Supporting Information

Chiral Separation of Diastereomers of the Cyclic Nonapeptides Vasopressin and Desmopressin by Uniform Field Ion Mobility Mass Spectrometry

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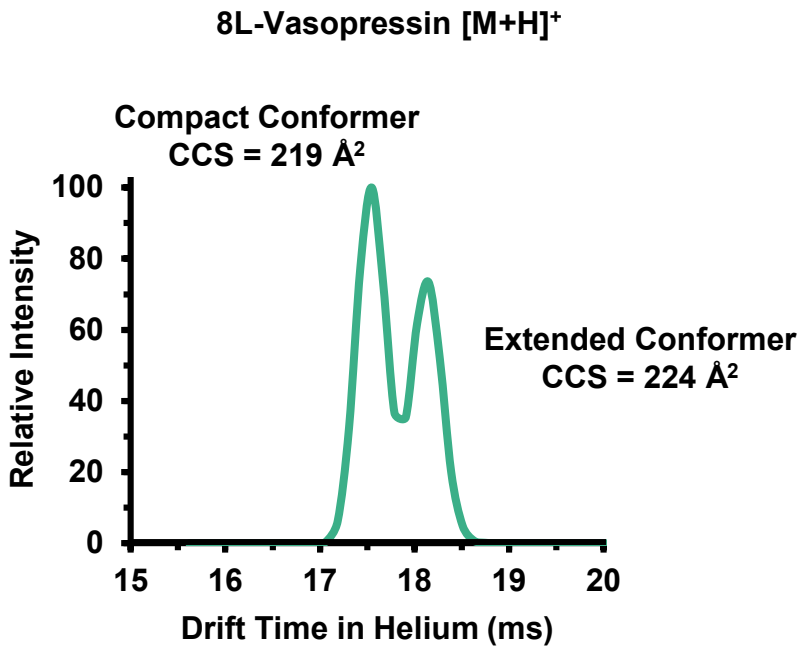
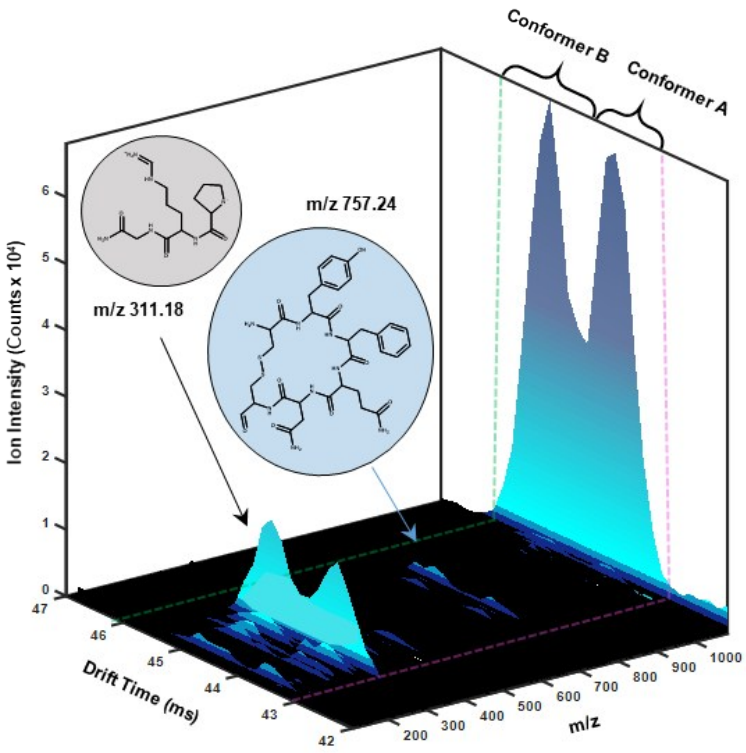
Nonapeptide		Ion Form	Mass (Da)	$^{DT}CCS_{N2}$ (Å^2)	$^{DT}CCS_{He}$ (Å^2)
8L-Vasopressin - A - B	8L-VP	$[M+H]^+$	1084	302.2 ± 0.2 310.4 ± 0.6	219 ± (0.9) 224 ± (1.4)
8D-Vasopressin	8D-VP	$[M+H]^+$	1084	309.8 ± 0.2	226 ± (1.1)
8L-Desmopressin	8L-DP	$[M+H]^+$	1069	307.8 ± 0.5	225 ± (1.2)
8D-Desmopressin	8D-DP	$[M+H]^+$	1069	314.4 ± 0.4	230 ± (1.1)

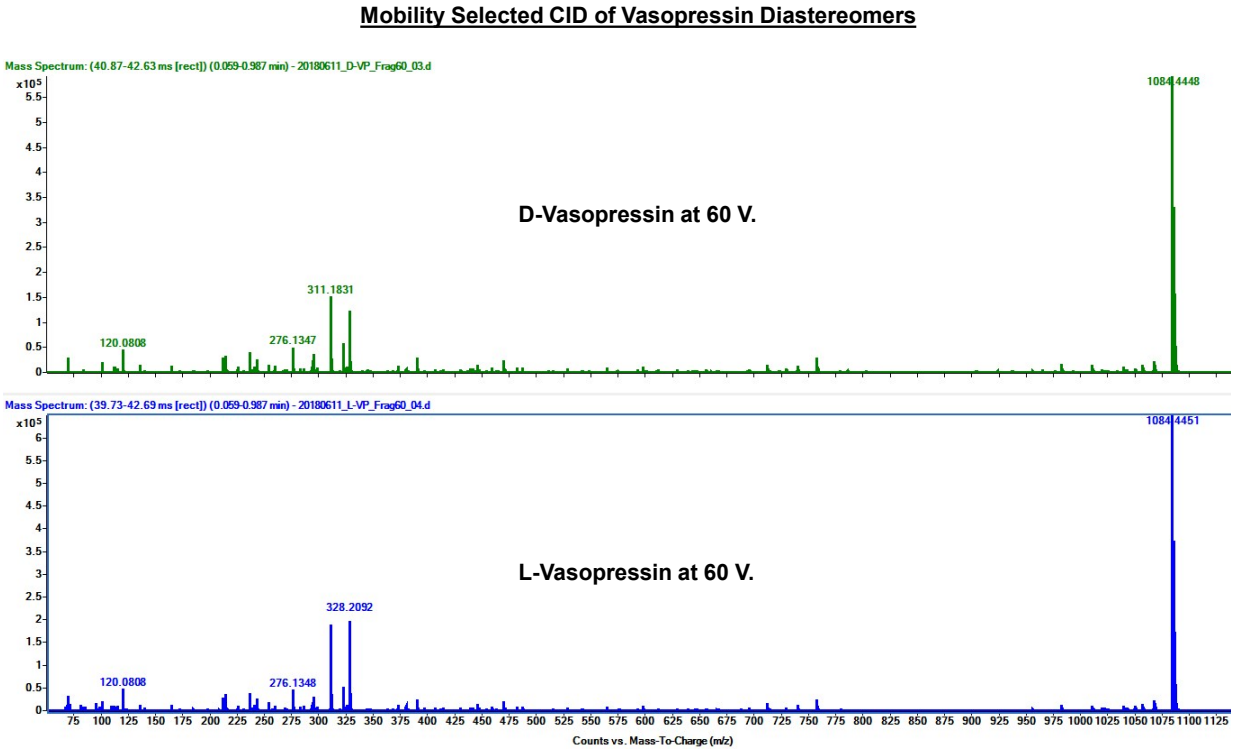
Table S1: A summary of the drift tube CCS measurements obtained in this study. The nitrogen values in this table correspond to three replicate measurements (N=3) from separate days. Helium measurements were from a single set of experiments (uncertainty given in parenthesis corresponds to the variance of each of the seven electric fields sampled in the stepped field method). The x axis was converted from drift time to CCS in the main text using the single field method described in a recent publication from Stow *et. al.* (*Anal. Chem.* **2017**, *89*, 9048-9055).



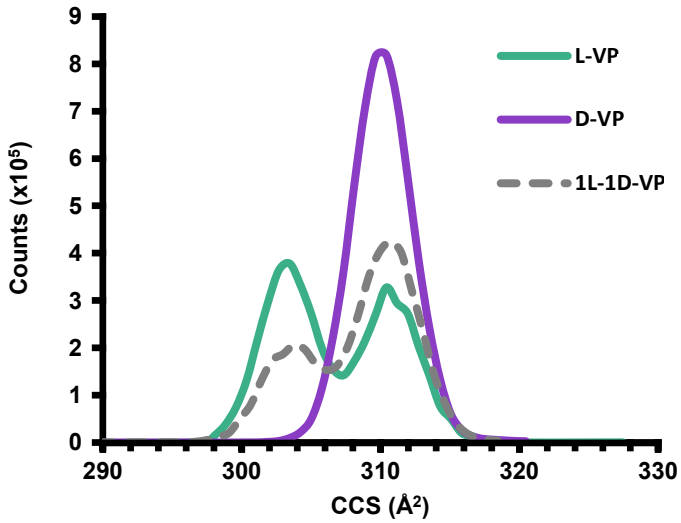
Supplemental Figure S1: Drift time distributions for singly-protonated 8L-vasopressin obtained when conducting the IM separation in helium drift gas. A with nitrogen drift gas (Figure 2B of the main text), two distinct conformational populations are observed in helium. These helium-based CCS measurements more closely align with the computational results.



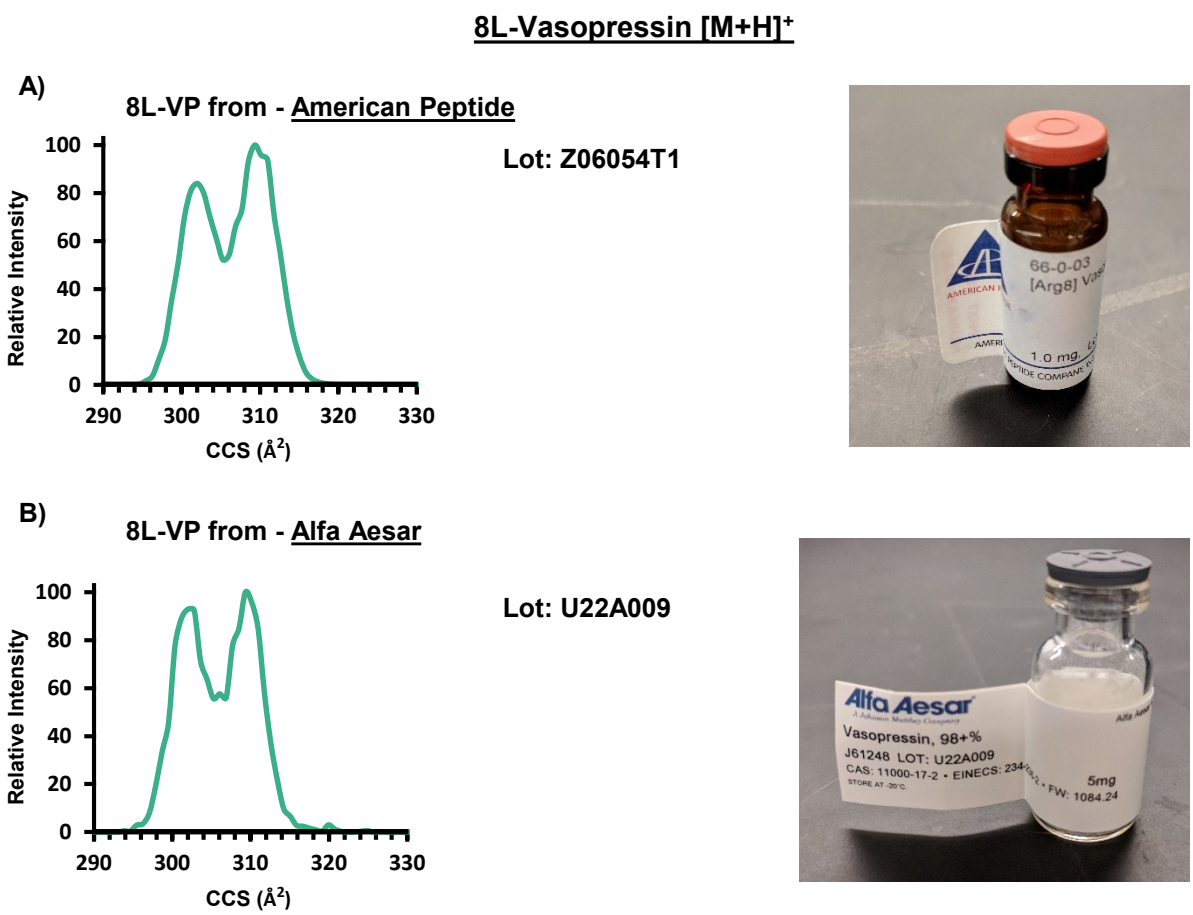
Supplemental Figure S2: Ion mobility-aligned fragmentation data for singly-protonated ($[M+H]^+$) 8L-vasopressin obtained using post IM collision-induced dissociation (i.e., IM/MS). Both conformers have the same fragmentation spectra, including fragment ion masses and relative abundances (c.f., Figure S2), providing increased confidence that both distributions at m/z 1084 are from the same parent ion, as opposed to a contaminant ion signal.



Supplemental Figure S3: Mobility selected mass spectra for 8D- (Top) and 8L- (Bottom) vasopressin diastereomers for 60 V (laboratory frame) CID. While both stereoisomers have the same fragmentation spectra, and hence does not eliminate the possibility of some "D" vasopressin as a contaminant in "L" VP from the manufacturer, this information provides additional confidence that the secondary conformer is not an unrelated molecule with the same molecular formula as 8L-VP.

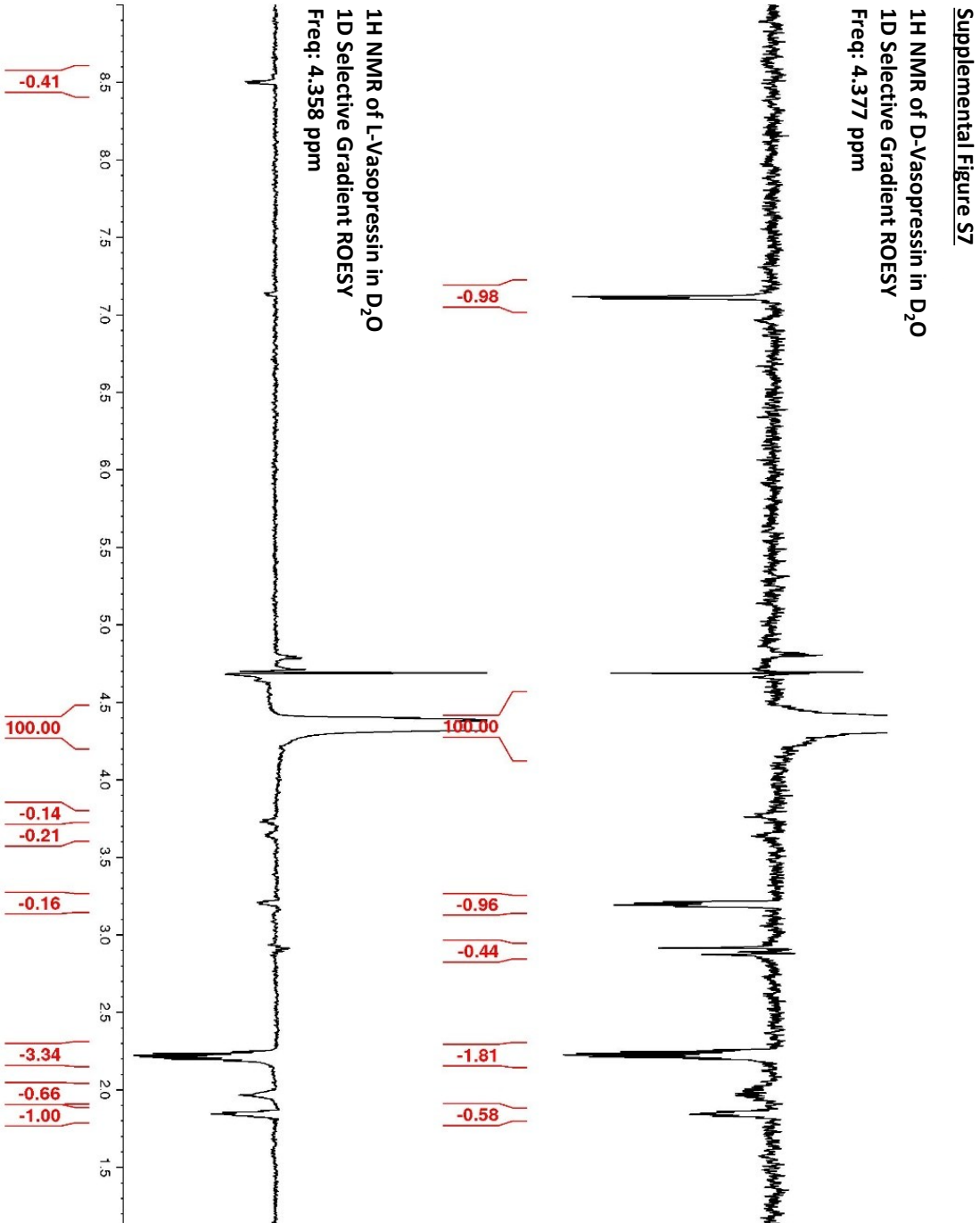


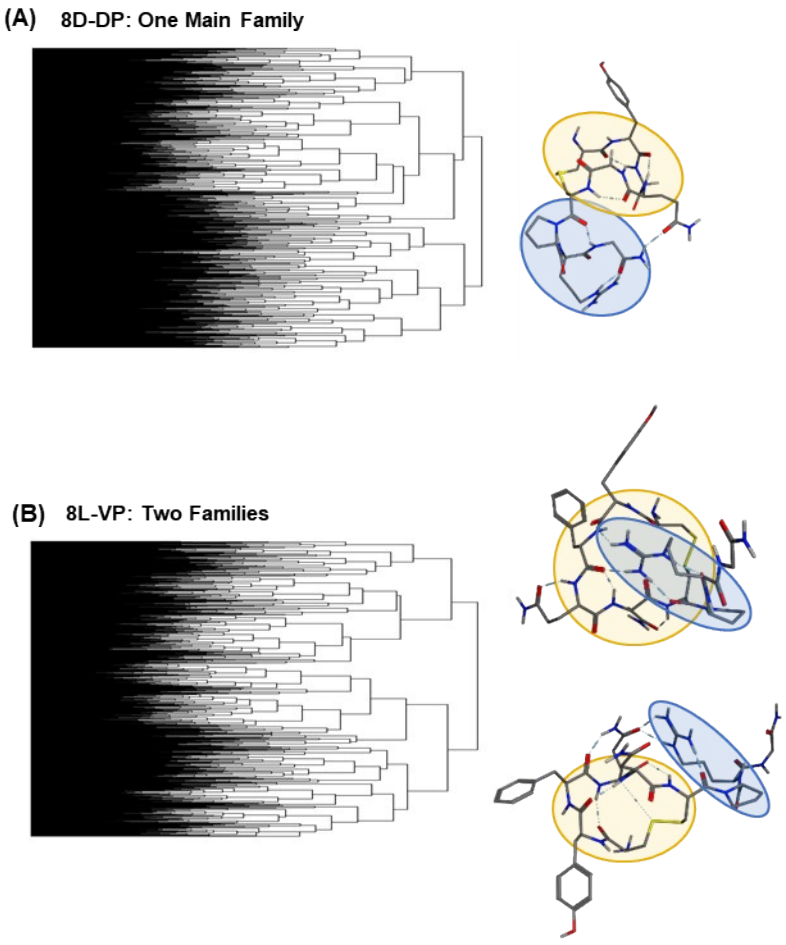
Supplemental Figure S4: Ion mobility spectra for the protonated $[M+H]^+$ Vasopressin diasteromers (L and D) in addition to a 1:1 mixture (gray dashed trace). The IM distribution for 8D-VP and the larger conformer of 8L-VP have essentially the same measured CCS. As more 8D-VP is added to a solution of 8L-VP (see gray trace), the distribution at higher CCS (*ca.* 310 Å^2) increases in abundance in comparison to the distribution of 8L-VP at lower CCS (*ca.* 304 Å^2). This observation helps support the claim that the two peaks observed for 8L-VP originate solely from the 8L-VP sample.



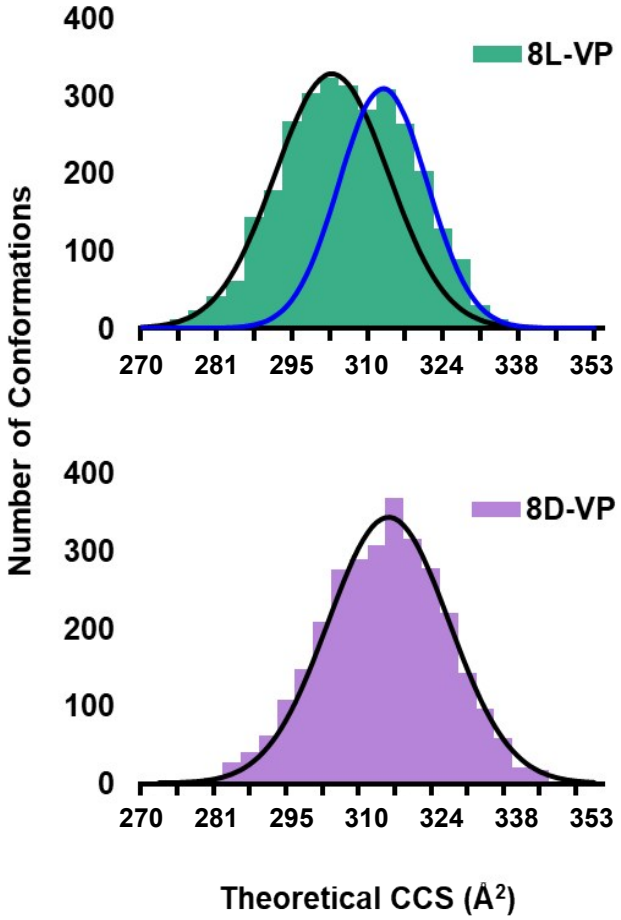
Supplemental Figure S5: To check the analytical purity of 8L-VP obtained from American Peptide **(A)** due to presence of two conformations (one of which possesses a similar CCS to the CCS distribution of 8D-VP), a second pure chemical standard of 8L-VP was purchased from a separate chemical vendor (Alfa Aesar, **B**). The spectra from both vendors have identical ion mobility distributions, and hence contamination of 8L-VP with quantities of 8D-VP is unlikely.

Supplemental Figures S6 & S7: To confirm enantiomeric purity of 8L-VP and to ensure the ion mobility distributions of Figure 2B arise solely from multiple gas phase conformations, rotating-frame nuclear Overhauser effect correlation spectroscopy (ROESY) was performed. The ^{1}H NMR spectra for both 8L-VP and 8D-VP were obtained on a Bruker 600 MHz NMR spectrometer equipped with a 5mm TXI cryogenically cooled NMR probe for enhanced sensitivity. The ^{1}H spectra were acquired with 128 scans to achieve maximum signal to noise ratio and the resulting spectra indicated pure analytical standards with no indication of contaminants or signs of a mixture (Figure S6). In order to examine the explicit differences in stereochemistry between 8L and 8D vasopressin, the proton corresponding to the stereocenter of the 8-arginine residue in ^{1}H NMR region of 4.37 ppm (blue ovals), was further investigated by 1D Selective Gradient ROESY for both diastereomers in order to observe the through-space interactions (Figure S7, referenced from Sikorska *et. al.*, *J. Pept. Sci.* **2008**, *14*, 76-84). After normalization of the excitation peak, each vasopressin diastereomer had different relative intensities for peaks showing nuclear Overhauser effect (NOI
8-arg
L- an





Supplemental Figure S8: RMSD clustering analysis performed on the 3,000 computationally-generated structures for each isomer. **(A)** 8D-Vasopressin exhibits one dominant family, while **(B)** 8L-Vasopressin has two main families. Representative low-energy structures are shown for each clustered structural population.



Supplemental Figure S9: Distribution of theoretical structures based on the calculated CCS. Here, normal distributions are overlaid across the histograms to illustrate that two structural families were observed for 8L-VP whereas only one primary structural family at higher CCS is observed for 8D-VP.