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# A Photocontrolled β-Hairpin Peptide

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**Abstract:** β-Hairpins constitute the smallest β-type structures in peptides and proteins. The development of highly stable, yet monomeric β-hairpins based on the tryptophan zipper motif was therefore a remarkable success [A. G. Cochran, N. J. Skelton, M. A. Starovasnik, *Proc. Natl. Acad. Sci USA* **2001**, *98*, 5578–5583]. We have been able to design, synthesize and characterize a hairpin based on this motif which incorporates an azobenzene-based photoswitch, that allows for time-resolved folding studies of β-structures with unprecedented time resolution. At room temperature the *trans*-azo isomer exhibits a mostly disordered structure; however, light-induced isomerization to the *cis*-azo form leads to a predominantly extended and parallel conformation of the two peptide parts, which are linked by the novel photoswitch, [3-(3-aminomethyl)-phenylazo]phenylacetic acid (AMPP). While in the original sequence the dipeptide Asn-Gly forms a type I′ β-turn which connects the two strands of the hairpin, this role is adopted by the AMPP chromophore in our photoresponsive β-hairpin that can apparently act as a β I′-turn mimetic. The β-hairpin structure was determined and confirmed by NMR spectroscopy, but the folding process can be monitored by pronounced changes in the CD, IR and fluorescence spectra. Finally, incorporation of the structurally and functionally important β-hairpin motif into proteins by chemical ligation might allow for the photocontrol of protein structures and/or functions.

**Keywords:** azobenzene · NMR spectroscopy · peptides · photochemistry · protein folding

## Introduction

Of the two major structural elements of proteins, α-helices have been thoroughly investigated for many decades, whereas defined models of β-sheets have been developed only in recent years.[1] In contrast to helices, where local ($i$,$i$+3) or ($i$,$i$+4) hydrogen bonds confer stability, β-strands require a structural context for stabilization. Tertiary structure is, therefore, the crucial prerequisite for the β-extended secondary structure. The smallest structural motif consisting of short β-strands is the β-hairpin motif, where two antiparallel strands are linked by a turn or small loop. In proteins β-hairpins often constitute binding epitopes and are involved in protein–protein or protein–DNA interaction.[2] While model α-helices have been studied extensively for decades, models for β-structure are less accessible. Besides the requirement for at least two β-strands, peptides with preference for an extended conformation are usually prone to aggregation. Additionally, small β-structures such as the β-hairpin are only marginally stable and more difficult to characterize spectroscopically than α-helices.[1] The development of highly stable, yet monomeric β-hairpins based on, for example, a designed tryptophan zipper motif[3] has been a major

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Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author: Four tables containing NMR resonance assignments and distance constraints for both *cis*-azo and *trans*-azo isomer of the AMPP-peptide.

step forward and allowed successful experimental and theoretical folding studies.[4] Molecular dynamics simulations using the folding@home approach[5] found overall folding rates in good agreement with experimental time-resolved temperature jump experiments.[4]

Following our previous strategy of combining small protein motifs with an azobenzene photoswitch,[6] the aim of the present study was the design of a tryptophan zipper-like β-hairpin with a turn containing azobenzene (Figure 1). The azobenzene chromophore offers the possibility to control the hairpin structure and initiate a folding or unfolding transition. High isomerization yield, remarkable photostability and ultra-fast kinetics (few ps) make azobenzene an ideal trigger for the use in time-resolved ultra-fast pump-probe spectroscopy of peptides. Small, well-defined model systems with an ultrafast trigger allow detailed folding studies to be carried out both experimentally and theoretically on the same molecules. Only the comparison of simulation and experiment permits assessment and improvement of the theoretical description on one side and a detailed interpretation of the observed rate constants on the other hand. Recently, we had been able to introduce the first model where ultrafast conformational dynamics of a peptide could be investigated by both femtosecond absorption spectroscopy and all-atom molecular mechanics simulations.[7]

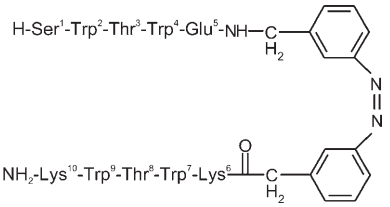



Figure 1. Structure of the β-hairpin peptide with the AMPP chromophore.

Herein we present the synthesis and characterization of a β-hairpin based on the tryptophan zipper motif which incorporates an azobenzene-based photoswitch and thus allows for time-resolved folding studies of β-structures with unprecedented temporal resolution. During the preparation of this manuscript a β-hairpin similar to ours has been published.[8] While both hairpin models share the same chromophore proposed by Kräutler et al.[9] the tryptophan zipper motif applied in our model compound resulted in good solubility of both the *cis*-azo and *trans*-azo isomers; this behavior allowed a structural characterization, whereas the amino acid sequence chosen by Hilvert and colleagues led to insolubility of the *trans*-azo isomer[8] possibly compromising its use in folding studies.

## Experimental Section

### Synthesis of the AMPP chromophore

**Materials**: All commercially available reagents and solvents were used as received. Reactions were monitored by thin-layer chromatography on precoated silica gel 60 F254 plates (Merck, Germany) and visualized using UV irradiation (254 nm). Flash chromatography was performed on silica gel 60 (Merck, Germany). Analytical RP-HPLC was carried out with Waters equipments on a XTerra C8 150×3.9 mm column (Waters, Germany) using a linear gradient of acetonitrile/2% $H_3PO_4$ from 5:95 to 90:10 in 15 min at a flow rate of 1.5 mL min$^{-1}$. ESI-MS was recorded on a Perkin–Elmer SCIEX API 165 spectrometer.

**(3-Aminobenzyl)carbamic 9*H*-fluoren-9-yl methyl ester (1)**: A solution of Fmoc-OSu (14.9 g, 44 mmol) in acetonitrile (100 mL) was added dropwise over a period of 30 min at room temperature to a stirred solution of (3-amino)benzylamine (44 mmol, 5 mL) and $Et_3N$ (6.1 mL, 44 mmol) in acetonitrile/DMF 10:1 (55 mL). After the reaction mixture was stirred for 1 h, the product was precipitated with water, filtered off and washed with methyl *tert*-butyl ether/trifluoroethanol 1:1 to give compound **1** as a white solid (8.73 g, 58%); analytical data were identical to those reported in ref. [8].

**3-Nitrosophenylacetic acid (2)**: $NH_4Cl$ (0.48 g, 9.0 mmol) and Zn powder (0.88 g, 13.5 mmol) were added portionwise at room temperature to a solution of 3-nitrophenylacetic acid (0.91 g, 5.0 mmol) in 2-methoxyethanol (25 mL). After 45 min stirring, the mixture was cooled to 0°C on an ice bath, and a solution of $FeCl_3 \cdot 6H_2O$ (2.12 g, 75 mmol) in ethanol/water 5:1 (30 mL) was added dropwise. After the reaction mixture was stirred for 1 h, the mixture was extracted, diluted with diethyl ether and the organic phase was washed three times with water and dried over $Na_2SO_4$. Flash column chromatography with ethyl acetate/hexane/acetic acid 100:5:1 furnished **2** (0.39 g, 47%) as a brownish solid which was used without further purification.

**3-[(3-*N*-Fmoc-aminomethyl)phenylazo]phenylacetic acid (3, Fmoc-AMPP-OH)**: Compound **1** (0.78 g, 2.25 mmol) was added portionwise to a solution of **2** (0.76 g, 4.55 mmol) in acetic acid (5 mL), and the resulting mixture stirred for 12 h at room temperature. The precipitate was filtered off and washed with diethyl ether to give the compound **3** (0.57 g, 51%) as a bright orange solid; HPLC: $t_r = 7.65$ min (*cis* isomer) and $t_r = 8.65$ min (*trans* isomer); analytical data were identical to those reported in ref. [8].

**H-Ser-Trp-Thr-Trp-Glu-AMPP-Lys-Trp-Thr-Trp-Lys-$NH_2$**: The peptide containing the AMPP building block was synthesized on a 0.25 mmol scale by standard protocols of Fmoc chemistry on an automated peptide synthesizer (PerSeptive Biosystems, Pioneer) with TentaGel S RAM resin (Rapp Polymere GmbH, Tübingen, Germany, 0.28 mmol g$^{-1}$) as solid support. The Fmoc-protected amino acids (Iris Biotech GmbH, Marktredwitz, Germany) were coupled in four-fold excess by HOBt/HBTU/DIEA 1:1:2 in NMP, while coupling of Fmoc-AMPP-OH proceeded manually under identical conditions. Fmoc cleavage was performed with 20% piperidine in NMP (3×1 min and 1×15 min). Cleavage from the resin and deprotection were carried out with TFA/water (95:5) in 2 h. The crude peptide was purified by RP-HPLC on a preparative column (VP 250/21 Nucleosil 100–5 C8, Macherey and Nagel, Düren, Germany) by eluting with a linear gradient from 20 to 65% acetonitrile in 0.1% aqueous TFA at a flow rate of 10 mL min$^{-1}$. Fractions containing homogeneous material were pooled and lyophilized (59 mg, 16%); HPLC: $t_r = 4.9$ min (*cis* isomer) and 5.3 min (*trans* isomer); ESI-MS: $m/z$: 845.0 $[M+2H]^{2+}$; $M_r$: calcd for $C_{87}H_{106}N_{20}O_{16}$: 1688.0; the $^1$H NMR (500 MHz in $CD_3OH$) spectra for the *cis*- and *trans* isomer were consistent with the assigned structure (see Tables S1 and S2 of the Supporting Information).

**Circular dichroism**: CD spectra in the 190–250 nm range were recorded on a JASCO J-715 spectropolarimeter equipped with a thermostated cell holder. Spectra were obtained at peptide concentrations between 25 and 90 μM employing quartz cells of 1 or 2 mm optical path length and are reported in terms of molar ellipticity per residue ($[\Theta]_R$). The concentrations were determined by UV absorption of the Trp residues and the AMPP chromophore. The CD spectra of the *cis*-azo isomer were recorded after irradiation at 360 nm until the photostationary state was reached (~80% *cis*-azo isomer as determined by $^1$H NMR experiments upon irradiation of a *trans*-azo isomer under identical conditions). The CD spectrum of the *trans*-azo isomer was recorded after thermal relaxation of the sample in the dark.

**Photoisomerization**: A xenon lamp 450 XBO (Osram, München) was used for irradiation at 360 or 430 nm (filter from Itos, Mainz) with a light intensity of ~20 mW in all experiments except for IR spectroscopy (see below). The photostationary states are reached after irradiation for ca 10 min at the required 360 and 430 nm wavelengths, respectively. A maximum of *cis*- to *trans*-azo ratios of 80:20 and 20:80 are obtained at the photostationary states as determined from integrals of well-separated resonances of both isomers in 1D $^1$H NMR spectra.

**Fluorescence spectroscopy**: Emission spectra in the 300–400 nm range were recorded upon excitation at 280 nm on a Perkin–Elmer LS50B fluorimeter (Perkin–Elmer Corp., Norwalk, CT, USA) at room temperature in aqueous solution (pH 3.6). Peptide concentration was 2 μM as determined by UV absorption of the Trp residues and the AMPP chromophore.

**IR spectroscopy**: IR spectra were obtained at 25 °C on 1 mM peptide samples dissolved in $CD_3OD$ (Merck, Darmstadt) with an IFS66 FTIR spectrophotometer from Bruker (Rheinstetten, Germany) using a cuvette with $CaF_2$ windows (path length 220 μm). Photoisomerization from *trans*-azo to *cis*-azo was achieved with light of 355 nm from a 1000 W HgXe high-pressure lamp (LOT, Darmstadt) combined with UG1 and WG 320 glass filters (Schott, Mainz). For the irradiation to the *trans*-azo form a Schott KL2500 LCD cold-light source together with the glass filters KG2 and GG 400 (Schott, Mainz) were used.

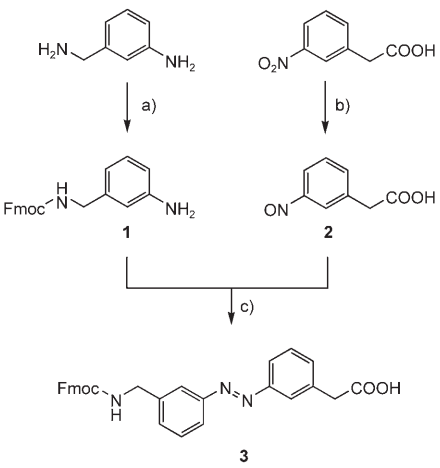
**NMR analysis**: NMR spectra of the hairpin peptide were recorded in water, $CD_3OH$ and $CD_3OD$ at −10, 0, 20, and 30 °C on a Bruker DRX 500 and an AV900 spectrometer equipped with pulsed-field-gradient (PFG) accessories and a cryoprobe in the case of the 900 MHz spectrometer. Resonance assignments of the *cis*- and *trans* isomer were performed according to the method of Wüthrich[10] and are reported in Table S1 and S2 of the Supporting Information. The 2D TOCSY was recorded with a spin-lock period of 70 ms using the MLEV-17 sequence for isotropic mixing.[11] For the *cis*-azo isomer 34 experimental distance constraints were extracted from 2D NOESY[12] and ROESY[13] experiments at 20 and 30 °C with mixing times of 150 to 200 ms (see Supporting Information, Table S3). For the *trans*-azo isomer 47 experimental distance constraints were derived from 2D ROESY experiments at 0 and 20° with mixing times of 100 and 150 ms (see Supporting Information, Table S4). For $^1$H diffusion measurements at 20 °C in $CD_3OH$ stimulated echo experiments with bipolar gradients and a diffusion time of 100 ms were performed in a pseudo 2D fashion by incrementing the gradient strength from 1 G cm$^{-1}$ to 60 G cm$^{-1}$ in 10 steps. The gradient strength was calibrated to a diffusion constant of $18\times10^{-10}$ m$^2$ s$^{-1}$ for $H_2O$ in $D_2O$ at 300 K. Only well-resolved signals were used for extracting diffusion constants from the mono-exponential signal decay.

**Structure calculation**: Structure calculations and evaluations were performed with the INSIGHTII 2000 software package (Accelrys, San Diego, CA) on Silicon Graphics O2 R5000 computers (SGI, Mountain View, CA). Hundred structures were generated from the distance-bound matrices. Triangle-bound smoothing was used. The NOE intensities were converted into interproton distance constraints using the following classification: very strong (vs) 1.7–2.3 Å, strong (s) 2.2–2.8 Å, medium (m) 2.6–3.4 Å, weak (w) 3.0–4.0 Å, very weak (vw) 3.2–4.8 Å and the distances of pseudo atoms were corrected as described by Wüthrich.[10] The structures were generated in four dimensions, then reduced to three dimensions with the EMBED algorithm and optimized with a simulated annealing step according to the standard protocol of the DG II package of INSIGHT II. All hundred structures were refined with a short MD-SA protocol: After an initial minimization, 5 ps at 300 K were simulated followed by exponential cooling to ~0 K during 10 ps. A time step of 1 fs was used with the CVFF force field while simulating the solvent $H_2O$ with a dielectric constant of 80.0. The experimental distance constraints were applied at every stage of the calculation with 50 kcal mol$^{-1}$ Å$^{-2}$. After simulated annealing with DISCOVER the structures were sorted according to final energies and the energy lowest were analyzed. No significant violations of experimental constraints occurred for any of the calculated *cis*-azo structures. However, for the *trans*-azo isomer distance constraints between the methylene group of the AMPP at the amino end and the adjacent phenyl ring had to be removed from the structure calculations, because they were mutually exclusive indicating fast conformational averaging (with rotation about the connecting bond) on the NMR time scale of milliseconds. The final *trans*-azo structural ensemble exhibited no significant or systematic violations of NMR constraints, but displayed two distinct conformational families differing in the relative orientation of the two peptide strands and the AMPP chromophore.

**Molecular modeling**: All calculations were performed with the DISCOVER program (Accelrys, San Diego, CA) on Silicon Graphics O2 R5000 computers. The force field CVFF with a time step of 1 fs and a cutoff of 12 Å for the non-bonded interactions was employed for all calculations. Periodic boundary conditions for a water box of size $40\times40\times30$ Å$^3$ containing ~1500 molecules served as explicit solvent model. Experimental NMR derived distance constraints were applied either all of the time with full strength (50 kcal mol$^{-1}$ Å$^{-2}$) or not at all. After minimization of the whole system molecular dynamics were started at 10 K for 10 ps. Heating to 100 K proceeded with a time constant of 1 ps for the coupling to the temperature bath during 10 ps (with NMR constraints) or 100 ps (unrestrained). The increase in temperature to 300 K was performed more slowly (5 ps time constant). In some cases even further heating to 500 K was applied. Individual conformations were saved each 5 ps.

## Results and Discussion

In the search for a suitable derivative of azobenzene our molecular modeling suggested that a recently proposed *meta*,*meta* substitution of the *cis* azobenzene[9] might be more suitable than our previously used AMPB,[6] to mimic the type I′ β-turn that is required for a β-hairpin. A spacing of the amino and carboxy function from the aromatic rings by single methylene groups not only simplifies chemistry by decoupling of the respective electronic systems, but also allows for the desired geometry. The N-Fmoc-protected photoswitch [3-(3-aminomethyl)phenylazo]phenylacetic acid (AMPP) was synthesized as outlined in Scheme 1 by a simplified procedure compared with that reported previously.[8] Its incorporation into the peptide sequence proceeded in a straightforward manner applying the methods reported for other azobenzene derivatives.[14] Despite the hydrophobicity




Scheme 1. Synthesis of the N-Fmoc-protected photoswitch [3-(3-aminomethyl)-phenylazo]phenylacetic acid (AMPP). a) Fmoc-OSu, $Et_3N$, RT; b) $NH_4Cl$, Zn powder, RT; then $FeCl_3$, 0 °C; c) AcOH.

of the chromophore the peptide is soluble at millimolar concentrations in water and methanol. A tendency towards aggregation was observed at high concentrations in aqueous solution, but NMR diffusion and dilution studies clearly showed that in methanol the peptide exists in a monomeric form. In fact, the diffusion constants at 20 °C in $CD_3OH$ of $1.9 \pm 0.1 \times 10^{-10}\,m^2 s^{-1}$ and $2.0 \pm 0.1 \times 10^{-10}\,m^2 s^{-1}$ for the *cis*-azo and *trans*-azo isomer, respectively, are independent of concentrations between 50 μM and 2 mM and are in agreement with a somewhat higher compactness for the *cis*-azo than for the *trans*-azo molecules in line with the conformational analysis.

**Circular dichroism**: The CD spectra of the AMPP-peptide in the predominantly *cis*-azo isomeric state compare well with those reported for tryptophan zippers[3] and are, therefore, indicative of differentially populated β-hairpin structures (Figure 2). The characteristic maximum at 228 nm

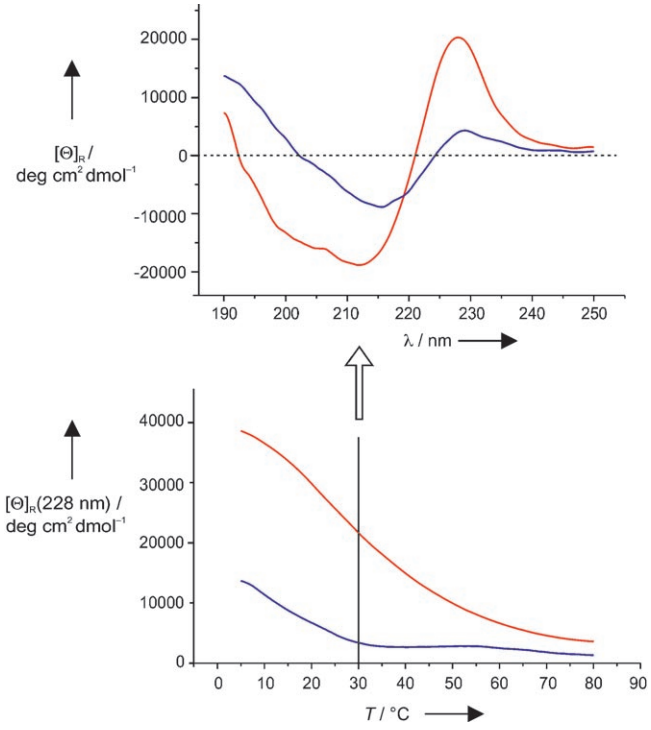



Figure 2. Top: CD spectra of AMPP-peptide in water (45 μM), pH 3.7, at 30 °C in the *cis*-azo (red) and *trans*-azo (blue) photostationary state. Bottom: Thermal unfolding monitored by the dichroic signal at 228 nm. The vertical line indicates the temperature that corresponds to the spectra shown in the upper panel.

originates from a stacking of the tryptophan residues and allows quantifying the folded fraction:[3] As some of the previously studied tryptophan zippers exhibit clear plateaus above and/or below the melting points in thermal unfolding, the CD intensity at 228 nm for a theoretically 100% folded sample can be deduced and used as basis for further calculations of fractions folded. While the original sequence (called "Trpzip2" in ref. [3]) is fully folded at room temperature and shows an unfolding midpoint at 72 °C,[3c] substitution of the turn residues Asn-Gly by AMPP destabilizes the hairpin considerably. The sensitivity of β-hairpins to the conformational propensities of the turn residues is well known[1] and was exploited in our photoresponsive peptide in order to optimize the influence of the chromophore on the conformation of the peptide. Comparison with the CD spectra of the original sequence (Figure 1 in ref. [3c]) allows for an estimate of the β-hairpin content of 50% at 5 °C in water for the *cis*-azo photostationary state generated by illumination at 360 nm. Irradiation at 430 nm reduces the folded fraction to 18%. At 30 °C the CD spectrum of the *trans*-azo isomer displays the typical characteristics of a β-extended conformation with a pronounced positive signal below 200 nm and a minimum close to 215 nm albeit of moderate intensity. However, the hairpin fold is almost completely lost as indicated by the very weak CD intensity at 228 nm (Figure 2, top). Contrarily, the *cis*-azo isomer is still significantly folded with a 25% hairpin content. The temperature dependent CD signal of the *cis*-azo isomer can be fit by a sigmoid curve, which suggests a two-state transition with a melting temperature of 25 °C. Importantly, the thermal unfolding as well as the isomerization transitions between *cis*- and *trans*-azo configuration are both fully reversible and CD spectra in the photostationary states before and after thermal excursions or after several isomerization events are identical within the limits of error (data not shown). Addition of methanol led to a linear increase in CD signal intensity in the range from 0 to 30% methanol (v/v) reaching plateau values at about 1:1 water/methanol mixtures with a CD spectrum (data not shown) that closely matches the CD curve of a fully folded tryptophan zipper in shape and intensity.[3] The stabilizing effect of methanol for β-hairpins has been observed before and has been attributed to a destabilization of the unfolded form.[15]

**Structural analysis of the *cis*-azo isomer**: NMR spectroscopy was employed for confirming the β-hairpin structure of the AMPP-peptide in the *cis*-azo configuration. As β-hairpin peptides are usually in a fast equilibrium between the folded and unfolded form, only an average resonance position is observed in NMR spectroscopy, which depends on the fraction of folded molecules.[1,3,15] Nevertheless, characteristic NOE values can be observed because of the strong bias of the average NOE towards short distances and, thus, folded forms.[16] Methanol was used as the solvent, as it is known to prevent aggregation and to stabilize the β-hairpin structure (see above). Extensive line broadening precluded NMR conformational analysis at low temperatures, but at 30 °C resonances of all amino acids could be observed and assigned for both the *cis*-azo and *trans*-azo isomer. CD measurements at this temperature in 100% methanol showed a hairpin content of 45% for the *cis*-azo photostationary state while the *trans*-azo isomer displayed only a very weak signal at 228 nm (data not shown). Characteristic NMR NOE values confirmed the hairpin structure for the *cis*-azo isomer and allowed calculation of an NMR structural ensemble

with a backbone rmsd of 1.3 Å for the ten energy lowest structures (Figure 3). While individual side chain conformations vary substantially, Figure 3 clearly shows the clustering

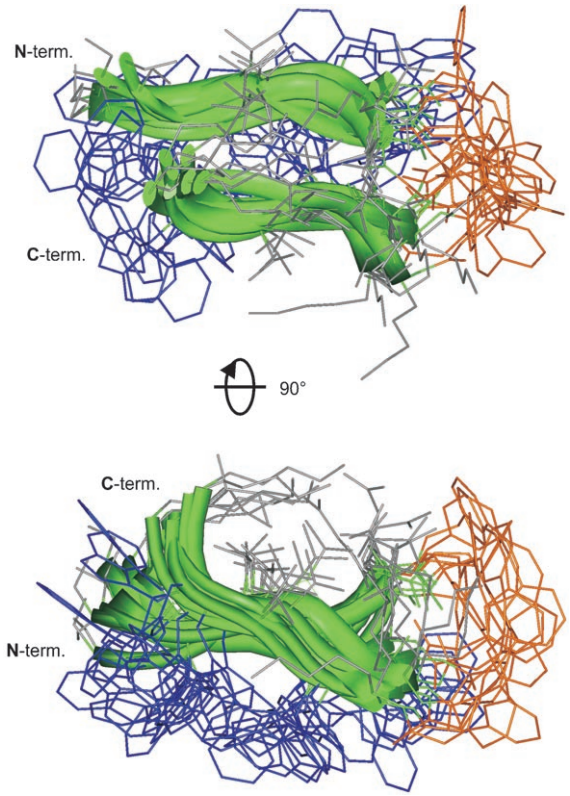



Figure 3. Superimposition on backbone atoms of the ten energy lowest NMR structures of the *cis*-azo isomer. The AMPP chromophore is colored in orange; green ribbons highlight the backbone conformation; side chains are depicted in blue for tryptophan residues and gray for all others; the two views are rotated by 90° around a horizontal axis.

of tryptophan side chains on one side of the β-hairpin and of the other side chains on the other side. Figure 4 depicts a comparison of the NMR structure of the AMPP-peptide in the *cis*-azo configuration with the NMR structure of the original tryptophan zipper peptide (PDB code: 1LE1). A remarkable agreement is observed for the antiparallel β-strands as well as for the tryptophan side chains. In the turn region only moderate differences between the Asn-Gly dipeptide of the original sequence and the AMPP chromophore are visible. While certainly not perfect, the novel AMPP $\omega$-amino acid is apparently able to act as a βI′-turn mimetic in the *cis*-azo isomeric form.

Molecular dynamics (MD) simulations were performed starting from individual conformations of the NMR structural ensemble to refine the structures (using the NMR derived distance constraints) or test their stability (unrestrained MD). In constrained simulations of 200 ps at 300 K no changes of overall conformation were observed, as anticipated, but the local geometry of the hydrogen bonds that would be expected for a typical β-hairpin was markedly improved. No transitions between the slightly different conformers of the NMR ensemble were observed and each structure remained in its original backbone conformation with changes only in the side chain orientations. Remarkably, the energy lowest NMR structure was also extremely stable in MD simulations without the experimental constraints. During 900 ps of unconstrained MD at 300 K followed by 500 ps at 500 K only minor alterations were observed. As before, a more regular β-hairpin geometry was adopted by the peptide and the corresponding hydrogen network was observed. No indication of unfolding could be seen during the 500 ps at 500 K.

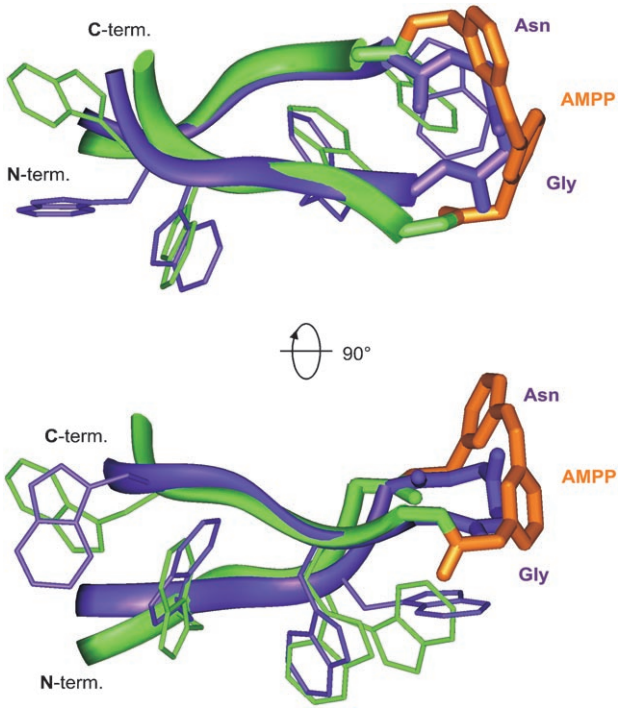



Figure 4. Backbone superimposition of the energy lowest NMR structure of the AMPP-peptide as *cis*-azo isomer (green ribbons) on the original tryptophan zipper (violet ribbons, PDB code: 1LE1). The AMPP chromophore is colored in orange and side chains of only the tryptophan residues are depicted for both peptides. The two views are rotated by 90° around a horizontal axis.

**Structural analysis of the *trans*-azo isomer**: The *trans*-azo AMPP is clearly not compatible with the βI′-turn geometry and, thus, prevents formation of a fully folded hairpin. Again distance constraints could be derived from NMR spectra, although the folded fraction is low at temperatures where resonance assignment is possible. Almost all constraints for structure calculations where obtained from ROESY spectra recorded at 20 °C. The resulting NMR structural ensemble displayed two clearly distinct conformational families with frequencies of 75 % for the first and 25 % for the second (Figure 5). Both minor and major conformation satisfied all 47 NMR-derived distance constraints that were used in the structure calculation. While the choice to superimpose structures on the second peptide strand (and

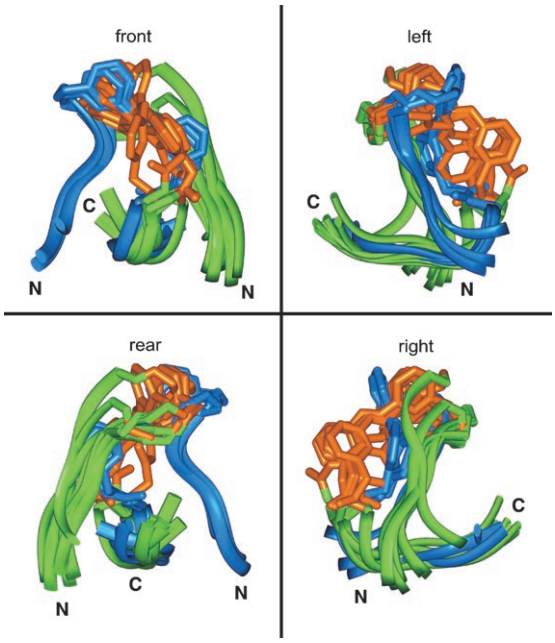



Figure 5. Superimposition on backbone atoms of the second peptide strand (Lys6 to Lys10) of the ten energy lowest NMR structures of the *trans*-azo isomer. For the major conformation the AMPP chromophore is colored in orange; green ribbons highlight the backbone conformation; the minor conformation is depicted in blue.

the AMPP) is somewhat arbitrary, the difference between the two conformations can clearly be seen: Depending on the orientation of the chromophore the first half of the peptide sequence may be positioned on either side of the second half. One has to keep in mind that the compact structures of Figure 5 are in fast exchange with disordered and, likely, more open structures that are not detected by NMR.

Figure 6 depicts a comparison of the energy lowest structure of the *trans*-azo and the *cis*-azo isomer. While the two five-residue strands are always in an extended conformation, the twisted antiparallel alignment observed for the *cis*-azo isomer transforms upon isomerization to the *trans*-azo state to an irregular arrangement, that for one *trans*-azo conformational family (depicted in Figure 6) is even close to a parallel orientation. All hydrogen bonds present in the β-hairpin are lost in the transition to the *trans*-azo configuration underlining the marked differences between the conformational properties of the two isomers. It is important to remember that for both isomeric forms substantial structural fluctuations do occur and that Figure 6 only displays one representative conformation, for sake of clarity, not the full NMR structural ensembles.

**Tryptophan fluorescence**: The stability of tryptophan zippers is mainly derived from the stacking interactions of the tryptophan side chains.[3] The enhancement of fluorescence emission that results from these interactions can be used to monitor the degree of folding. In fact, a strong difference between the two isomers was observed (Figure 7). The

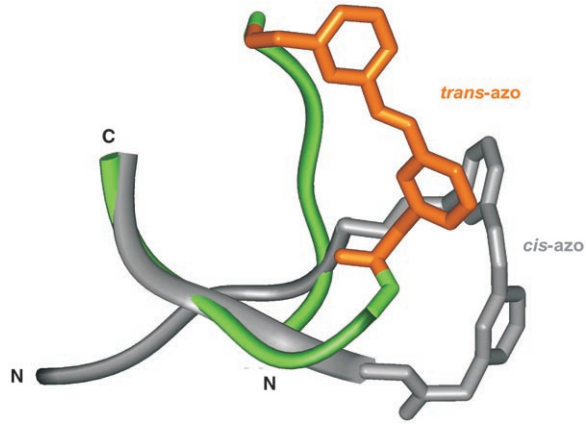



Figure 6. Comparison of *trans*-azo (green/orange) and *cis*-azo isomer (gray).

*trans*-azo isomer of the AMPP-peptide exhibits a reduction of the fluorescence intensity by almost 50% compared with the *cis*-azo isomer due to the lower degree of hairpin structure and, possibly, energy transfer to the AMPP chromophore. Since it is known that the tryptophan fluorescence strongly depends on its surroundings, the pronounced change in fluorescence intensity is a sensitive probe for correct packing of the tryptophan side chains in the β-hairpin. Complementary to fluorescence as a means of observing side chain interactions, IR spectroscopy can detect backbone conformations and hydrogen bonding.

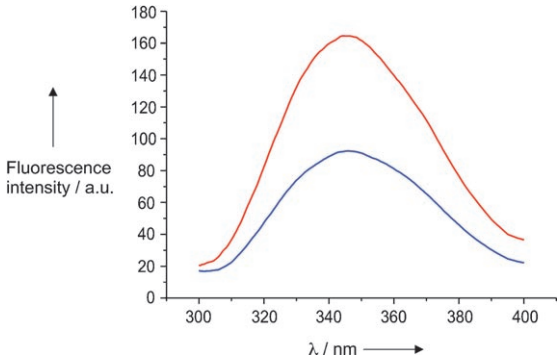



Figure 7. Tryptophan fluorescence before (*trans*-azo, blue curve) and after irradiation at 360 nm (*cis*-azo, red curve); excitation wavelength: 280 nm.

**Fourier-transform IR spectroscopy**: The results from FTIR absorption experiments are presented in Figure 8. The upper panel shows the absorption spectrum of the AMPP-peptide dissolved in deuterated methanol at 25 °C (solvent contributions subtracted), which in the amide I band is characterized by a pronounced peak at 1675 $cm^{-1}$ and a broad shoulder extending to 1620 $cm^{-1}$. Upon irradiation at 355 nm and thus increase of the *cis*-azo isomer, an absorption increase at 1640 $cm^{1}$ and a decrease at 1660 $cm^{-1}$ is observed, which was fully reversed at irradiation at $\lambda > 400$ nm

(Figure 8, bottom). These absorption changes reflect the structural changes of the peptide. The increased absorption around 1640 $cm^{-1}$ in the *cis*-azo state is in the spectral range that is characteristic for β-sheet[17] and β-hairpin[18] absorption and confirms the interpretation of increased β-hairpin formation when the chromophore is in the *cis*-azo configuration. Weaker absorption changes at smaller wave numbers are due to changes of the amide II absorption and of the chromophore. The strong absorption changes in the amide I range show, that ultrafast time-resolved IR spectroscopy can be used for the direct observation of the fastest parts of the light-induced folding and unfolding processes of the newly developed chromopeptides.

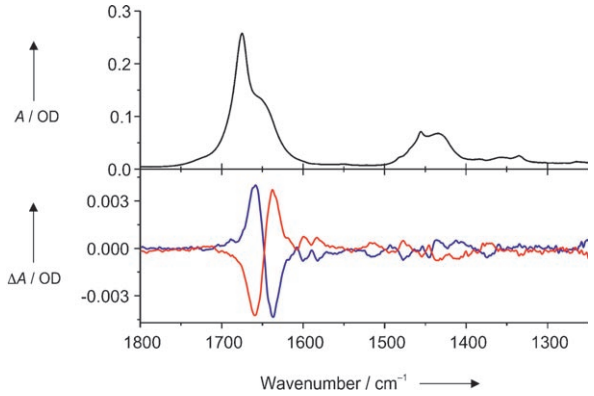



Figure 8. Top: FTIR-absorption spectrum of the AMPP-peptide at 25 °C; bottom: Absorption difference spectra induced by irradiation at 355 nm (*trans* → *cis*, red curve) and at $\lambda > 400$ nm (*cis* → *trans*, blue curve). The strong absorption changes around 1650 $cm^{-1}$ are due to the different β-hairpin content of the *cis*-azo and *trans*-azo isomer.

## Conclusion

In summary, we have designed, synthesized and characterized a β-hairpin peptide where the degree of folding is controlled by the novel AMPP chromophore that can mimic a type I′ β-turn in the *cis*-azo isomeric state. The β-hairpin structure of the *cis*-azo isomer was confirmed and determined by NMR spectroscopy, while the *trans*-azo isomer exhibits more disordered and irregular conformations. Light-induced folding can be monitored by pronounced changes in the CD, IR and fluorescence spectra allowing for time-resolved folding studies of β-structures with unprecedented time resolution. Furthermore, ligation methods, which are now routinely applied in biochemistry, might allow incorporation of such photosensitive β-hairpins in natural protein structures resulting in proteins with built-in light switches.

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