Electronic Supplementary Material (ESI) for Analyst.

Toward Sequencing of Electrostatic Interactions between Kdo_2-Lipid A and Cationic Antimicrobial Peptides via Ultraviolet Photodissociation Mass Spectrometry

Christopher M. Crittenden, Lindsay J. Morrison, Mignon D. Fitzpatrick, Allison P. Myers, Elisa T. Novelli, Jake Rosenberg, Lucas D. Akin, Sorin Srinivasa, Jason B. Shear, Jennifer S. Brodbelt[*]

Department of Chemistry, University of Texas at Austin, Austin, Texas 78712,

[*] Phone: (512) 471-0028, E-mail: brodbelt@cm.utexas.edu

Supporting Information

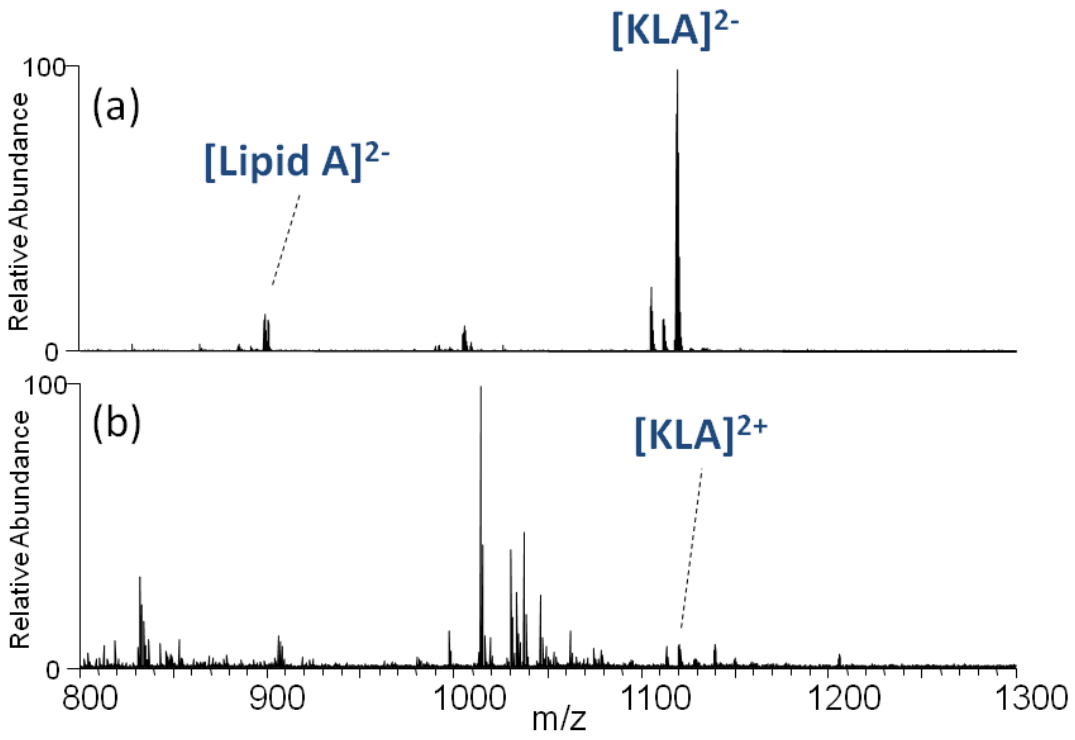

Figure S1: MS1 spectra of Kdo_2-lipid A (10 μM) in the (a) negative and (b) positive ion modes, sprayed in 5 mM ammonium acetate. Hexaacyl bisphosphorylated lipid A (*m/z* 898) is observed in low abundance in the negative ion mode.

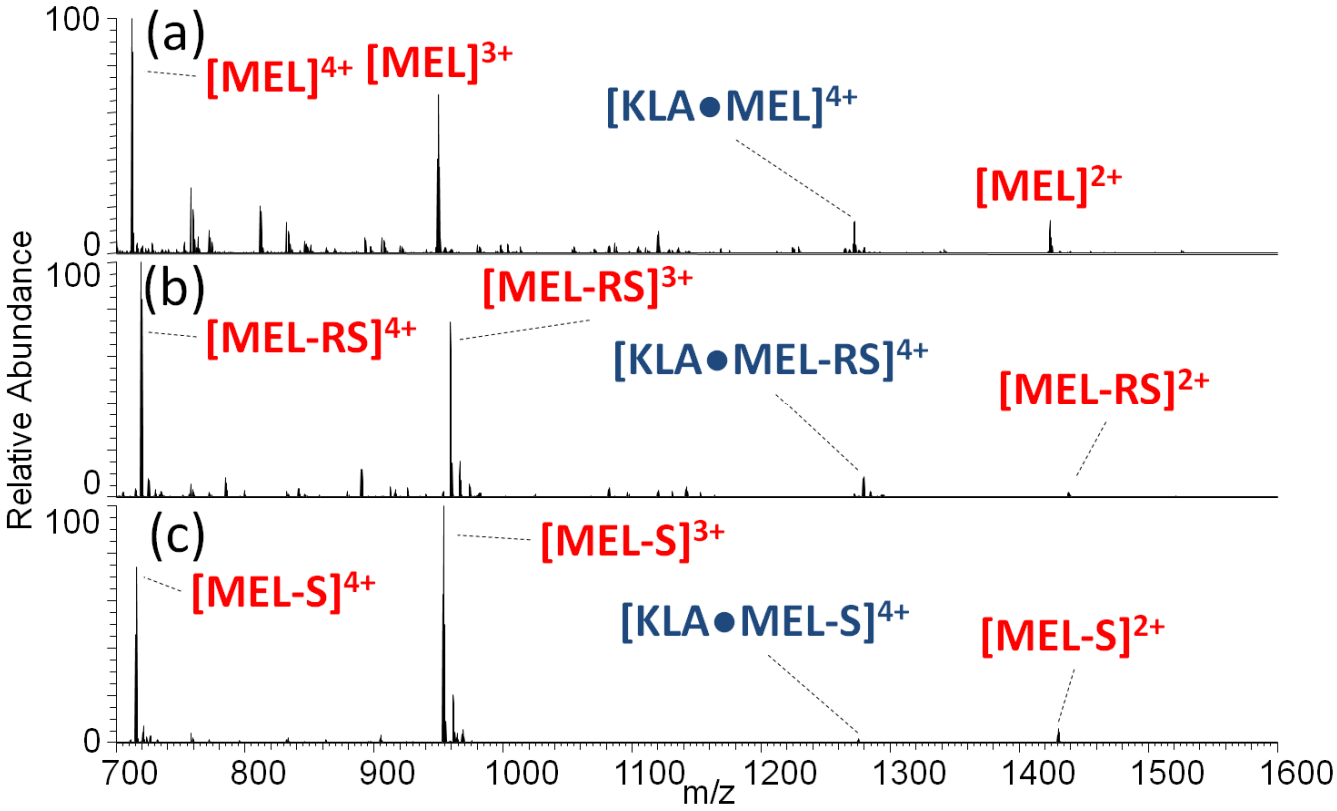

Figure S2: MS1 spectra of solutions containing Kdo_2-lipid A and (a) melittin, (b) melittin-RS or (c) melittin-S. Each solution contains 20 μM KLA and 4 μM peptide in 5 mM ammonium acetate.

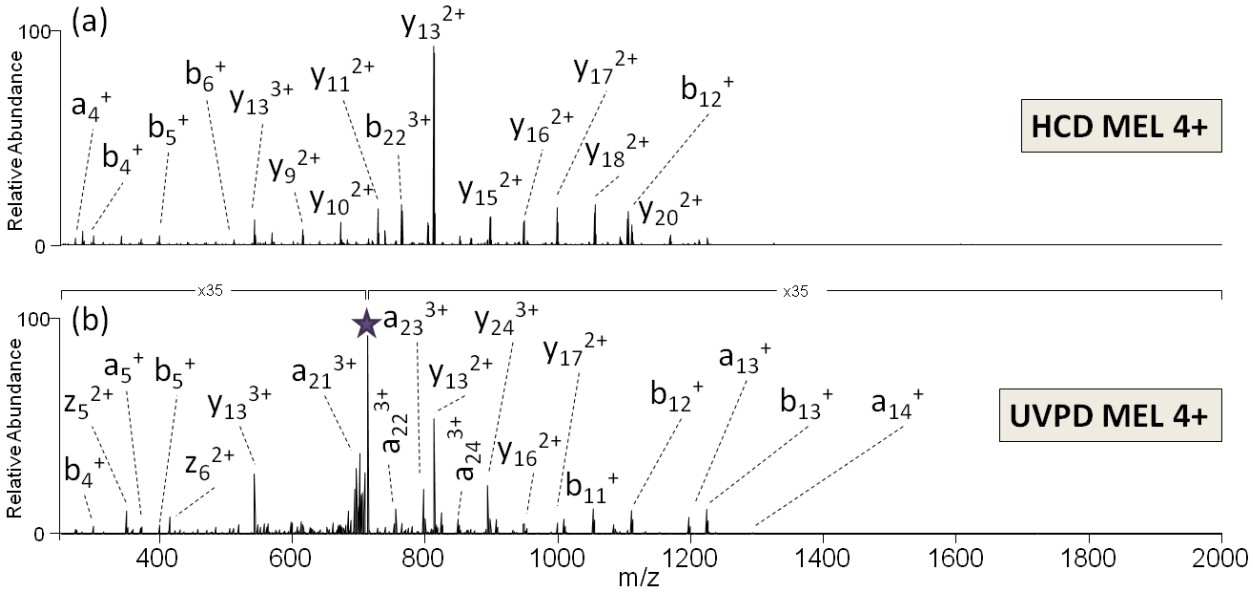

Figure S3: MS/MS spectra of melittin (4+). (a) HCD (NCE 35) generates an array of *b* and *y* product ions. (b) UVPD (1 pulse at 3.5 mJ) generates *a*, *b*, *c*, *x*, *y*, and *z* product ions. The precursor ion is indicated with a purple star.

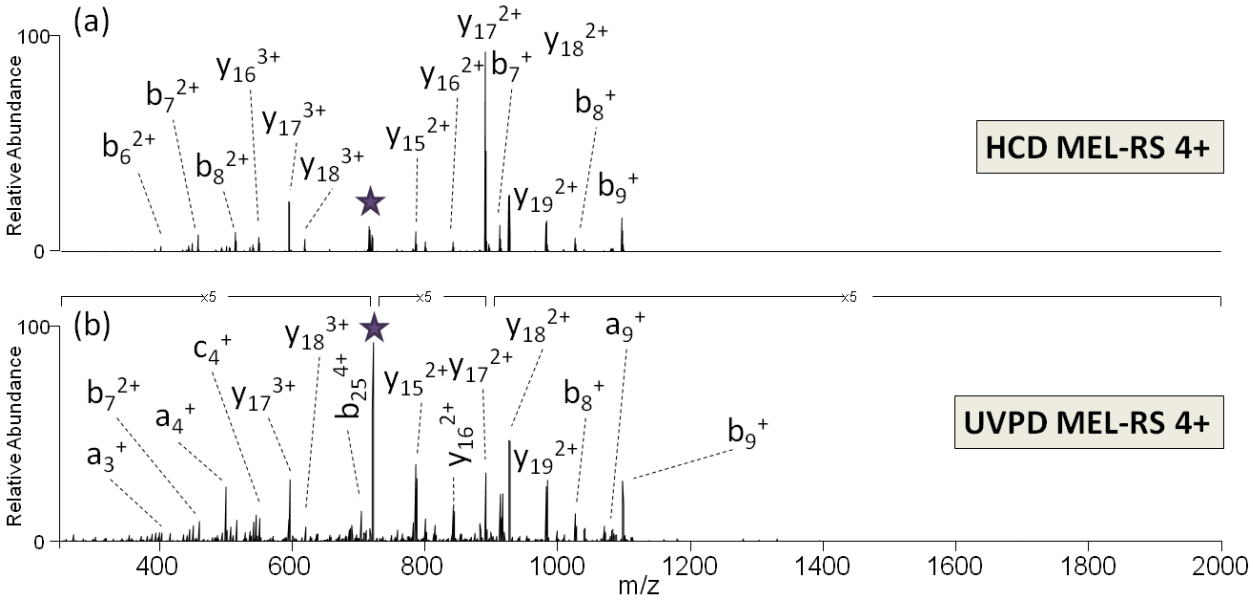

Figure S4: MS/MS spectra of melittin-RS (4+). (a) HCD (NCE 35) generates an array of *b* and *y* product ions. (b) UVPD (1 pulse at 3.5 mJ) generates *a*, *b*, *c*, *x*, *y*, and *z* product ions. The precursor ion is indicated with a purple star.

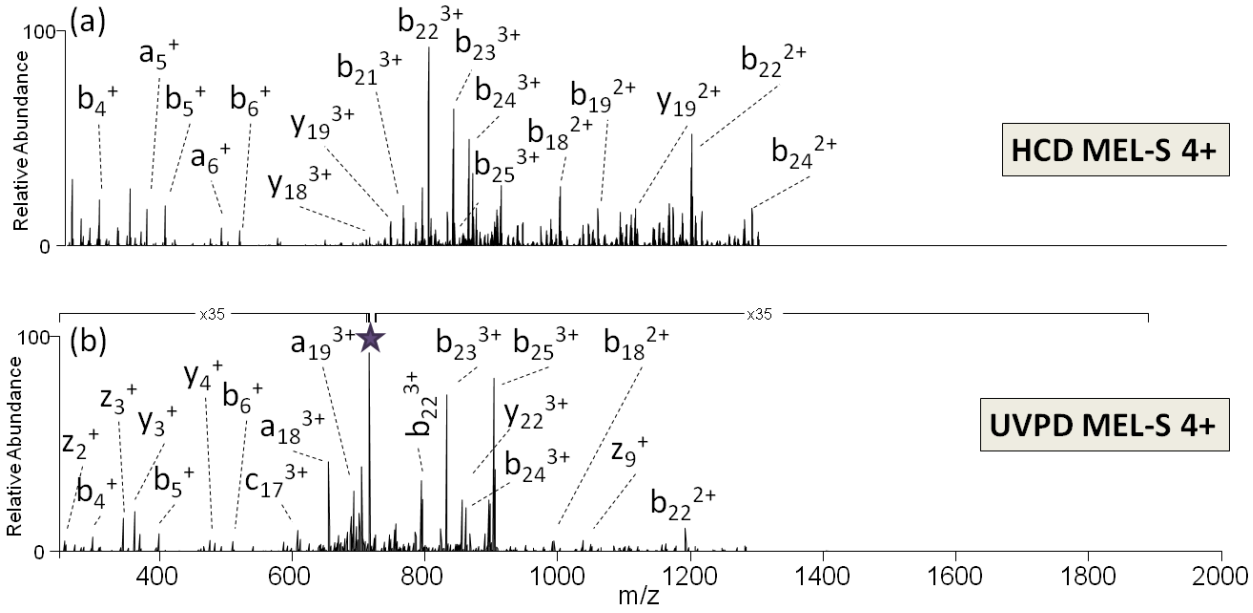

Figure S5: MS/MS spectra of melittin-S (4+). (a) HCD (NCE 35) generates an array of *b* and *y* product ions. (b) UVPD (1 pulse at 3.5 mJ) generates *a*, *b*, *c*, *x*, *y*, and *z* product ions. The precursor ion is indicated with a purple star.

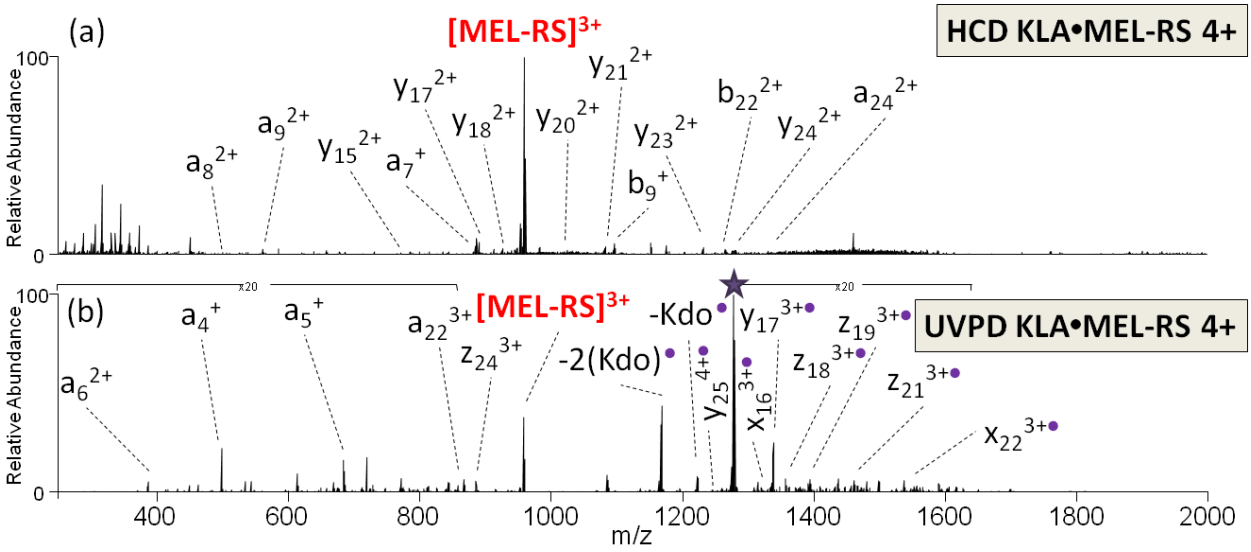

Figure S6: MS/MS spectra of the Kdo$_2$-lipid A•melittin-RS complex (4+). (a) HCD (NCE 35) generates an array of apo-peptide ions. (b) UVPD (1 pulse at 3.5 mJ) produces both apo- and holo-peptide ions (holo-ions are denoted with a superscript purple dot).

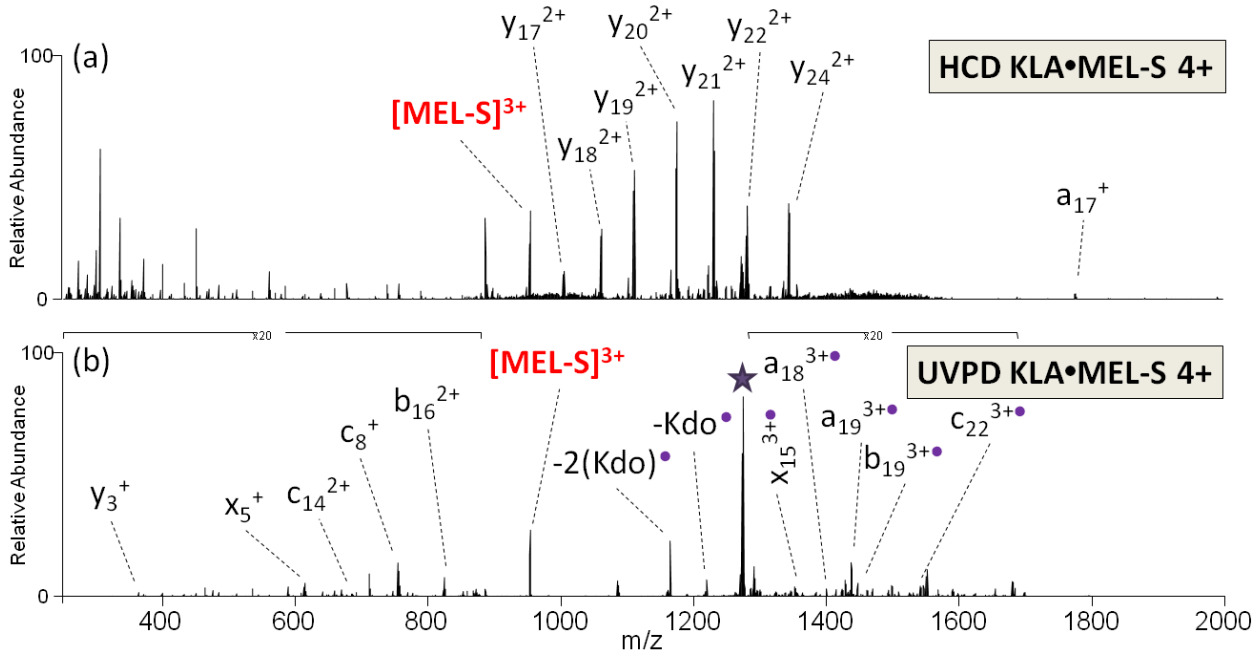

Figure S7: MS/MS spectra of the Kdo_2-lipid A•melittin-S complex (4+). (a) HCD (NCE 35) generates an array of apo-peptide ions. (b) UVPD (1 pulse at 3.5 mJ) produces both apo- and holo-peptide ions (holo-ions are denoted with a superscript purple dot).

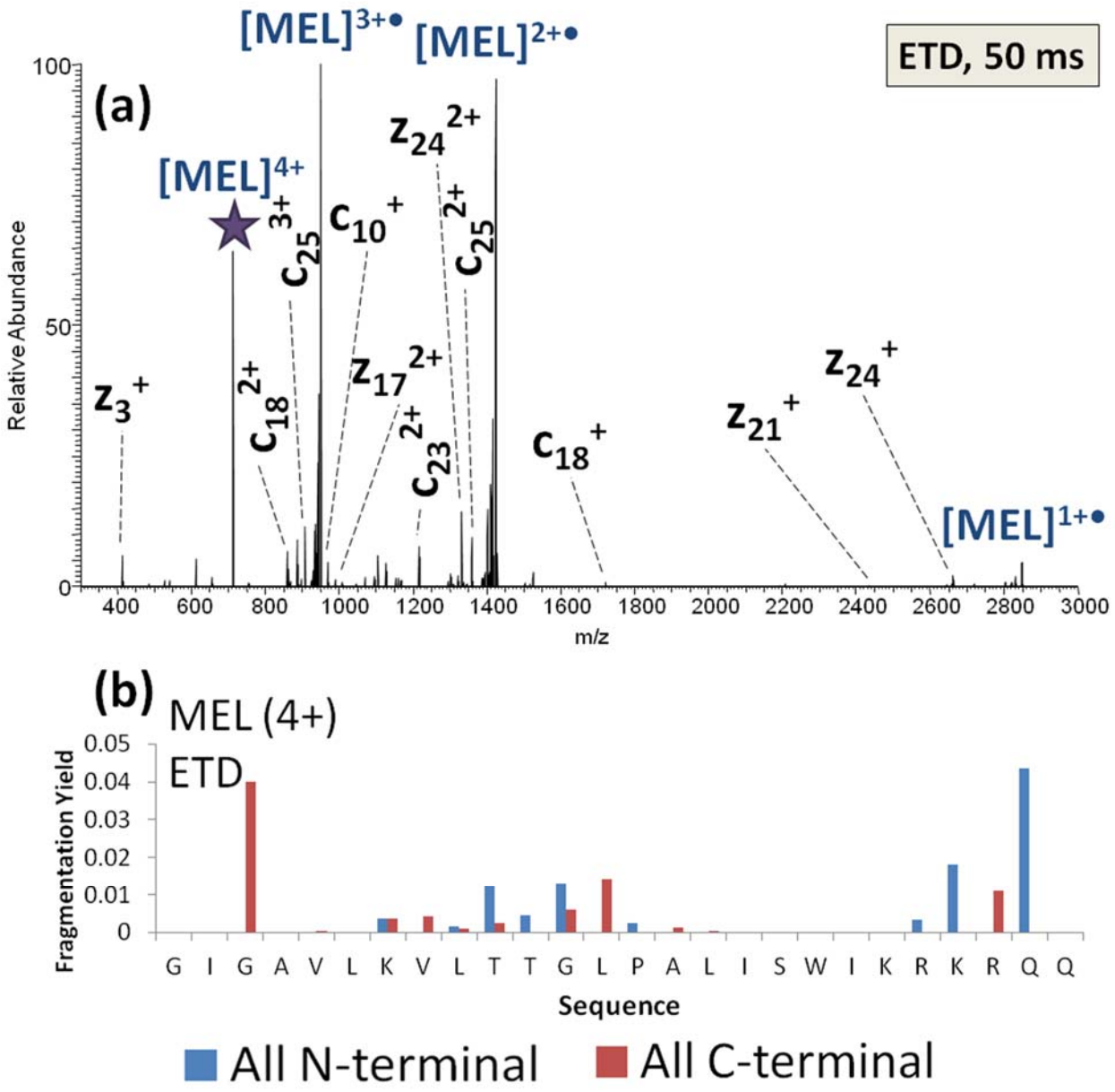

Figure S8: (a) ETD (50 ms activation time) of 4+ melittin in 5 mM ammonium acetate reveals diagnostic c- and z- sequence ions. (b) Normalized fragmentation yield of N-terminal ions (blue) and C-terminal ions (red) identified for ETD of melittin.

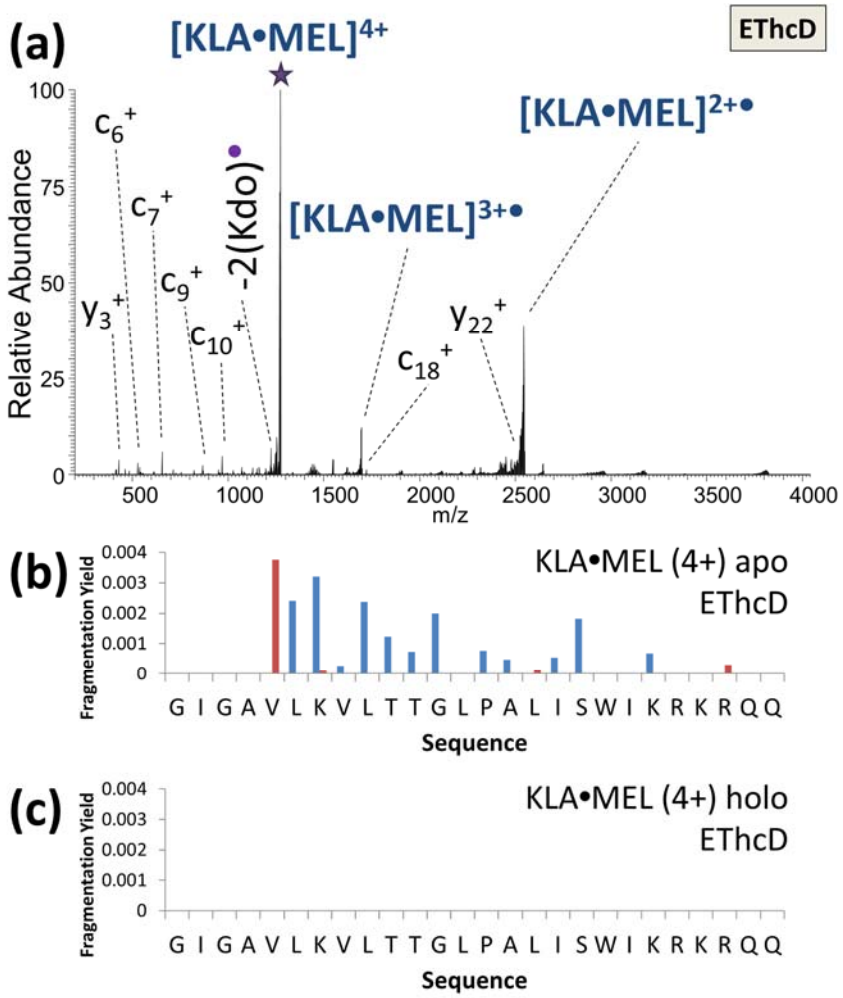

Figure S9: (a) MS/MS spectrum for EThcD (50 ms ETD period with supplemental HCD of 30 NCE) of the Kdo_2-lipid A•melittin complex (4+). (b,c) Normalized fragmentation yields showing (b) apo-peptide sequence ions and (c) holo-peptide sequence ions. N-terminal ions (all *a*, *b*, *c* ions) are denoted in blue and C-terminal ions (all *x*, *y*, *z* ions) are denoted in red.

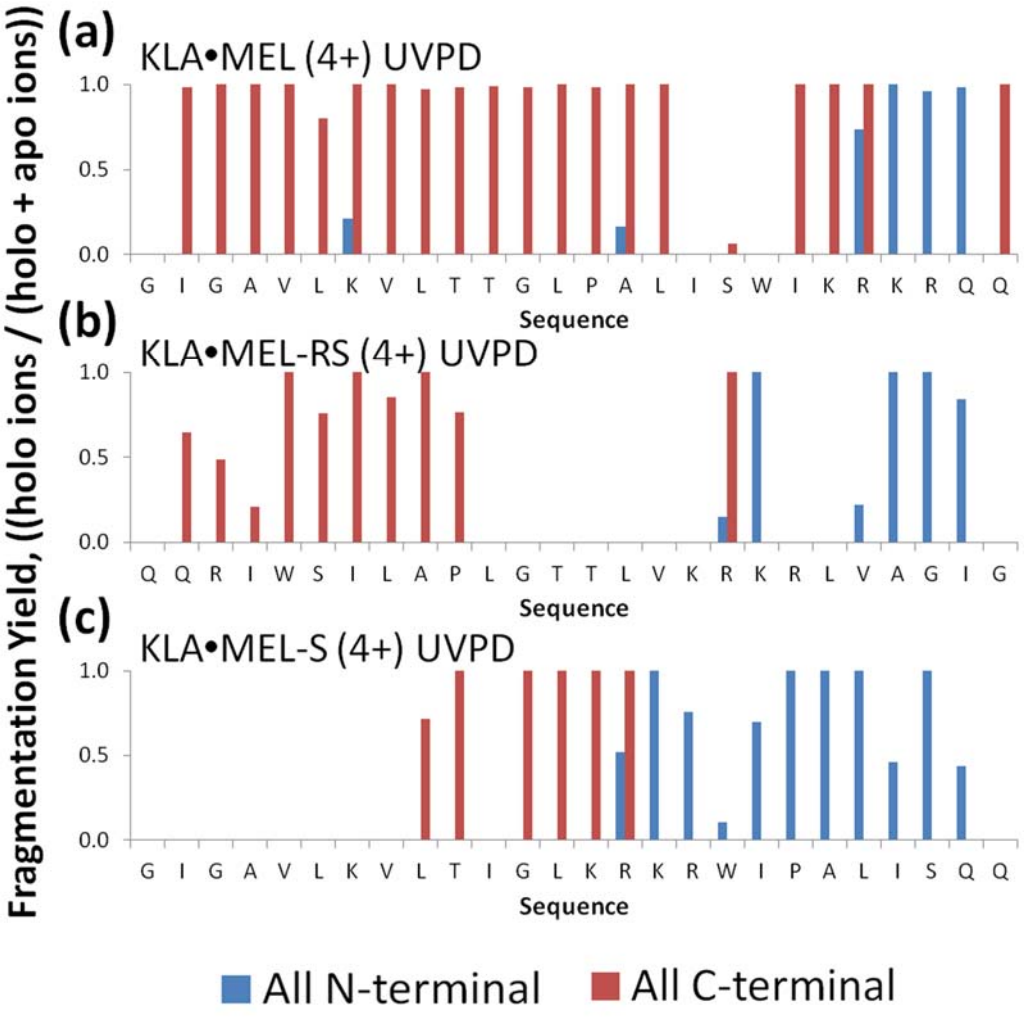

Figure S10: UVPD fragmentation yields in terms of the ratios of abundances of the holo (KLA-containing) fragment ions divided by the summed abundances of the holo and apo fragment ions for a given sequence position for Kdo_2-lipid A•peptide complexes (4^+) for (a) melittin, (b) melittin-RS, and (c) melittin-S.

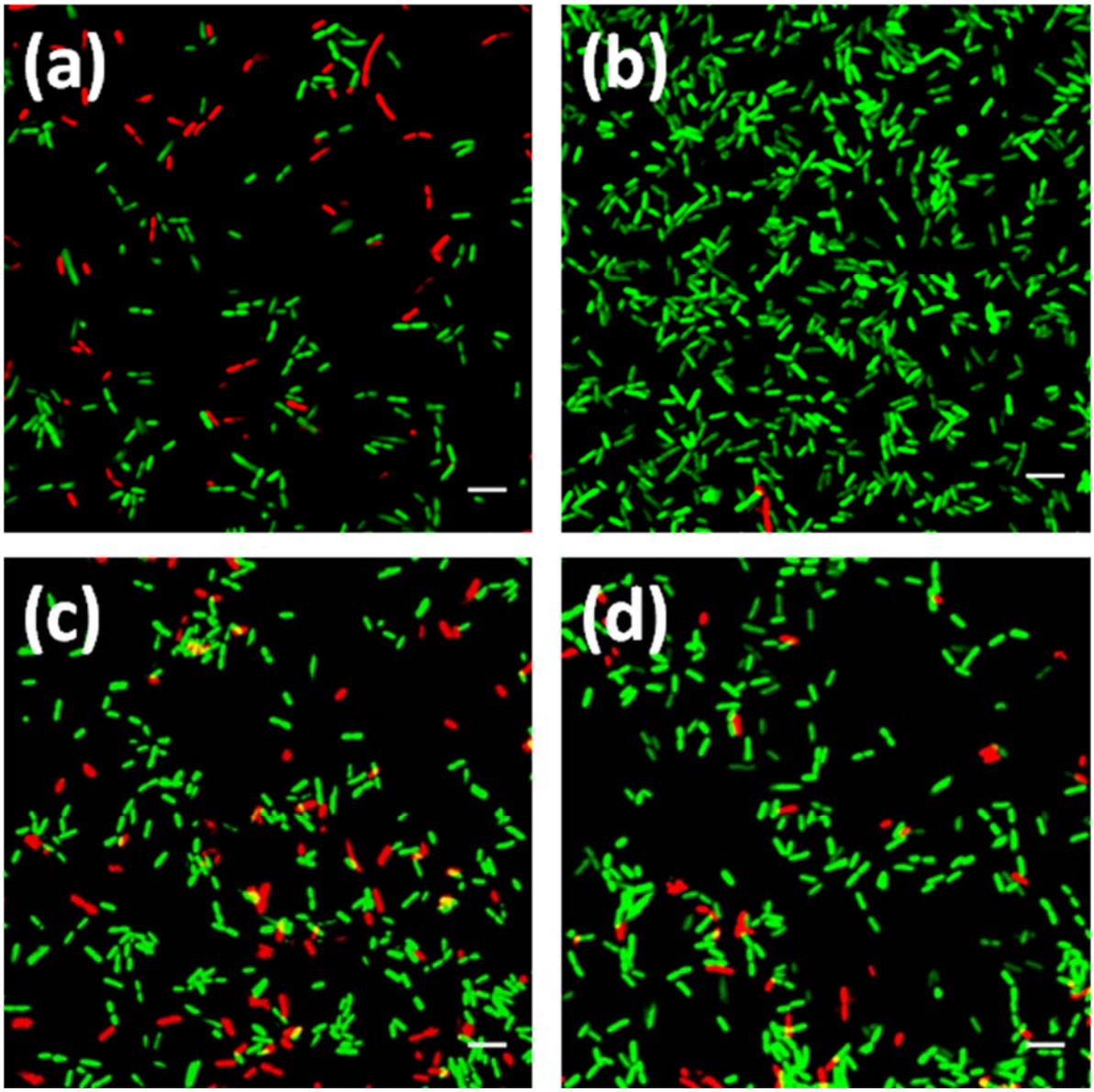

Figure S11: Confocal microscopy images demonstrating the mortality rates of *Pseudomonas aeruginosa* based on 12.5 μg/mL dosing of (a) MEL, (b) MEL-RS, (c) MEL-S, and (d) MEL-NoKRKR. Scale bars in the bottom right of each image are 5 μm. Green (GFP fluorescence) indicates metabolically active (i.e., live) cells; red reveals where cellular membranes have been damaged, allowing propidium iodide to enter the (dead) cells.

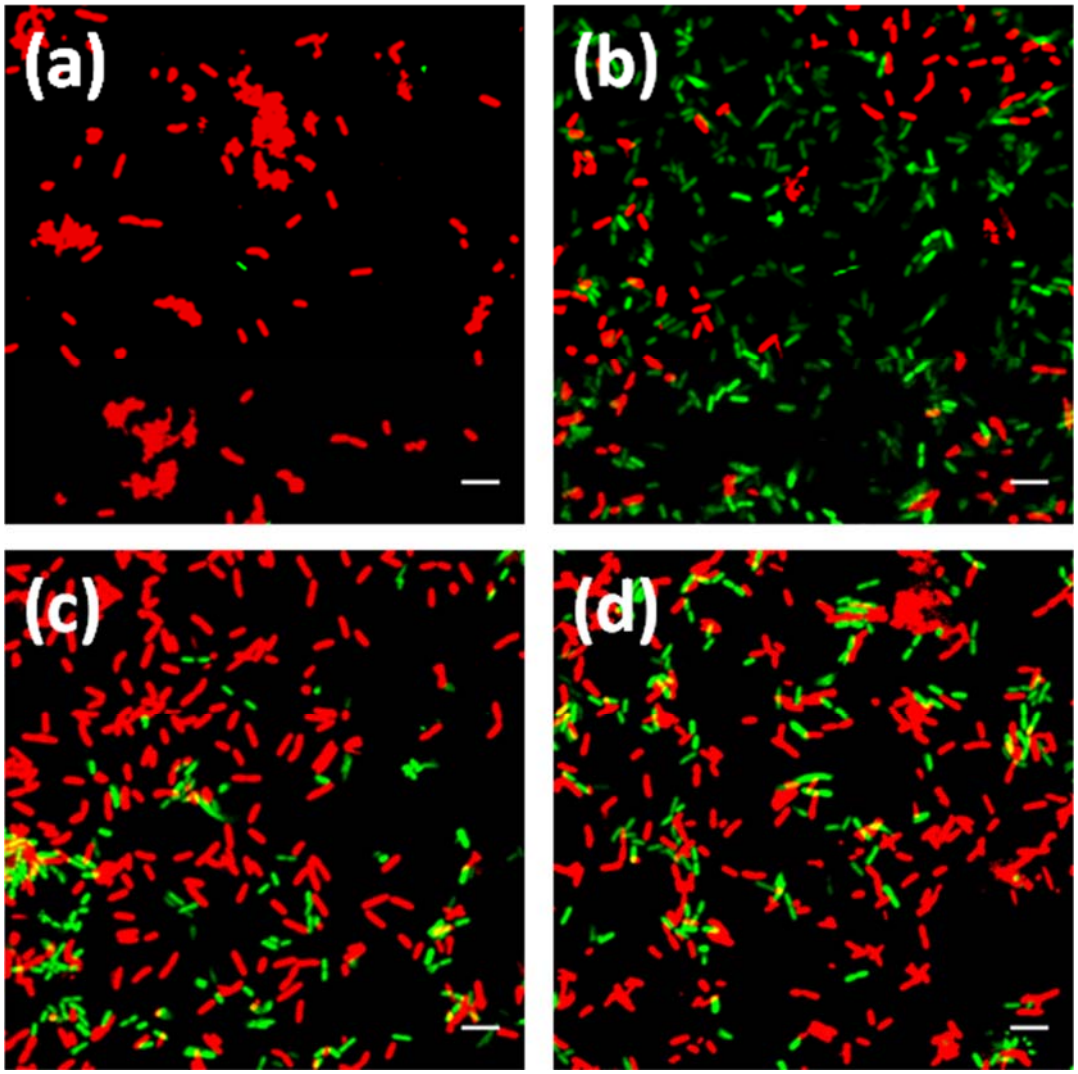

Figure S12: Confocal microscopy images demonstrating the mortality rates of *Pseudomonas aeruginosa* based on 50.0 μg/mL dosing of (a) MEL, (b) MEL-RS, (c) MEL-S, and (d) MEL-NoKRKR. Scale bars in the bottom right of each image are 5 μm. Green (GFP fluorescence) indicates metabolically active (i.e., live) cells; red reveals where cellular membranes have been damaged, allowing propidium iodide to enter the (dead) cells.

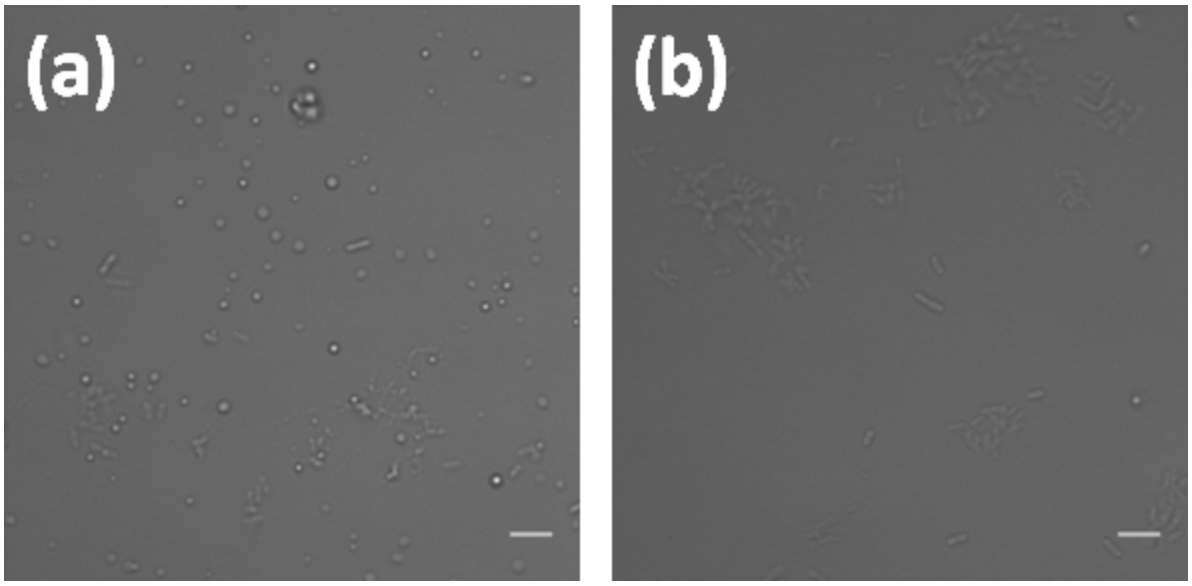

Figure S13: Bright field images showing (a) lysed cells clustering and (b) debris from lysed cells on the coverslip from dosing with 50 μg/mL MEL. Scale bars in the bottom right of each image are 5 μm.

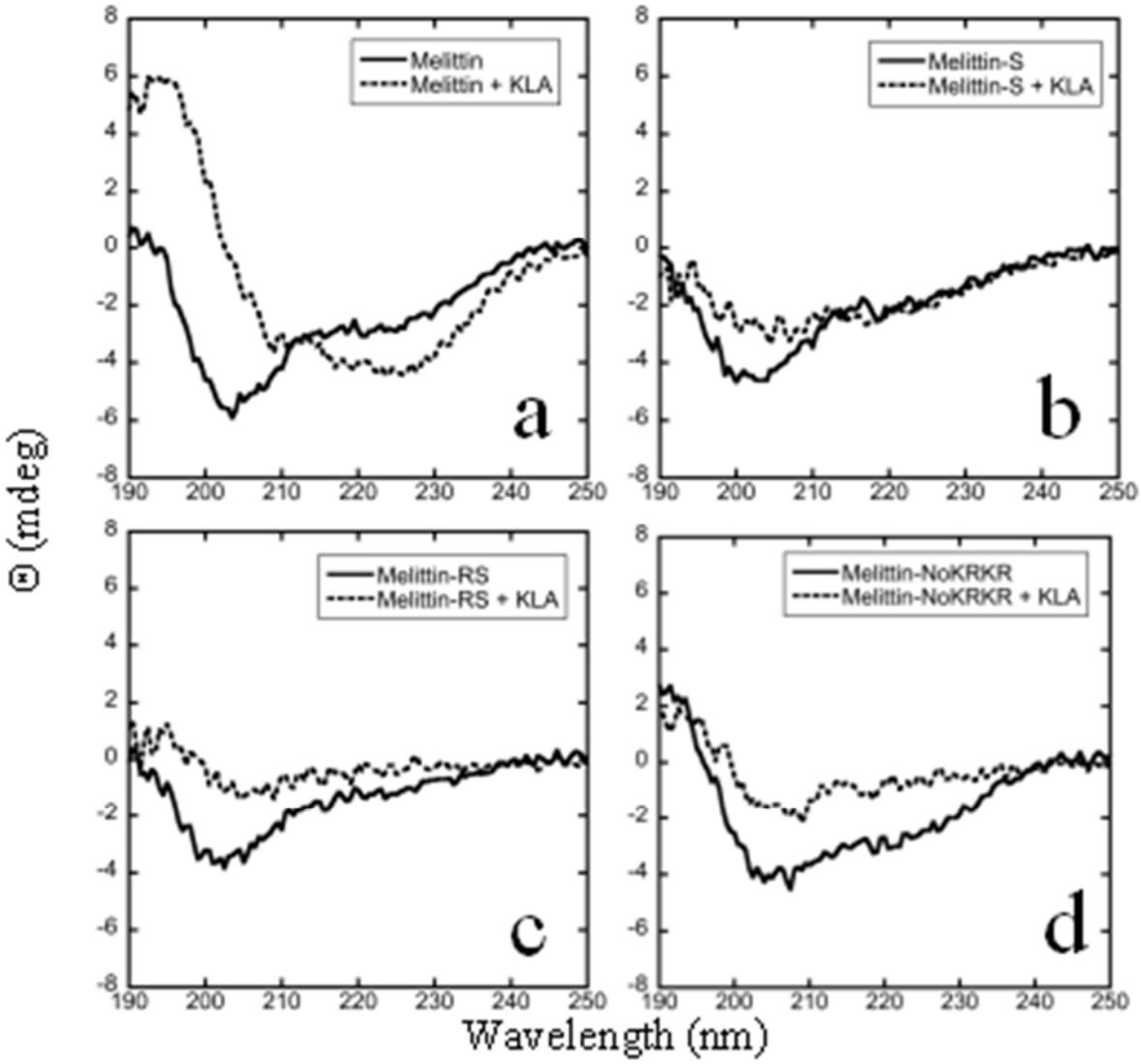

Figure S14: CD spectra of 20 μM peptide (a) melittin, (b) melittin-S, (c) melittin-RS, and (d) melittin-NoKRKR alone (solid lines) and with 100 μM KLA (dotted lines) in 5 mM ammonium acetate at pH 7.0.

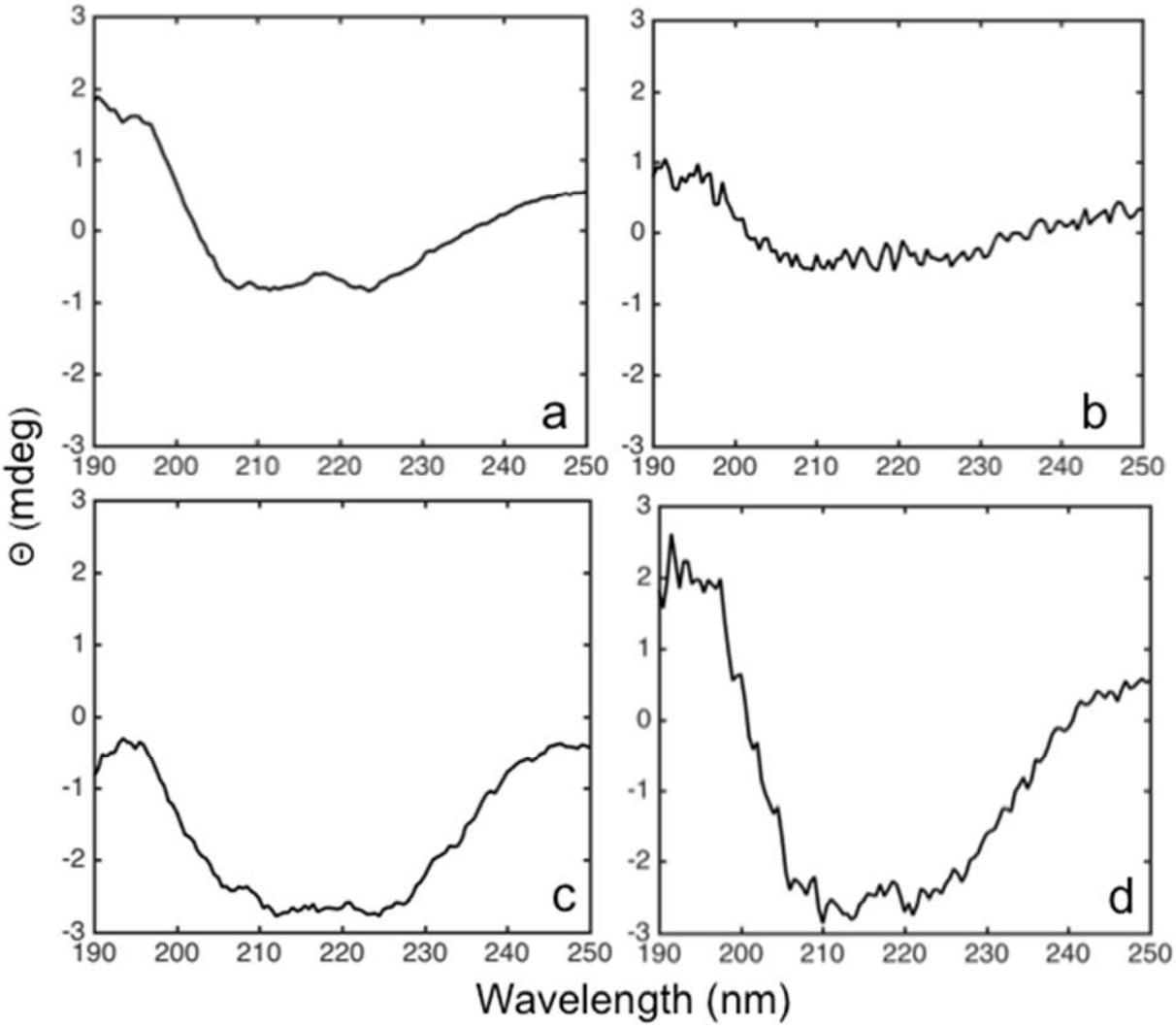

Figure S15: Background CD spectra used for peptide conformational studies. (a) 10 mM potassium phosphate, (b) 5 mM ammonium acetate, (c) 10 mM potassium phosphate + 100 mM KLA, (d) 5 mM ammonium acetate + 100 mM KLA.

Table S1: Secondary structure content (%) for each of the peptides in solution without KLA and with KLA from CD experiments in 10 mM potassium phosphate at pH 7.0, as predicted via the BeStSel server.

Secondary Structure Motif	Melittin		Melittin-S		Melittin-RS		Melittin-noKRKR	
	Unbound (no KLA)	With KLA	Unbound (no KLA)	With KLA	Unbound (no KLA)	With KLA	Unbound (no KLA)	With KLA
Helix (Regular)	18.0	6.5	10.4	8.7	0.0	29.0	14.3	31.3
Helix (Distorted)	11.1	1.5	8.7	5.0	0.0	16.2	8.1	20.6
Anti-Parallel β-Sheet (left-twisted)	0.0	0.0	0.0	0.0	2.6	0.0	0.0	4.2
Anti-Parallel β-Sheet (relaxed)	0.0	8.5	0.0	8.9	22.4	0.0	0.0	0.0
Anti-Parallel β-Sheet (right-twisted)	11.9	1.4	18.1	0.0	21.2	8.6	3.4	0.0
Parallel β-Sheet	1.4	19.3	0.0	16.4	0.0	0.5	15.4	12.0
Turn	14.6	18.0	13.1	14.8	12.5	9.0	14.8	6.6
Others	43.0	44.7	49.7	46.2	41.2	36.6	44.0	25.4

Table S2: Secondary structure content (%) for each of the peptides in solution without KLA and with KLA from CD experiments from CD experiments in 5 mM ammonium acetate at pH 7.0, as predicted via the BeStSel server.

Secondary Structure Motif	Melittin		Melittin-S		Melittin-RS		Melittin-noKRKR	
	Unbound (no KLA)	With KLA	Unbound (no KLA)	With KLA	Unbound (no KLA)	With KLA	Unbound (no KLA)	With KLA
Helix (Regular)	4.9	10.2	4.9	0.0	2.4	0.0	7.6	0.6
Helix (Distorted)	6.9	6.5	6.9	6.9	3.1	0.0	7.0	0.0
Anti-Parallel β-Sheet (left-twisted)	0.0	0.0	0.0	0.0	1.0	4.0	1.7	4.2
Anti-Parallel β-Sheet (relaxed)	7.9	8.9	7.9	7.4	12.2	18.7	10.7	17.2
Anti-Parallel β-Sheet (right-twisted)	17.0	0.0	17.0	12.2	18.8	18.3	17.5	16.9
Parallel β-Sheet	1.3	13.8	1.3	9.3	0.0	0.0	0.0	0.0
Turn	16.7	15.9	16.7	15.7	16.2	14.7	14.1	1.3
Others	45.2	44.7	45.2	48.5	46.3	44.3	41.5	45.9