Supporting Information for

Structural analyses of isolated cyclic tetrapeptides with varying amino acid residues

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a) R2PI spectrum of CP\textsubscript{Glu}

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e) Possible conformers and calculated IR spectra of CP\textsubscript{Glu} including a water solvent shell (B3LYP-D3/TZVP, $\epsilon = 78.39$)

f) NMR characterization of CP\textsubscript{Glu}

g) Mass spectrum of CP\textsubscript{Glu}
cyclo[L-Tyr(Me)-D-Pro-L-Ala-D-Pro] (CP$_{\text{Ala}}$)

a) R2PI spectrum of CP$_{\text{Ala}}$

Figure S1. One-color R2PI spectrum of CP$_{\text{Ala}}$ recorded in the region of 34783-36364 cm$^{-1}$. 
b) Possible doubly hydrogen-bonded structures and calculated IR spectra of \( \text{CP}_{\text{Ala}} \) (B3LYP-D3/TZVP)

Figure S2. Possible structures of \( \text{CP}_{\text{Ala}} \) with a doubly hydrogen-bonded binding motif (A type) based on DFT calculations (B3LYP-D3/TZVP). (Hydrogen bond lengths of these structures (given in Å): \( A \) (1.991 and 2.010), \( A_2 \) (2.00 and 1.991) and \( A_3 \) (2.009 and 2.031).)

Figure S3. Comparison between the experimental IR/R2PI spectrum and calculated frequencies obtained from DFT calculations (B3LYP-D3/TZVP) of selected conformers of \( \text{CP}_{\text{Ala}} \) (cf. Figure S2). For a better illustration the calculated intensities in the amide A region were doubled. (Details about the used mode-specific scaling factors cf. main paper.)

By comparing the harmonically calculated frequencies of \( A, A_2 \) and \( A_3 \) of \( \text{CP}_{\text{Ala}} \) with the experimental IR/R2PI spectrum it can be concluded that all spectra are very similar, but with respect to the NH stretching vibrations conformer \( A \) fits slightly better to the experimental frequencies than \( A_2 \) and \( A_3 \). Beside the calculated frequencies also energetic aspects have to be taken into account. Conformer \( A \) is energetically more favored than \( A_2 \) and \( A_3 \) which further supports the assignment of conformer \( A \). It should be remarked that the presence of \( A_2 \) and \( A_3 \) cannot completely be excluded. Therefore, conformers \( A_2 \) and \( A_3 \) may coexist with conformer \( A \).
c) Possible conformers of \textbf{CP}_{\text{Ala}} and calculated IR spectra \textit{without} dispersion corrections (B3LYP/TZVP)

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{FigureS4.png}
\caption{Possible structures of \textbf{CP}_{\text{Ala}} based on DFT calculations without dispersion corrections (B3LYP/TZVP). (A represents a structure with two internal hydrogen bonds (2.022 Å and 2.024 Å), B and D with one hydrogen bond (2.311 Å and 2.046 Å) and C without any hydrogen bond.)}
\end{figure}
Comparison between the experimental IR/R2PI spectrum and calculated frequencies obtained from DFT calculations without dispersion interactions (B3LYP/TZVP) of selected conformers of CPₐₐ (cf. Figure S4). Mode-specific scaling factors of 0.9567 for hydrogen-bonded NH stretching vibrations, 0.9613 for non-hydrogen-bonded NH stretching vibrations and 0.99 for the amide I-III region were used. For a better illustration the calculated intensities in the amide A region were doubled.

The comparison of the DFT calculations without dispersion corrections (B3LYP/TZVP) and the experimental IR/R2PI spectrum again yield the assignment of the doubly hydrogen-bonded conformer A comparable to the calculations including dispersion corrections.
d) Possible conformers and calculated IR spectra of CP$_{\text{Ala}}$ calculated at the ri-MP2/SVP level of theory

Figure S6. Possible structures of CP$_{\text{Ala}}$ based on ri-MP2/SVP calculations. (A represents a structure with two internal hydrogen bonds (1.927 Å and 1.968 Å), B and D with one hydrogen bond (2.216 Å and 1.980 Å) and C without any hydrogen bond.)

All structural binding motifs (A, B, C and D; Figure 3) obtained from the DFT calculations with dispersion corrections (B3LYP-D3/TZVP) were taken as starting geometries for the MP2 optimizations using the approximate resolution of identity (ri) methods together with a SVP basis set. These calculations were realized with Turbomole V7.1 [1,2]. The relative energies are corrected by the zero-point energy and refer to the most stable conformer.

The ri-MP2/SVP optimized structures (Figure S6) basically yield the same geometry as the DFT/B3LYP-D3/TZVP optimizations. Furthermore, a comparison of the calculated frequencies (ri-MP2/SVP) and experimental spectra of CP$_{\text{Ala}}$ (Figure S7) again leads to the assignment of the doubly hydrogen-bonded conformer A comparable to the DFT calculations including and excluding dispersion corrections. Thus an overall agreement between different theoretical methods is obtained. This agreement is a further support that DFT calculations with dispersion corrections (Grimme D3 [3]) and a triple-zeta basis set are a very efficient tool to obtain relative energies of different conformers with reliable frequencies.
Figure S7. Comparison between the experimental IR/R2PI spectrum and calculated frequencies obtained from ri-MP2/SVP calculations of selected conformers of CP$_{\text{Ala}}$ (cf. Figure S6). Mode-specific scaling factors of 0.9412 for NH stretching vibrations and 0.9492 for the amide I-III region were used (for scaling factors see Ref. 4; the factor of 0.9492 is an average of the CO stretching and NH bending scaling factors of 0.9415 and 0.9568, respectively). For a better illustration the calculated intensities in the amide A region were doubled.
e) Possible conformers and calculated IR spectra of $\text{CP}_{\text{Ala}}$ including a water solvent shell (B3LYP-D3/TZVP, $\varepsilon = 78.39$)

![Figure S8](image.png)

**Figure S8.** Possible structures of $\text{CP}_{\text{Ala}}$ in the energetic order based on DFT calculations (B3LYP-D3/TZVP, $\varepsilon = 78.39$). (A represents a structure with two internal hydrogen bonds (2.010 Å and 2.040 Å), B and D with one hydrogen bond (2.258 and 2.034 Å) and C without any hydrogen bond.)

Solvation effects were taken into account using the polarized continuum model (PCM) implemented in Turbomole's solvation program COSMO$^{[5,6]}$ (conductor-like screening model). This method determines the electrostatic interaction of a molecule with a solvent which treats the solvent as a continuum with a permittivity $\varepsilon$. These calculations were realized with a combination of Gaussian09$^{[7]}$ and Turbomole$^{[1,2]}$ (for details see main paper section III) using the B3LYP/TZVP level of theory including dispersion corrections (Grimme D3$^{[6]}$). The energy values were corrected by the zero-point energy and refer to the most stable conformer.

For $\text{CP}_{\text{Ala}}$ water is used as solvent with a permittivity of 78.39 (B3LYP-D3/TZVP) basically yielding the same results as without solvent effect, i.e. structure A turns out to be the most stable arrangement.
Figure S9. Calculated frequencies obtained from DFT calculations (B3LYP-D3/TZVP, \( \varepsilon = 78.39 \)) of selected conformers of CP\textsubscript{Ala} (cf. Figure S8). Mode-specific scaling factors of 0.9563 for hydrogen-bonded NH stretching vibrations, 0.9608 for non-hydrogen-bonded NH stretching vibrations and 0.985 for the amide I-III region were used. For a better illustration the calculated intensities in the amide A region were doubled.
f) NMR characterization of CP_{Ala}

**Figure S10**: $^1$H-NMR (600 MHz, DMSO-d$_6$) of CP_{Ala}.

**Figure S11**: $^{13}$C-NMR (151 MHz, DMSO-d$_6$) of CP_{Ala}.
g) Mass spectrum of $\text{CP}_{\text{Ala}}$

**Figure S12:** Mass spectrum of $\text{CP}_{\text{Ala}}$ with two fragments ($^* = 231 \text{ m/z}$ and $+ = 331 \text{ m/z}$). The parent mass signal of $\text{CP}_{\text{Ala}}$ obtained at a sample oven temperature of about 120 °C is constant while the IR/R2PI spectra are recorded.
cyclo[L-Tyr(Me)-D-Pro-L-Glu(Me)-D-Pro] \((\text{CP}_{\text{Glu}})\)

a) R2PI spectrum of \(\text{CP}_{\text{Glu}}\)

**Figure S13.** One-color R2PI spectrum of \(\text{CP}_{\text{Glu}}\) recorded in the region of 35461-36364 cm\(^{-1}\).
b) Possible doubly hydrogen-bonded structures and calculated IR spectra of CP\textsubscript{Glu} (B3LYP-D3/TZVP)

![Possible structures of CP\textsubscript{Glu} with doubly hydrogen-bonded binding motifs (A and B type) based on DFT calculations including dispersion corrections (B3LYP-D3/TZVP).](image)

(Hydrogen bond lengths of these structures (given in Å): A (1.988 and 2.022), A\textsubscript{2} (1.944 and 2.018), A\textsubscript{3} (1.995 and 1.997), B (1.961 and 2.264, 2.108), B\textsubscript{2} (2.267 and 1.987, 2.161) and B\textsubscript{3} (2.304 and 2.015, 2.122).)

**Figure S14.** Possible structures of CP\textsubscript{Glu} with doubly hydrogen-bonded binding motifs (A and B type) based on DFT calculations including dispersion corrections (B3LYP-D3/TZVP).
Figure S15. Comparison between the experimental IR/R2PI spectrum and calculated frequencies obtained from DFT calculations (B3LYP-D3/TZVP) of selected conformers of CP$_{Glu}$ (cf. Figure S14). For a better illustration the calculated intensities in the amide A region were doubled. (Details about the used mode-specific scaling factors cf. main paper.)

By comparing the harmonically calculated frequencies of CP$_{Glu}$ with the experimentally obtained vibrations A2, A3, B2 and B3 do not explain the IR spectrum as well as the A and B structures. In case of conformers A2 and A3 the calculated vibrations are above 1750 cm$^{-1}$ (originating from the carbonyl group of the Glu side chain) and conformers B2 and B3 have predicted frequencies at about 1675 cm$^{-1}$ which are not observed in the experimental spectrum. Moreover these conformers are energetically not favored.
c) Possible conformers of $\text{CP}_{\text{Glu}}$ and calculated IR spectra without dispersion corrections (B3LYP/TZVP)

**Figure S16.** Possible structures of $\text{CP}_{\text{Glu}}$ based on DFT calculations without dispersion corrections (B3LYP/TZVP). $A$ and $B$ represent structures with two internal hydrogen bonds (2.019 Å and 2.015 Å for $A$, 2.006 Å and 2.370 Å for $B$), additionally $B$ forms a further hydrogen bond to the CO side chain of the glutamic acid with a bond-length of 2.147 Å. $C$ and $E$ contain one hydrogen bond (bond-lengths 2.294 Å and 2.173 Å) and $D$ has one internal hydrogen bond (2.142 Å) as well as one bond to the CO side chain of the glutamic acid (bond-length 2.241 Å).

A binding motif of $\text{CP}_{\text{Glu}}$ without hydrogen bonds ($F$ type) was not found according to these DFT calculations without dispersion corrections within a relative energy of 6000 cm$^{-1}$. 
Figure S17. Comparison between the experimental IR/R2PI spectrum and calculated frequencies obtained from DFT calculations without dispersion corrections (B3LYP/TZVP) of selected conformers of CP_Glu (cf. Figure S16). For a better illustration the calculated intensities in the amide A region were doubled. The following mode-specific scaling factors are used: 0.9567 for hydrogen-bonded NH stretching vibrations, 0.9613 for non-hydrogen-bonded NH stretching vibrations and 0.99 for the amide I-III region. For a better illustration the calculated intensities in the amide A region were doubled.

Comparison of the DFT calculations without dispersion corrections (B3LYP/TZVP) and the experimental IR/R2PI spectrum again lead to the assignment of the doubly hydrogen-bonded conformers A and B comparable to the calculations including dispersion corrections.
d) Possible conformers and calculated IR spectra of $\text{CP}_{\text{Glu}}$ calculated at the ri-MP2/SVP level of theory

**Figure S18.** Possible structures of $\text{CP}_{\text{Glu}}$ based on ri-MP2/SVP calculations. $\text{A}$ and $\text{B}$ represent structures with two internal hydrogen bonds ($1.928 \text{ Å}$ and $1.978 \text{ Å}$ for $\text{A}$, $1.949 \text{ Å}$ and $2.276 \text{ Å}$ for $\text{B}$), additionally $\text{B}$ forms a further hydrogen bond to the CO side chain of the glutamic acid with a bond-length of $2.003 \text{ Å}$. $\text{C}$ and $\text{E}$ contain one hydrogen bond (bond-lengths $2.253 \text{ Å}$ and $2.092 \text{ Å}$) and $\text{D}$ has one internal hydrogen bond ($1.984 \text{ Å}$) as well as one bond to the CO side chain of the glutamic acid (bond-length $1.911 \text{ Å}$).

All structural binding motifs ($\text{A}$, $\text{B}$, $\text{C}$, $\text{D}$, $\text{E}$ and $\text{F}$; Figure 5) obtained from the DFT calculations with dispersion corrections (B3LYP-D3/TZVP) were taken as starting geometries for the MP2 optimizations using the approximate resolution of identity (ri) methods together with a SVP basis set. These calculations were realized with Turbomole V7.1. The relative energies are corrected by the zero-point energy and refer to the most stable conformer.

The ri-MP2/SVP optimized structures (Figure S18) basically yield the same geometry as the DFT/B3LYP-D3/TZVP optimizations only the relative energies change. Furthermore, a comparison of the calculated frequencies (ri-MP2/SVP) and experimental spectra of $\text{CP}_{\text{Glu}}$ (Figure S19) again lead to the assignment of the doubly hydrogen-bonded conformers $\text{A}$ and $\text{B}$. Both conformers are stabilized by two internal hydrogen bonds (within the cyclic peptide ring) but conformer $\text{B}$ additionally contains a hydrogen bond between the carbonyl group of the Glu side chain and the NH$_{\text{Glu}}$ group comparable to the DFT calculations including and excluding dispersion corrections. It should be remarked that in case of the DFT-D3 calculations the assigned conformers $\text{A}$ and $\text{B}$ are the most stable structures whereas in case of the ri-MP2 method structure $\text{C}$ is more stable than conformer $\text{B}$ although the
frequencies still indicate that this structural arrangement has to be assigned to the IR spectrum (similar to the DFT calculation). Like in the case of CP_{Ala} this agreement is a further support that DFT calculations with dispersion corrections (Grimme D3 [3]) and a triple-zeta basis set are a very efficient tool to obtain relative energies of different conformers with reliable frequencies.

Figure S19. Comparison between the experimental IR/R2PI spectrum and calculated frequencies obtained from ri-MP2/SVP calculations of selected conformers of CP_{Glu} (cf. Figure S18). Mode-specific scaling factors of 0.9412 for NH stretching vibrations and 0.9492 for the amide I-III region were used (for scaling factors see Ref. 4; the factor of 0.9492 is an average of the CO stretching and NH bending scaling factors of 0.9415 and 0.9568, respectively). For a better illustration the calculated intensities in the amide A region were doubled.
e) Possible conformers and calculated IR spectra of CP$_{\text{Glu}}$ including a water solvent shell (B3LYP-D3/TZVP, $\varepsilon = 78.39$)

![Possible structures of CP$_{\text{Glu}}$ in the energetic order based on DFT calculations (B3LYP-D3/TZVP, $\varepsilon = 78.39$).](image)

**Figure S20.** Possible structures of CP$_{\text{Glu}}$ in the energetic order based on DFT calculations (B3LYP-D3/TZVP, $\varepsilon = 78.39$). A and B represent structures with two internal hydrogen bonds (2.010 Å and 2.037 Å for A, 1.968 Å and 2.106 Å for B), additionally B forms a further hydrogen bond to the CO side chain of the glutamic acid with a bond-length of 2.263 Å. C and E contain one hydrogen bond (bond-lengths 2.319 Å and 2.092 Å) and D has one internal hydrogen bond (2.009 Å) as well as one bond to the CO side chain of the glutamic acid (bond-length 1.895 Å).

Similar to CP$_{\text{Ala}}$ solvation effects for CP$_{\text{Glu}}$ were also taken into account by using the PCM model implemented in Turbomole’s solvation program COSMO$^{[5,6]}$ (for details about the calculation procedure see section CP$_{\text{Ala}}$/e)). Water is used as solvent with a permittivity of 78.39 (B3LYP-D3/TZVP) yielding a structure with one internal hydrogen-bond as most stable arrangement. This is in contrast to the results of CP$_{\text{Ala}}$ for which the most stable arrangement of the non-solvated cyclic peptide remains the most stable one upon solvation. This difference results from the additional carbonyl group in CP$_{\text{Glu}}$ (compared to CP$_{\text{Ala}}$) which can be very efficiently solvated by water molecules. Thus the variation of the side-chain can affect structural preferences significantly with respect to solvation.
Figure S21. Calculated frequencies obtained from DFT calculations (B3LYP-D3/TZVP, $\varepsilon = 78.39$) of selected conformers of CP\textsubscript{Glu} (cf. Figure S20). Mode-specific scaling factors of 0.9563 for hydrogen-bonded NH stretching vibrations, 0.9608 for non-hydrogen-bonded NH stretching vibrations and 0.985 for the amide I-III region were used. For a better illustration the calculated intensities in the amide A region were doubled.
f) NMR characterization of \( \text{CP}_{\text{Glu}} \)

**Figure S22**: \(^1\text{H-NMR (400 MHz, DMSO-}d_6\text{)}\) of \( \text{CP}_{\text{Glu}} \).

**Figure S23**: \(^{13}\text{C-NMR (101 MHz, DMSO-}d_6\text{)}\) of \( \text{CP}_{\text{Glu}} \).
g) Mass spectrum of $\text{CP}_{\text{Glu}}$

**Figure S24:** Mass spectrum of $\text{CP}_{\text{Glu}}$. The parent mass signal of $\text{CP}_{\text{Glu}}$ obtained at a sample oven temperature of about 150 °C is constant while the IR/R2PI spectra are recorded.
References


