Supplementary Information

Membrane-selective Nanoscale Pores in Liposomes by a Synthetically Evolved Peptide: Implications for Triggered Release

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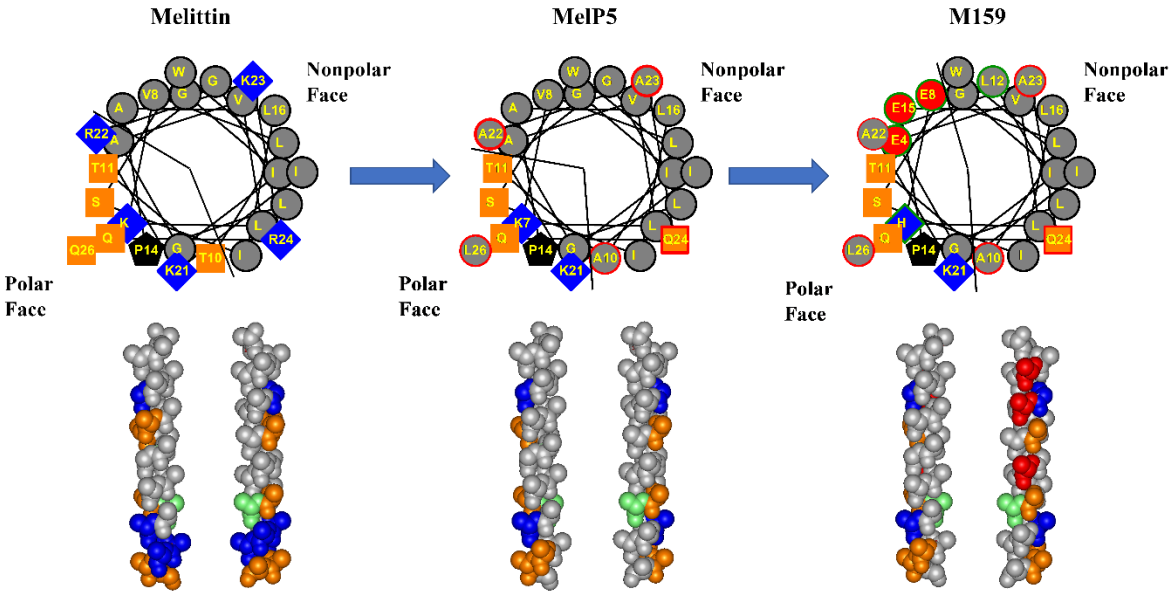


Figure S1. Overview of the synthetic molecular evolution leading to the discovery of the macrolittins. In the first generation, we created a library based on the non-selective, transient pore-former melittin, from Honeybee venom, and used a screen based on lipid vesicle leakage to select highly potent equilibrium pore forming peptides. The most active gain-of-function variants is called MelP5.[1] In the second generation we made a library based on MelP5 and selected for highly potent macromolecular poration in lipid vesicles, thereby identifying the macrolittins.[2] Helical Wheel projections of the peptides are shown, in which acidic (red), basic (blue) and polar (yellow) residues highlight amphipathicity.

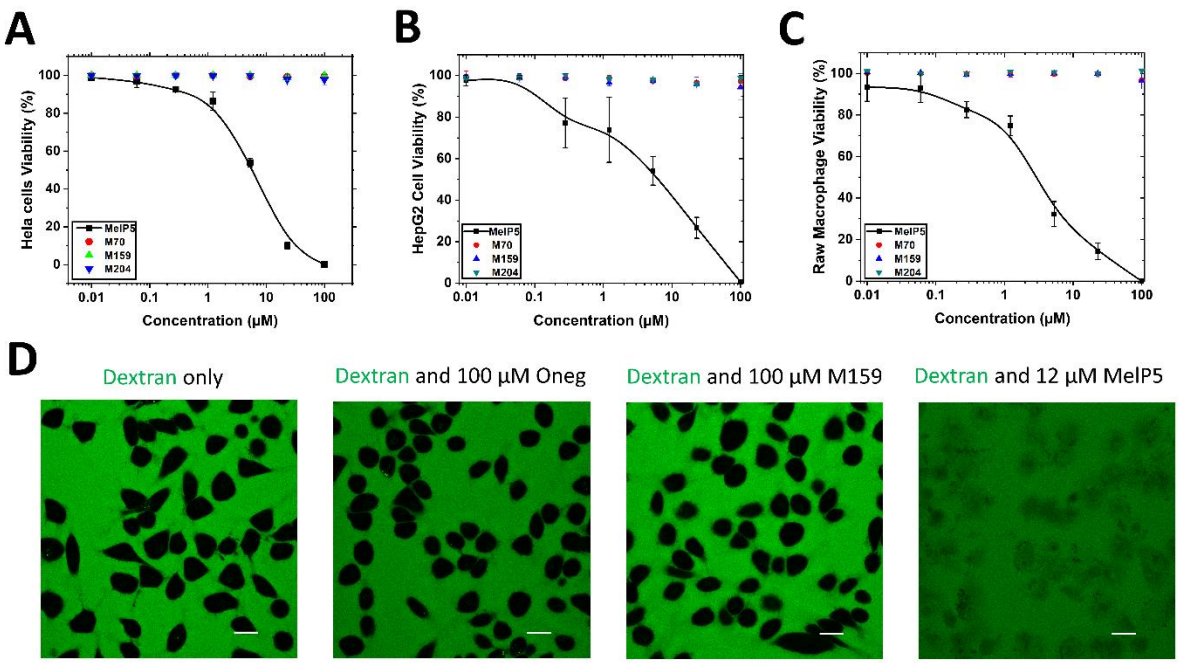


Figure S2. Effect of macrolittins and MelP5 on mammalian cells. (**A**-**C**) Different concentrations of MelP5 and three macrolittins were incubated with HeLa cells (**A**), HepG2 cells (**B**) and raw macrophages (**C**) at around 80% cell confluency for 3 h. After 24 h, cells were subjected to a cell toxicity assay using Alamar Blue reagent. (**D**) HeLa cells were incubated with 10 kDa AF-labeled dextran which marks the external space between cells, and peptides at around 80% cell confluency for 30 min. Then cells were observed using confocal microscopy (from left to right: dextran alone; dextran and Oneg; dextran and M159; dextran and MelP5). Intact cells are black because the dextran is excluded. Scale bar = 20 μm.

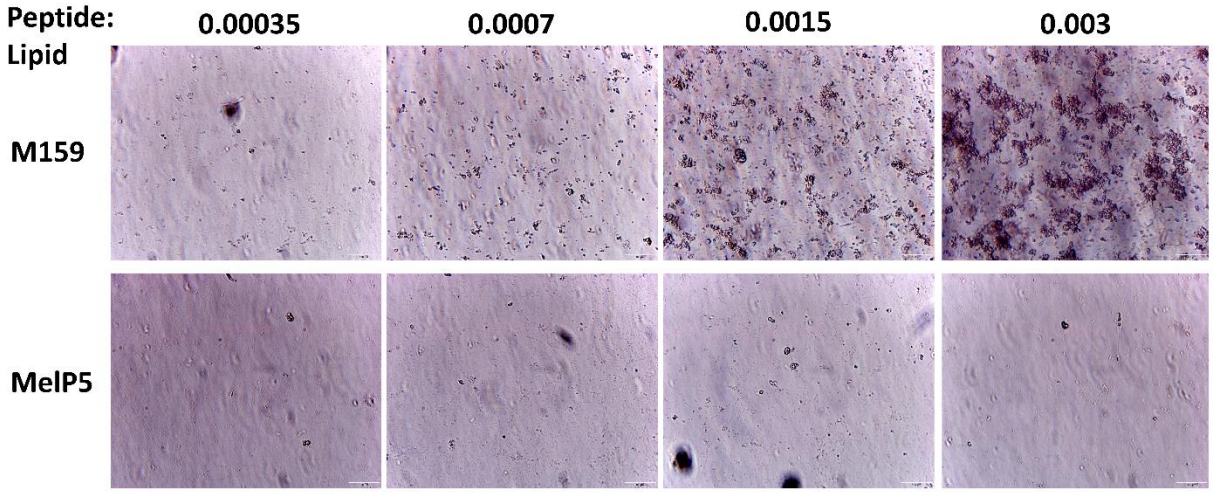


Figure S3. M159-induced aggregation of POPC liposomes. POPC liposomes were incubated with M159 or MelP5 at different P:L for 3 h, and the mixtures were observed in a light microscope. (upper panel: M159 treatment; bottom panel: MelP5 treatment). Peptide-to-lipid ratios are shown at the top (scale bar = 100 μm)

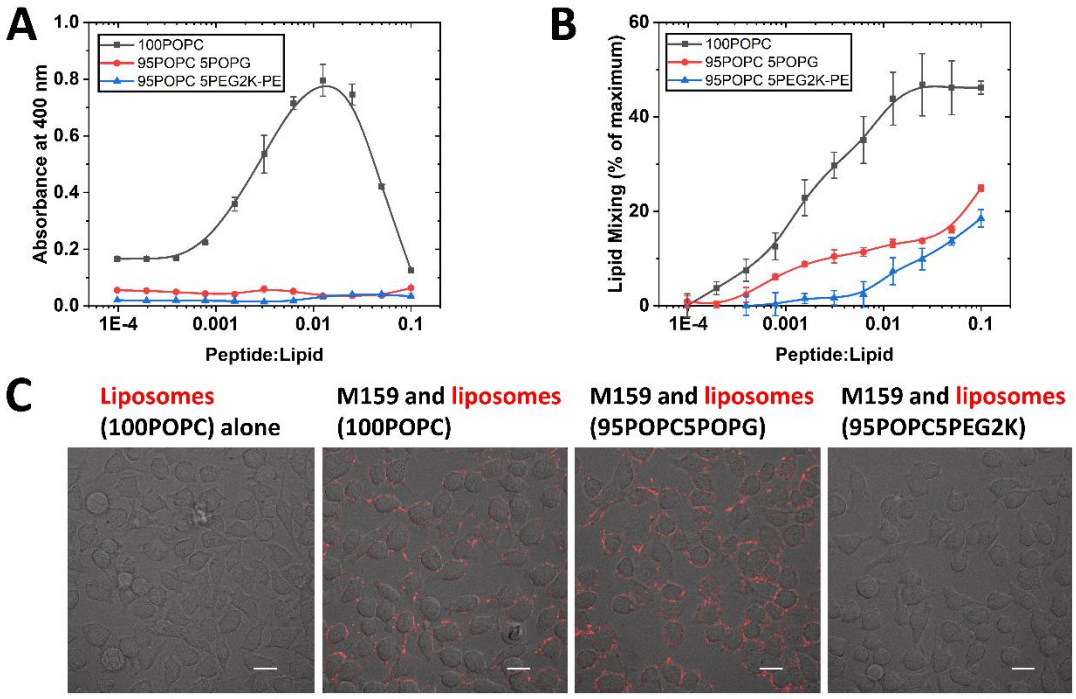
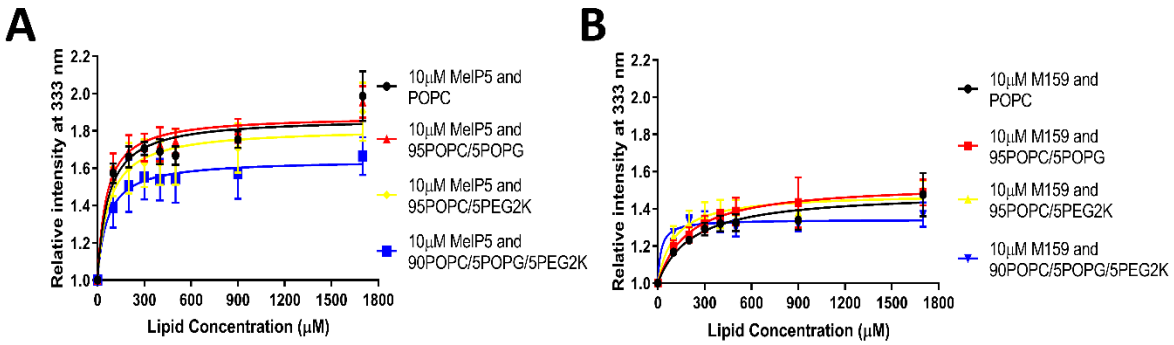


Figure S4. Inhibition of liposomal aggregation and fusion. (**A**) Light scattering of different composition (100% POPC, 95% POPC and 5% POPG, 95% POPC and 5% PEG2K-PE) liposomes with M159 treatments. 2 mM liposomes were incubated with M159 for 3 h at different P:L. (**B**) FRET between dual labeled liposomes and non-labeled liposomes (100% POPC, 95% POPC and 5% POPG, 95% POPC and 5% PEG2K-PE). 0.4 mM liposomes containing 0.5% NBD-PE and 0.5% rhodamine-PE dyes were mixed with 2 mM unlabeled liposomes and were incubated with M159 for 3 h at different P:L. (**C**) 32 μM M159 were incubated with different compositions of 1.6 mM 0.1% rhodamine-labeled liposomes in the presence of HepG2 cells at around 80% cell confluency for 30 min. Cells were washed and observed in a confocal microscope (from left to right: 100% POPC alone; 100% POPC and M159; 95% POPC, 5% POPG and M159; 95% POPC, 5% PEG2K and M159 (ex/em = 512/560 nm). Scale bar = 20 μm.



C

	MelP5 and POPC	MelP5 and 95POPC/5 POPG	MelP5 and 95POPC/5 PEG2K	MelP5 and 90POPC/5 POPC/5PE G2K	M159 and POPC	M159 and 95POPC/5 POPG	M159 and 95POPC/5 PEG2K	M159 and 90POPC/5 POPG/5PE G2K
Kp	812526	981728	747990	871018	247790	282672	527879	323900
Std. Error	231056	252779	285734	367285	59949	67374	150770	409000

Figure S5. MelP5 or M159 binding to lipid bilayers, as indicated by tryptophan fluorescence intensity increase upon liposome additions. Different liposomes (100% POPC; 95% POPC, 5% POPG; 95% POPC, 5% PEG2K-PE; 90% POPC, 5% POPG, 5% PEG2K-PE) binding assay of 10 μM MelP5 (**A**) and 10 μM M159 (**B**) at a series of lipid concentrations. The relative fluorescence intensities were normalized to the maximal value (333nm) divided by tryptophan fluorescence intensity without lipid addition using a fluorimeter. Curves were fitted to Eq (4) to determine the partition coefficient (K_p), which is shown in (**C**).

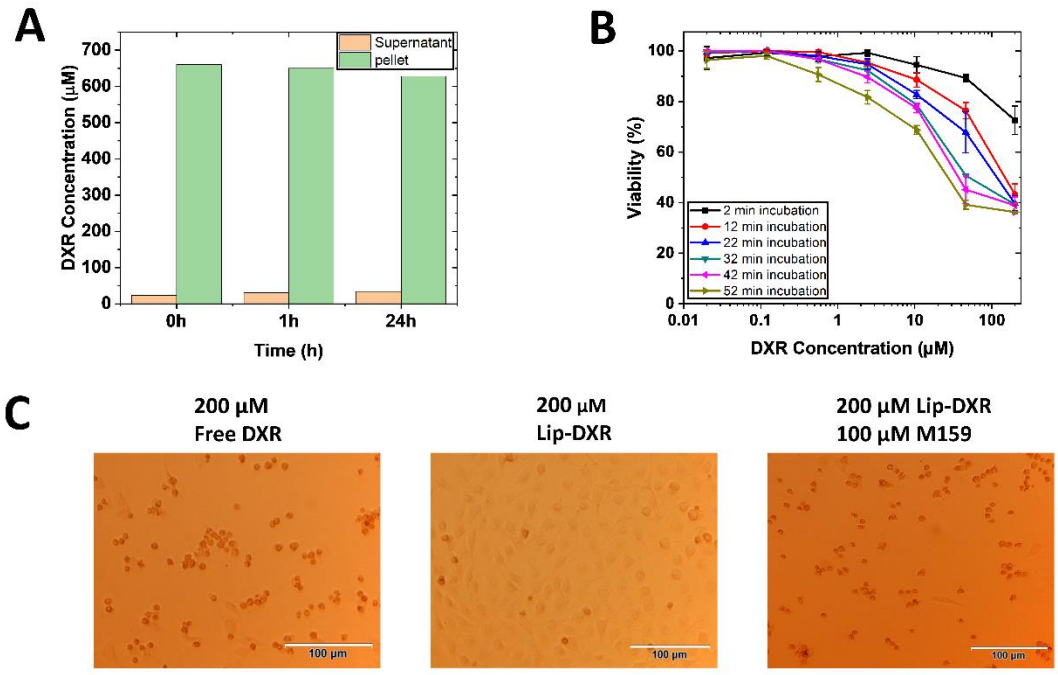


Figure S6. M159 and liposomes containing DXR in the presence of HeLa cells. (**A**) The stability of liposome-DXR. Doxorubicin was encapsulated into liposomes containing 70% POPC and 30% cholesterol by remote loading method. After preparation, at specific time points, liposome-DXR was ultracentrifuged (130000 x g) and DXR concentrations in the supernatant and in liposomes (pellet) were measured using a nanodrop. (**B**) Increasing concentrations of free DXR (0.02 to 200 μM) were incubated with HeLa cells at around 80% cell confluency for different time points (2 to 52 min). Cells were washed and subject to Alamar blue assay for viability measurements. (**C**) HeLa cells were treated with 200 μM free DXR (upper), 200 μM liposome-DXR (middle) and 200 μM liposome-DXR plus 100 μM M159 (bottom) at around 80% cell confluency for 15 min respectively. After 24 h, cell morphology was observed by an optic microscope. Scale bar = 100 μm.

Supplementary Video

2 mM NBD labeled 100% POPC liposomes was imaged by confocal microscope immediately after 60 μM M159 was added in liposome solution. The resultant video consists of 80 images from 0 minute to 40 minutes after M159 addition in a time-lapse manner. (30 seconds per image)

References

[1] G. Wiedman, T. Fuselier, J. He, P. C. Searson, K. Hristova, W. C. Wimley, *J Am Chem Soc* **2014**, *136*, 4724-4731.

[2] S. Li, S. Y. Kim, A. E. Pittman, G. M. King, W. C. Wimley, K. Hristova, *J Am Chem Soc* **2018**, *140*, 6441-6447.