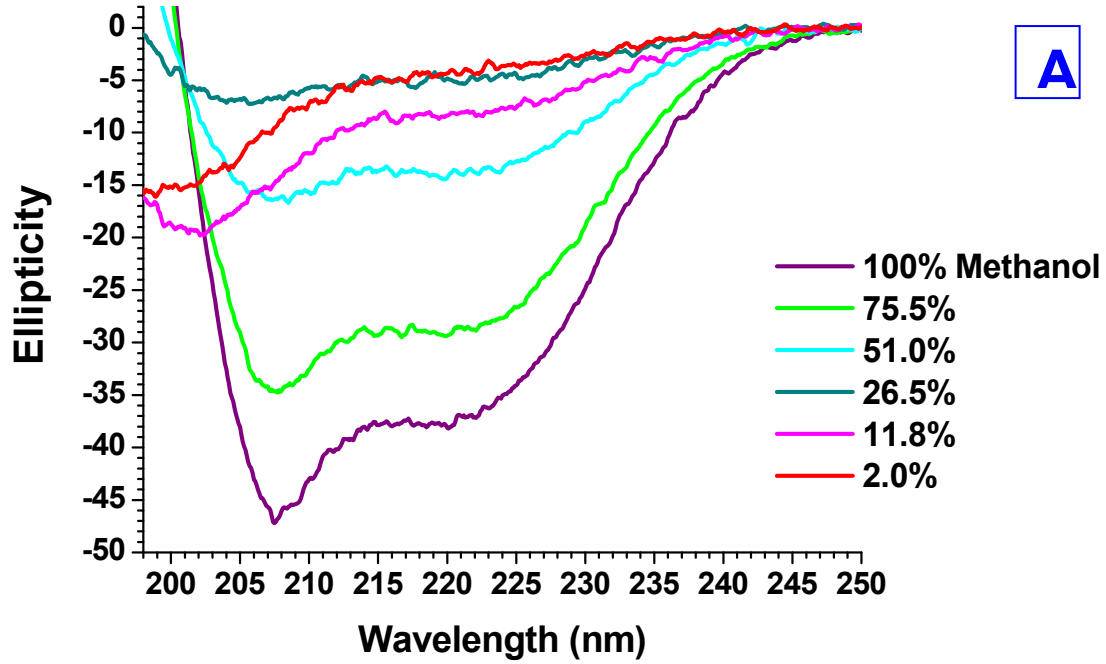
Supplementary Data

Evidence for α-helices in the gas phase: A case study using Melittin -honey bee venom

Figure S1 (A) CD chromatograms of melittin with increasing methanol content and (B) a plot of ellipticity at 222nm. The dashed line is included to guide the eye.

Figure S1 illustrates the shift in ellipticity to produce the typical α-helical signature with increasing methanol content. This is exemplified by plotting the values at 220nm. There is no evidence of helical conformation with 12% methanol and below. However, there is an apparent transformation when increasing the methanol to 27%. The random coil signature appears to be less predominant inferring a potential shift in structural conformation. At 50% and above, the α-helix clearly becomes increasingly prevalent.

All data was acquired using a Jasco J-810 Cicular Dichroism Spectrometer. Melittin stock (10 mg/mL) was diluted to 0.15 mg/mL (52.8 µM). Spectra were acquired in continuous mode from 250 – 190 nm (band width of 1nm), averaging 3 scans at 20 nm/min. The following solvent conditions were used to induce various conformational states: 2% - 100% methanol in 10 mM ammonium acetate, pH 6.8.



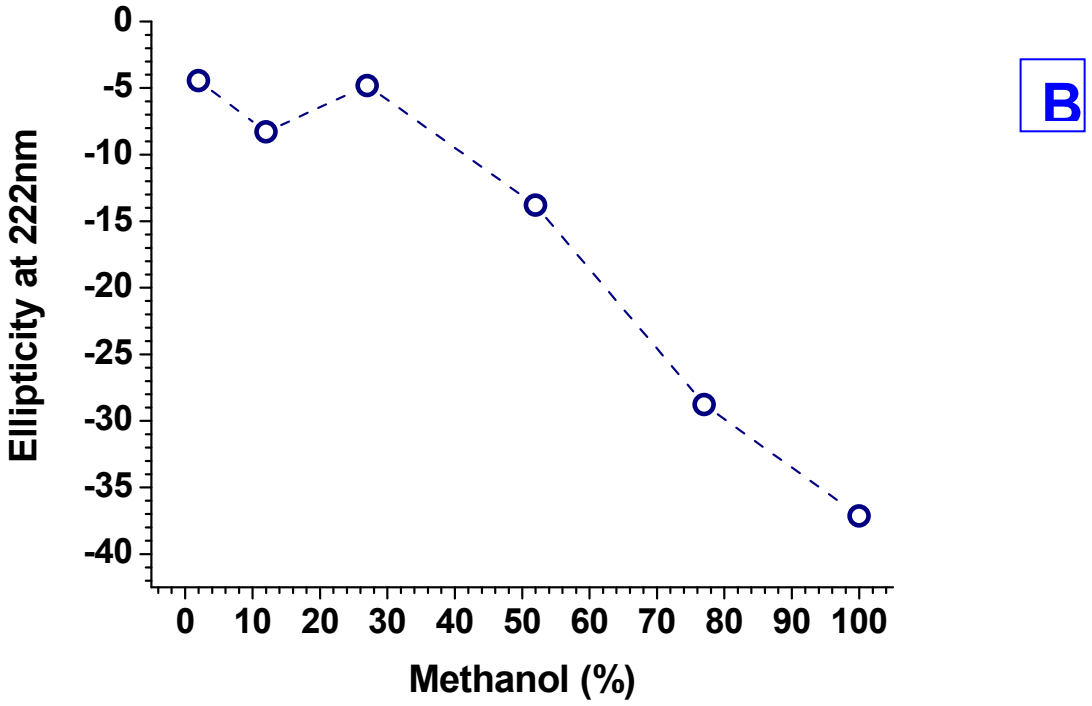


Figure S2 Typical Mass Spectrum of Melittin, this was obtained following nano-ESI of a solution at a concentration of 50 uM from a solution that was 50:50: methanol:water. This was obtained using the nanoESI on the LCQ (Thermo Scientific). Similar data were obtained on the mobility capable Q-TOF.

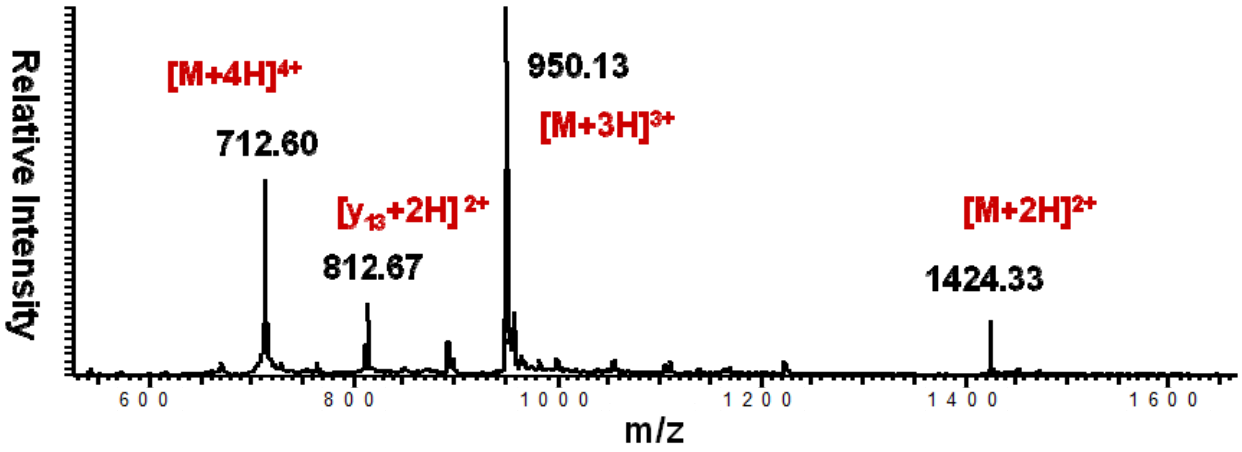


Figure S2

Mass Spectrum of melittin following nano-ESI from 100% methanol at 350 uM. Obtained on the mobility capable Q-TOF. Some of the assigned species were not of sufficient intensity to perform IM-MS analysis.

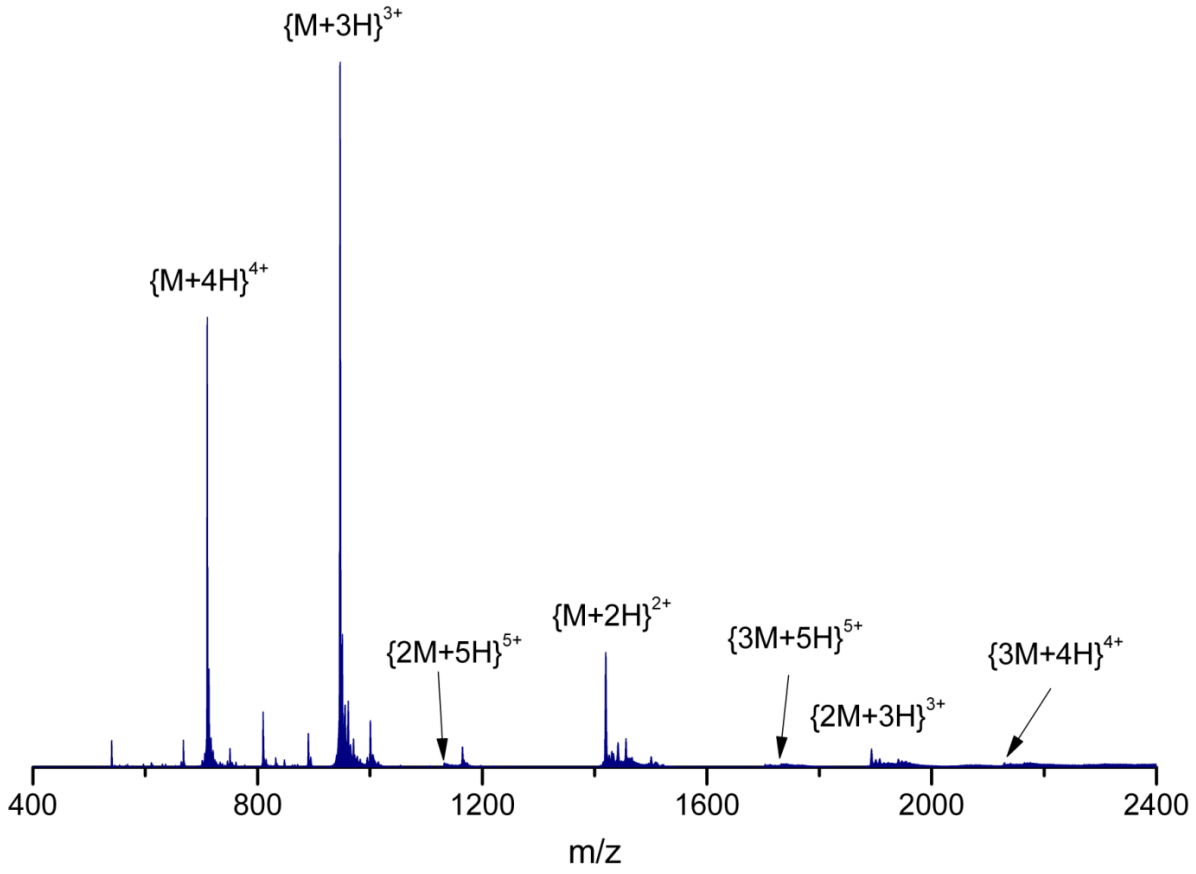
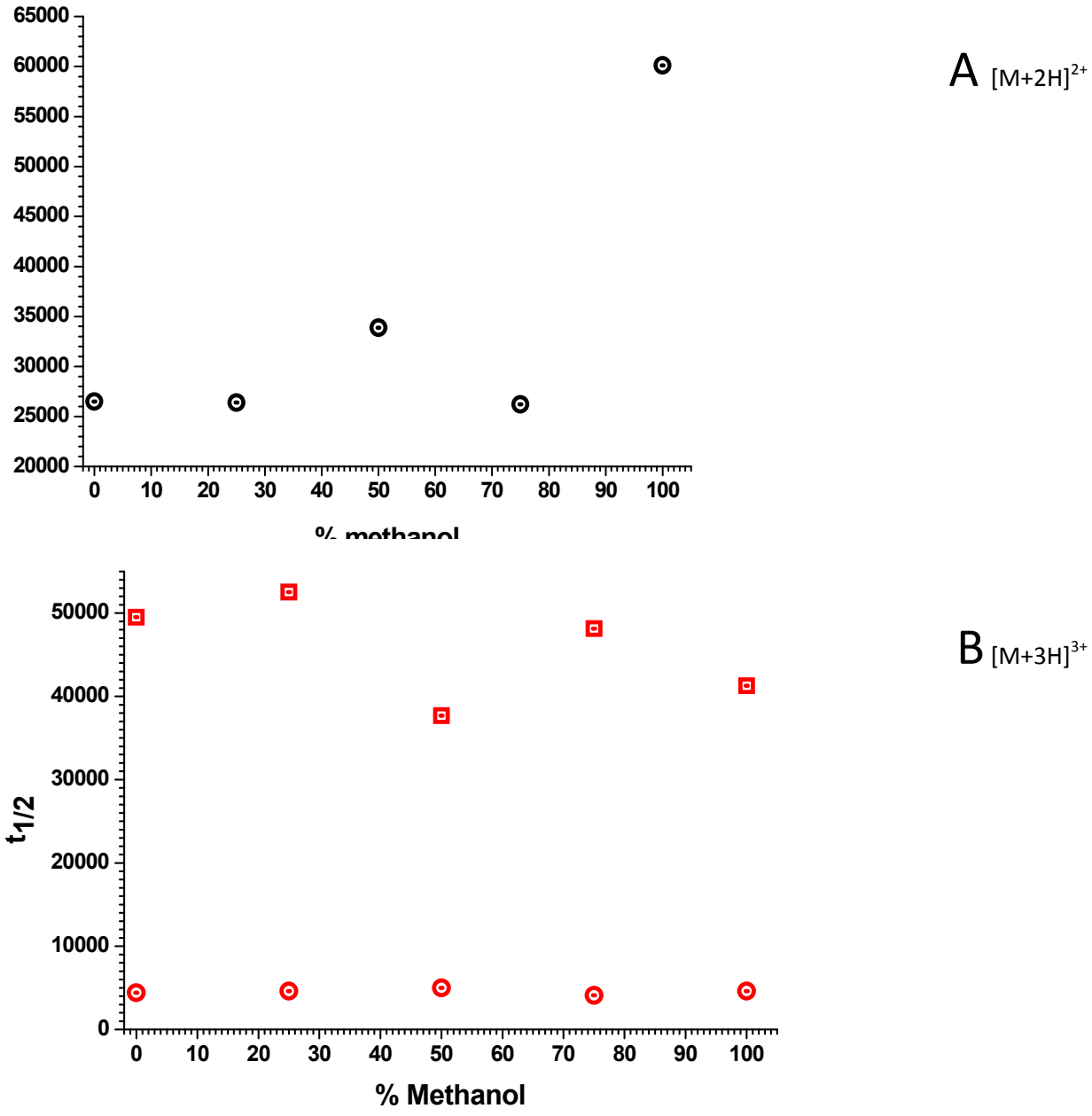


Figure S3: Half lives ($t_{1/2}$) for the HDX exchange rates for $[M+2H]^{2+}$ (A) and $[M+3H]^{3+}$ (B) following HDX for 10 seconds from samples sprayed from varying concentrations of methanol.

Here subtle differences as a function of solvent which are not apparent in Figure 3 are evident. The rate of exchange decreases linearly with increase in methanol content from 25%. As the data in Figure S3A demonstrates, the differences between samples is small but becomes exaggerated when expressed as hydrogen depletion.

$[M+3H]^{3+}$ half lives are plotted in S3B and infer the presence of two mobile proton populations.



Methanol

Capillary Temperature	$[M+3H]^{3+}$						$[M+2H]^{2+}$			
	10 s				90 s		10 s		90 s	
	(i)		(ii)							
	t ½	-k	t ½	-k	t ½	-k	t ½	-k	t ½	-k
200°C	7223.3	9.60×10^{-5}	33324.4	2.08×10^{-5}	22733.6	3.05×10^{-5}	37732.6	1.84×10^{-5}	44805.9	1.55×10^{-5}
125°C	27440.5	2.43×10^{-5}			24202.1	2.86×10^{-5}	115910.9	5.98×10^{-6}	182263.3	3.80×10^{-6}

Aqueous

Capillary Temperature	$[M+3H]^{3+}$						$[M+2H]^{2+}$			
	10 s				90 s		10 s		90 s	
	(i)		(ii)							
	t ½	-k	t ½	-k	t ½	-k	t ½	-k	t ½	-k
200°C	7145.8	9.70×10^{-5}	42265.1	1.64×10^{-5}	27670.5	2.51×10^{-5}				
125°C	25662.6	2.70×10^{-5}			25530.3	2.72×10^{-5}	103609.4	6.69×10^{-6}	148584.6	4.67×10^{-6}

Table S-1 ***Comparison of 100% methanol and 100% aqueous half lives (sec***) and rates (sec^{-1}) ***for activation times of*** 10 and 90 s

Methanol

Capillary Temperature	$[M+3H]^{3+}$						$[M+2H]^{2+}$			
	10 s				90 s		10 s		90 s	
	(i)		(ii)							
	t ½	-k	t ½	-k	t ½	-k	t ½	-k	t ½	-k
125ºC	9624.4	7.20×10^{-5}	43321.7	1.60×10^{-5}	22060.7	3.14×10^{-5}	61668.0	1.12×10^{-5}	83171.0	8.33×10^{-6}
80ºC	8724.3	7.95×10^{-5}	55899.0	1.24×10^{-5}	28108.2	2.47×10^{-5}	69245.5	1.00×10^{-5}	94049.8	7.37×10^{-6}
60ºC	330384.7	2.10×10^{-6}			32944.3	2.10×10^{-5}				

Aqueous

Capillary Temperature	$[M+3H]^{3+}$						$[M+2H]^{2+}$			
	10 s				90 s		10 s		90 s	
	(i)		(ii)							
	t ½	-k	t ½	-k	t ½	-k	t ½	-k	t ½	-k
125ºC	13580.5	5.10×10^{-5}	43321.7	1.60×10^{-5}	20985.4	3.30×10^{-5}	75572.1	9.17×10^{-6}	71194.3	9.74×10^{-6}
80ºC	44318.9	1.56×10^{-5}			38788.3	1.79×10^{-5}	288691.0	2.40×10^{-6}	202378.7	3.43×10^{-6}
60ºC	38919.0	1.78×10^{-5}			33977.8	2.04×10^{-5}				

Table S2 Comparisons of half lives ***and rates*** using nanoESI calculated over ***1***0 and ***9***0 s activation time