Supporting Information for:

Native Mass Spectrometry of Antimicrobial Peptides in Lipid Nanodiscs Elucidates Complex Assembly

Lawrence R. Walker,[1] Elaine M. Marzluff,[2] Julia A. Townsend,[1] William C. Resager,[1] and Michael T. Marty[1]*

[1] Department of Chemistry and Biochemistry, The University of Arizona, Tucson, AZ 85721
[2] Department of Chemistry, Grinnell College, Grinnell, IA 50112
*Email: mtmarty@emaill.arizona.edu

SUPPLEMENTAL FIGURES

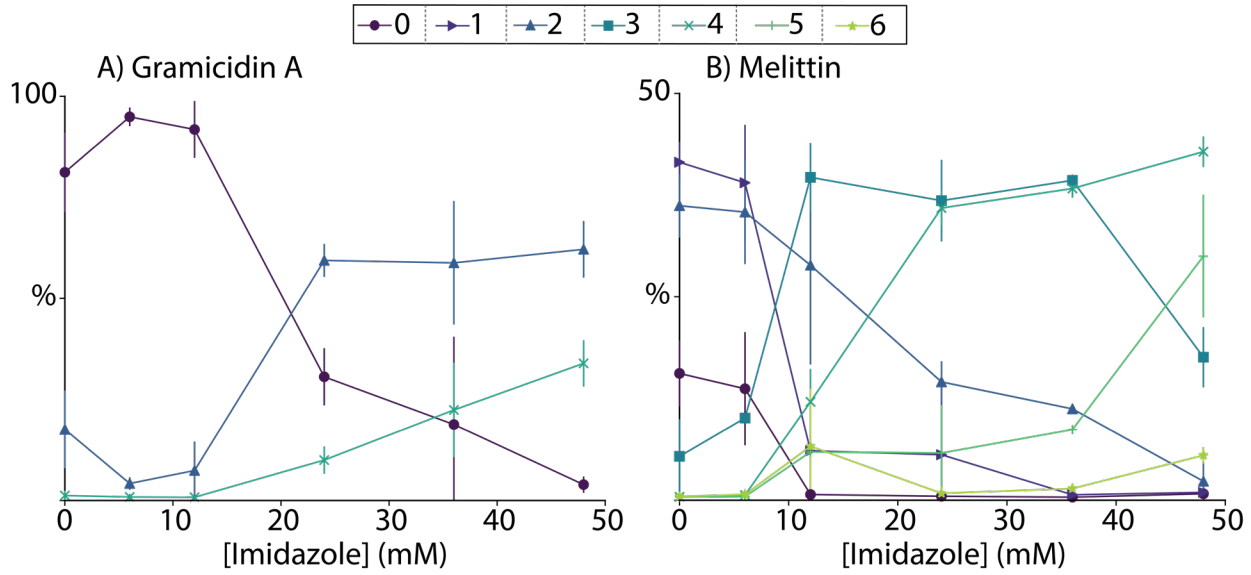

Figure S1. The effect of imidazole concentration on relative intensities of incorporated GA (A) or melittin (B) peptides (0–6) in DMPG nanodiscs at a 6/1 added GA/nanodisc ratio or 12/1 melittin/nanodisc ratio. As the concentration of imidazole was increased, higher numbers of incorporated peptides were observed.

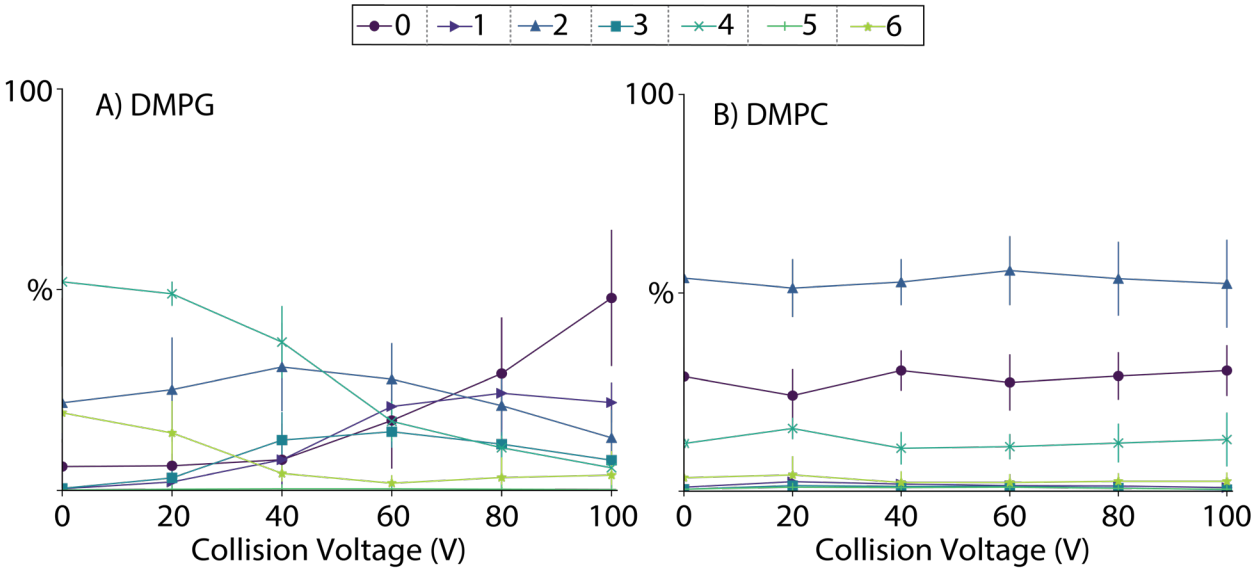

Figure S2. The effect of in-source trapping collision voltage on the measured number of incorporated gramicidin A for (A) DMPG nanodiscs (at 9/1 ratio of GA/nanodisc) and (B) DMPC nanodiscs (at 6/1 ratio of GA/nanodisc).

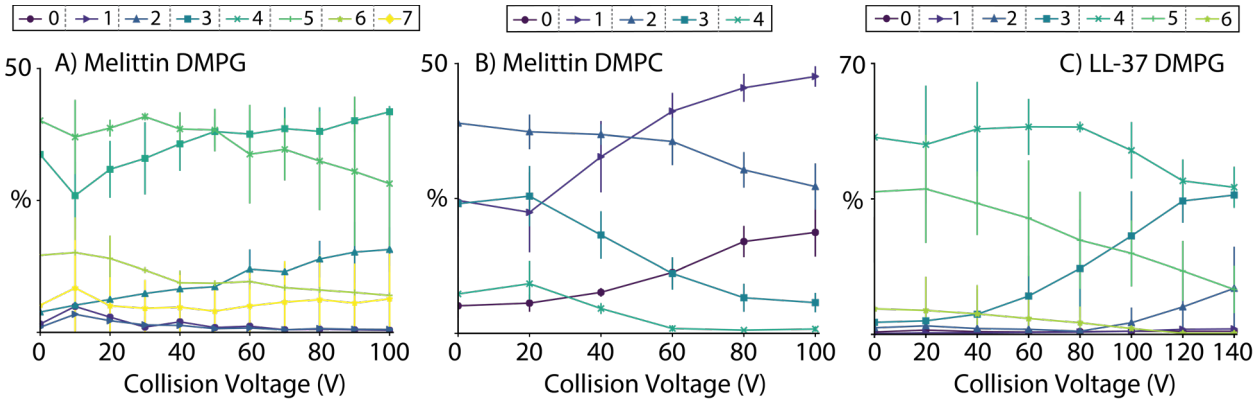

Figure S3. The effect of in-source trapping collision voltage on the measured number of incorporated peptides at 12/1 peptide/nanodisc ratio for (A) melittin in DMPG nanodiscs; (B) melittin in DMPC nanodiscs; and (C) LL-37 in DMPG nanodiscs.

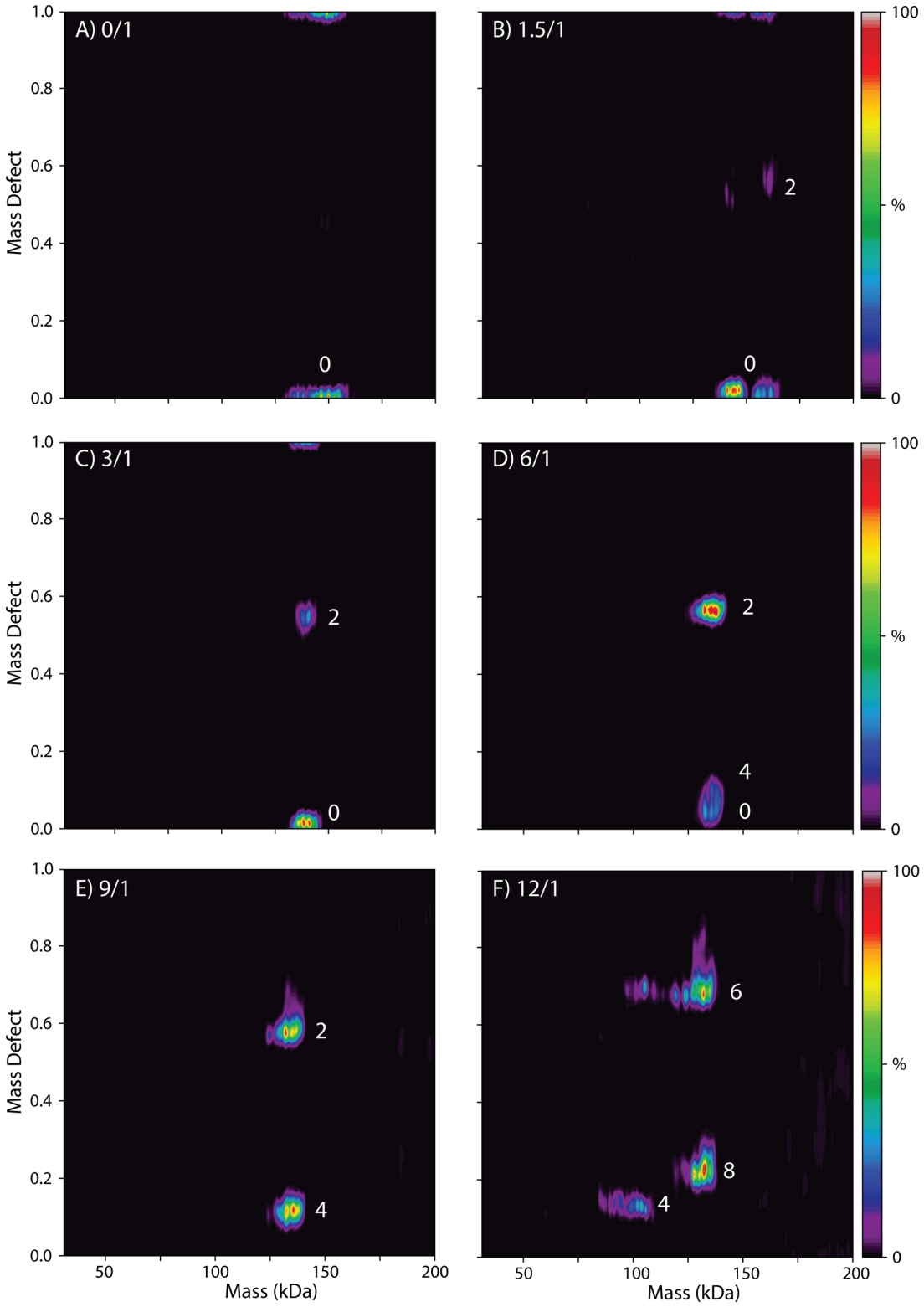

Figure S4. Representative mass defect plots from a titration of GA into DMPC nanodiscs at different molar ratios of GA/nanodisc (annotated as A-F). The number of incorporated GA peptides for each set of peaks is labelled.

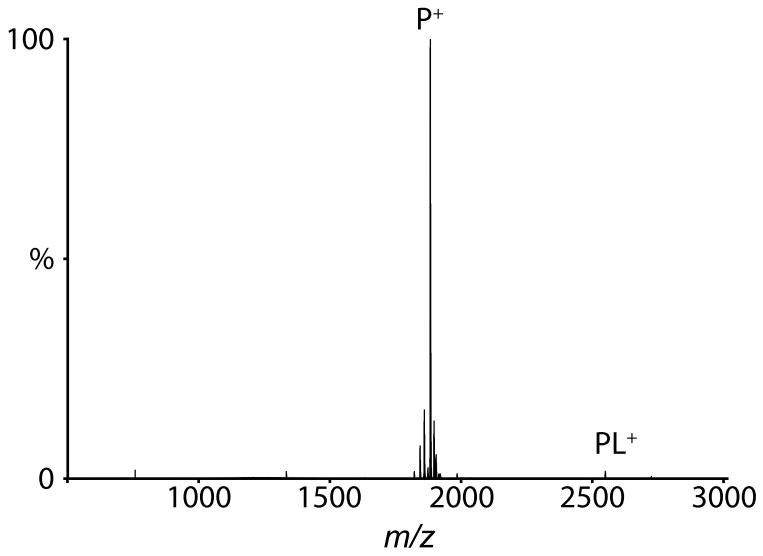

Figure S5. Mass spectrum of gramicidin A ejected from DMPG nanodiscs isolated in the quadrupole and activated with 50 V in the HCD cell. The primary species detected is free peptide (P), but a small peak for a peptide-lipid complex (PL) is observed. The molar ratio of added GA/nanodiscs was 9/1.

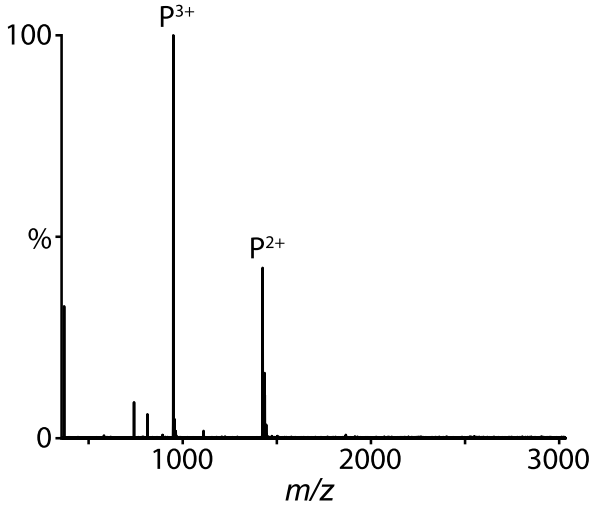

Figure S6. Melittin diluted to 25 μM in 0.2 M ammonium acetate at pH 6.8 (13% methanol by volume) with no nanodiscs present and 25 mM imidazole. Only monomeric melittin peptide (P) was observed.

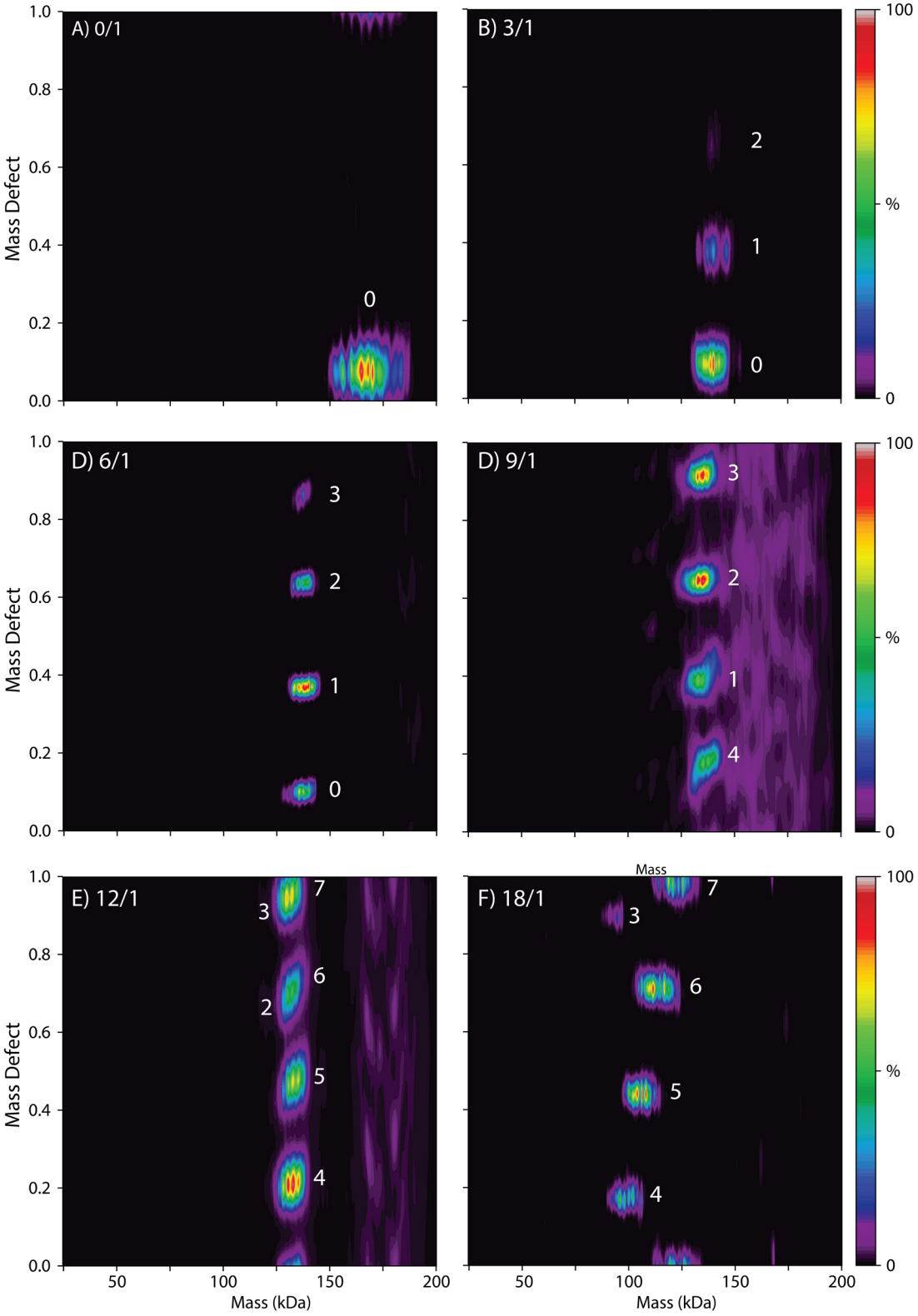

Figure S7. Representative mass defect plots from a titration of melittin into DMPG nanodiscs at different molar ratios of melittin/nanodisc (annotated as A-F). The number of incorporated peptides for each set of peaks is labelled.

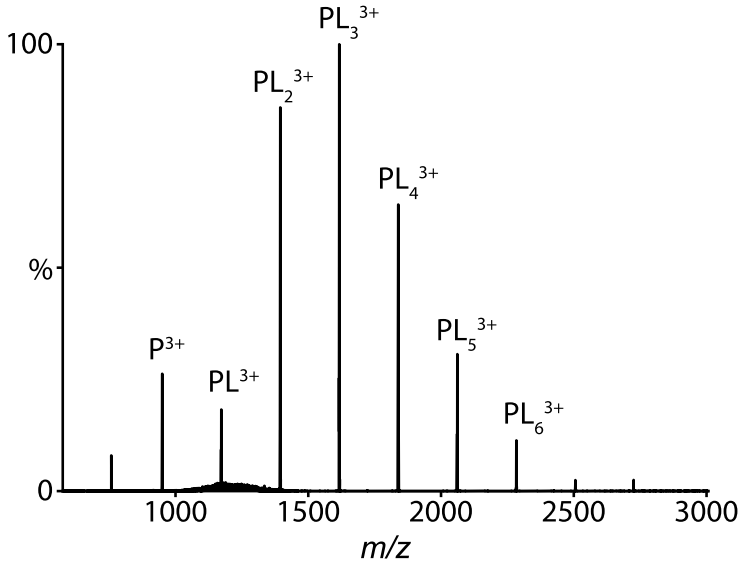

Figure S8. Mass spectrum of melittin ejected from DMPG nanodiscs isolated in the quadrupole and activated with 50 V in the HCD cell. Ejected melittin is monomeric but retains several bound lipids. The stoichiometry of the peptide (P) and bound lipids (L) are annotated along with the charge state. The molar ratio of added melittin/nanodiscs was 9/1.

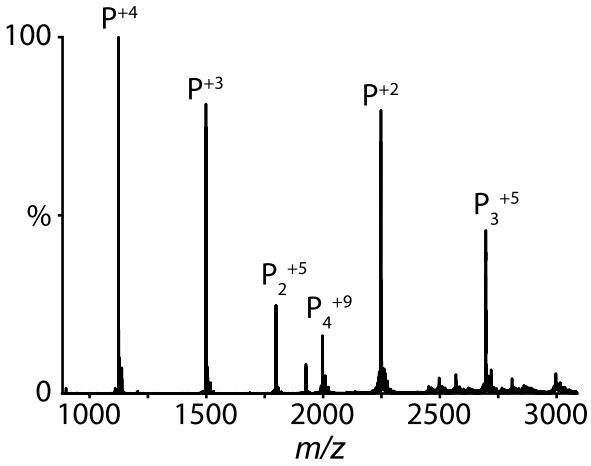

Figure S9. Mass spectrum of LL-37 diluted to 25 μM in ammonium acetate with imidazole. The stoichiometry of the peptide (P) and charge states are annotated.

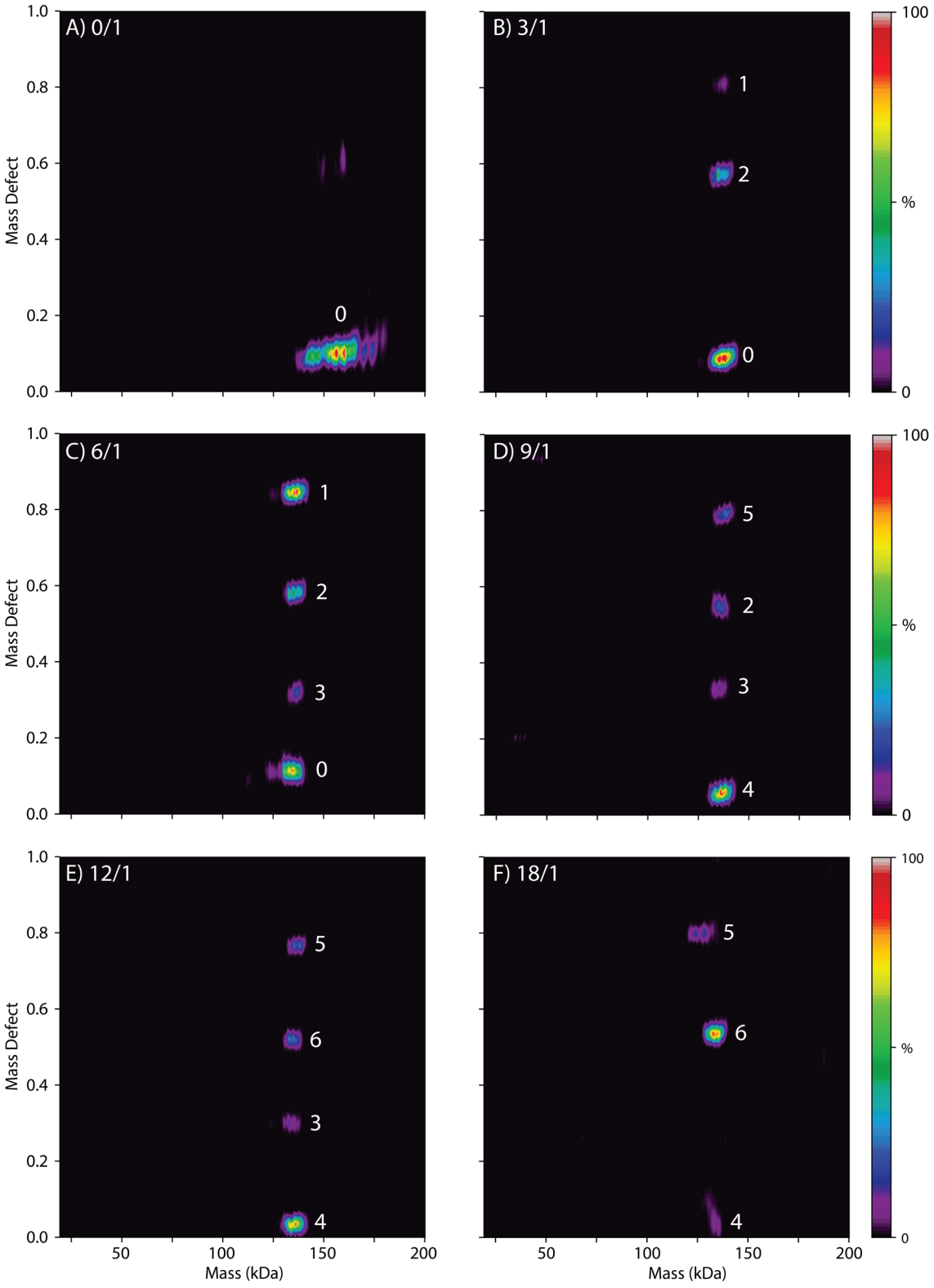

Figure S10. Representative mass defect plots from a titration of LL-37 into DMPG nanodiscs at different molar ratios of LL-37/nanodisc (annotated as A-F). The number of incorporated peptides for each set of peaks is labelled.

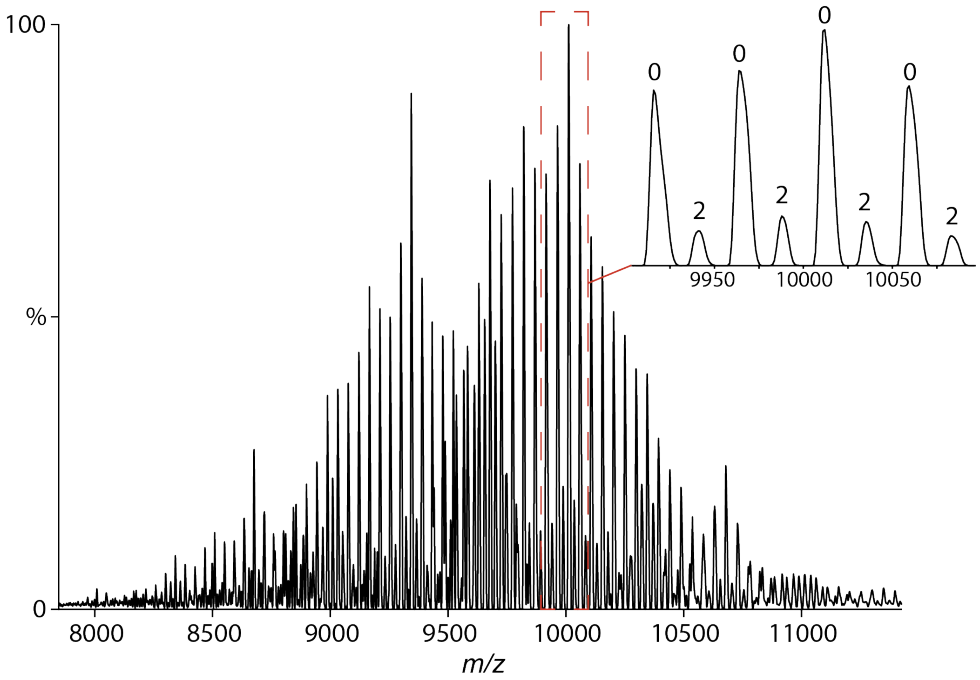

Figure S11. Raw mass spectrum of LL-37 nanodiscs with 3/1 LL-37/nanodisc. The zoomed inset shows two distinct peak series: one for empty nanodiscs and one with two incorporated LL-37 peptides.

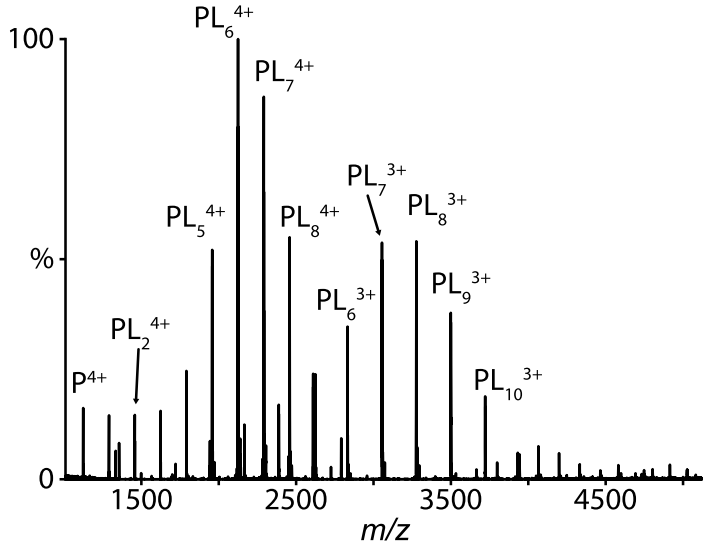

Figure S12. LL-37 peptides ejected from DMPG nanodiscs isolated in the quadrupole and activated with 50 V in the HCD cell. The stoichiometry of peptide (P) and bound lipids (L) are annotated along with the charge states. The molar ratio of added LL-37/nanodiscs was 9/1.

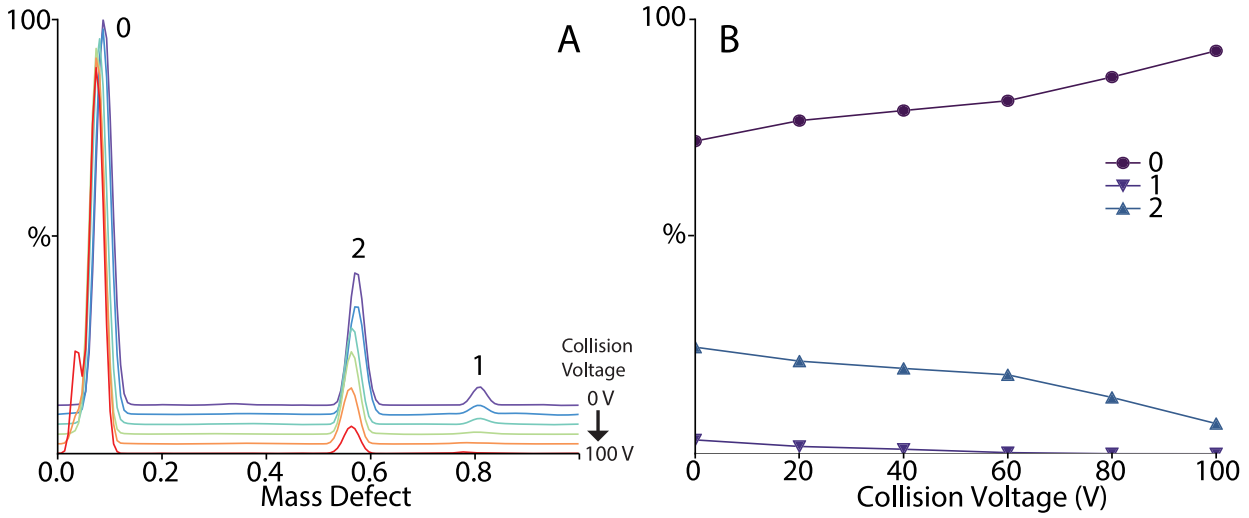

Figure S13. Dissociation of LL-37 from DMPG nanodiscs at a 6/1 peptide/nanodisc ratio. (A) Mass defects show primarily 0 or 2 LL-37 incorporated at this ratio. The peaks for 2 incorporated peptides decrease compared to the peak for 0 incorporated. (B) Relative intensities of each species in (A) as a function of collision voltage. The shoulder at a mass defect of 0.05 at 100 V is due to monomeric MSP belts that have dissociated from the complex.

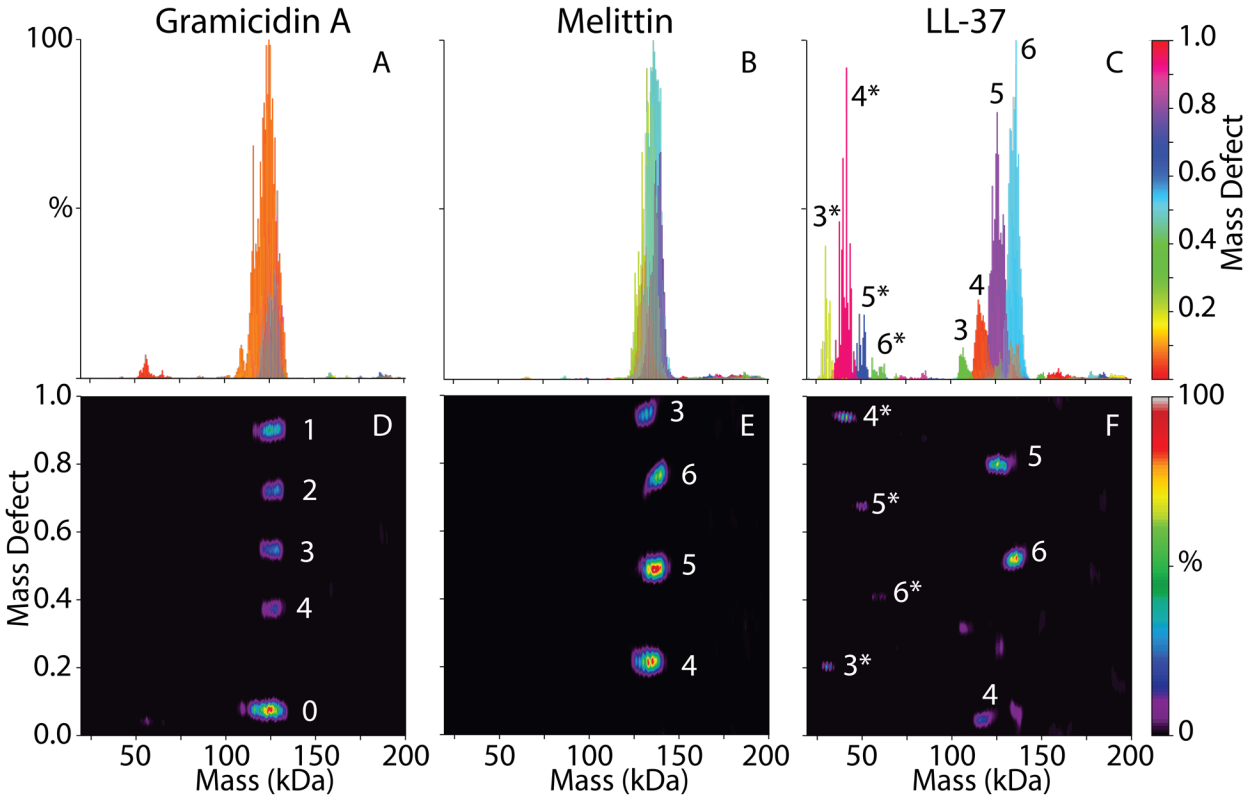

Figure S14. Comparison of the deconvolved mass distributions coloured by mass defect (A–C) or plotted versus mass defect (D–F) for DMPG nanodiscs with added GA (A, D), melittin (B, E), or LL-37 (C, F) activated with 80 V in the in-source trapping region. The number of peptides

incorporated are annotated for each mass defect and * marks peptides with bound lipids but no MSP belts. The molar ratios of peptide/nanodiscs were 9/1 for GA, 12/1 for melittin, and 12/1 for LL-37.

SUPPLEMENTAL TABLES

Peptide	Sequence	Charge	Molecular Weight (Da)	Mass Defect Values	
				DMPC	DMPG
Gramicidin A	HCO-VGALAVVVWLW-LWLW-NHCH$_2$CH$_2$OH	0	1882.3	0 – 0.03 1 – 0.80 2 – 0.58 3 – 0.36 4 – 0.13 5 – 0.91 6 – 0.68 7 – 0.46 8 – 0.24 9 – 0.01 10 – 0.79 11 – 0.57 12 – 0.34	0 – 0.10 1 – 0.92 2 – 0.74 3 – 0.57 4 – 0.39 5 – 0.21 6 – 0.03 7 – 0.85 8 – 0.68 9 – 0.50 10 – 0.32 11 – 0.14 12 – 0.96
Melittin	GIGAVLKVLTTGL-PALISWIKRKRQQ	+5	2846.5	0 – 0.03 1 – 0.20 2 – 0.40 3 – 0.60 4 – 0.80 5 – 1.00 6 – 0.19 7 – 0.39	0 – 0.10 1 – 0.27 2 – 0.54 3 – 0.81 4 – 0.07 5 – 0.34 6 – 0.61 7 – 0.88
LL-37	LLGDFFRKSKEKIGKEFK-RIVQRIKDFLRNLVPRTES	+6	4493.3	0 – 0.03 1 – 0.63 2 – 0.26 3 – 0.88 4 – 0.51 5 – 0.14 6 – 0.77 7 – 0.40	0 - 0.10 1 – 0.74 2 – 0.48 3 – 0.21 4 – 0.95 5 – 0.69 6 – 0.43 7 – 0.17

Table S1. Peptide sequences, solution charges, molecular weights, and mass defect values.