Supporting Information for

Helical Structure of Recombinant Melittin

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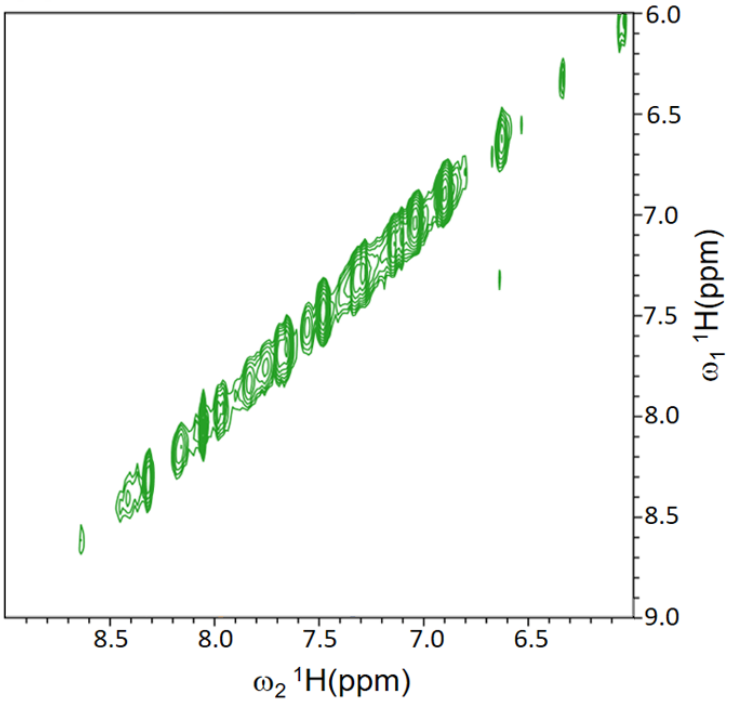

Figure S1. 2D ^{1}H-^{1}H ROESY of melittin at 298 K (related to Figure 1). The spectrum was recorded at 298 K with a mixing time of 200 ms. 50 μM melittin was prepared in NMR buffer with 30% v/v TFE. No cross peaks were observed after collection of 128 transients.

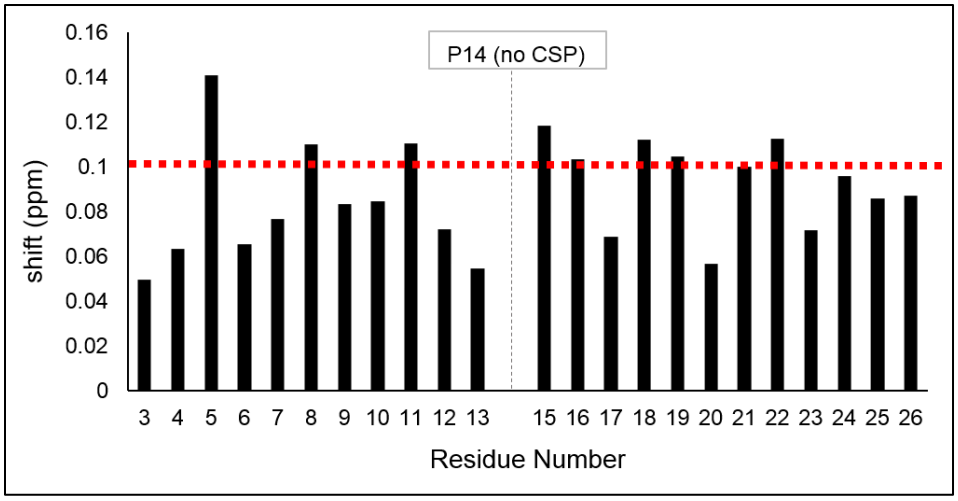

Figure S2. Increasing solvent viscosity causes minimal chemical shift perturbations in melittin (related to Figure 2). Chemical shift perturbations (CSPs) induced by adding 10% w/v glycerol to melittin in 30% v/v TFE and lowering the sample temperature from 298 K to 285 K. The CSPs were calculated from the ^{15}N and ^{1}H chemical shifts of melittin taken from ^{1}H-^{15}N HSQC spectra shown in Figure 2. Residues 1-2 and 14 are excluded from the analysis because their NH peaks are not visible in the ^{1}H-^{15}N HSQC spectra. Residue 14 is proline, which does not have an NH peak. The threshold at 0.1 ppm is indicated by a red broken line.

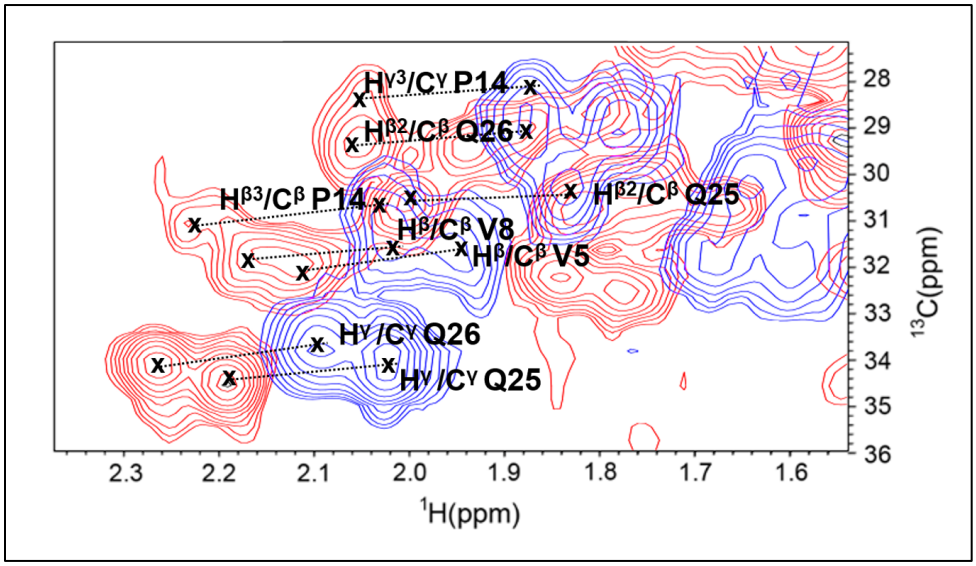

Figure S3. Transfer of melittin assignments (related to Figure 2). A representative 2D slice of the HCCH-TOCSY spectrum (red contours) acquired at 298 K is overlaid with the corresponding 2D slice of the ^{1}H-^{13}C NOESY spectrum (blue contours) acquired at 285 K with a mixing time of 200 ms. The sample (50 µM [*U*-^{13}C,^{15}N]-labeled melittin) was prepared in 10 mM potassium phosphate at pH 7.0 with 30% v/v deuterated TFE, 10% v/v D_2O, and 10% w/v deuterated glycerol.

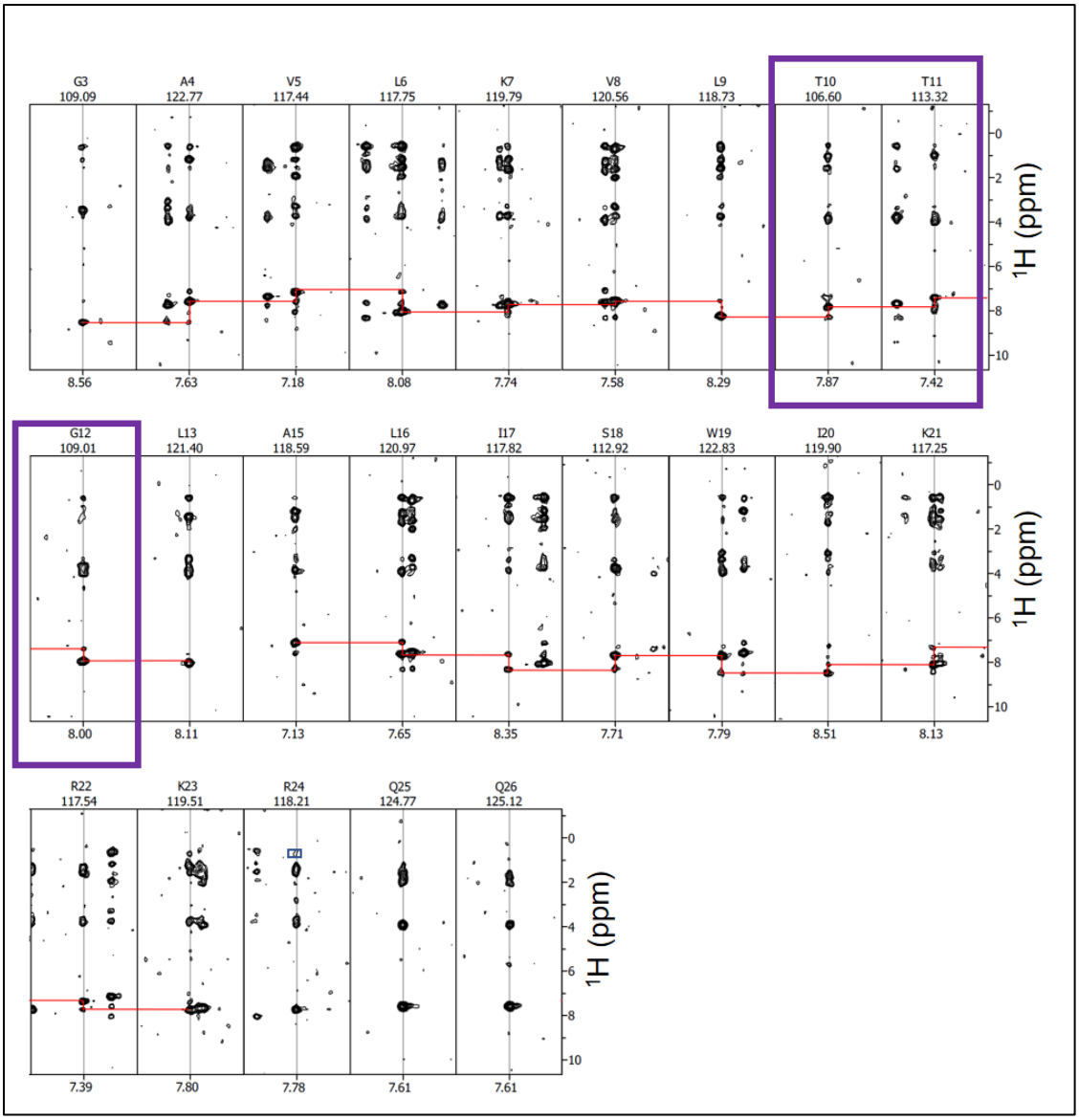

Figure S4. Evidence for helical structure in melittin (related to Figure 3). Selected $\omega_2(^1H^N)$-$\omega_3(^1H^{NOE})$ regions from a 3D ^1H-^{15}N NOESY spectrum of [*U*-^{13}C,^{15}N]-labeled melittin at $\omega_1(^{15}N^H)$ chemical shifts. The spectrum was acquired with a mixing time of 200 ms. The strips show sequential NOEs involving backbone amide protons of residues 3-23, connected by red lines. Residues belonging to the hinge region are enclosed in violet boxes.

Table S1. List of hydrogen bonds[a] in PDB entries 2MLT, 2MW6, 3QRX, and 6DST

2MLT chain A	2MLT chain B	2MW6[b]	3QRX	6DST[b]
Ile2-Leu6	Ile2-Leu6	Ile2-Leu6	Ile2-Leu6	
Gly3-Lys7	Gly3-Lys7	Gly3-Lys7	Gly3-Lys7	
Ala4-Val8	Ala4-Val8	Ala4-Val8	Ala4-Val8	Ala4-Val8
Val5-Leu9	Val5-Leu9		Val5-Leu9	
Leu6-Thr10	Leu6-Thr10		Leu6-Thr10	
				Lys7-Thr10
Lys7-Thr11	Lys7-Thr11			
			Lys7-Gly12	
	Val8-Gly12			Val8-Gly12
Val8-Leu13			Val8-Leu13	
	Leu9-Leu13			
				Thr10-Leu13
			Thr11-Ala15	Thr11-Ala15
Gly12-Leu16	Gly12-Leu16		Gly12-Leu16	Gly12-Leu16
Leu13-Ile17	Leu13-Ile17		Leu13-Ile17	Leu13-Ile17
Pro14-Ser18	Pro14-Ser18	Pro14-Ser18	Pro14-Ser18	
Ala15-Trp19	Ala15-Trp19	Ala15-Trp19	Ala15-Trp19	Ala15-Trp19
Leu16-Ile20	Leu16-Ile20	Leu16-Ile20	Leu16-Ile20	
Ile17-Lys21	Ile17-Lys21	Ile17-Lys21		Ile17-Lys21
Ser18-Arg22	Ser18-Arg22	Ser18-Arg22		Ser18-Arg22
Trp19-Lys23	Trp19-Lys23	Trp19-Lys23		
Ile20-Arg24	Ile20-Arg24	Ile20-Arg24		
Lys21-Gln25	Lys21-Gln25	Lys21-Gln25		

a) Hydrogen bonds were determined in DSSP[1, 2] using a cut-off bond energy of -1.0 kcal/mol.

b) Hydrogen bonds are listed if they are present in at least 70% of conformers of the ensemble

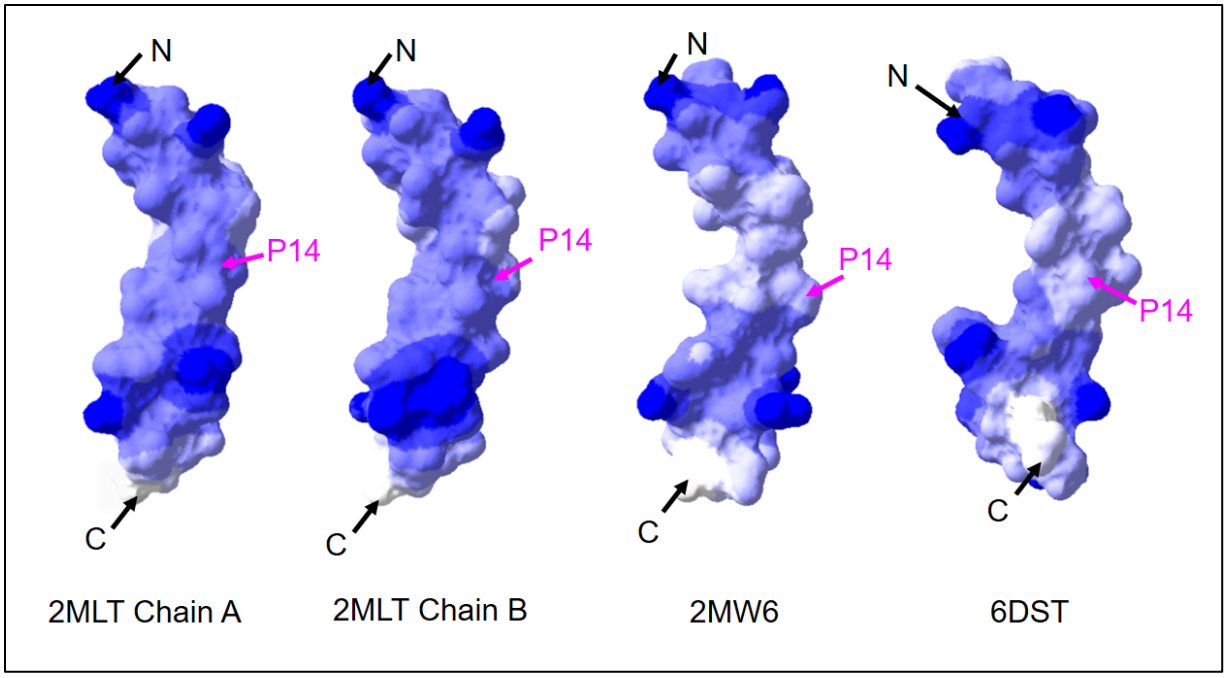

Figure S5. Electrostatic potential is mapped onto the molecular surfaces of the crystal structure of tetrameric melittin (PDB ID: **2MLT** chains A and B), the representative NMR structure in methanol (PDB ID: **2MW6**), and the representative NMR structure in TFE/water/glycerol (PDB ID: **6DST**). The structure in **3QRX** was omitted from the comparison because it lacks residues 1, 22-26. Uncharged surfaces are shown in white and positively charged surfaces are rendered in blue, while no negatively charged surfaces (red) are present. Black arrows point to N- and C-terminal residues, and magenta arrows indicate the position of Pro14. (The orientations of the melittin structures shown here are consistent with the backbone traces in Figure 6A).

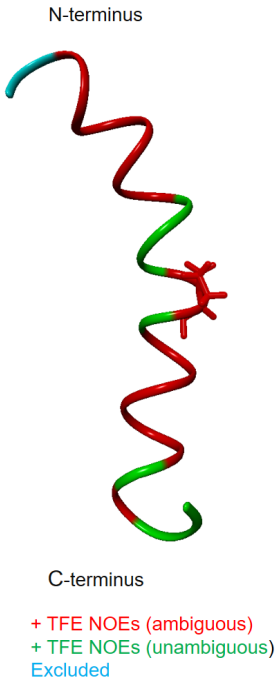

Figure S6. Map of TFE-melittin interactions on the 3D solution NMR structure of melittin. The representative structure shown here (model 10) is taken from the ensemble of solution NMR structures in Figure 3A-B, rotated by 90 degrees for ease of viewing. Residues colored red (3-9, 13-14, 16-21, 23) may or may not have surfaces within ~5 Å of the TFE proton resonating at 0.6 ppm. The ambiguity arises from the possibility of overlap of the methyl peaks of melittin with a TFE peak at ~0.6 ppm. Residues colored green (10-12, 15, 22, 24-26) have NOESY strips clearly showing weak intermolecular NOEs at ~0.6 ppm. G1-I2, colored light blue, were excluded from the analysis because they lacked ^{1}H-^{15}N NOESY data.

References

[1] Kabsch, W., and Sander, C. (1983) Dictionary of protein secondary structure: Pattern recognition of hydrogen-bonded and geometrical features, *Biopolymers 22*, 2577-2637.

[2] Joosten, R. P., te Beek, T. A. H., Krieger, E., Hekkelman, M. L., Hooft, R. W. W., Schneider, R., Sander, C., and Vriend, G. (2011) A series of PDB related databases for everyday needs, *Nucleic Acids Res. 39*, D411-D419.