Native like helices in a specially designed β peptide in the gas phase

Supporting Information

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Peptide synthesis

Peptides were synthesized on a 0.05 mmol scale by manual solid-phase peptide synthesis on Lysine-preloaded Wang resin applying a standard Fmoc/t-Bu protocol[1] as follows: Fmoc-amino acids (eight eq. relative to resin loading for Fmoc-Ala-OH and two eq. for (R)-(Fmoc-amino)-2-methylpropionic acid, β^2hAla) were preactivated for seven minutes using equimolar amounts of N,N'-diisopropylcarbodiimide and 1-hydroxybenzotriazole hydrate in two to three mL N,N-dimethylformamide and then added to the resin. Reaction times varied from one hour to "over night". Coupling efficiency was monitored by the Kaiser-Test.[2] Fmoc-deprotection was achieved by treatment with piperidine and DBU (2 % v/v each) in DMF (4 x 5 min). All peptides were acetylated N-terminally using freshly distilled acetic anhydride (10 % v/v) and N,N-diisopropylethylamine (10 % v/v) in DMF. Following cleavage from the resin (95 % trifluoroacetic acid, 2 % triisopropylsilane, and 2 % water) and evaporation, the residues were dissolved in water, extracted three times with diethylether, lyophilized and purified by HPLC applying a gradient from 5 % to 50 % acteonitrile in 30 minutes. The combined fractions were lyophilized and identified by ESI-MS. Yield (TFA salt): Ac-Ala$_6$-Lys-OH: 8 %, calc. [M+H]$^+$ = 615.3465, found: 615.3573; Ac-β^2hAla$_6$-Lys-OH: 28 %, calc. [M+H]$^+$ = 699.4401, found: 699.4465.

Experimental Vibrational Spectra

Samples were dissolved in water/methanol v/v 50/50 and 1% acetic acid. Subsequently, the samples were mixed to yield a solution containing 100 μM of each peptide. Figure 1 shows the experimental raw data of the IRMPD spectra. The IRMPD spectra shown in the main manuscript are smoothed as explained in the molecular dynamics section of this Supporting Information.
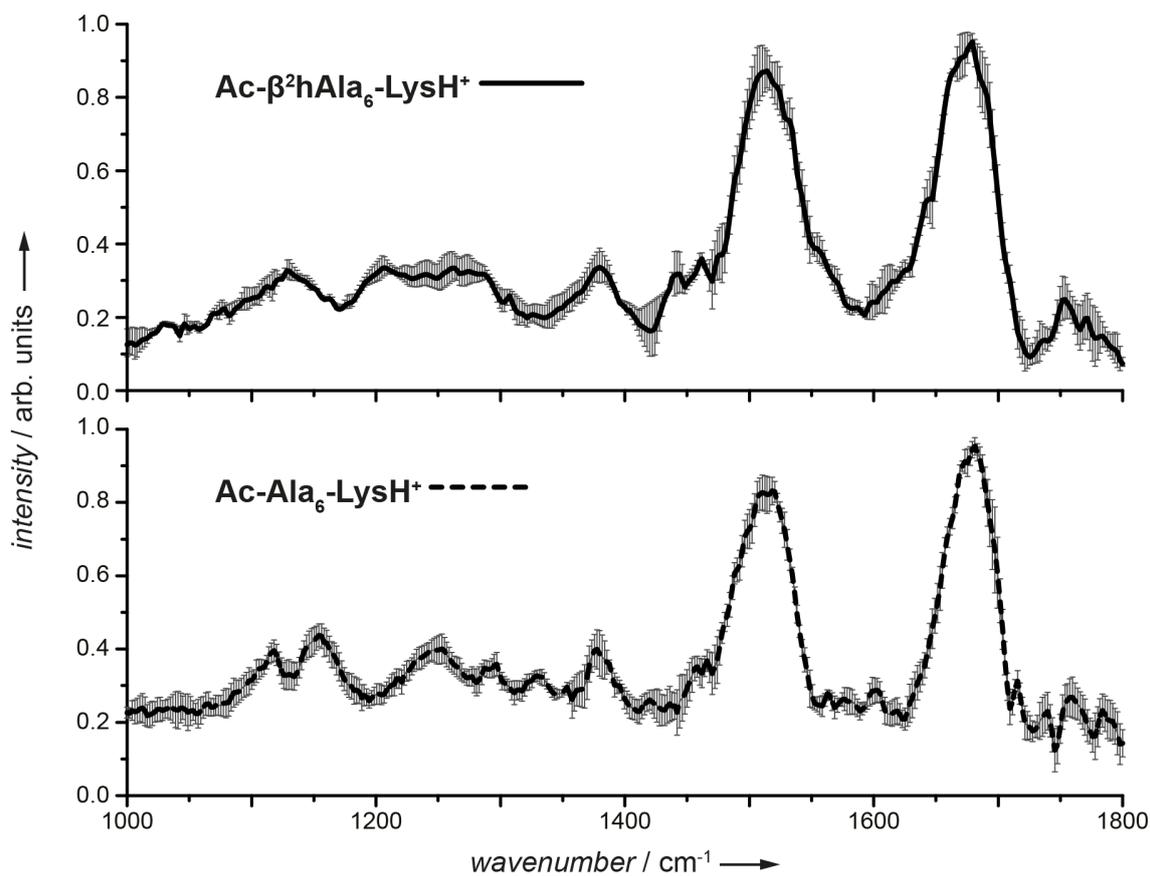
Figure 1: Experimental infrared multiphoton dissociation (IRMPD) spectra for Ac-β^2hAla$_6$-Lys(H$^+$) and Ac-Ala$_6$-Lys(H$^+$). The standard deviation of the measurements is given by grey vertical whiskers. The resulting spectra are averaged over multiple measurements. Measurements were repeated differently in different wavenumber regions for the two systems. Ac-β^2hAla$_6$-Lys(H$^+$): 1000 to 1440 cm^{-1} repeated 2 times; 1440 to 1800 cm^{-1} repeated 3 times. Ac-Ala$_6$-Lys(H$^+$): 1000 to 1100 cm^{-1} repeated 2 times; 1100 to 1440 cm^{-1} repeated 3 times; 1440 to 1800 cm^{-1} repeated 4 times.

Ion Mobility - Mass Spectrometry

Samples were dissolved in water/methanol v/v 50/50 and mixed together to yield a concentration of $100\,\mu$M for each peptide. 1% of acetic acid was added to help the ionization process. Collision cross sections (CCSs) were calculated from arrival-time distributions (ATD) as reported previously.[3] At constant temperature and pressure, the velocity v_d of the ions in the IMS cell is directly proportional to their mobility K and the applied electric field E (eq. 1).

$$v_d = KE \tag{1}$$

The drift time t_d that ions need to traverse a drift cell of length L is proportional to the inverse mobility as well as the inverse electric field (eq. 2). Consequently, the mobility of a given ion is typically determined by plotting the drift time *vs.* the inverse drift voltage and subsequent linear regression. The intercept t_0 of the fit corresponds to the time required to transport the ions from the end of the drift region into the mass analyzer.

$$t_d = \frac{L}{KE} + t_0 \tag{2}$$

From the obtained mobilities, absolute CCSs were calculated using the Mason-Schamp equation (eq. 3).[4]

$$\mathrm{CCS} = \frac{3e}{16N}\sqrt{\frac{2\pi}{\mu k_B T}}\frac{1}{K} \tag{3}$$

where N is the drift-gas number density, μ the reduced mass of the ion and the drift-gas molecule, k_B the Boltzmann constant and T the temperature. The given CCSs represent averages of at least two independent measurements.

Computational Section

For the β-peptide Ac-$(\beta^2\text{hAla})_6$-LysH$^+$, we employed the basin hopping algorithm that is implemented in Tinker[5,6] with 35 search directions, an energy window of $50\,\mathrm{kcal\,mol^{-1}}$, and an energy convergence criterion of $0.0001\,\mathrm{kcal\,mol^{-1}}$. Furthermore, we employed

replica-exchange molecular dynamics (REMD) simulations with the Gromacs program.[7] The simulations yielded an overall sampling time of $8\,\mu$s distributed over 16 replicas, each evolving in time for $500\,$ns in a temperature range between 300 K and 915 K. The integration time step was $0.5\,$fs and swaps between neighboring temperatures were attempted every $500\,$fs (every 1000 steps). We extracted snapshots in $2\,$ps intervals from the 300 K trajectory and sorted them into clusters according to their root mean square deviation (RMSD) using a cutoff criterion of $0.05\,$nm.[8]

Resulting structures were relaxed by density-functional theory (DFT) calculations employing the PBE functional[9] that was corrected for long-range dispersion interactions[10] (PBE+vdW). All electronic structure theory calculationswere performed with the FHI-aims program package which employs numeric atom-centered orbitals as basis sets.[11] Replica-exchange AIMD simulations starting from representative structures of the H12, H16, and H20 helices. The total sampling times were $486\,$ps, $576\,$ps, and $558\,$ps, respectively, each of them distributed over 18 replicas in a temperature range between 300 K and 687 K. We used a time step of $1\,$fs and swaps between replicas were attempted every $100\,$fs. Structure snapshots of all replicas were taken after each ps and post-relaxed with PBE+vdW. In summary, 14,739 PBE+vdW relaxations of candidate structures of the β-peptide Ac-$(\beta^2$hAla$)_6$-LysH$^+$ were performed. The initial geometry optimizations and the replica-exchange AIMD simulations were performed with Tier-1 basis sets.[11] The following post-relaxations of all minima within a relative energy window of $38.6\,$kJmol^{-1}, the harmonic free energy calculations, and AIMD simulations of selected conformers were performed with the Tier-2 basis sets and tight computational settings.[11] A free-energy correction that includes vibrational free energies in the harmonic approximation and rotational contributions in the rigid-rotor approximation, both computed with PBE+vdW and tight computational settings[11] at $T = 300\,$K, were applied. Additionally, we tested modifications of the theory towards a higher-level functional, PBE0,[12] and with the improved many-body description of the long-range dispersion,[13] similar to a recent study of the validity of exchange-correlation functionals and dispersion corrections for the prediction of peptide secondary structures.[14]

Lowest energy structures

The conformers for Ac-Ala$_6$-Lys(H$^+$) were obtained from Ref. 15. The total energies and relative energies for the conformers depicted in the main article are given in Table 1. They were calculated using "tight" computational settings and tier-2 basis sets.[11] Free energies were obtained in the harmonic oscillator-rigid rotor approximation. The conformers discussed in the main paper constitute the lowest free-energy (300 K) helical representatives and non-helical (compact) motifs. Structural data of the conformers displayed in the manuscript is available for download at the following locations :

`http://www.fhi-berlin.mpg.de/~baldauf/data/AcA6K.tgz`

`http://www.fhi-berlin.mpg.de/~baldauf/data/AcB6K.tgz`

best open `http://www.fhi-berlin.mpg.de/~baldauf/data` in a browser and right-click/save-as the respective compressed archive.

Table 1: Relative energies for Ac-Ala$_6$-Lys(H$^+$)[15] and Ac-β^2hAla$_6$-Lys(H$^+$), PBE+vdW

Conformer	F_{vib}(300K) (eV)	F_{rot}(300K) (eV)	ΔE_{tot} (kJ/mol)	ΔF(300K) (kJ/mol)
Ac-Ala$_6$-Lys(H$^+$)[15]
compact	19.058462	-0.452656	0.0^a	0.0
α-helix	18.954459	-0.456808	5.5	-4.9
Ac-β^2hAla$_6$-Lys(H$^+$)
compact	23.517325	-0.463184	0.0^b	0.0
H12	23.210270	-0.472776	28.6	-2.0
H16	23.304278	-0.466813	29.1	8.2
H20	23.406058	-0.462802	12.0	1.3

^{a} Total energy for reference: -58033.567461 eV
^{b} Total energy for reference: -64446.677137 eV

Theoretical vibrational spectroscopy

MD runs (with the PBE+vdW functional) were performed for 25 ps (NVE ensemble after at least 5 ps of thermalization at 300 K) with a time step of 0.75 fs. We used "tight" computational settings and tier2 basis sets.[11] The infrared spectra were calculated from the Fourier transform of the dipole time derivative autocorrelation function obtained from the first-principles MD simulations. To account for broadening effects that occur

in experiment the IR spectra were convoluted with a Gaussian function with a variable width of $\sigma = 0.5\%$ of the wavenumber.

As the R_P factor is sensitive to small kinks in the spectra, the experimental spectra had to be smoothed before the comparison to the theoretical data. In order not to over-smooth them, they were first interpolated on a 2 cm^{-1} grid before smoothed twice using a three-point-formula. Afterwards the spectra were interpolated on a fine 0.5 cm^{-1} grid. Figure 2 shows the simulated spectra computed for selected conformers of Ac-β^2hAla$_6$-Lys(H$^+$) in the wavenumber range from 0 to 4000 cm^{-1}.

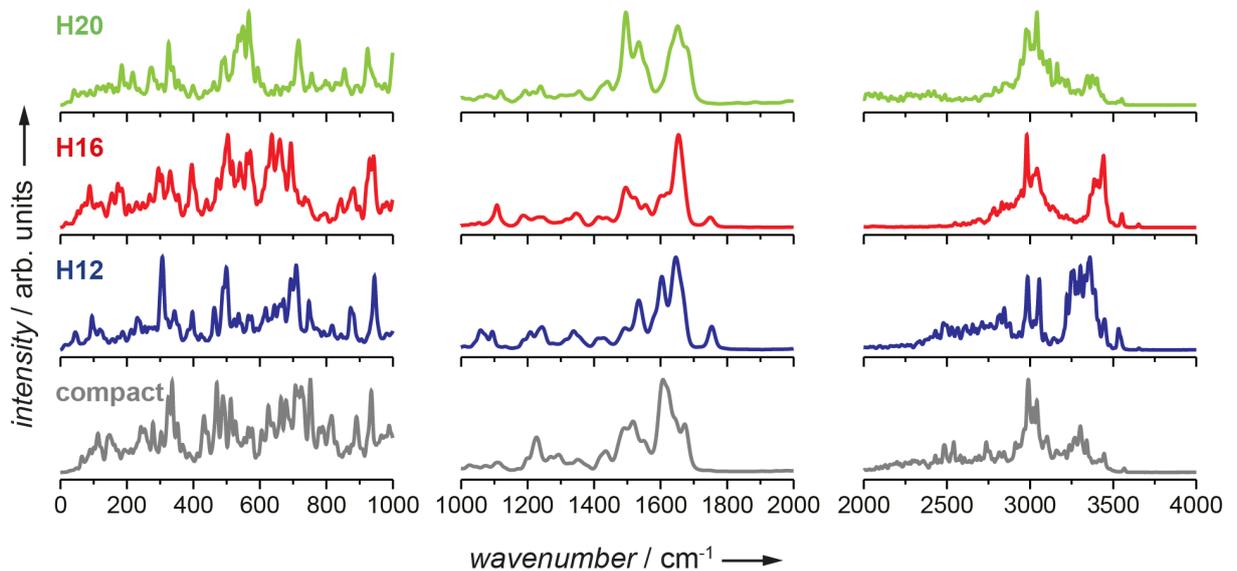
Figure 2: Vibrational spectra derived from AIMD simulations for the helical conformers H12, H16, and H20 and for a compact conformer of Ac-β^2hAla$_6$-Lys(H$^+$) in the wavenumber range from 0 to 4000 cm^{-1}.

For a quantitative comparison we employed the Pendry reliability factor,[16] which has been successfully used in the context of IR spectroscopy before.[17,18] Perfect agreement yields $R_P = 0$ while no correlation between the spectra yields $R_P = 1$. R_P is calculated including a rigid shift Δ_x of the theoretical spectrum against the experimental spectrum. This accounts for a small mode softening due to the functional and the negligence of nuclear quantum effects. Additionally, we employed a shift Δ_y of the normalized intensity axis to account for offsets in the experimental spectra. The R-factors were calculated in the wavenumber region between 1100 and 1720 cm^{-1}.

Simulating collision cross sections (CCSs)

CCSs in the main article were computed based on the projection approximation method by Wyttenbach *et al.*[19] (chosen accuracy: 0.2%). For comparison we also provide here results obtained with the trajectory method (TM).[20–22] 500,000 trajectories were simulated per single conformer, atomic charges were derived as Hirshfeld charges[23] from the PBE electron densities of the respective conformers. Table 2 gives a comparison of the CCS values for the DFT-optimized conformers obtained based on the PA and TM methods and the experimentally derived value. Due to random numbers used in both methods, repeated runs can lead to slightly different results.

Table 2: Comparison of calculated CCS with the projection approximation (PA) and the trajectory method (TM).

| | Ac-β^2hAla$_6$-Lys(H$^+$) | | | | | | Ac-Ala$_6$-Lys(H$^+$) | |
	A	B	H12	compact	H20	H16	α-helix	compact
PA (Å^2)	183	187	203	183	182	191	180	172
TM (Å^2)	-	-	204	182	182	193	181	171
Exp. (Å^2)	190						180	

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