Supplementary Material

Hidden complexity in membrane permeabilization behavior of antimicrobial polycations

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Definition of binding constants

The molar ratio partitioning constant, K_P, was defined as:

$$K_P = \frac{[PL]}{[P]_{free}\,[L]_{total}}$$

$$[P]_{free} = [P]_{total} - [PL]$$

where $[P]$, $[L]$ and $[PL]$ are the concentrations of polymer subunits, lipids and polymer subunits bound to lipids in the sample cell, respectively.

The single set of sites binding constant, K_B, was defined as:

$$K_B = \frac{[PL]}{[P]_{free}\,[B]_{free}}$$

$$[B]_{free,B} = b\,[L]_{total,anionic} - [PL]$$

where $[B]_{free,B}$ is the concentration of free binding sites, and $[L]_{total,anionic}$ refers to the total concentration of negatively charged lipids in the sample cell.

Additional ITC data

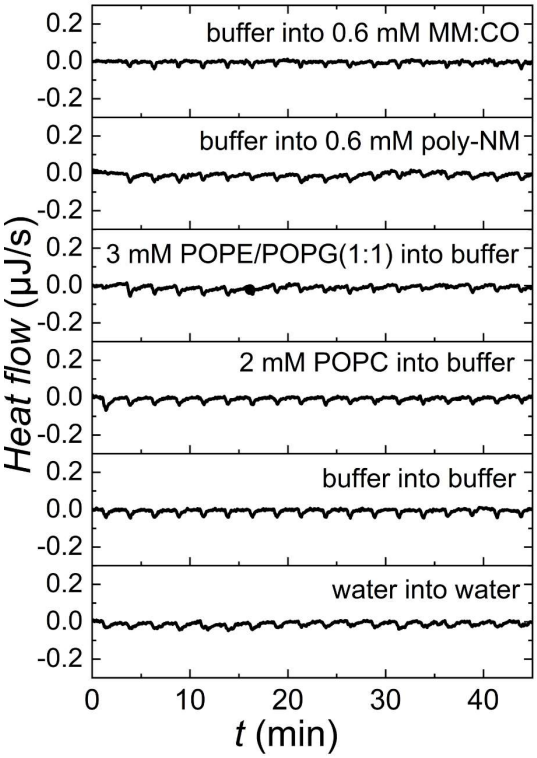
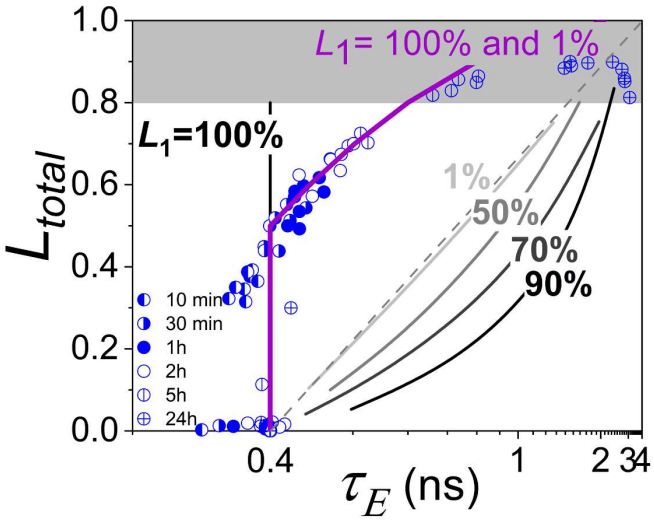


Figure S 1 Reference injection of ITC data: injection of liposomes into buffer and the injection of buffer into smAMP. Note that the individual injection volumes were 10 µL (only 3 to 5 µL in the smAMP binding experiments). (MOPS buffer: 25 mM MOPS, 130 mM NaCl, pH 7.0, 25 °C.) (MOPS buffer: 25 mM MOPS, 130 mM NaCl, pH 7.0, 25 °C.)

Superposition of two types of leakage events

To us, the most simple model that can describe the data in **Fehler! Verweisquelle konnte nicht gefunden werden.** H assumes very strong leakage events (L_1 = 100%) that occur alone in the first ten minutes of incubation with additional very weak leakage events (L_1 ~ 1%) only starting to occur and



reoccur afterwards.

Figure S 2: Figure 4H with an additional theoretical curve (purple, solid line) calculated assuming leakage events with L_1 = 100% and additional weak leakage events characterized by L_1 = 1% that occur first after 10 minutes of incubation, i.e. after 50% of total leakage had already happened. Both types of leakage events reoccur thereafter. Total leakage above ~ 80% become indistinguishable to the fit and are considered complete (grey area).

Additional data of GUV leakage

A comparison of the apparent cumulative leakage kinetics of LUVs and those GUVs that retain and intact rim (Figure S 33) shows similar behavior. Substantial and fast leakage of GUVs induced by MMCO happened in the first hour and leakage did not increase much after that. While leakage induced by poly-NM also follows a more continuous time course in good agreement with the experiments on LUVs. This indicates again that the mechanism of leakage does differ between MMCO and poly NM independently of vesicle size. More quantitative analysis is not warranted as the GUV samples could not be stirred.

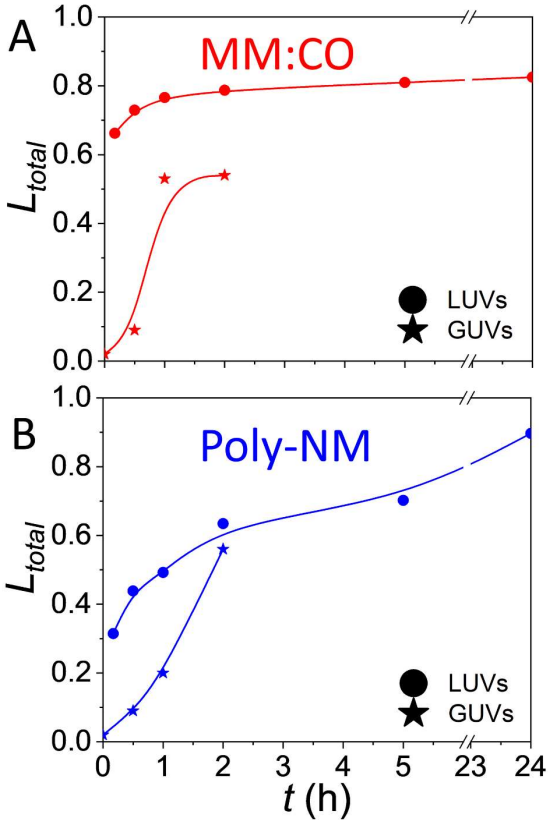
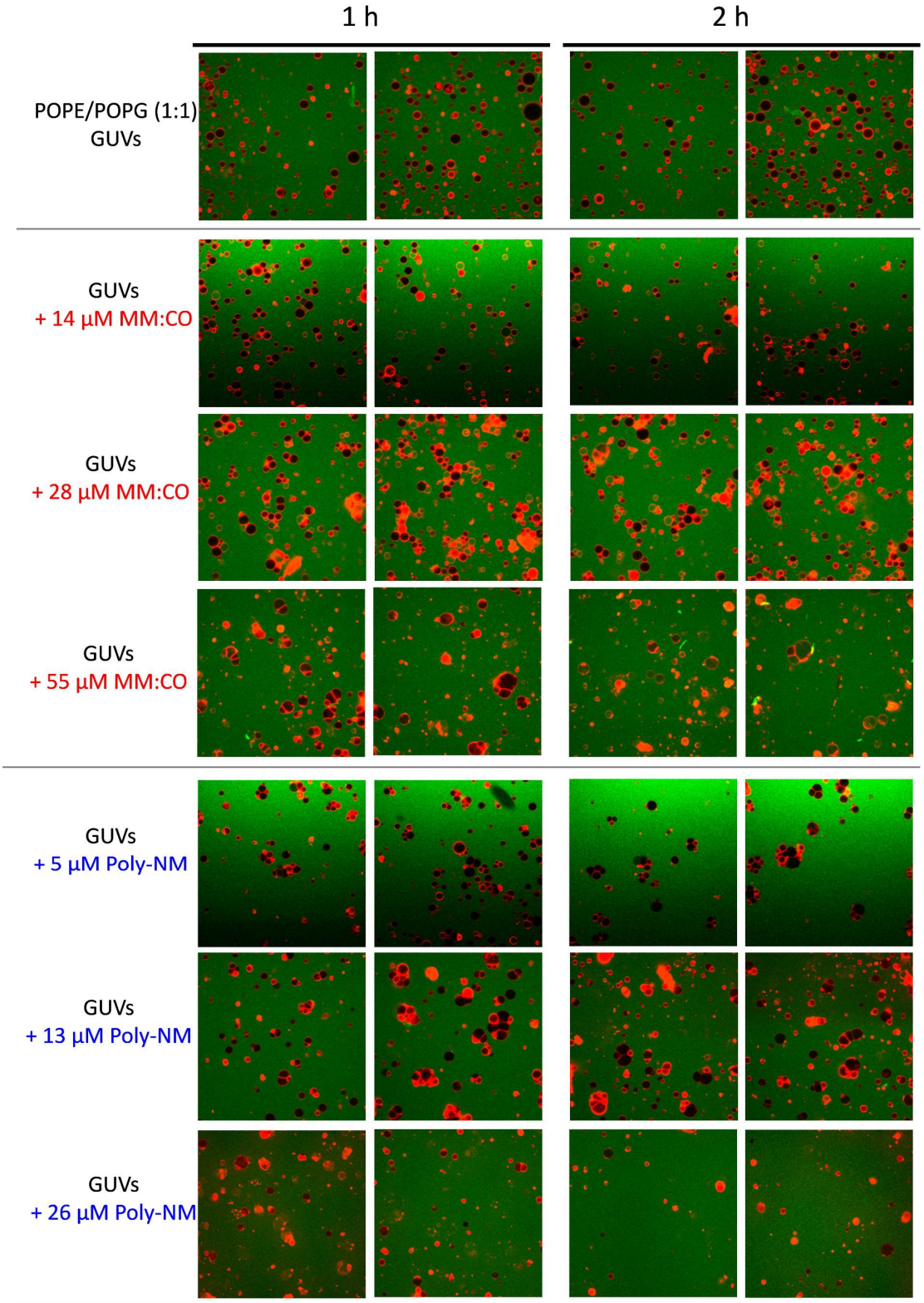


Figure S 3 Total leakage induced by 28 µM MMCO (A, red) or 13 µM Poly-NM (B, blue) in POPE/POPG 1:1 GUVs (stars) and in LUVs (circles) as a function of incubation time.

In Figure S 4, the same GUV microscopy images are given as in **Fehler! Verweisquelle konnte nicht gefunden werden.** together with additional examples with lower or higher smAMP concentrations as indicated. Generally, the same effects as discussed in the main text occur: vesicle adhesion/aggregation, leakage, or complete disintegration of GUVs upon addition of smAMPs.



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in MOPS buffer (25 mM MOPS, 130 mM NaCl, pH 7.0) containing 1 µM Atto488 at room temperature. SmAMP concentrations are given per subunit.