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Supporting Information

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Amide to Ester Substitution in Coiled Coils – The Effect of H-Bond Elimination on Protein Structure Formation

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A. - *Peptide synthesis and characterization*

The peptide and depsipeptides were synthesized using Boc-SPPS.[1, 2] The optimized protocols for the machine assisted synthesis (433A Peptide Synthesizer, Applied Biosystems) will be reported elsewhere [J. Spengler, B. Koksch, F. Albericio, manuscript in preparation]. The compounds were purified by semipreparative HPLC and characterized by analytical HPLC and MALDI. Analytic HPLC uses a HPLC Waters 1525, an automatic injector 717 plus and a detector UV-Vis Waters 2487. The column Nucleosil C_{18} (250 x 4 mm) was run with acetonitrile (0.036% TFA) and water milipore (0.045% TFA). Data were managed with Breeze v3.20 software. In semipreparative scale, the HPLC used was the model Waters 600, the automatic injector Waters 2700, the detector UV-Vis Waters 2487. Samples were collected with the Waters Fraction Collector II. The column Symmetry C_{18} (100 x 30 mm) was run with acetonitrile (0.05% TFA) and water millipore (0.1% TFA). Data were managed with MassLynux 3.5 software. For MALDI-analysis, the samples were prepared with the ACH-matrix (alpha-cyano-4-hydroxy cinnamic acid, Aldrich). The spectra were obtained with a 4700 Proteomics Analyzer (Applied Biosystems).

peptide	MALDI
pp	2892.26 $M+H^+$ (exact mass: 2891.73)
L5λ	2893.93 $M+H^+$ (exact mass: 2892.72)
A10α	2893.58 $M+H^+$ (exact mass: 2892.72)
K11κ	2893.49 $M+H^+$ (exact mass: 2892.72)
L12λ	2893.87 $M+H^+$ (exact mass: 2892.72)
A13α	2894.01 $M+H^+$ (exact mass: 2892.72)
L22λ	2893.39 $M+H^+$ (exact mass: 2892.72)
L12λ A13α	2894.93 $M+H^+$ (exact mass: 2893.70)

B. - *CD Spectroscopy*

All spectra were measured on a Jasco J-810 spectropolarimeter at peptide concentrations of 6 mg/mL in 100 mM sodium phosphate buffer at pH 7.4 and 20°C. A high peptide concentration was chosen, because at lower concentrations depsi peptides only rarely folded and had only a partially α-helical structure. Small variations in the peptide concentrations did not change the CD-spectra or the denaturation curves. The mean residue molar ellipticity was calculated using the following equation:

$$[\Theta] = (\Theta_{obs} \times MRM)/(10 \times l \times c)$$

Where Θ_{obs} is the observed ellipticity expressed in mdeg, *MRM* is the total molecular mass (TFA-salt) of the peptide divided by the number of amino acid residues, *l* is the optical path length in cm (0.01 cm), and *c* is the final peptide concentration in mg/mL.

C. - *Chemical denaturation experiments*

For all chemical denaturation experiments, the change in the ellipticity was followed at 222 nm, which is indicative for the helical content of peptides. An equilibration time of 3 h was used. Increasing the equilibration time above 3 h did not change the curve. GuHCl was purchased from Fluka. Concentration of the GuHCl stock solution was determined by the refractive index.[3] The denaturation curves were fitted by using the program Sigma Plot 10.0 (sigmoidal - 5 parameter; exponential rise to max. - double 5 parameter).

Thermodynamic Analysis[4, 1, 5]

$N \leftrightarrow U$ $\qquad$ $f_N = ([\Theta] - [\Theta]_U) / ([\Theta]_N - [\Theta]_U)$

$K_U = f_U/f_N = f_U/1\text{-}f_U$ $\qquad$ $K_U = \exp(-\Delta G_U/RT)$

$\Delta G_U = -RT \cdot \ln(K)$ $\qquad$ $\Delta G_U = -RT \cdot \ln(f_U/1\text{-}f_U)$

The linear dependence of ΔG_U on denaturant concentration observed in the transition region continues to zero concentration and fit data to an equation of the form[6]:

$\Delta G_U = \Delta G_U^{H2O} - m\,[GuHCl]$

$\Delta G_U^{H2O} = \Delta G_U + m\,[GuHCl]$

$\Delta G_U^{H2O} = -RT \cdot \ln(f_U/1\text{-}f_U) + m\,[GuHCl]$

At $D_{50\%}$ follows $f_U = 1\text{-}f_U$ $\quad \Rightarrow \quad$ $\Delta G_U^{H2O} = m\,[GuHCl\,(D_{50\%})]$

$\Rightarrow$ Calculation of the $\Delta\Delta G_U^{H2O}$ values [7]:

$\Delta\Delta G_U^{H2O}{}_{(2)} = [(m_{(PP)} + m_{(2)}) / 2] * [D_{50\%\,(2)} - D_{50\%\,(PP)}]$

D. - *Size Exclusion Chromatography*

Size exclusion chromatography of the parent peptide (pp) was accomplished with an Äkta FPLC system equipped with a Superdex 30 column (HiLoad 16/60 prep grade) from Amersham Biosciences. Elution buffer was 10 mM sodium phosphate pH 7.4 and the flowrate was 1.2 mL/min. Peptide absorbance of 0.5 mg pp was registered at 254 nm.

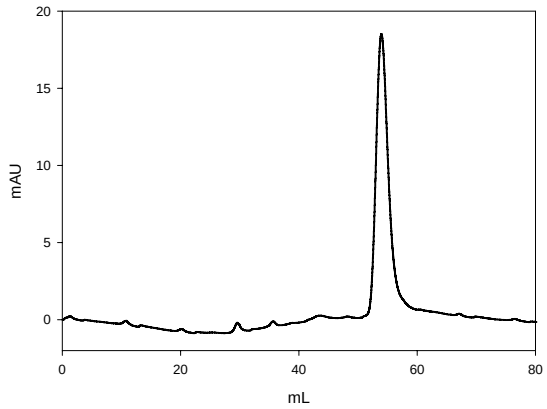


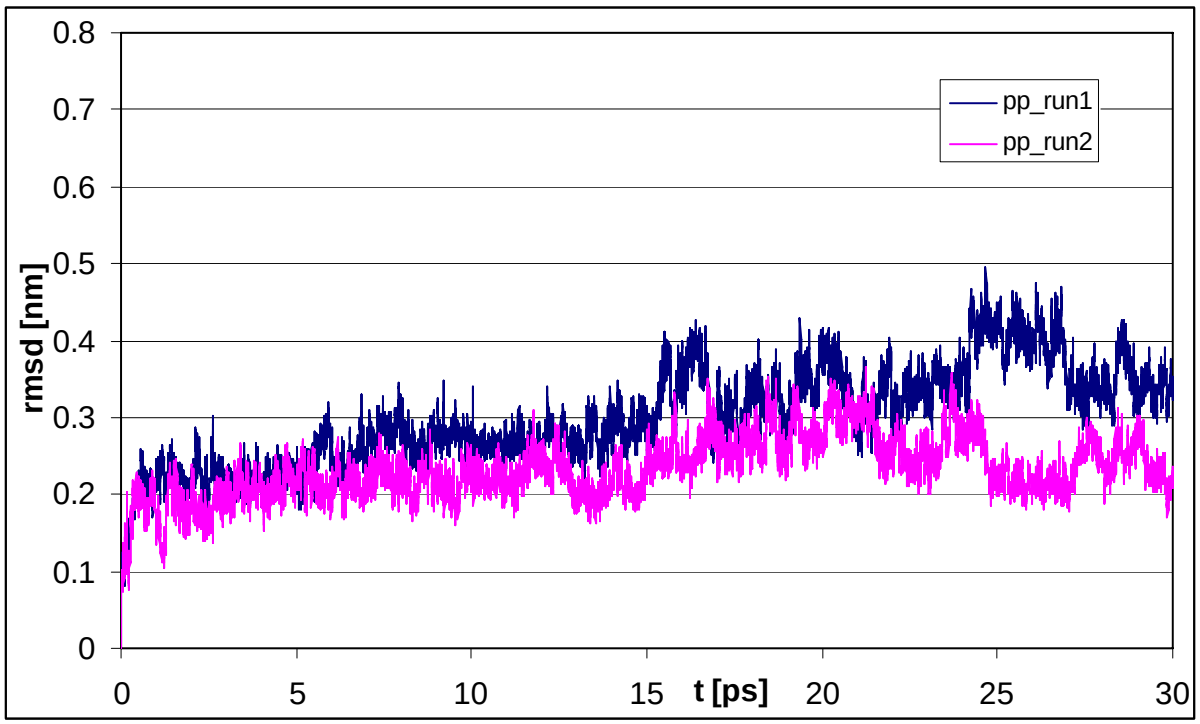
Figure S1: Size exclusion chromatogram of the pp.

E. – *Molecular Dynamics Simulations*

The MD-simulations were carried out with the Gromacs suite[8] using the Gromos 53a6 force field.[9] A twin range cut-off for van der Waals (0.9/1.4 nm) and a smooth particle mesh Ewald algorithm for Coulomb interactions (switching distance of 0.9 nm) were used. The neighbor lists were updated every 0.01 ps.[10] Temperature (320 K) and pressure (1 bar) were kept constant by Berendsen weak coupling, with coupling constants of 0.1 ps for the temperature and 1 ps for the pressure.[11] Constraints were applied to the bonds of the peptide with the LINCS algorithm.[12] The coiled coil dimers were solvated in dodecahedric boxes with around 9,500 SPC water molecules, periodic boundary conditions were applied.

The simulations were performed on servers with 2 Opteron Dual Core processors and a clockspeed of 2.2 GHz; each ns of simulation required about 9 h of computation time.

To ensure the validity of the MD simulations the trajectory of pp was recalculated with different initial velocities. The shown trajectory pp_run1 is the one shown in the paper in Figure 2b, pp_run2 was recalculated. One clearly sees the, to a certain level, random based nature of MD simulations. Nevertheless, it is clearly shown that both simulations correlate very well.



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