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Supporting Material

Pardaxin permeabilizes vesicles more efficiently by pore formation than by disruption

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Supplementary Information

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MATERIALS AND METHODS:

Peptide synthesis: Pardaxin (sequence GFFALIPKIISSPLFKTLLSAVGSALSSSGGQE) was synthesized on an automatic CEM liberty microwave assisted peptide synthesizer by solid-phase synthesis using standard Fmoc chemistry on Wang resin. Wang resin was used with the C-terminal Glu residue coupled to the linker group at a concentration of 0.66 mmol Glu/g_{resin}. The resin was initially swelled with DCM for 30 min, followed by 5 repeating washes with DMF. The Fmoc protective group was removed with 20% piperidine solvated in DMF. Fmoc-amino acids were coupled to the resin bound peptide in 4 times molar excess together with 4 times molar excess of HBTU and 8 times molar excess of DIPEA solvated in DMF. The resin was washed with DMF and the procedure was repeated for each residue. The Lysine side chains had BOC as a protective group. The crude peptide mix was run on a on a Dionex Ultimate 3000 HPLC system with a Reverse Phase semi-preparative C16 column and an AJO-7371 guard column in an AJO-7220 guard column system. Prior to each purification, the column was equilibrated with 15% acetonitrile. After loading the peptide to the column, an initial step with 15% acetonitrile was run for 2 min followed by a gradient from 15% acetonitrile from 15% to 99.9% acetonitrile over a period of 30 min. The column was allowed to run another 30 min at 99.9 % acetonitrile followed by a gradient from 99,9% acetonitrile to 15% acetonitrile over a period of 30 min. The eluent solutions all contained 0.1 % TFA. The fractions containing peptides were freeze-dried and the peptide dissolved in the appropriate buffer.

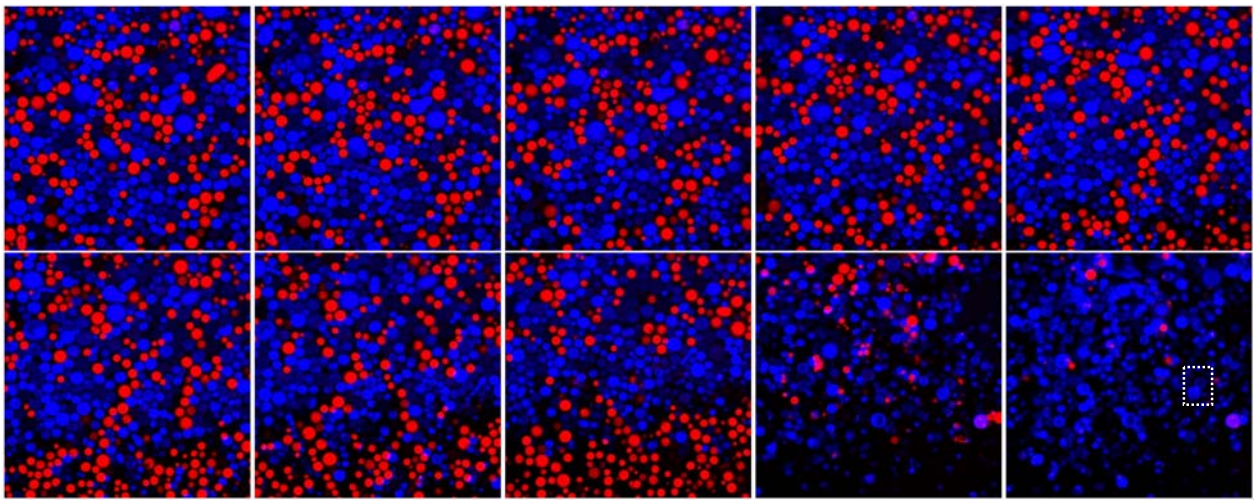
Preparation of Giant Unilamellar Vesicles (GUVs): GUVs were prepared from chloroform stocks containing 10 g/L lipid as well as 0,01 g/L DiI. ~10 µl of chloroform lipid stocks were spread on each platinum electrode. The chamber was incubated at 50 °C for 2 h to evaporate any remaining solvents and then filled with a solution of 200 mM sucrose containing $Alexa^{488}$ or $Alexa^{633}$ hydrazide. The platinum wires were connected to a function generator (Digimess FG 100, Grundig Instruments, Nürnberg, Germany), and a low-frequency alternating field sinusoidal wave function with a frequency of 10 Hz and amplitude of 1,5 V was applied for 90 min followed by 30 min at 1 Hz and amplitude of 3 V. The GUV´s were removed from the platinum electrodes by gently pipetting up and down. The excess free fluorophor was removed by running the GUV´s over a PD10 column pre-equilibrated with 200 mM glucose. The eluent from the column was collected in 0.5 ml fractions; samples were selected on the basis of absorption of free fluorophor and transferred to an eight-well microscopy chamber (Lab-Tek Brand Products, Naperville, IL). Tris pH 8.0 was added to a final concentration of 10 mM and the GUVs were left overnight at 4 °C to allow them to sediment at the bottom of the viewing chamber prior to viewing.

Solid-state NMR measurements: Solid-state NMR experiments were performed on a Bruker Avance 400 (9.4 T) spectrometer using a 5 mm Bruker triple-resonance probe for static-sample experiments. The ^{1}H RF field strength was 35 kHz for the 90° excitation pulse while it was reduced

to 20 kHz during the 3 ms cross-polarization (CP) contact period as well as during the t_1 period of the PISEMA experiment. The ^{15}N RF field strength was 20 kHz during CP and t_1 of the PISEMA experiment (19). SPINAL-64 (20) with an rf field strength of 20 kHz was employed for decoupling during 20 ms detection. The PISEMA experiment employed 45 t_1 increments, each acquired using 64 scans. Upon processing, the scaling factor of $\sqrt{2/3}$ in the indirect dimension was accounted for by increasing the spectral width accordingly. Optimal spectral quality was obtained at 32°C for samples containing only PC lipids and at 30°C for samples containing PG lipids.

Supplementary Figure S1:

Action of Pardaxin on a mixed population of two different vesicles. Confocal Laser Scanning Microscopy images of vesicle contents release from Giant Unilamellar Vesicles consisting either of 100% DOPC (blue, filled with Alexa 488) or 20% DOPG and 80% DOPC (red, filled with Alexa 633) after addition of Pardaxin, followed over 36 minutes. Each image represent ~ a 4 min interval. Peptide was injected at $t = 0$. The dashed box in the last frame is magnified in the zoom below. Clearly the contents of the DOPC:PG vesicles disappear over the time course of the experiment, which is compatible with the lysis of the vesicles observed in Fig. 5. The pure DOPC vesicles release their contents more slowly, probably due to preferential binding of Pardaxin to the DOPC:PG vesicles, and the emptied vesicles (highlighted in the zoom) retain a halo at the edges, probably due to slow diffusion at the membrane.



Zoom after 36 minutes: