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Review Commentary *Cis/trans* configurations of the peptide C—N bonds: isomerization and photoswitching

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ABSTRACT: *Cis/trans* isomerization of peptide C—N bonds is involved in the configurational changes and are definitive for the bioactivity of peptides and proteins. The basic molecular character and origin of the restricted rotation of the peptide C—N bonds are briefly introduced, as well as the methods used for study of the *cis/trans* isomerization, such as the chymotrypsin-coupled assay, pH-mediated solvent jump, and UV-resonance Raman spectroscopy, etc. The control of the peptide bond configuration with photoresponsive (photochromic) groups such as azobenzene or thioamide bonds is also reviewed. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: Cis/trans isomerization; rotation barrier; peptide thioamide bond; peptidyl–prolyl bond; azobenzene; photoswitching; photoresponsiveness

INTRODUCTION

Conformation of peptides and proteins is responsible for their biological activity.[1,2]In a peptide chain, there are three kinds of bonds, for which the rotations are indicated by angles ψ_i, ϕ_I, and ω_i, respectively. A representative segment of peptide chain and the rotation of the peptide bonds are illustrated in Fig. 1.[3]

Specific torsion angles are found for the regular structures of the peptides or proteins. For instance, the right-handed α-helix is characterized with ψ angle of -47°, whereas the left-handed α-helix is with a ψ angle of $+47^\circ$.[3]

The rotation about the C_i—N_{i+1} bond (ω_i) is intrinsically hindered, due to its double-bond character. The *cis/trans* isomerization mainly involves this bond and its rotation is more definitive than the other bonds in the peptide chain on the conformational shuffling of the biopolymers.[1,2,4–6] Control of the *cis/trans* configuration of peptide amide bond will modulate the activity of biopolymers (Fig. 2).

Many efforts have been made to study the *cis/trans* isomerization of peptide C—N amide bonds and to control its configuration, such as the photoswitch method,[5,7] or the configuration restriction by synthetic approach.[7k] Moreover, a lot of model compounds have been used to investigate the mechanism of catalyzed or aided folding of protein, namely the *cis/trans* isomerization of the peptide amide bonds.[8]

In this commentary review, a brief summary will be given on the chemical nature of amide bonds, the transition between its *cis* and *trans* configurations, the experimental methods to study this isomerization, and finally on the control of configurations of peptide bonds by external stimulus, especially the photoswitch method.

THE AMIDE BOND AND ITS RESTRICTED ROTATION: *CIS/TRANS* ISOMERIZATION

The amide group is characterized by short C—N bond lengths, co-planarity and restricted rotation about the C—N bond (Fig. 3). [2,8,9] The substantial double-bond character of the C—N bond is the origin of its hindered rotation. [2,8]

Rotation barrier ($\Delta G^{\neq}$) of the peptide amide bonds and isomerization rate constants (k_{obs}) can be correlated with the Eyring equation [Eqn (1)].

$$\ln\frac{k_{obs}}{T} = -\frac{\Delta H^{\neq}}{RT} + \frac{\Delta S^{\neq}}{R} + \ln\frac{k_B}{h} \qquad (1)$$

where k_{obs} stands for the observed first-order isomerization rate constant (in s^{-1}), T is the experimental temperature (in K), R is universal gas constant, k_B is the Boltzmann constant, and h is the Planck constant. The rotational barriers were calculated according to the Gibbs

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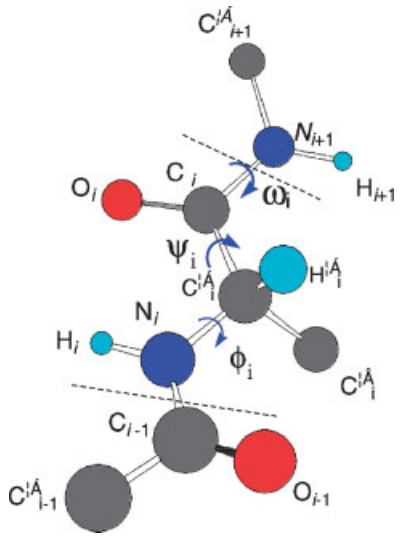

Figure 1. Perspective view of a section of polypeptide chain including two peptide units. The limits of a residue are indicated by dashed lines and recommended notation for atoms and torsion angles are indicated. The chain is shown in a fully extended conformation ($\psi_i = \phi_i = \omega_i = 180°$)[3]

equation [Eqn (2)]. Basically a higher rotation barrier is accompanied with smaller isomerization rate constants and *vice versa*.

$$\Delta G^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq} \qquad (2)$$

Isomerization of the *cis*/*trans* conformers of normal secondary peptide amide bond in dipeptides exhibits a rotational barrier of less than 75 kJ mol^{-1} (with rate constant smaller than 1.0 s^{-1} at room temperature).[10] With such a low rotation barrier, usually the secondary amide bonds are not involved in the critical kinetic traps of the energy landscape for protein folding. However, the *cis*/*trans* configurational heterogeneity and the rotation barrier are more significant for imino acids like proline.[1,2] This tertiary peptide amide bond is termed as peptidyl–prolyl bond (-Xaa-Pro-, where Xaa stands for arbitrary amino acid residues, Fig. 4).

Usually the *trans* conformers of peptide amide bonds are thermodynamically more stable than the *cis* conformers because the steric hindrance of the groups flanking the C—N bonds is released in the *trans* conformers. For the equilibrated secondary peptide bonds, the *cis* conformers are in a very low concentration (~1%). For the tertiary peptide bonds (peptidyl–prolyl bonds), both *cis* and *trans* conformers have similar steric

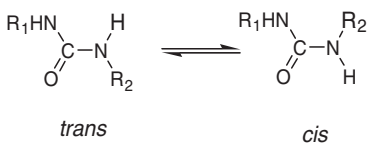

Figure 2. *Trans* ($\omega = 180°$) and *cis* ($\omega = 0°$) configurations of the amide bond in a polypeptide chain

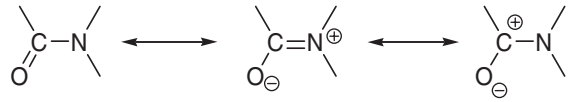

Figure 3. Resonance structures of the amide bond

hindrance, thus, the *cis* and *trans* conformers coexist in almost equal amounts in the unstructured proteins or peptides (*cis* contents up to 50%).[2] Furthermore, the rotation of the peptidyl–prolyl bond is characterized with a higher rotation barrier, the average activation enthalpy is about 80 kJ mol^{-1}, *versus* a value less than 70 kJ mol^{-1} for secondary peptide bonds in dipeptides.[10] The rate constants for *cis*/*trans* isomerization of peptidyl–prolyl bonds range from 0.1 to 0.01 s^{-1}. Due to its slow isomerization kinetics, the peptidyl–prolyl bonds are the origin of kinetic barriers for protein folding and it is not surprising that nature has developed catalyts which are specific and efficient for this kind of hindered rotations, such as the peptidyl–prolyl *cis*/*trans*-isomerases, which are highly conserved and ubiquitous rotamase enzymes.[1,2,6]

METHODS TO STUDY THE *CIS*/*TRANS* ISOMERIZATION OF THE PEPTIDE AMIDE BONDS

As the configuration of peptide bonds is definitive for the activity of the biomolecules such as peptides or proteins,[2] investigation of this equilibrium as well as its shifting is important. Hence, we will discuss various methods used to study the equilibrium of the native peptide bonds, the photoresponsive peptide derivatives, such as the peptides containing photochromic moiety, or more recently the intrinsic photoresponsive thiopeptide bonds.

Some of the representative methods used in the study of *cis*/*trans* isomerization of peptide amide bond are the chymotrypsin-coupled assay,[11–14] pH-mediated solvent jump,[10] NMR spectroscopy (magnetization transfer or the saturation transfer method),[15–19] UV-resonance Raman spectroscopy,[20–22] low temperature vibrational spectroscopy such as the time resolved IR spectroscopy, etc.[23,24]

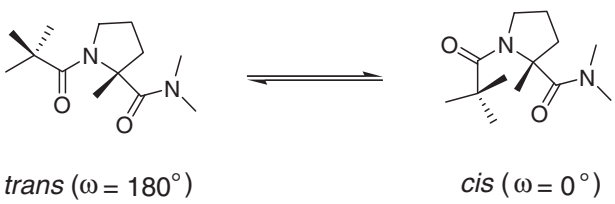

Figure 4. *Cis* and *trans* isomers of prolyl peptide bonds. In the *trans* form, the two flanking C atoms around the C–N bond are on the opposite side of the rotating C–N bond (dihedral angle $\omega = 180°$). In the *cis* form, the two flanking C atoms are on the same side of the C–N bond (dihedral angle $\omega = 0°$)

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Chymotrypsin-coupled assay: *Cis/trans* isomerization of the peptidyl–prolyl bonds

Cis/trans isomerization of imidic peptide bonds preceding proline, that is, -Xaa-Pro-, is an essential process for unfolded or denatured protein to gain its right conformation and thus the desired activity. This kind of isomerization has been extensively studied, mainly with the chymotrypsin coupled assay, developed by Fischer and coworkers *et al.*[1,2,11–14,25,26] This enzyme-coupled assay exploits the configuration specificity of chymotrypsin-mediated hydrolysis of the amide bonds,[11b] so that only the peptide isomers with *trans* conformers at the P2–P3 position can be hydrolyzed.[11a] For example, a short peptide of Suc-Val-Pro-Phe-*p*NA (where *p*NA stands for 4-nitroaniline) is hydrolyzed by chymotrypsin between the Phe and *p*NA amino acid residue only if the Val-Pro peptide bond is in the *trans* configuration. When the reaction is started by mixing the substrate and chymotrypsin together, a burst phase first appeared, due to the instant and complete hydrolysis of the existing *trans* isomers in the equilibrated solution, thereafter only the *cis* isomers are left. During the relatively slow re-equilibration of the conformers (the *cis* → *trans* transition), the newly formed *trans* conformers will be hydrolyzed instantly and the kinetics can be analyzed by following UV absorption of the released *p*NA at 390 nm. It should be pointed out that in this case the hydrolysis kinetics is, and should be, much faster than isomerization rate constants. A typical UV–Vis absorption time curve following the hydrolysis is illustrated in Fig. 5.[27]

By observing the kinetics with UV–Vis absorption of the released *p*NA at 390 nm, the *cis* to *trans* isomerization rate constants can be determined as $k_{cis \rightarrow trans} = 5.3 \times 10^{-3}\ s^{-1}$.[27] This method has been used widely in the study of isomerization of peptidyl–prolyl peptide bonds (-Xaa-Pro-). However, it is not suitable for the study of *cis/trans* isomerization of normal secondary peptide bonds, which are not the substrates for chymotrypsin.

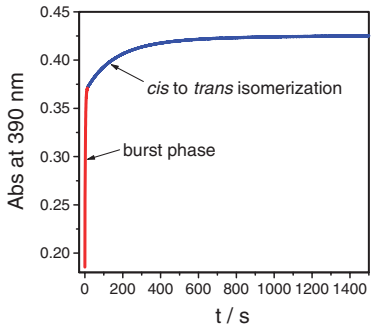

Figure 5. Cleavage of *p*NA moiety from peptide of Ala-Ala-Pro-Phe-*p*NA with chymotrypsin. Starting the reaction by injection of substrate peptide into the chymotrypsin solution in HEPES buffer (pH7.8, 10 °C), then monitor UV absorption of the solution at 390 nm. The burst phase (red line) represents the instant hydrolysis of the pre-existing *trans* conformers. Thereafter the *cis* → *trans* re-equilibration of the Ala-Pro tertiary amide bond (blue line) is characterized by a slower kinetics[27]. This figure is available in colour online at www.interscience.wiley.com/journal/poc

pH-mediated solvent jump method

The ionization state of the unprotected peptide is pH-dependent. As the static attraction of the terminal groups, which is determined by the ionization state (Fig. 6a), imposes an effect on the *cis/trans* equilibrium, thus the *cis/trans* configurational equilibrium of the unprotected peptide can be shifted by variation of the pH of the peptide solution, hence the re-equilibration of the *cis/trans* conformers can be studied by a pH-mediated solvent jump method.[10] Also demonstrated in Fig. 6 is the effect of ionization state on the energy of the *cis* and *trans* configurations and transition state. For the zwitterionic forms of the peptides, the rotation barrier increased but the free energy difference (ΔG°) between the *cis* and the *trans* conformers decreased (Fig. 6b), thus, the zwitterionic forms are characterized with a slow *cis/trans* isomerization and a higher *cis* content in the equilibrated peptides solution.[10,28] The relationship between the pH of the solution and the *cis/trans* isomerization rate constants can be correlated with the modified Henderson–Hasselbalch equation.[10,28]

To study the *cis/trans* isomerization of peptide bonds with pH-mediated solvent jump method, it is essential to find a suitable spectroscopic signal to follow the re-equilibration process. Recently it was found that UV-Vis absorption of the peptide amide bonds are conformer specific.[10] Thus, the *cis/trans* isomerization of the secondary peptide bonds can be easily monitored by UV-Vis absorption. Although the perturbation of the equilibrium with pH-mediated solvent jump is small, as well as UV absorption changes, yet such a convenient experimental approach will greatly facilitate the related research.

The pH-dependency of the *cis/trans* isomerization rate constants of the secondary peptide bonds (Gly-Gly) studied with pH-mediated solvent jump is illustrated in Fig. 7.[10] Due to the fast kinetics of the *cis/trans* isomerization of the secondary peptide bonds, the experiment has to be carried out with the stopped-flow instrument, to ensure the mixing process of two different solutions to be finished within a short period of time on much smaller timescale than that of the *cis/trans* isomerization kinetics of the peptide bonds.

It is supposed that the zwitterionic form shows the strongest attraction between the terminal groups, thus isomerization in the neutral pH range will cross a higher barrier than in acidic or basic pH ranges (Fig. 6). Smaller isomerization rate constant was found for Gly-Gly in neutral pH range. In Fig. 7, the pK_a values for carboxyl and the amino groups are 3.1 and 8.08, respectively).[10]

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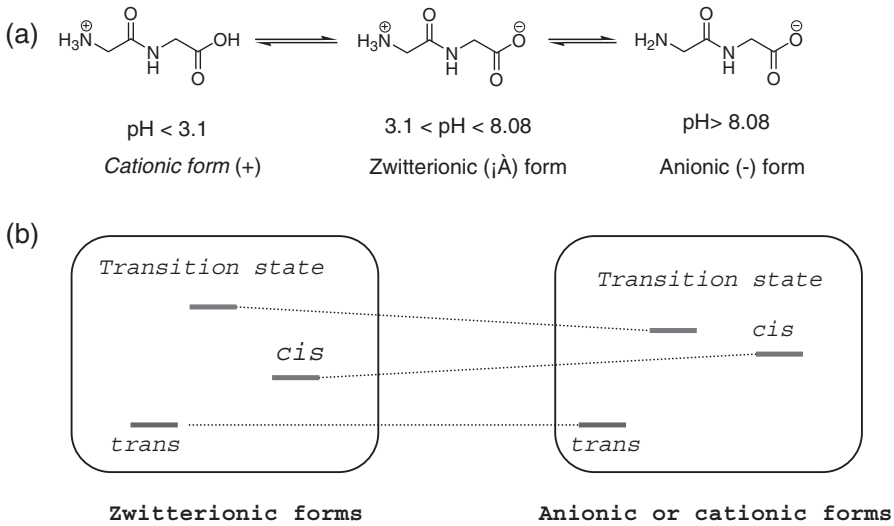

Figure 6. (a) pH effect on the ionization state of the unprotected peptides Gly-Gly. Note the ionization state of the unprotected peptides is dependent on the pK_a values of the amino and carboxyl groups.[10] (b) Schematic representation of the effect of the ionization state of the peptides on the energy of the *cis* (red) and *trans* (green) configurations and transition state of the *cis/trans* isomerization.

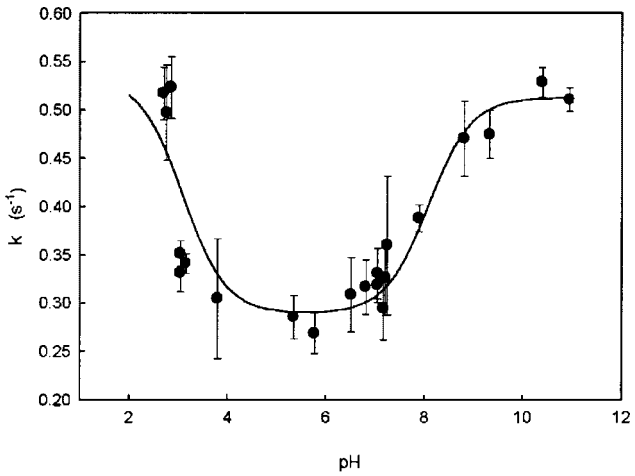

Figure 7. Effect of pH on the *cis/trans* isomerization rate constants ($k_{c/t}$) of the amide bond in dipeptide Gly-Gly. The solid line is the calculated curve. Reprinted with permission from Ref. 10. Copyright © (2001) American Chemical Society

The activation parameters of isomerization ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) can be obtained by studying the temperature dependence of the *cis/trans* isomerization kinetics. The rotational barrier ($\Delta G^{\ddagger}$) is low for the secondary peptide bonds, thus isomerization rate constant is too large to allow any *cis/trans* conformation–bioactivity correlation studies. For example, at neutral or slightly acidic pH, the $\Delta G^{\ddagger}$ value is 75.3 kJ mol^{-1} for Gly-Gly, accordingly the rate constant is 0.289 s^{-1}. Therefore the *cis* conformers, generated by whatever method, will isomerize to the *trans* conformers within a short period of time which is less than 5 s.

UV-resonance raman spectroscopy

This method was used for measurements of the *cis/trans* isomerization rate constants as well as for *cis/trans* conformers' ratio determinations at the equilibrated state, as each isomers gives different Raman scattering intensity.[20–22]

The *trans* → *cis* isomerization of NMA and Gly-Gly is achieved with laser irradiation at 206.5 nm. The contents of the *cis* conformers in the equilibrated mixture was determined from the Raman scattering intensities at 1492 and 1487 cm^{-1} for *N*-methylacetamide (NMA) and Gly-Gly, respectively.[20] The *cis* → *trans* back-isomerization rate constant at room temperature is about 2.3 s^{-1} for NMA and the corresponding activation barrier is $E_a = 57.8 \pm 3.3$ kJ mol^{-1}. For Gly-Gly, a barrier of $E_a = 46.1 \pm 2.9$ kJ mol^{-1} was observed and isomerization rate constants range from 0.288 to 0.675 s^{-1} (pH-dependent).[10] The temperature dependence of the relative *cis* and *trans* contents (determined with the respective Raman scattering intensity) is used to determine the Gibbs free energy gaps between the two ground state isomers. An energy gap of 10.9 ± 1.7 kJ mol^{-1} for NMA and 13.0 ± 2.1 kJ mol^{-1} for Gly-Gly were determined (the *trans* isomers are more stable). Photoisomerization quantum yields for *trans* → *cis* transition are 0.12 ± 0.02 and 0.075 ± 0.01 for NMA and Gly-Gly, respectively. However, short UV irradiation wavelength is required for the excitation of the normal peptide amide bonds, thus photodecomposition is inevitable.

NMR methods

With the NMR method, there is no need for an external stimuli to shift the existing equilibrium of the *cis* and the *trans* conformers of the peptide. For example, the rotation of the Ala-Tyr unit in various solvents has been studied by the NMR line-shape analysis, from which isomerization rate constants can be derived.[16] Peptide bonds adjacent to the aromatic amino acid were found to be with a *cis*

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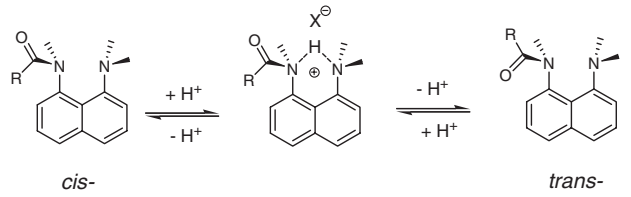

Figure 8. Catalysis of *cis*/*trans* isomerization over the amide C–N bond by intramolecular hydrogen bond formation (X = anions of weak organic acids)[17]

isomer population ranging from 0.1 to 1% depending on the temperature. The rate constant of the *trans* → *cis* isomerization for zwitterionic Ala-Tyr is $2.4 \times 10^{-3}\,s^{-1}$ at 298 K, but the reverse *cis* → *trans* isomerization is much faster ($0.6\,s^{-1}$). It is also found that extending the peptide chain in both directions of the Ala-Tyr moiety leads to a decrease of both the *cis* content and the rotation barrier of the *cis* → *trans* isomerization. The linear Arrhenius plots gave E_a value of $76.7 \pm 1.5\,kJ\,mol^{-1}$ for the dipeptide Ala-Tyr, whereas this value is decreased to $64.6 \pm 1.5\,kJ\,mol^{-1}$ in the pentapeptide of Ala-Ala-Tyr-Ala-Ala.

Besides the peptides, the amide bonds in some small organic molecules also demonstrated restricted rotations. The *cis*/*trans* isomerization of these amide bonds have been also extensively studied with NMR method.[17,18,29–38] With the magnetization saturation transfer experiment, it is proposed that hydrogen bond donation to the amide nitrogen by charged proton donors may play a significant role in the enzymatic catalysis of amide isomerization.[17] Stoichiometric and even catalytic quantities of weak acids in aqueous solution can efficiently catalyze the amide isomerization (Fig. 8). The presence of an intramolecular hydrogen bond has been proposed to play also a key role in the catalysis of amide isomerization by peptidyl–prolyl isomerases (PPIases).[2,6b]

Theoretical calculations

Besides the experimental approach, theoretical calculations provide a complementary insight into isomerization of peptide bonds.[39–41] For example, the rotational barriers for *N,N*-dimethylformamide and *N,N*-dimethylacetamide have been investigated by calculations at the G2(MP2) level. An examination of the geometries of amides revealed that the lower barrier for acetamide resulted mainly from a ground state methyl–methyl repulsive interaction.[42]

Furthermore, with *ab initio* molecular orbital calculations, the rotational barriers of thioformamide and formamide were also compared and the higher rotation barrier for thioamide was explained. [42b] The amino group of thioformamide is less 'floppy' than that of ordinary amides. The change in charge density at sulfur atom on rotation of the amino group in thioformamide is much greater than that at the oxygen atom in formamide. Therefore, the traditional amide resonance concept is more appropriate for thioamides than for normal amides. The small difference in electronegativity between carbon and sulfur and the larger size of sulfur are the major factors that allow charge transfer from nitrogen atom to sulfur atom in thioamides. Thus, a high rotation barrier is resulted in thiopeptide bond than the normal peptide bonds. Such a minor difference may impose significant effect on the related properties of the amide bond, which are also found in urea's reactivity. For example, the affinities of the urea and thiourea derivatives towards anions like fluoride (F^-), differ drastically.[43]

PHOTOSWITCHING OF THE PEPTIDE CONFIGURATION

It is known that the *cis*/*trans* configurations of peptide amide bond is photoswitchable.[20–22] However, as the *cis*/*trans* isomerization is fast and the rotation barrier is low, therefore, photoswitch of the normal peptide amide bond is not significant for the conformation modulation of the peptides and proteins.

In order to make the conformation of the peptide or protein photoswitchable, usually a photoresponsive group is introduced, either incorporated or appended to the main chain of the peptide. The azobenzene group has been widely used for this purpose.[5,44–48] In this case, however, isomerization constraint of the azobenzene group is transmitted to the peptide chain via flexible bonds, therefore, it is impossible to predict which of the amide bonds undergoes isomerization. For the photoswitch of the native peptide amide bond (and also for the thiopeptide bond, see Section 'Photoswitching the thiopeptide C—N bonds'), however, it is the rotation of the C—N amide bonds (ω) that initiates the conformational changes of the peptide chain so that the isomerizing site can be precisely controlled.

Control of the peptide conformation with azobenzene moiety

To modulate the polypeptide conformation using photochromic moieties, azobenzene unit has been successfully incorporated into peptides to control the conformations.[5,44–50]

For example, by incorporating azobenzene group into a short peptide, the resulted monocyclic (4-amino)phenylazobenzoic acid (APB) peptide folds into a well-defined lowest energy structure as a *trans*-azo isomer (Fig. 9).[44a] Upon photoisomerization, the *trans*-azo isomer is transformed to the *cis* configuration, and then relaxes into a less restricted *trans* configuration.

The *trans* conformers give an intense absorption band at 350 nm, whereas the *cis* conformers absorb at 440 nm.

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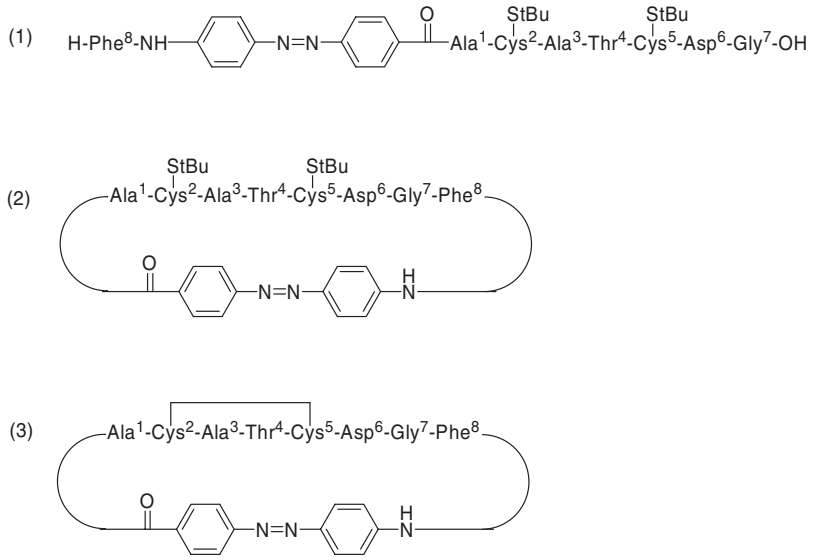

Figure 9. Structure of the *S-tert*-butylthio-protected linear (**1**) and monocyclic (**2**) as well as of the disulfide-bridged bicyclic (**3**) APB-peptide. Reprinted with permission from Ref. 44a. Copyright © (2000) John Wiley & Sons, Inc.

Isomer specific UV–Vis absorption enables photoswitching of APB-peptide. With irradiation at 350 nm, some of the *trans* isomers will be switched to the *cis* configuration. Conversely, the *cis* conformers can be switched back to the *trans* conformers by irradiation at 440 nm.

With NMR analysis of the APB-peptides **2**, the signals of the *trans* and *cis* conformers can be resolved from each other. A full assignment of the NMR of the peptide conformers can be carried out with the Nuclear Overhauser Effect Spectroscopy (NOESY) spectral analysis. Inter-proton distance and dihedral angle were obtained to construct the preferred conformations in solution (Fig. 10).[45] Photomodulation of the conformation is also found for the APB-peptide **3**.

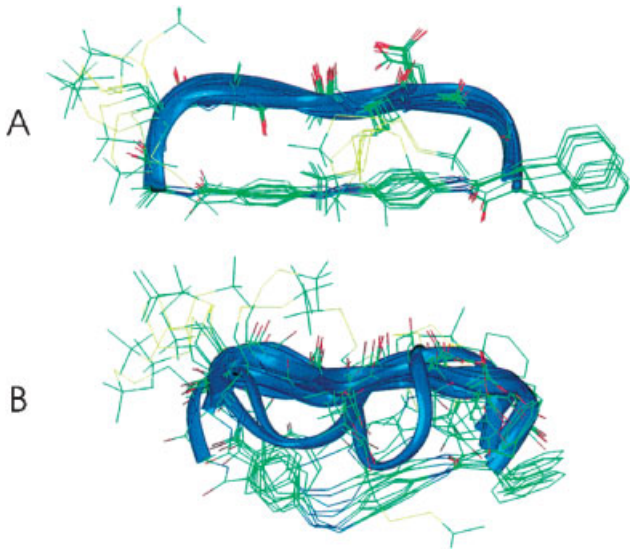

Figure 10. Ensembles of 10 lowest energy structures of the monocyclic APB-peptide **2** in (A) the *trans*-azo and (B) the *cis*-azo configuration. Reprinted with permission from Ref. 44a. Copyright © (2000) John Wiley & Sons, Inc.

Similarly photoswitch of a photochromic moiety incorporated in the polymer backbone can induce a conformational change.[49] For structure **4** (Fig. 11),[49b] isomerization of azobenzene moiety can be directly coupled to polymer backbone to control the conformation of the polymer. The azo-modified polymers were soluble in hexafluoro-2-propanol (HFP). Circular Dichroism (CD) spectra demonstrated that the macromolecules adopted a disordered structure in HFP, with azo unit in either the *cis* or *trans* configuration. This means isomerization of the azobenzene moiety cannot generate an effective constraint on the polymer. However, when appropriate amounts of methanol or 1,2-dichloroethane were added to the HFP solutions, irradiation at 340 and 417 nm produced reversible formation of a helical and random coil structure of the macromolecules (Fig. 12).[49]

Upon irradiation at 417 nm, the CD spectra of the modified polymer changed dramatically, due to the conformation changes of the polymer caused by isomerization

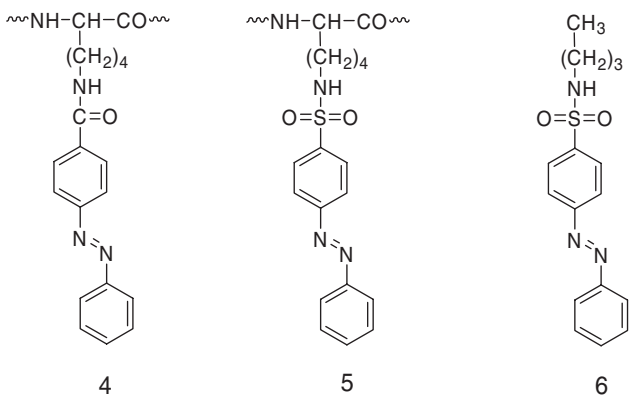

Figure 11. Structures of the azobenzene appended peptides **4**, **5** and the low molecular weight compound **6**. Reprinted with permission from Ref. 49b. Copyright © (1996) American Chemical Society

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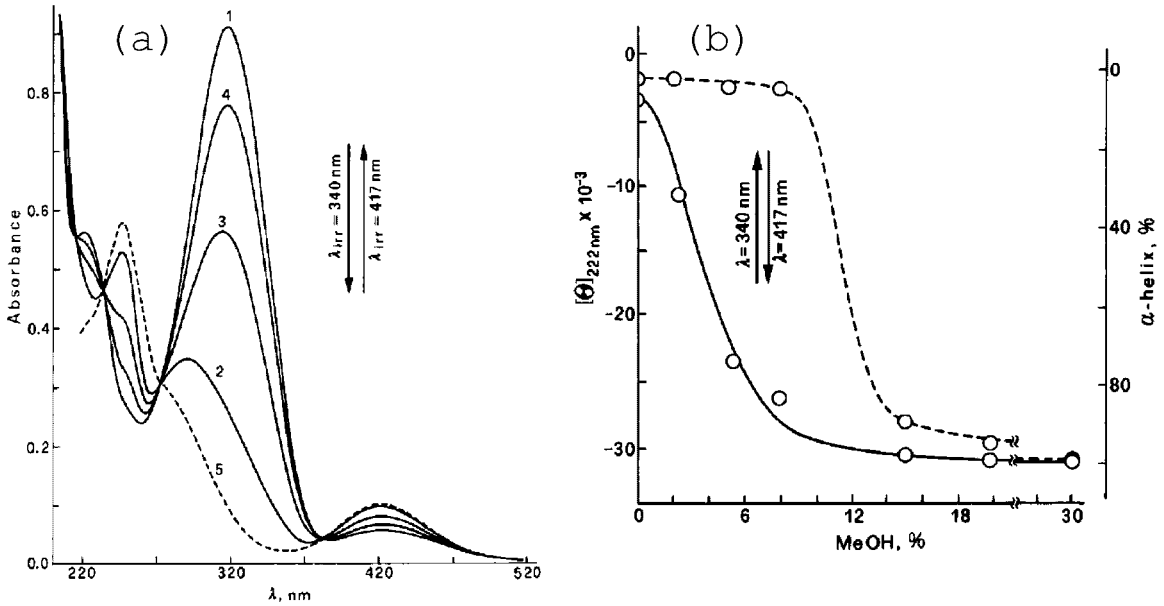

Figure 12. (a) Absorption spectra of compound **5** and **6** in hexafluoro-2-propanol (HFP). 1: Dark-adapted polymer. 2: Photostationary state upon irradiation at 340 nm. 3: Intermediate spectrum before reaching the photostationary state. 4: Photostationary state upon irradiation at 417 nm. 5: Pure-*cis* isomer of **6**; (b) ellipticity of **5** at 222 nm in HFP/MeOH solvent mixtures and α-helix content percentage, as a function of methanol concentration, for the samples irradiated at 417 (——) and 340 nm (- - -). For the compound numbers, refer to Fig. 11. Reprinted with permission from Ref. 49b. Copyright © (1996) American Chemical Society

of the azobenzene moiety. Under 417 nm irradiation, the content of the α-helix increases from zero to 80%, furthermore, the backward transition is also photoswitchable: with irradiation of the α-helix enriched polymer solution at 340 nm, the photogenerated α-helix disappeared. It was also found the α-helix content is pH-dependent (Fig. 12b).[49]

Woolley and coauthors[51] reported a water-soluble, thio-reactive azobenzene compound which can be easily used for peptide modification by reaction with the —SH groups of peptides. Sulfonic groups are introduced to improve the solubility of the modified peptide in aqueous solution (Fig. 13a). This thio-reactive azo compound can react with cysteine. It was found that the thermodynamically stable *trans* form can stabilize the helix structure of the peptide(Fig. 13b). With photoswitch to the *cis* conformer (85% at the photostationary state), however, isomerization constraint destabilizes the helix and unfolding of the peptide is resulted. The content of the helical forms is decreased from 65 to 35% with photoswitch of the azobenzene moiety from *trans* to *cis* configuration. Drastic changes in UV–Vis absorption and CD spectra of the peptide upon photoswitching are also observed.[51]

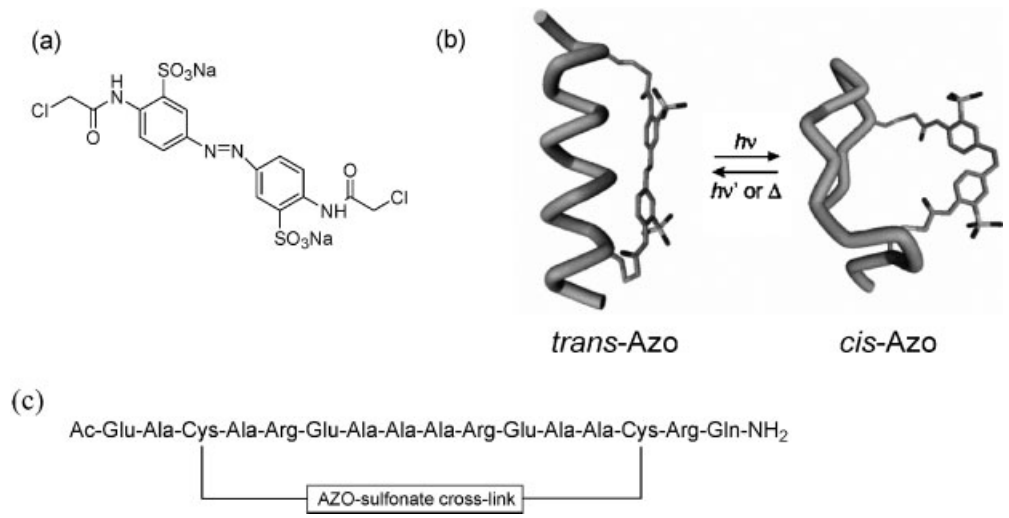

Figure 13. (a) Structure of the thio-reactive azobenzene derivative; (b) *trans*- and *cis*-azobenzene isomers of the cross-linked peptide; (c) the sequence of the cross-linked peptide. The cysteine side-chains and cross-linker are shown as sticks. Reprinted with permission from Ref. 51. Copyright © (2003) American Chemical Society

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Besides the *cis/trans* isomerization rates, the photostability, and the thermal relaxation kinetics of the bistable states, the excitation wavelength of isomerization is also of great importance because using short excitation wavelengths gives rise to many problems, for example, the poor ability to penetrate biological tissue, or damage to the protein or DNA. A photoresponsive azobenzene moiety with longer excitation wavelength was developed recently (Fig. 14).[52] The characteristic absorption of compound **7** is about 480 nm, longer than that of the previously reported photoresponsive azobenzene moieties.

Based on molecular modeling and the CD spectra (Fig. 15), it is predicted that the *cis* isomer of the azobenzene unit will induce random coiling conformation, while the *trans* isomer of the azobenzene will induce α-helix conformation. With cross-linked JRK-7 peptide (Fig. 14b), however, a different result was observed, that is, the *cis* isomer of the azobenzene moiety induces higher α-helix content.

Beside the azobenzene groups, other photochromic moieties,[5a] such as the spiropyrans,[53] were also used for photoswitching the peptide conformers.

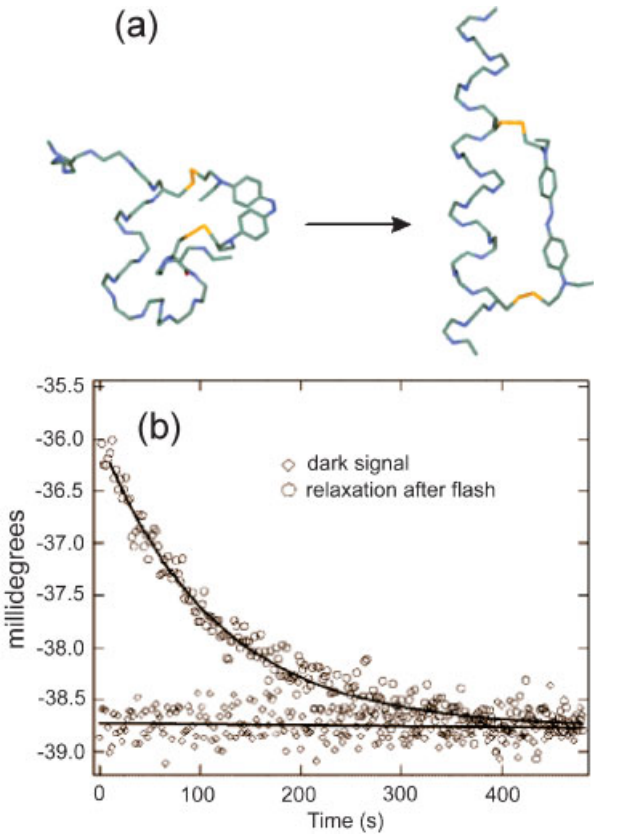
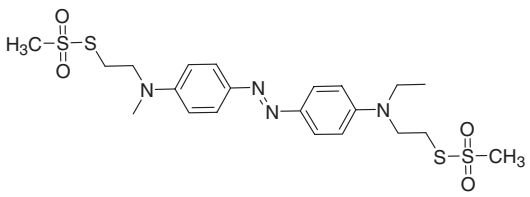


Figure 15. (a) *Cis* to *trans* relaxation of the azobenzene moiety in cross-linked FK-11 peptide associated with an increase in peptide helix content; (b) time-dependent Circular Dichroism (CD) signal at 225 nm of the cross-linked FK-11 observed during the *cis* to *trans* relaxation. Reprinted with permission from Ref. 52. Copyright © (2006) American Chemical Society

Photoswitching the thiopeptide C—N bonds

Thiopeptide bond has attracted considerable interest because of its effects of conformation restriction, enhanced proteolytic stability, and modulable activity and selectivity on the peptide or proteins. It has been shown that the thioxylated peptide bond is photoswitchable and that the *cis* conformer can be generated by irradiation.[54] For the thioxylated peptidyl–prolyl bond, the increase in *cis* conformers can also be achieved by a



7

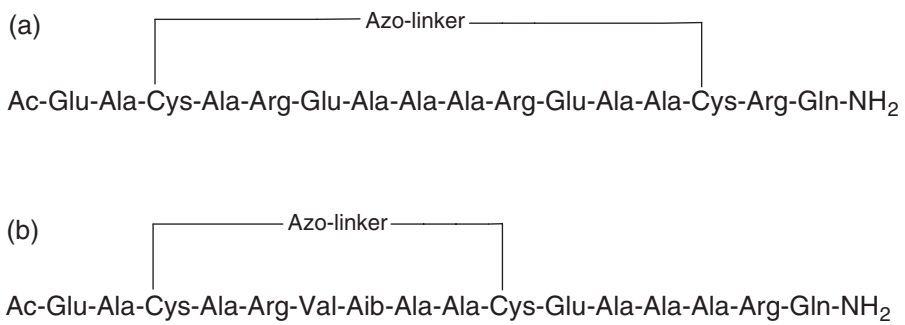


Figure 14. Azobenzene dye **7** and the structure of the azobenzene cross-linked (a) FK-11 and (b) JRK-7 peptide. Reprinted with permission from Ref. 52. Copyright © (2006) American Chemical Society

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slow thermal equilibration process,[55] which does not take place for secondary peptide bonds. For the latter, a synthetically demanding conformation–restriction method has to be used to obtain the thermodynamically unfavorable *cis* conformers.[56,57] It should be pointed out that the starting point for the *cis*/*trans* equilibration of the peptide amide bonds, either the normal amide bonds or the thioamide bonds, is the thermodynamically stable *trans* conformer. For the secondary amide bonds, as the *trans* conformers are less sterically hindered (the energy of the *cis* configuration is much higher than the *trans* conformers), hence the *cis* contents in the equilibrated solution are very low. For the tertiary peptide bonds, however, the *cis* and *trans* conformers have the similar energy, hence, the content of the *cis* isomer will increase by a slow kinetics in solution.[55]

Recently, it was observed that the secondary thiopeptide bond is dual-directional photoswitchable,[28,58] that is, it can be photoswitched from *trans* configuration to *cis* configuration and *vice versa*; this is different from the reported *N*-alkyl thiopeptide bonds.[59] Much slower isomerization rate constants compared to those of the normal peptide amide bonds were observed for the thiopeptides.[28] The rotation barrier of the secondary thiopeptide bond is determined as about 85 kJ mol^{-1} and the rate constants for the *cis*/*trans* isomerization are on the scale of $10^{-3}\,s^{-1}$. Thus, in principle the thiopeptide can be used for the conformer–bioactivity correlation study.[28,58,59] Except the unique example with thiopeptidyl–prolyl bond in a dipeptide,[55] this is nearly an unexploited field.

Similar to the normal secondary amide bonds, the *cis*/*trans* equilibration of the secondary thiopeptide bond can also be shifted with the pH-mediated solvent jump method.[28]

Different from the normal amide bond, for which the photodecomposition is considerable due to the short excitation wavelength, the absorption of the thiopeptide is red-shifted to 270 nm, thus no serious photodecomposition was observed during the photoswitching studies.[28,58,59]

Fischer and coworkers reported photoswitching of a tetrathiopeptide of endomorphine Tyr-Ψ[CS-N]-Pro-Trp-Phe-NH_2 (**8**) and Tyr-Ψ[CS-N]-Pro-Phe-Phe-NH_2 (**9**).[59] Although UV–Vis absorption spectra did not yield significant variation, yet with CD spectra, it was demonstrated that the thiopeptidyl–prolyl bond is significantly photoswitchable (Fig. 16). The proof of the photogenerated *cis* conformers was unambiguously observed with Capillary Electropherogram (CE), in which the *cis* and the *trans* conformers of the tetrathiopeptide were separated. The *cis* isomers were isolated as pure species by HPLC, this success to observe and isolate the thermodynamically unstable *cis* conformers is due to its slow relaxation (lifetime is about 1 h at room temperature).[59]

Different from the peptidyl–prolyl thiopeptide bonds,[59] UV–Vis absorption of *cis* and *trans* conformers of the

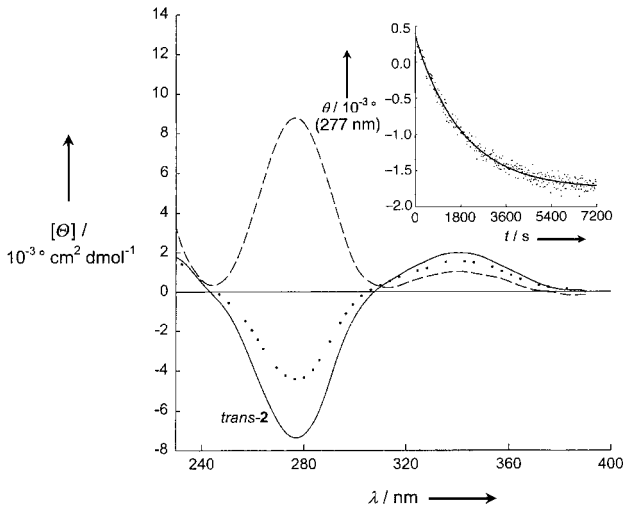

Figure 16. CD spectra of the *cis*- and *trans*-conformers of peptide Tyr-Ψ[CS-N]-Pro-Phe-Phe-NH_2 (**9**) with irradiation. Equilibrated peptide **9** (...), *trans*-**9** (——), and *cis*-**9** (-----). Inset: The *cis*/*trans* isomerization of peptide **9** monitored with the ellipticity at 277 nm ($k_{obs} = (5.38 \pm 0.02) \times 10^{-4}\,s^{-1}$). Sodium phosphate buffer (0.01 M, pH 7.4), 2×10^{-4} M, 313 K. Reprinted with permission from Ref. 59. Copyright © (2000) John Wiley & Sons, Inc.

secondary thiopeptide Phe-Ψ[CSNH]-Ala are drastically different,[28,58] which makes a dual-directional photoswitch possible (Fig. 17) because suitable wavelength can be found to excit *trans* and *cis* conformers separately. Capillary electrophoresis (CE) was also used to observed the *cis* conformers generated by photoswitch. It was also found that the *cis* conformers can be photoswitched back to the *trans* conformers.[58] As it is shown that secondary

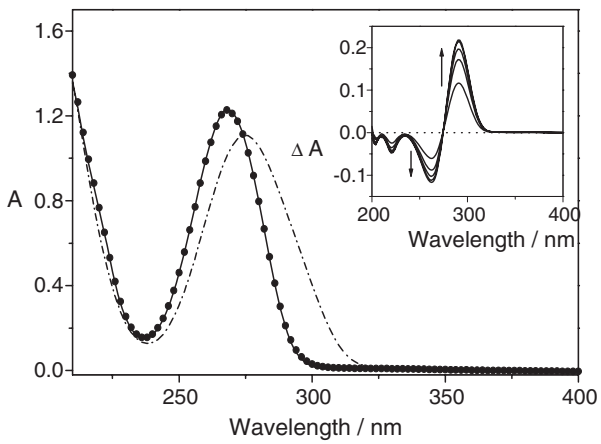

Figure 17. UV–Vis absorption spectra of peptide Phe-Ψ[CSNH]-Ala with UV irradiation. Peptide (1.4×10^{-4} mol dm^{-3}) in 5.0×10^{-2} mol dm^{-3} sodium phosphate buffer (pH 7.0), 289 K. Before irradiation (solid line), after 3 min of irradiation at 254 nm (dot-dash line), re-equilibrated peptide after three cycles of irradiation-re-equilibration (solid circles). Inset: difference UV spectrum during irradiation (*cis* isomer has a stronger absorbance in the 275–325 nm region). Reproduced by permission from Ref. 58 Copyright © (2003) Royal Chemical Society

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peptide bonds are also important for the folding of protein, thus the photoswitch of the secondary thiopeptide bonds offers an alternative for the efficient photomodulation of the activity of peptides or proteins. It has been shown that the activity of the thioxylate-RNase can be photomodulated.[28,60] We propose that conformation of the modified RNase is photomodulable but no study has been carried out on the lowest energy structure of the *cis*- and *trans*-configurations.

Very recently a study on the photoswitch of the *cis*/*trans* isomerization of the thioamide bond in *N*-methylthioacetatamide with transient IR spectroscopy and theoretical calculations has shown that the photoswitch is a fast process; the formation time of the *cis* configuration is about 8–9 ps.[24,40]

Isomerization of thioamide bonds was also studied with transient resonance Raman spectroscopy.[61] It was shown that the transition from *trans* to *cis* is a fast process on timescale of 5 ns. Isomerization is likely to take place after intersystem crossing of the excited states from the $^1\pi^*\pi$ to the $^1\pi^*n_s$ state, although the possibility of direct isomerization from $^1\pi^*\pi$ state was not completely ruled out.[61] The reason for the discrepancy of isomerization rate constants determined by the different methods is unclear.

For the azobenzene-based photoswitchable system, it is impossible to predict which peptide bond in the main chain of the polymer/peptide will be switched. With the photochromic thiopeptide bonds, the photoswitch site on the main chain of the peptide can be precisely controlled. At the same time, the perturbation of the structure of peptide is minimal compared to the use of other photochromic moieties, [5b,40] because in the thioamide peptide bond there is only one substitution of an oxygen atom by sulfur.

SUMMARY AND PERSPECTIVES

The basic property of the amide bonds is its hindered rotation about the C—N bond, due to the delocalization of the carbonyl group over the C—N bond. This restricted rotation caused conformation heterogeneity of the peptides or proteins and *cis*/*trans* isomerization of the peptide bonds are important for the biological processes involving peptide or protein, such as folding and unfolding of proteins. The methods used for the study of the *cis*/*trans* isomerization of the peptide amide bonds were discussed. Many efforts have been made to photomodulate the conformation of polypeptides. It was demonstrated that the azobenzene moiety incorporated in the peptide can switch its conformation efficiently. With novel photochromic groups, such as thioamides, that show longer excitation wavelength, high isomerization yields, fast isomerization kinetics and stable *cis* conformers, more applications could be made to control the conformation as well as the bioactivities of peptides or proteins.

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