Gain-of-function analogs of the pore-forming peptide melittin selected by orthogonal high-throughput screening

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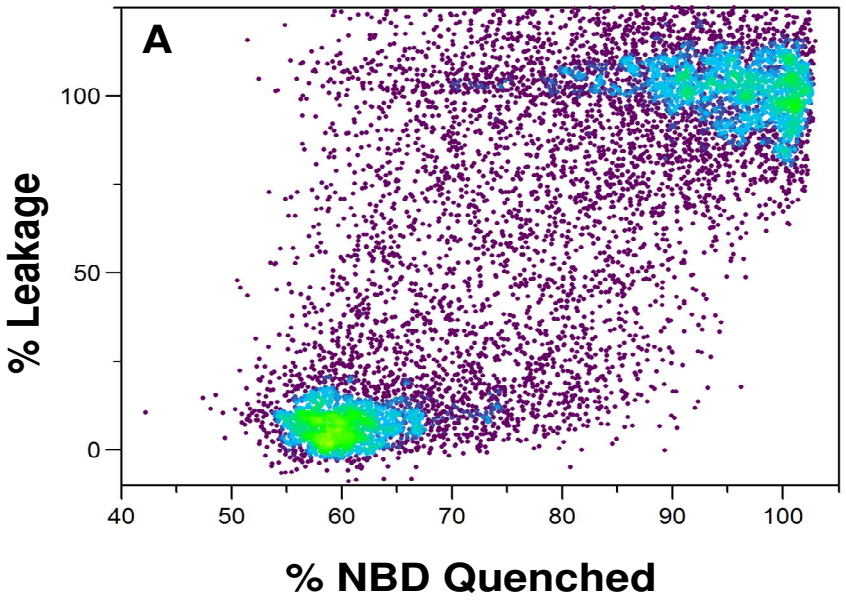
Supporting Information



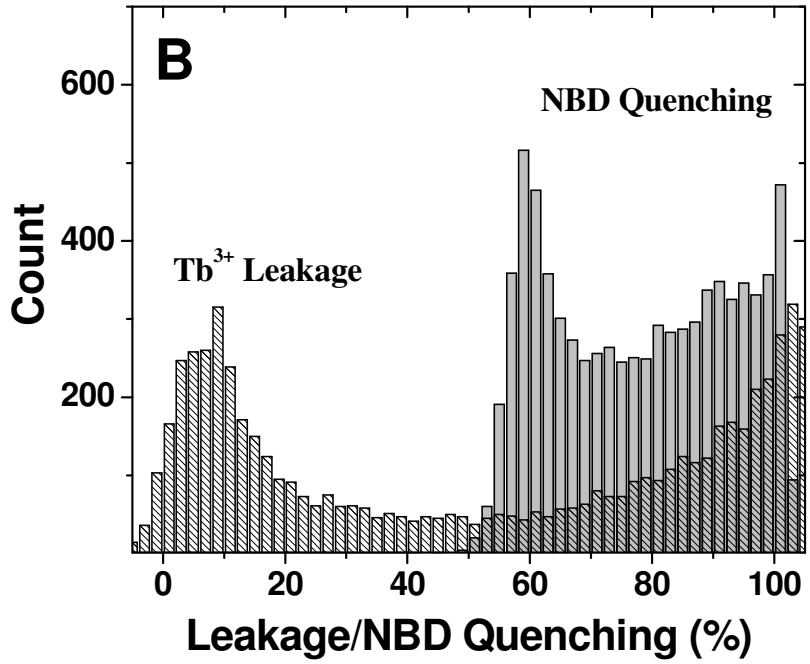


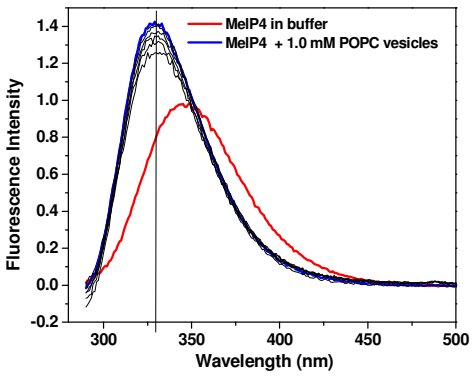
Figure S1. High-throughput screen for loss of function variants of melittin. In addition to the screen at high stringency (P:L = 1:1000), each library member was also screened at the same peptide concentration against a very low lipid concentration to give P:L =

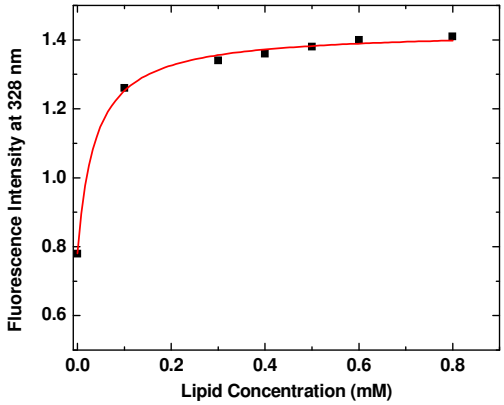
1:20. Note that there is a significant number of negative peptides in the distribution with leakage < 20% and NBD quenching < 65%.

Peptide Partitioning into Lipid Vesicles

Mole-fraction partition coefficients (K_x) were determined for all peptides binding to POPC and 10% POPG bilayers using fluorescence titration as described in detail elsewhere(*1-3*). Briefly, tryptophan fluorescence emission was measured on a fluorescence spectrophotometer with excitation at 270 nm and emission from 300 to 500 nm. Peptide fluorescence was measured in a titration experiment at least 15 min. after addition of an aliquot of phospholipid vesicles. Binding is observed as an increase in fluorescence intensity and a shift of the emission maximum to a shorter wavelength. Titrations of liposomes into buffer were used for background corrections, and titration into a solution of free tryptophan, which does not bind to liposomes, was used as an intensity control. Partition coefficients, K_x, were determined by non-linear regression.

The binding of melittin and the variants were measured by tryptophan fluorescence titration(*3*). The detailed results, shown in supplemental Table S1, indicate that melittin and the variants bind similarly to PC and to PG-containing vesicle membranes. The wavelength of tryptophan emission maximum of bound peptides, indicative of the polarity of the environment, was between 328 and 337 nm, with an average of about 332 nm which is very similar to the value of 330-331 for melittin itself. Thus the depth of membrane insertion of the variants is similar to that experienced by melittin; inserted into the interfacial zone of the membrane, but not deeply buried or completely excluded from contact with water.

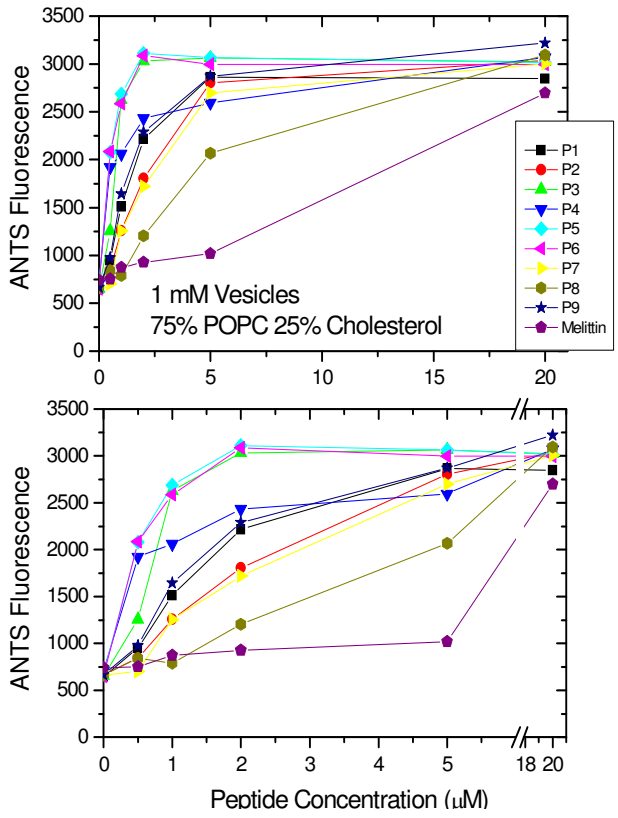




Supplemental Figure S2. Example membrane binding data. **Left**: Upon titration of lipid into a solution of peptide, the tryptophan fluorescence intensity increases and shifts to lower wavelengths, indicating a partitioning into the hydrophobic environment of the bilayer. **Right:** A plot of intensity at one wavelength against lipid concentration allows the determination of a mole fraction partition coefficient by curve fitting.

Leakage Studies

Leakage is reported in the manuscript as the concentration of peptide that causes 50% leakage in 1 mM lipid vesicles, or LIC_{50}. Leakage was measured by incubating 1 mM lipid vesicles with increasing amounts of peptide and measuring Tb^{3+}/DPA or ANTS/DPX fluorescence.



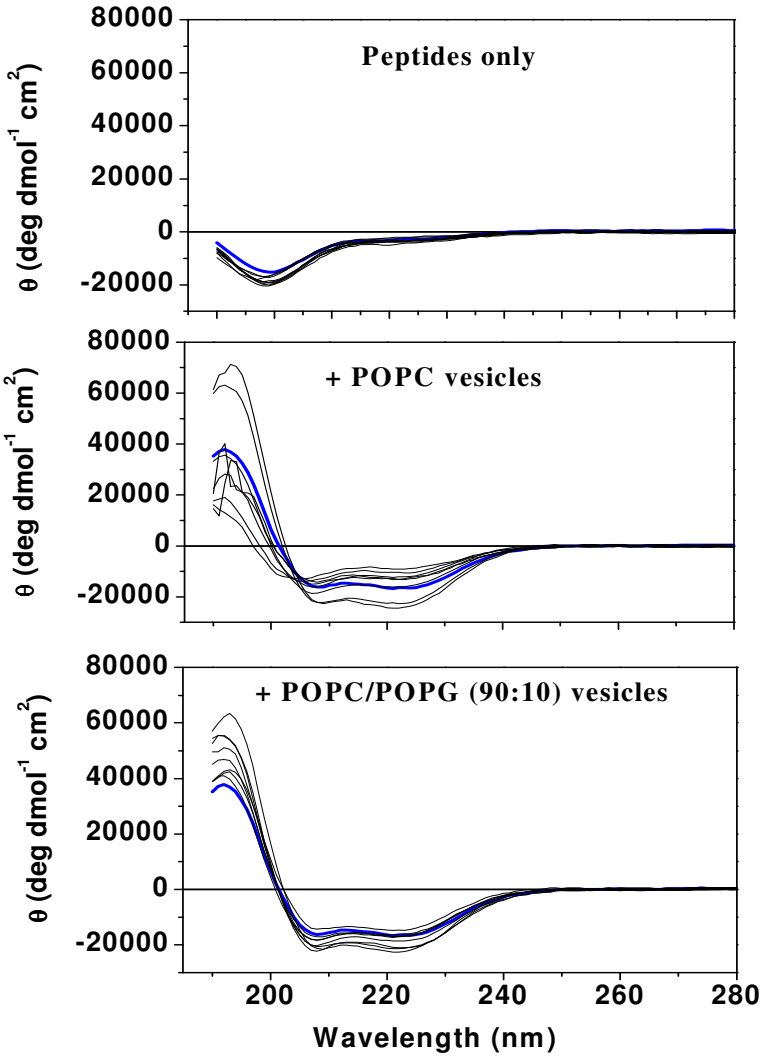
Supplemental Figure S3. Example leakage experiment, raw data. Increasing peptide concentrations were added to 1 mM lipid vesicles containing the entrapped fluorophore ANTS and its quencher DPX. Leakage was allowed to proceed for 3 hours and then the fluorescence of ANTS was measured in all experimental samples and in samples that had been lysed with a high concentration of melittin. Leakage versus concentration is plotted (as in this example data set) and LIC_{50} is determined by curve fitting to a hyperbolic/sigmoidal function. In this example data set, we show the same data with and without a break in the X-axis. The data shown here are for vesicles made with 75% POPC and 25% cholesterol. The two panels are the same data shown with and without a X-axis break. The leakage curves for all lipid compositions and for Tb^{3+}/DPA as well as ANTS/DPX had the same features.

Circular Dichroism

Circular dichroism was used to study the secondary structure of melittin-derived peptides in solution. Measurements were made in the absence and in the presence of lipid vesicles. Like melittin, the selected peptides are random coil in water and in low ionic strength buffer. In the presence of membranes, all of the peptides adopted α-helical secondary. In bilayers, most of the variants have slightly higher helical content than melittin, consistent with the better amphipathicity of the variant sequences. However, neither membrane binding nor the percent helicity are correlated with differences in pore-forming activity. In terms of membrane binding and secondary structure, the melittin analogs are very similar to melittin. Differences in activity between them are not due to large changes in solution behavior, membrane binding or secondary structure. Instead, differences between the parent melittin and the gain-of-function analogs are due to differences in the pore state of the peptide.

Circular dichroism was measured with a Jasco J-810 Spectropolarimeter using 1 mm rectangular quartz cuvettes. CD spectra were measured for solutions of 25 μM peptide in buffer and then after addition of either 1.0 mM POPC LUVs, or in 1.0 mM POPC:POPG (9:1) LUVs. Fractional helical content (f_α) was estimated according to the equation $f_\alpha = (\Theta_{obs} - \Theta_{RC}) / (\Theta_H - \Theta_{RC})$, where θ_{OBS} is the observed mean-residue molar

ellipticity at 222 nm. θ_H and θ_{RC} are the predicted mean-residue molar ellipticity value for 100% helix (-34,500) and 100% random coil (-5,600) at 20 °C. Percent helicity of bound peptide takes into account the fraction of bound peptide (f_B) of each peptide, calculated with each partition coefficient (K_x), the 1.0 mM lipid in each sample, and the fractional helical content (f_α) : % helicity of bound peptide = [(1/f_B) * f_α] x 100%



Supplemental Figure S4. Solution Circular Dichroism. **Top:** CD of melittin and all selected variants in buffer show that all peptides are random coil in buffer. The spectrum for melittin is shown in blue. **Middle:** Upon addition of 1 mM POPC vesicle, the circular dichroism shows α-helical content for all peptides ranging from about 30% for Mel-P1 to about 70% helix for Mel-P5 (See table below for values). The spectrum for melittin is shown in blue. **Bottom:** Upon addition of 1 mM POPC/POPG vesicles, the solution circular dichroism shows α-helical content for all peptides. The differences in

helix content are smaller in POPG-containing vesicles in which all peptides are 50-70% helical (See table below for values). The spectrum for melittin is shown in blue.

Binding and Structure in POPC Vesicles					
Peptide	Delta G	λ max	Fbound	F alpha	Falpha/bound
Melittin	-8.21	331	0.95	0.35	0.37
Mel-P1	-7.00	330	0.71	0.28	0.40
Mel-P2	-7.00	333	0.71	0.47	0.67
Mel-P3	-8.63	333	0.97	0.33	0.33
Mel-P4	-8.10	334	0.94	0.76	0.81
Mel-P5	-8.21	335	0.95	0.71	0.74
Mel-P6	-9.11	335	0.99	0.50	0.51
Mel-P7	-8.53	333	0.97	0.41	0.42
Mel-P8	-7.86	334	0.91	0.38	0.42
Mel-P9	-7.78	332	0.90	0.41	0.45

Binding and Structure in POPC/POPG Vesicles					
Peptide	Delta G	λ max	Fbound	F alpha	Falpha/bound
Melittin	-8.59	330	0.98	0.52	0.53
Mel-P1	-7.02	330	0.71	0.63	0.87
Mel-P2	-7.93	335	0.92	0.51	0.55
Mel-P3	-9.29	331	1.00	0.66	0.67
Mel-P4	-7.28	330	0.80	0.49	0.61
Mel-P5	-9.72	328	1.00	0.70	0.70
Mel-P6	-9.54	336	1.00	0.52	0.52
Mel-P7	-8.72	333	0.98	0.59	0.60
Mel-P8	-8.06	337	0.93	0.52	0.55
Mel-P9	-9.10	330	0.99	0.54	0.54

error	+/- 0.4	+/- 3 nm	+/- 0.1	+/- 0.1	+/- 0.14

Supplemental Table S5. Binding and secondary structure. Binding of all peptides was measured as shown in Figure S2 to POPC vesicles and to vesicles with 10% POPG. Free energy is calculated as $\Delta G = -RT\ln(Kx)$ where Kx is the mole fraction partition coefficient. λmax is the wavelength of the maximum in the tryptophan fluorescence emission spectra. Fraction bound is calculated for 1 mM lipid concentration as used in CD experiments. Fraction helix is calculated from the maximum ellipticity(*4*). Fraction α/Fraction bound is the helicity of the bound peptide. Typical standard deviations in the measured values are given in the last row.

References

1. Wimley, W. C. (2010) *Adv. Exp. Med. Biol. 677*, 14-23.
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3. Ladokhin, A. S., Jayasinghe, S., and White, S. H. (2000) *Anal. Biochem. 285*, 235-245.
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