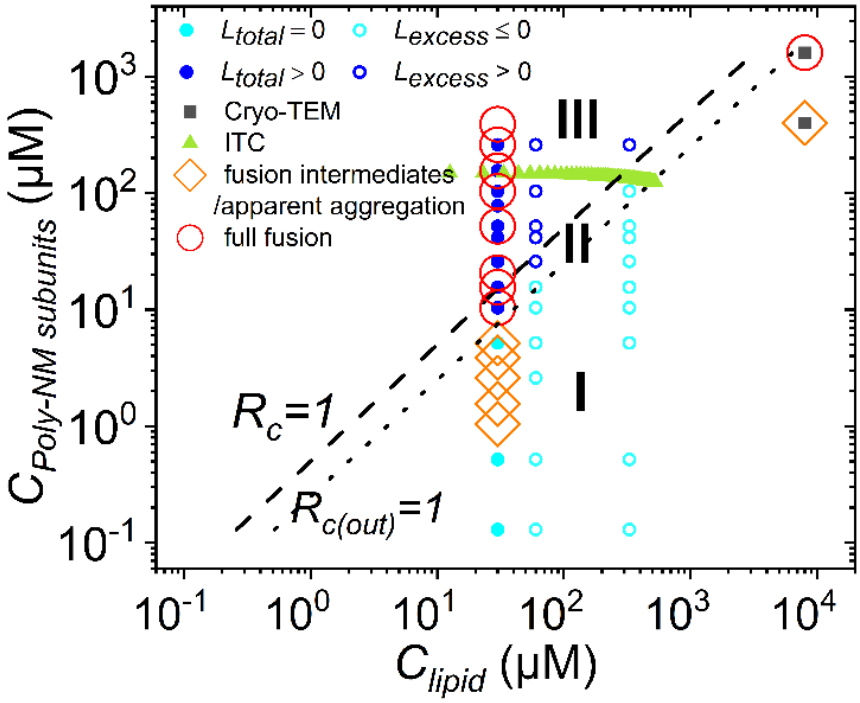

Figure S 12 Overview of experimental conditions and poly-NM induced behavior of POPE/POPG vesicles in various polymer and lipid concentration ranges. Experimental methods as indicated. Vesicle fusion intermediates/apparent aggregation or full fusion is judged by increased particle sizes, content mixing or concluded from the cryo-TEM images. We use total leakage (L_{total}) for the normal leakage experiment (without additional vesicles) and excess leakage (L_{excess}) for the leakage with additional vesicles. The line representing the theoretical charge saturation ratio (R_c = 1) for the entire lipid layers is dashed and for the outer lipid layer is dotted. (25 mM MOPS; 130 μM NaCl; pH 7.0; 25 °C).

References

(1) Shi, S.; Quarta, N.; Zhang, H.; Lu, Z.; Hof, M.; Šachl, R.; Liu, R.; Hoernke, M. Hidden complexity in membrane permeabilization behavior of antimicrobial polycations. *Physical chemistry chemical physics: PCCP* **2021**, *23* (2), 1475–1488. DOI: 10.1039/d0cp05651k.