HOKKAIDO UNIVERSITY

Title	Ultrafast photoisomerization and its single-shot pump pulse efficiency of trans-azobenzene derivative : Compound for photosensitive DNA
Author(s)	Chen, Tao; Yamaguchi, Atsushi; Igarashi, Kazumasa et al.
Citation	Optics Communications, 285(6), 1206-1211 https://doi.org/10.1016/j.optcom.2011.10.032
Issue Date	2012-03-15
Doc URL	https://hdl.handle.net/2115/48559
Type	journal article
File Information	OC285-6_1206-1211.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

*Manuscript
Click here to view linked References

Ultrafast photoisomerization and its single-shot pump pulse efficiency of *trans*-azobenzene derivative: compound for photosensitive DNA

Tao Chen[a,c,]*, Atsushi Yamaguchi[a], Kazumasa Igarashi[a], Naoya Nakagawa[a], Hidenori Nishioka[b], Hiroyuki Asanuma[b] and Mikio Yamashita[a,]**

[a]*Department of Applied Physics, Graduate School of Engineering, Hokkaido University and Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Kita-13, Nishi-8, Kita-ku, Sapporo 060-8628, Japan*

[b]*Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University and Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Nagoya 464-8603, Japan*

[c]*School of Electronic & Information Engineering, Xi'an Jiaotong University, Xi'an, 710049 China*

*Corresponding author to provide: Tel: +81-11-7066705, Fax: +81-11-7067887

Email:*taochen@stu.xjtu.edu.cn，**mikio@eng.hokudai.ac.jp；

Abstract: The femtosecond photoisomerization processes of *trans* (T) 4-carboxy-2',6'-dimethylazobenzen, which has been employed recently as an efficient photoregulator of DNA hybridization, were clarified by the rate equation analysis of measured transient absorbance changes with (350 nm) and without (380 nm) ground-state absorption of both the reactant (T) and photoproduct (*cis*: C) isomers under S_2^T-band excitation (360 nm, 150 fs pump): after excitation to the S_2^T state with a 450-fs lifetime, ~1.5% of the T-molecules in the S_2^T state are isomerized to the

C-form within ~ 6 ps through the intermediate state (so called bottleneck state), but most of those return back to the T ground-state S_0^T via the internal conversion processes with an ultrafast kinetic rate of 2.2×10^{12} s^{-1}. Moreover, the rate equation analysis enables us to determine the T-to-C photoisomerization rate $\eta^{T,C}$ per pump pulse to be 0.0011 at the pump energy of 80 nJ from the amplitude $A_{3,350}$ of the offset component in the 350-nm probe signal, and to obtain the photoisomerization quantum yield $\Phi^{T,C}$ =0.094. The latter value is slightly lower than that of T-azobenzene, and well agrees with that ($\Phi^{T,C}$=0.097) measured by the conventional CW irradiation method using a photostationary state.

Keywords: Photoisomerization, femtosecond phenomena, photosensitive DNA material.

1. Introduction

Recently, various photosensitive DNAs involving azobenzene (Az) compounds have been investigated for effective photoregulation of DNA hybridization, and it has been found that 4-carboxy-2',6'-dimethylazobenzene (AzD: Fig. 1) is one of the most effective photoregulator with the highest thermal stability of the *cis* (C)-form [1,2]. *Trans* (T)-to-C isomerization of the AzD is induced by ultraviolet (UV: wavelength λ=300~380 nm) lamp irradiation (S_2-band excitation) for several minutes, so that the dissociation of AzD-bound DNA duplexes is photoregulated. While, by visible (λ=400~500 nm) lamp irradiation (S_1-band excitation) C-to-T isomerization is induced, so that the formation of AzD-bound DNA duplexes is photoregulated. However, the ultrafast photoisomerization processes of the AzD compound are

unknown except for our study of the C-AzD compound [3], although, for Az related compounds, extensive studies for clarification of ultrafast photoisomerization mechanisms such as rotation, inversion and concerted inversion processes have been carried out [4-7].

On the other hand, recently selective quantum control of photoisomerization processes of organic (e.g. stilbene [8]) and biological molecules (e.g. rhodopsin [9]) has been demonstrated using a femtosecond optical-pulse shaping technique [10]. We believe that the application of this technique by shaped pulse excitation of a single shot for the above mentioned DNA-photoregulation processes offers a new tool for the biotechnology and DNA-nanotechnology [11]. This is because its instant efficient control technique may enable us to manipulate quasi-simultaneously and site-selectively biomolecular functions at many local desired points of a high order molecular-structure system. For this purpose, it is necessary to reveal the ultrafast photoisomerization processes of the newly synthesized photoregulation molecule AzD. Particularly, it is important to shed light on the photoisomerization single-shot efficiency. In this paper, for the T-AzD solution we characterize the photoisomerization rate $\eta^{T,C}$ per femtosecond pump pulse (the ratio of the number of photoproduct isomer molecules to that of reactant isomer molecules per pump pulse) based on clarification of the electronic excited-state dynamics. Furthermore, we determine the quantum yield $\Phi^{T,C}$ of photoisomerization from the femtosecond signal of the transient absorbance change, which shows an excellent agreement with the result obtained independently by the technique using a photostationary state under

CW lamp irradiation [12]. Our research aim is different from a lot of studies for Az and related molecules [4-7], where the relation between S_1 or S_2 band excitation and photoisomerization mechanisms of the rotation, inversion and concerted inversion processes has been investigated.

2. Experimental

2.1 Sample solution

AzD was synthesized and purified according to refs. [1,2]. The spectroscopy-grade ethanol (Tokyo Kasei Co.) was employed as the solvent. The pure T-AzD solution (5×10^{-3} mol/L) as an initial state of the sample was prepared by the 400-500 nm lamp irradiation (a 500-W Hg lamp with filters) for 10 hours at 90 °C to establish a photostationary T-form. Similarly, the pure C-AzD solution was prepared by the 330-360 nm lamp irradiation for 3 hours at room temperature to establish a photostationary C-form. Those purities were confirmed by NMR spectroscopy and the measurement of the absorption spectrum (Fig. 1) for the T-AzD solution (more than 98% T-form) and the C-AzD solution (more than 90% C-form). The vertical axis in Fig. 1 is denoted by the corresponding absorption cross section σ^m (m=T or C). The half-life time of the thermal isomerization of the C-AzD solution is ~10 hours at even 60 °C, indicating the excellent thermal stability of the ten times larger than that of the C-Az solution [1,2].

In the femtosecond transient absorption measurement, we paid much attention to always keep AzD the T-form as an initial state at each pump pulse (the 1-kHz repetition rate) for accurate characterization of the photoisomerization rate $\eta^{T,C}$ per

pump pulse, as follows: the solution was pumped through two different cells in a closed circulation flow system during the pump-probe experiment. The first 100-μm cell was used for the establishment of a photostationary form of the desired isomer by exposing the cell to the incoherent light with the corresponding spectral range from 400 to 500 nm for the T-isomer. The second cell with an optical path length *d* of 1 mm was used for the transient absorption measurement. The flow rate of the AzD solution in the second cell was high enough to exchange the volume excited by the femtosecond pulse between successive excitation.

2.2 Femtosecond transient absorption experiment and CW experiment

Under the sample condition mentioned in the previous subsection, when the 360 nm femtosecond pulse pumps the T-AzD solution at a 1-kHz repetition rate, only T-to-C photoisomerization occurs but C-to-T photoisomerization does not occur because only the T-molecules in the ground state exist and are excited to the second electronic excited state S_2^T with the much larger absorption cross section $\sigma_{0,2}^{'T}(\lambda_{pump})$ compared with that of C-molecules at each photo-excitation. This corresponds to the already reported fact that the 330 to 380 nm irradiation (S_2-band excitation) enables us to dominantly isomerize to the C-form even for the AzD solution of the T- and C-mixture state [1,2]. While the 400 to 500 nm CW irradiation (S_1-band excitation) dominantly isomerizes to the T-form for the T- and C-mixture solution, suggesting that S_1-band excitation for the T solution does not effectively isomerize to the C-form.

The femtosecond transient absorption measurement was carried out using the wavelength-tunable optical source based on Ti-sapphire laser-amplifier pulses (30 fs,

800 nm, 2.5 mJ) at a 1-kHz repetition rate [3]: after the amplified pulses were input to an Ar-filled hollow fiber for a spectral broadening by self-phase modulation, the chirped output pulses were compensated for by visible chirped mirrors. The compensated beam was split into two beams, and then those were independently frequency-doubled by two 500-μm thick β-barium borate (BBO) crystals. The two second-harmonic (SH) beams were again chirp-compensated for by two pairs of UV chirped mirrors. As a result, 360-nm, 80-nJ, 150-fs pulses were generated from one SH beam as pump pulses. Similarly, 350-nm, 10-nJ, 150-fs pulses or 380-nm, 10-nJ, 150-fs pulses were generated from the other SH beam as probe pulses by turning the BBO-crystal angle. The pump beam was chopped and the linear polarization of the probe beam was set to become the magic angle of 54.7° using an UV polarizer to avoid the reorientation effect. The probe signal passing through the second sample cell was detected as a function of the delay time by a photomultiplier with optical filters and the following lock-in-amplifier, and was recorded by a personal computer. The reason why we choose the 350-nm probe and the 380-nm probe is as follows: since at 350 nm the difference $\Delta\sigma_{0,2}^{T,C}$ between the ground-state absorption cross section $\sigma_{0,2}^{T}$ of the reactant T-isomer and that $\sigma_{0,2}^{C}$ of the photoproduct C-isomer is large (see Fig.1), the 350-nm probe provides information about the ground-state population dynamics of the photoproduct isomer as well as the excited-state and ground state population dynamics of the reactant isomer, while the 380-nm probe provides selectively information about the dynamics of only the excited-state population of the reactant isomer, because of no ground-state absorption for both the

isomers.

In the CW experiment, by the conventional method proposed by Zimmerman *et. al* [12], we also measured the quantum yield $\Phi^{T,C}$ of photoisomerization for the T-AzD solution. For the completely stirred solution with an optical density 0.6 at 360 nm, light induced absorption changes were recorded as a function of the irradiation time until the establishment of a photostationary state by a 300 W Xenon light source with a 360-nm filter. The absorbance change of the AzD solution filled in a 1 cm square cell with a volume of 3.5 ml was recorded immediately after each irradiation time (5 or 15 minutes) by a JASCO V-550 spectrophotometer (18 s recorded time).

3. Result of transient absorption measurement and determination of photoisomerization rate per pump pulse

Figure 2 shows the typical transient absorbance changes $\Delta OD_j(t)$ (j=350 or 380 nm), measured by the 350-nm probe (black inversed triangle) and the 380-nm probe (red circle) under 360-nm pulse excitation for the T-AzD solution. The 350-nm probe signal $\Delta OD_{350}(t)$ shows a negative decay (transient photobleaching) with a fast component of ~sub ps, an intermediate component of ~5 ps and a constant component (we call it offset; small positive component of around the zero-fs region was confirmed to be due to the two-photon absorption of the solvent). We confirmed that the offset was observed even at the delay time of 100 ps. This result corresponds to the negative decay reported in the experiment of the 303-nm S_2^T-band pump and 303-nm probe for a T-Az solution [13]. While, the 380-nm signal $\Delta OD_{380}(t)$ shows a positive decay (transient absorption recovering) with a fast component of ~sub ps and

an intermediate component of ~5 ps but no offset which was confirmed even at the delay time of 100 ps. This latter result corresponds to the positive decay with no offset component, reported in the 303-nm pump and 367-nm probe experiment for the T-Az solution, which has no ground-state absorption around at 367 nm [13]. The similar behaviors of the absorbance change were also reported for a *trans* 4-(dimethylamino)azobenzene (T-DMAAz) solution under the 400-nm pump (S_2^T-band excitation), where as the probe wavelength changes from 400 to 500 nm towards no ground-state absorption, the amplitude of the negative offset becomes from non-zero to zero [14].

To quantitatively understand the measured results of the transient absorbance change, we carried out the rate equation analysis describing the population dynamics after optical pulse excitation $I_{pump}(t)$ (photon energy $\hbar\omega_{pump}$) in the similar way to ref. [3] (see Appendix A). The electronic-state energy diagram on the basis of the model with the intermediate-state $I_1^{T,C}$ corresponding to the "bottleneck state" S_2^* in refs. [13,15], and the employed notations for the kinetic rate constants $k_{i,j}^{m,n}$ (i,j=0, 1 or 2, m,n=T, I or C) and the absorption cross sections $\sigma_{i,j}^{m,n}$ including the excited-state ones [13,16] are shown in Fig. 3. We assume that the intermediate state in the π-π* excitation (360 nm pumping) is different from that in the n-π* excitation as well as the difference of the initial isomer. This is because the practical potential-energy barrier [17] from the different excited state S_i^m (i=1 or 2, m=T or C) to the isomerization pathway is different in each case, depending on the isomerization mechanisms such as rotation, inversion or concerted inversion processes [4-7,18].

Some of the molecules excited to the singlet-state S_2^T by optical pulse pumping are relaxed to the ground state S_0^T by internal conversion. The others are transferred to the intermediate state $I_1^{T,C}$ with a relatively small potential energy barrier to yield new C-isomers [17] and then are relaxed to the ground state S_0^C of the new isomer. That is, for simplification, we assume that the molecules returning to the ground state S_0^T of the initial T-isomer are mostly via the internal conversion processes (S_2^T-to-S_0^T transition) but hardly via the intermediate state $I_1^{T,C}$ (e.g. $k_{1,0}^{I,C} \gg k_{1,0}^{I,T}$).

Using solutions of the rate equation, which describe the time dependent population $n_i^m(t)$ (i=0, 1 or 2; m=T, I or C) in the states S_0^T, S_2^T, $I_1^{T,C}$ and S_0^C, we can obtain the 350-nm probe signal $\Delta OD_{350}(t)$ for the 360-nm pump as follows:

$$\Delta OD_{350}(t) = A_{1,350}\exp(-t/\tau_1) + A_{2,350}\exp(-t/\tau_2) + A_{3,350} \tag{1}$$

$$A_{1,350} = CP_{0,2}^T n_0^T(0)[(\sigma_{2,n}^T - \sigma_{0,2}^T) - d_1(\sigma_{1,n}^I - \sigma_{0,2}^T) + d_1\Delta\sigma_{0,2}^{C,T}\tau_1/\tau_2] \tag{2}$$

$$A_{2,350} = CP_{0,2}^T n_0^T(0) d_1(\sigma_{1,n}^I - \sigma_{0,2}^C) \tag{3}$$

$$A_{3,350} = CP_{0,2}^T n_0^T(0) k_{2,1}^{T,I}\tau_1\Delta\sigma_{0,2}^{C,T} = Cn_0^C(\infty)\Delta\sigma_{0,2}^{C,T} \tag{4}$$

where $d_1 = k_{2,1}^{T,I}/(k_{2,0}^T + k_{2,1}^{T,I} - k_{1,0}^{I,C})$, $\tau_1 = 1/(k_{2,0}^T + k_{2,1}^{T,I})$, $\tau_2 = 1/k_{1,0}^{I,C}$, $\Delta\sigma_{0,2}^{C,T} = \sigma_{0,2}^C - \sigma_{0,2}^T$ at 350 nm, $C = d/\ln 10$, and the effective excitation probability

$$P_{0,2}^T = \sigma_{0,2}^{'T}(\lambda_{pump})\int I_{pump}(t)dt/(\hbar\omega_{pump}). \tag{5}$$

Moreover, we can obtain the corresponding 380-nm probe signal by putting the $\sigma_{0,2}^T = 0$, $\sigma_{0,2}^C = 0$ and $\Delta\sigma_{0,2}^{C,T} = 0$ with $A_{1,380} = A_{1,350}$ and $A_{2,380} = A_{2,350}$ because of no ground state absorption at 380 nm for both the isomers, as seen from Fig. 1:

$$\Delta OD_{380}(t) = A_{1,380}\exp(-t/\tau_1) + A_{2,380}\exp(-t/\tau_2). \tag{6}$$

These equations well explain the experimental results, as follows: (i) Eq. (1)

predicts that the 350-nm signal has two decay components and the offset, while Eq. (6) predicts that 380-nm signal has only two decay components. These agree with the measured results which are shown in Fig. 2. Therefore, the measured time constant τ_1 of the fast component and that τ_2 of the slow component represent the lifetime of the second excited state S_2^T and that of the intermediate state $\mathrm{I}_1^{T,C}$, respectively. In addition, the τ_2 indicates the birth time of the newly yielded isomer (the photoproduct). (ii) Eq. (4) predicts that the amplitude $A_{3,350}$ of the offset is proportional to the difference $\Delta\sigma_{0,2}^{C,T}$ (at the probe wavelength of 350 nm) between the ground-state absorption cross section $\sigma_{0,2}^{C}$ of the photoproduct isomer and that $\sigma_{0,2}^{T}$ of the reactant isomer. Also, it suggests that the offset is negative because of $\Delta\sigma_{0,2}^{C,T}$ =-5.4×10^{-17} cm^2, which also agrees with the measured result. Moreover, (iii) Eq. (4) predicts that the absolute amplitude $\left|A_{3,350}\right|$ of the offset is proportional to the number $\mathrm{n}_0^C(\infty)$ of photoproduct molecules per volume since $\mathrm{n}_0^C(\infty) = P_{0,2}^T \mathrm{n}_0^T(0) k_{2,1}^{T,I} \tau_1$. This enables us to obtain the photoisomerization rate $\eta^{T,C}$ per pump pulse for the T-to-C reaction:

$$\eta^{T,C} = \mathrm{n}_0^C(\infty)/\mathrm{n}_0^T(0) = \left|A_{3,350}\right| / (C n_0^T(0) \left|\Delta\sigma_{0,2}^{C,T}\right|) = P_{0,2}^T k_{2,1}^{T,I} \tau_1 . \quad (7)$$

Furthermore, (iv) Eqs. (2), (3), (4) and (7) predict that the signs of all the amplitudes $A_{1,350}$, $A_{2,350}$, $A_{3,350}$, and $A_{1,380}$ depend on the difference between the related absorption cross-sections, except for the $A_{2,380}$ which is always positives. Therefore, from the observed sign of each component we can compare the magnitudes of the unknown excited-state absorption cross-sections with those of the ground-state absorption cross-sections.

Using Eqs. (1) and (6) and the instrumental response measured from the two-photon absorption of the solvent, we fitted numerically all the measured absorbance changes $\Delta OD_j(t)$ (j=350 or 380) to obtain the lifetimes, the amplitudes $A_{i,j}$ (i=1, 2 or 3) and the kinetic rate constants. The results are summarized in Table I. We find that the lifetime τ_1 of the second excited state S_2^T of the T-isomer is 450 fs and the lifetime τ_2 of the excited intermediate state $I_1^{T,C}$ is 5.6 ps. The former lifetime is longer than that of the corresponding state of the T-Az in hexane ($\tau_1 = 110$ fs) reported in ref. [19], which was measured from the fluorescence decay after femtosecond pulse pumping at 280 nm. This difference may be due to the fact that the T-AzD molecule is more planar than the T-Az molecule. The latter lifetime suggests that the C-isomer is yielded within about 6 ps after excitation by a 360-nm femtosecond pulse. These results give useful information for a coherent control experiment of T-to-C photoisomerization of AzD to decide a suitable delay time (longer than ~6 ps) of the 350-nm probe pulse after excitation by a 360-nm shaped pulse. We can also estimate the kinetic rate constant $k_{2,0}^T$ =2.2×10^{12} (s^{-1}) from the S_2^T state to the S_0^T state, the kinetic rate constant $k_{2,1}^{T,I}$ =3.4×10^{10} (s^{-1}) from the S_2^T state to the $I_1^{T,I}$ state, and the transfer rate $T_{2,1}^{T,I} = 0.015$ ($= \tau_1 k_{2,1}^{T,I}$) using Eq. (4) and the measured value $|A_{3,350}|$ =8 mOD. Furthermore, for the relation between different absorption cross sections we find as follows: $\sigma_{0,2}^T > \sigma_{2,n}^T$ and $\sigma_{0,2}^T > \sigma_{0,2}^C > \sigma_{1,n}^I$ at 350 nm. The former $\sigma_{0,2}^T > \sigma_{2,n}^T$ corresponds to the already reported result for the T-Az solution [13,16].

Finally, we obtain the important parameter $\eta^{T,C}$ per femtosecond pump pulse

for T-to-C of the T-AzD solution. By substituting the S_0^T-to-S_2^T effective excitation probability $P_{0,2}^T$=0.073 and the S_2^T-to-$I_1^{T,C}$ transfer rate $T_{2,1}^{T,I}$=0.015 into Eq. (7), we obtain the photoisomerization rate $\eta^{T,C}$ to be 0.0011 at the 360 nm pump with pulse energy of 80 nJ. In addition, this equation suggests that the theoretical maximum value of $\eta^{T,C}$ is 0.015. We expect that this value can be increased by the coherent control technique with a shaped pump pulse, which will enable us to increase the rate $k_{2,1}^{T,I}$ and decrease the rate $k_{2,0}^{T}$, such as photoisomerization control of stilbene molecules [8].

4. Discussion

Here we discuss the origin of the offset A_3 (in the following sections, we denote $A_{i,350}$ as A_i: i=1, 2 or 3) of the 350-nm probe signal, by comparing with the already reported results of different Az-related molecules [13,14,20-24]: The similar negative offset was observed for the T-Az solution [13], the T-DMAAz solution [14], the capped T-AzD1 (*trans*-**1** in ref. [20]) solution and the *trans* 4-(4'-aminophenylazo)benzoic acid sodium salt (T-AzD2) solution [21] under the condition of S_2^T-band excitation and the probe wavelength of the S_2^T band. In addition, the positive offset was also observed for those solutions under the condition of S_2^T-band excitation but the probe wavelength of the S_1^T band. Furthermore, for the C-Az solution [22] and the C-AzD solution [3] the negative or positive offset was observed under the condition of S_1^C-band excitation, depending on the probe wavelength of the S_1^C band or that of S_2^C band. However, for those offsets two-different assignments were done: (i) is due to the appearance of the *photoproduct*

isomer not via (or via) the “hot-band” vibrational state of the electronic ground state with the depletion of the *reactant isomer* after ultrafast photoisomerization [13,20-22,24]. (ii) is due to the formation of the “hot-band” vibrational state in the electronic ground state of the *reactant isomer* after ultrafast internal conversion from the excited state S_i^T (i=1 or 2) or that of both the *reactant isomer* and the *photoproduct isomer* [4,14,23]. We assign that the offset observed for the T-AzD solution under the condition of S_2^T-band excitation is due to the former without accompanying the “hot-band” state because of the following facts: ① The offset was still observed even at long delay time of 100 ps, which is much longer than the lifetime (ten to several ten picoseconds [24]) of the “hot-band” state. The result that the T-AzD solution has no “hot-band” vibrational state in the photoproduct (C-isomer) ground state may be related to the unique properties that thermal isomerization of C-to-T hardly occurs in the C-AzD solution [1,2]. ② Only the direct experimental evidence of the formation of the “hot band” was offered by the transient mid-infrared vibrational spectroscopy after ultrafast photoisomerization by femtosecond visible pulse excitation, where the “hot-bands” state was observed in the electronic ground state of the photoproduct isomer from the Az solution but not in that of the reactant isomer [24]. ③ In general the offset due to the latter transient “hot bands” should not depend on the sign and the magnitude of the difference $\Delta\sigma_{0,2}^{C,T}$ between the absorption cross section (at the probe wavelength) of the electronic ground state of the reactant isomer and that of the photoproduct isomer. In addition, in some cases the rise-up behaviors of the positive direction in the formation processes of the offset

should appear as the transient absorption during the ultrafast internal conversion relaxation. However, our experimental and analytical results (see Eq. (4)) showed that the offset amplitude decreases in proportional to $\Delta\sigma_{0,2}^{C,T}$ including the influence of its sign and the offset does not appear in the 380-nm probe signal where $\Delta\sigma_{0,2}^{C,T} = 0$.

Next, we discuss the relation between the offset A_3 and the well-known photoisomerization quantum yield $\Phi^{T,C}$. A photoisomerization quantum yield $\Phi^{m,n}$ (m,n=T,C or C,T) is defined by the ratio of change of the number of reactant isomer molecules per time and volume, $dN_0^m(t)/dt$ (m=T or C), to the amount of photons absorbed by reactant molecules per time and volume, $I_{abs,m}(t)$ [12,25]. Here, N_0^m is in mole·molecules per liter (mol·molecules/L) and hence $N_0^m = 1000 \times n_0^m / N_{AV}$ since n_0^m is in numbers of reactant molecules per cubic centimeters (N_{AV} is Avogadro's number). $I_{abs,m}(t)$ is in mole·photons per liter and time (mol·photons/L·s). Usually a typical photoisomerization reaction system in a dilute confined system with one pure isomer as an initial state is considered, where T-to-C and C-to-T reactions occur simultaneously by CW incoherent-light irradiation at a fixed wavelength λ_{pump} under the completely stirring but not flowing, and consequently establish a photostationary state. However, in the femtosecond pump and probe experiment where each pump pulse excites only the existing molecules of the T- (or C-) isomer and the photoproduct C- (or T-) molecules are removed before the subsequent pump pulse comes to the T- (or C-) AzD solution in the fast flowing system at a low (1-kHz) repetition rate of the pulses (see section 2), only one photoisomerization reaction at the fixed wavelength λ_{pump}, for example, T-to-C occurs. Accordingly, the

photoisomerization quantum yield $\Phi^{T,C}$ is described by the following equation:

$$\frac{dN_0^T(t)}{dt} = -\Phi^{T,C} I_{abs,T}(t) . \tag{8}$$

Since the left term is approximated by $-N_0^C(\infty)/\Delta t = -1000A_3/(C \times \Delta\sigma_{0,2}^{C,T} \times N_{AV} \times \Delta t_p)$ and $I_{abs,T}(t)$ in the right term is approximated by

$$1000 \times (A_1 + A_2 + A_3)/[(\sigma_{2,n}^T - \sigma_{0,2}^T)/\sigma_{0,2}^{'T}(\lambda_{pump}) \times \Delta t_p \times l_p \times C \times N_{AV}] \tag{9}$$

from Eqs. (2)~(5) of the rate equation analysis (see Appendix B), where l_p is the pump pulse length in centimeter. The quantum yield $\Phi^{T,C}$ is expressed by

$$\Phi^{T,C} = A_3/(A_1 + A_2 + A_3) \times [(\sigma_{2,n}^T - \sigma_{0,2}^T)/\Delta\sigma_{0,2}^{C,T}] \times l_p \times n_0^T(0) \times \sigma_{0,2}^{'T}(\lambda_{pump}) . \tag{10}$$

Here we assume that $\sigma_{2,n}^T \ll \sigma_{0,2}^T$ (see section 3) which corresponds to the result reported for the excited-state absorption of the T-Az solution in refs. [13,16] and $l_p \times n_0^T(0) \times \sigma_{0,2}^{'T}(\lambda_{pump})$ =0.58 ($\sigma_{0,2}^{'T}(\lambda_{pump})$ =3.2×10^{-17} cm^2, $n_0^T(0)$ =3.0×10^{18} cm^{-3}, l_p=6.03×10^{-3} cm). Eq. (10) is favorably compared with the simplified estimation equation of $\Phi^{T,C}$ employed for the T-Az solution at the 303 nm pump pulse in ref. [13] which is $\Phi^{T,C} = A_3 \times \sigma_{0,2}^T/[(A_1 + A_2 + A_3) \times \Delta\sigma_{0,2}^{T,C}]$. This difference may arise from the points that in the simple analysis in ref. [13] the pump effect including the absorption cross-section difference between the pump wavelength and the probe wavelength and the effect of the excited-state absorption processes are not considered. Substituting value of $A_3/(A_1+A_2+A_3)$=0.16 from the measured femtosecond transient absorbance change and the values of $\sigma_{0,2}^T$ = 5.54×10^{-17} cm^2 and $\Delta\sigma_{0,2}^{T,C}$ =5.45×10^{-17} cm^2 at the 350-nm probe wavelength from the measurement of steady-state absorption spectrum (see Fig. 1) into the above equation, we obtain $\Phi^{T,C}$ =0.094 at the 360-nm pump wavelength for the T-AzD solution. This value is slightly smaller than that of

the T-Az solution [12]. We confirmed that this result well agrees with the $\Phi^{T,C}$ =0.097, which was independently measured at 360 nm by the widely employed CW-irradiation method for determination of photoisomerization quantum yield using the photostationary state. This agreement supports our assignment for the offset.

5. Conclusion

We clarified the femtosecond photoisomerization processes of *trans* 4-carboxy-2',6'-dimethylazobenzene (AzD) for the first time, which has been employed recently as an effective photoregulator of DNA hybridization with the highest thermal stability for ground-state isomerization, on the basis of the interpretation of the 350-nm transient absorbance signal showing a three-component decay and the 380-nm one showing a two-component decay by the rate-equation analysis: some molecules (1.5%) of the *trans*-molecules excited to the S_2^T state with a lifetime of 450 fs become the *cis* form within ~6 ps through the intermediate state $\mathrm{I}_1^{T,C}$, but most of *trans*-molecules return back to the *trans* ground S_0^T state via the ultrafast internal conversion process at a rate of ~ 2.2×10^{12} s^{-1}. The amplitude $A_{3,350}$ of the constant component of the 350-nm probe signal, which is proportional to the difference $\Delta\sigma_{0,2}^{C,T}$ between ground-state absorption cross sections of both the isomers, enables us to determine the T-to-C photoisomerization rate $\eta^{T,C}$ per femtosecond pump pulse at 360 nm to be 0.0011 at 80-nJ pulse energy. Furthermore, we confirmed that the photoisomerization quantum yield $\Phi^{T,C}$ =0.094 obtained from the amplitude ratio $A_3/(A_1+A_2+A_3)$ well agrees with that $\Phi^{T,C}$ =0.097 from the conventional CW irradiation method using a photostationary state, which is slightly smaller than that of

the T-Az solution.

Acknowledgments

This research was supported by Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology. T. Chen would like to thank the support of government scholarship from China Scholarship Council.

Appendix A: Derivation of Equations (I)

Here we derivate Eqs. (1) and (6), which describe the transient absorbance changes of the 350 nm probe and the 380 nm probe at 360 nm excitation.

We consider the excitation of *trans*-molecules by a 360 nm delta-function pulse at time t=0, which produces $n_2^T(0)\,(=P_{0,2}^T n_0^T(0))$, the number of the molecules per unit volume (the population) in the second excited S_2^T state. Here, $n_0^T(0)$ is the number of molecules per unit volume in the S_0^T state and $P_{0,2}^T = \sigma_{0,2}^{'T}(\lambda_{pump})\int I_{pump}(t)dt/(\hbar\omega_{pump})$ is the effective excitation probability per unit volume, where $I_{pump}(t)$ and $\hbar\omega_{pump}$ are the light intensity and the photon energy of the pump pulse, respectively. According to the schematic energy diagram of Fig. 3, the rate equations are given as follows (t>0):

$$\frac{dn_2^T(t)}{dt} = -(k_{2,0}^T + k_{2,1}^{T,I})n_2^T(t)\,, \tag{A.1}$$

$$\frac{dn_1^I(t)}{dt} = k_{2,1}^{T,I} n_2^T(t) - k_{1,0}^{I,C} n_1^I(t)\,, \tag{A.2}$$

$$\frac{dn_0^C(t)}{dt} = k_{1,0}^{I,C} n_1^I(t)\,, \tag{A.3}$$

where $n_2^T(t)$, $n_1^I(t)$ and $n_0^C(t)$ are the time-dependent populations in the S_2^T, I_1^I and S_0^C states at t>0, respectively. Solving Eqs. (A.1)-(A.3) with the above-mentioned initial condition of $n_2^T(0)$ and $n_1^I(0) = n_0^C(0) = 0$ at t=0, we can

obtain the following time behaviors of the populations in the different states:

$$n_2^T(t) = P_{0,2}^T n_0^T(0)\exp[-(k_{2,0}^T + k_{2,1}^{T,I})t], \tag{A.4}$$

$$n_1^I(t) = P_{0,2}^T n_0^T(0)\frac{k_{2,1}^{T,I}}{(k_{2,0}^T + k_{2,1}^{T,I}) - k_{1,0}^{I,C}}\{\exp(-k_{1,0}^{I,C}t) - \exp[-(k_{2,0}^T + k_{2,1}^{T,I})t)]\}, \tag{A.5}$$

$$n_0^C(t) = P_{0,2}^T n_0^T(0)\frac{k_{2,1}^{T,I}k_{1,0}^{I,C}}{(k_{2,0}^T + k_{2,1}^{T,I}) - k_{1,0}^{I,C}}\{\frac{\exp[-(k_{2,0}^T + k_{2,1}^{T,I})t)]-1}{k_{2,0}^T + k_{2,1}^{T,I}} - \frac{\exp(-k_{1,0}^{I,C}t)-1}{k_{1,0}^{I,C}}\}. \tag{A.6}$$

Furthermore, the time-dependent ground state population $n_0^T(t)$ in the S_0^T state at t>0 is obtained by the conservation law of the number of molecules. To obtain the equation describing the transient absorbance change, we assume that the probe pulse is represented by a delta-function and is weak enough not to affect the population change.

For the 350 nm probe, the time dependent absorbance change. $\Delta OD_{350}(t)$ is given by $\Delta OD_{350}(t) = C[\sum \sigma_i n_i(t) - \sigma_{0,2}^T n_0^T(t)]$, where $\sum \sigma_i n_i(t)$ means the summation of $\sigma_i n_i(t)$ of all the related states because the ground state absorption ($\sigma_{0,2}^T \neq 0, \sigma_{0,2}^C \neq 0$) occur at 350 nm (see blue dashed lines in Fig. 3). Here, C (=d/log$_e$10) is a constant, and d is the optical pass length of the samples solution. As a result, $\Delta OD_{350}(t)$ is described by Eq. (1)

For the 380 nm probe, the time dependent absorbance change $\Delta OD_{380}(t)$ is given by $\Delta OD_{380}(t) = C\sum \sigma_i n_i(t)$, where $\sum \sigma_i n_i(t)$ means the summation of $\sigma_i n_i(t)$ of the only related excited states since no ground state absorption ($\sigma_{0,2}^T = 0, \sigma_{0,2}^C = 0$) occurs at 380 nm unlike the case of the 350 nm probe (see red dashed lines in Fig. 3). Accordingly, $\Delta OD_{350}(t)$ is described by Eq. (6)

Appendix B: Derivation of Equations (II)

The left term in Eq. (8) is approximated by limit $h \to 0$:

$$\lim_{h\to 0}(N_0^T(t+h)-N_0^T(t))/h \approx (N_0^T(\infty)-N_0^T(0))/\Delta t_p = -N_0^C(\infty)/\Delta t_p \,. \qquad \text{(B.1)}$$

Because the pump pulse duration $\Delta t_p = 150$ fs is enough short compared with the birth time of the C-molecules (~6 ps) and the number of the product C-molecules per pump pulse is enough small compared with the number of the reactant T-molecules (~0.1%) (see section 3). Also, $I_{abs,T}(t)$ in the right term in Eq. (8) is favorably approximated as follows [12,25]: $I_{abs,T}(t) = I_0(1-10^{-E(t)}) \approx I_0(1-10^{-E(0)}) \approx I_0$, where I_0 is the amount of photons of the input pulse per unit time and volume, $E(t) = \varepsilon_T^{'} \times N_0^T(t) \times d \approx \varepsilon_T^{'} \times N_0^T(0) \times d = E(0)$ and $\varepsilon_T^{'}$ is the molar extinction coefficient at the pump wavelength. This is because $N_0^T(t) \approx N_0^T(0)$, $E(0)$=4.2 and $10^{-E}\approx 0$. We employ $\varepsilon_T^{'}$ (and $\sigma_{0,2}^{'T}(\lambda_{pump})$) for the extinction coefficient (and the absorption cross section) at the pump wavelength but not the probe wavelength. As a result, Eq. (8) is rewritten by

$$\Phi^{T,C} = N_0^C(\infty)/(\Delta t_p \times I_0) \,. \qquad \text{(B.2)}$$

On the other hand, from the result of the rate equation analysis (see Eqs. (2)-(5) in section 3) we find that $N_0^C(\infty) = 1000 \times A_3/(C \times \Delta\sigma_{0,2}^{C,T} \times N_{AV})$ and

$$I_0 = 1000 \times (A_1 + A_2 + A_3)/[(\sigma_{2,n}^T - \sigma_{0,2}^T) \times \sigma_{0,2}^{'T}(\lambda_{pump}) \times \Delta t_p \times l_p \times C \times N_{AV})] \qquad \text{(B.3)}$$

where l_p is the pump pulse length in centimeter. Therefore, we obtain Eq. (10) in the text.

References

[1] H. Nishioka, X. Liang, H. Kashida, H. Asanuma, Chem. Commun. (Cambridge) (2007) 4354-4356.

[2] H. Nishioka, X. Liang, H. Asanuma, Chem. Eur. J. 16 (2010) 2054-2062.

[3] A. Yamaguchi, N. Nakagawa, K. Igarashi, T. Sekikawa, H. Nishioka, H. Asanuma, M. Yamashita, Appl. Surf. Sci. 255 (2009) 9864-9868.

[4] T. Fujino, T. Tahara, J. Phys. Chem. A 104 (2000) 4203-4210.
[5] C. -W. Chang, Y. -C. Lu, T. -T. Wang, E. W. -G. Diau, J. Am. Chem. Soc. 126 (2004) 10109-10118.
[6] Y. -C. Lu, E. W. -G. Diau, J. Phys. Chem. A 109 (2005) 2090-2099.
[7] A. Cembran, F. Bernardi, M. Garavelli, L. Gagliardi, G. Orlandi, J. Am. Chem. Soc. 126 (2004) 3234-3243.
[8] M. Greenfield, S. D. McGrane, D. S. Moore, J. Phys. Chem. A 113 (2009) 2333-2339.
[9] A. C. Florean, D. Cardoza, J. L. White, J. K. Lanyi, R. J. Sension, P. H. Bucksbaum, PNAS 106 (2009) 10896-10900.
[10] T. Tanigawa, Y. Sakakibara, S. Fang, T. Sekikawa, M. Yamashita, Opt. Lett. 34 (2009) 1696-1698.
[11] Y. Ito, E. Fukusaki, J. Mol. Catal. B 28 (2004) 155-166.
[12] G. Zimmerman, L. Y. Chow, U. J. Paik, J. Am. Chem. Soc. 80 (1958) 3528-3531.
[13] I. K. Lednev, T. -Q. Ye, R. E. Hester, J. N. Moore, J. Phys. Chem. 100 (1996) 13338-13341.
[14] S. G. Mayer, C. L. Thomsen, M. P. Philpott, P. J. Reid, Chem. Phys. Lett. 314 (1999) 246-254.
[15] I. K. Lednev, T. -Q. Ye, P. Matousek, M. Towrie, P. Foggi, F. V. R. Neuwahl, S. Umapathy, R. E. Hester, J. N. Moore, Chem. Phys. Lett. 290(1998), 68-74.
[16] T. Fujino, S. Y. Arzhantsev, T. Tahara, Bull. Chem. Soc. Jpn 75 (2002) 1031-1040.
[17] L. Wang, W. Xu, C. Yi, X. Wang, J. Mol. Graph. Mod. 27 (2009) 792-796.
[18] C. R. Crecca, A. E. Roitberg, J. Phys. Chem. A 110 (2006) 8188-8203.
[19] T. Fujino, S. Y. Arzhantsev, T. Tahara, J. Phys. Chem. A 105 (2001) 8123-8129.
[20] I. K. Lednev, T. -Q. Ye, L. C. Abbott, R. E. Hester, J. N. Moore, J. Phys. Chem. A 102 (1998) 9161-9166.
[21] J. Wachtveitl, T. Nagele B. Puell, W. Zinth, M. Kruger, S. R. Bohner, D. Oesterhelt, L. Moroder, J. Photochem. Photobiol. A 105 (1997) 283-288.
[22] T. Nagele, R. Hoche, W. Zinth, J. Wachtveitl, Chem. Phys. Lett. 272 (1997) 489-495.
[23] H. Satzger, C. Root, M. Braun, J. Phys. Chem. A: Chem 108 (2004) 6265-6271.
[24] P. Hamm, S. M. Ohline, W. Zinth, J. Chem. Phys. 106 (1997) 519-529.
[25] G. Gauglitz, in: H. Durr and H. B.-Laurent (Eds.), Photochromism: Molecules and systems, Elsevier, Amsterdam, 1990, pp. 883-902.

Figure Caption

Fig. 1.(Color on line) The absorption cross section (left) and molar extinction coefficient (right) of the *trans* and *cis* 4-carboxy-2',6'-dimethylazobenzenes in ethanol. Inset: The molecular structures of *trans* and *cis* forms.

Fig. 2.(Color on line) Measured transient absorbance change for *trans* 4-carboxy-2',6'-dimethylazobenzenes in ethanol at 360 nm pump. The probe wavelengths are 350 (black inversed triangle and red solid curve) and 380 nm (red circle and black solid curve). The solid curves are the fitted theoretical results.

Fig. 3.(Color on line) Schematic energy diagram of electronic states with relaxation and photoisomerization processes for 4-carboxy-2',6'-dimethylazobenzenes.

Table(s)

Table I: Lifetime τ_i, relative amplitudes a_i, kinetic rate constants $k_{i,j}^{m,n}$ and photoisomerization rate per pump pulse $\eta^{T,C}$ and photoisomerization quantum yield $\Phi^{T,C}$ of 2',6'-dimethylazobenzene in ethanol.

Probe (nm)	τ_1 (fs)	τ_2 (ps)	a_1^* (%)	a_2^* (%)	a_3^* (%)	$k_{2,0}^{T}$ (s^{-1})	$k_{2,1}^{T,I}$ (s^{-1})	$k_{1,0}^{I,C}$ (s^{-1})	$\eta^{T,C}$	$\Phi^{T,C}$
350	450	5.6	52	32	16	2.2×10^{12}	3.4×10^{10}	1.8×10^{11}	0.0011	0.094
380	450	5.6	54	46	-					

* $a_i = A_i/(A_1 + A_2 + A_3)$, i=1, 2, 3.

Figure1
Click here to download high resolution image

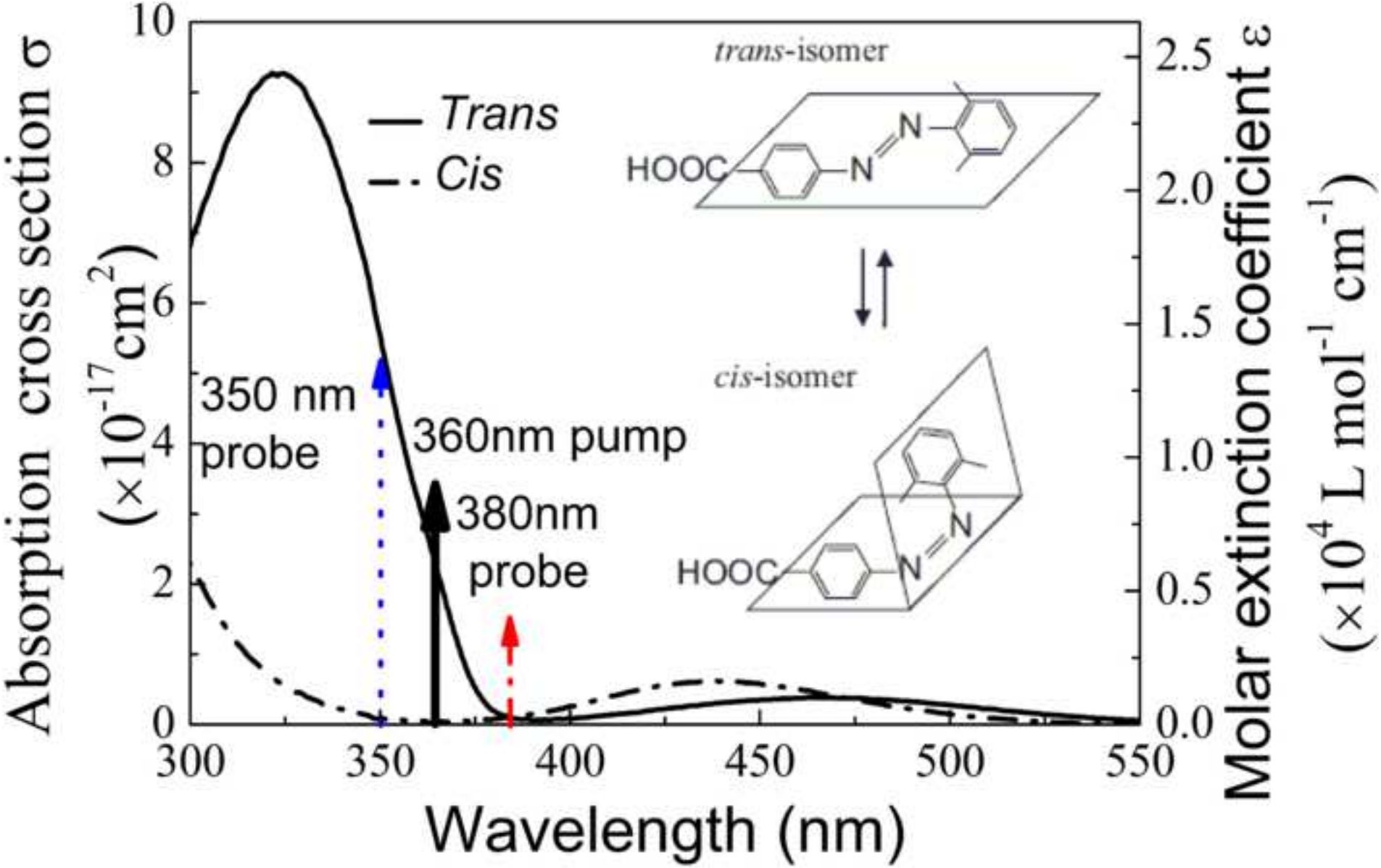

Figure2
Click here to download high resolution image

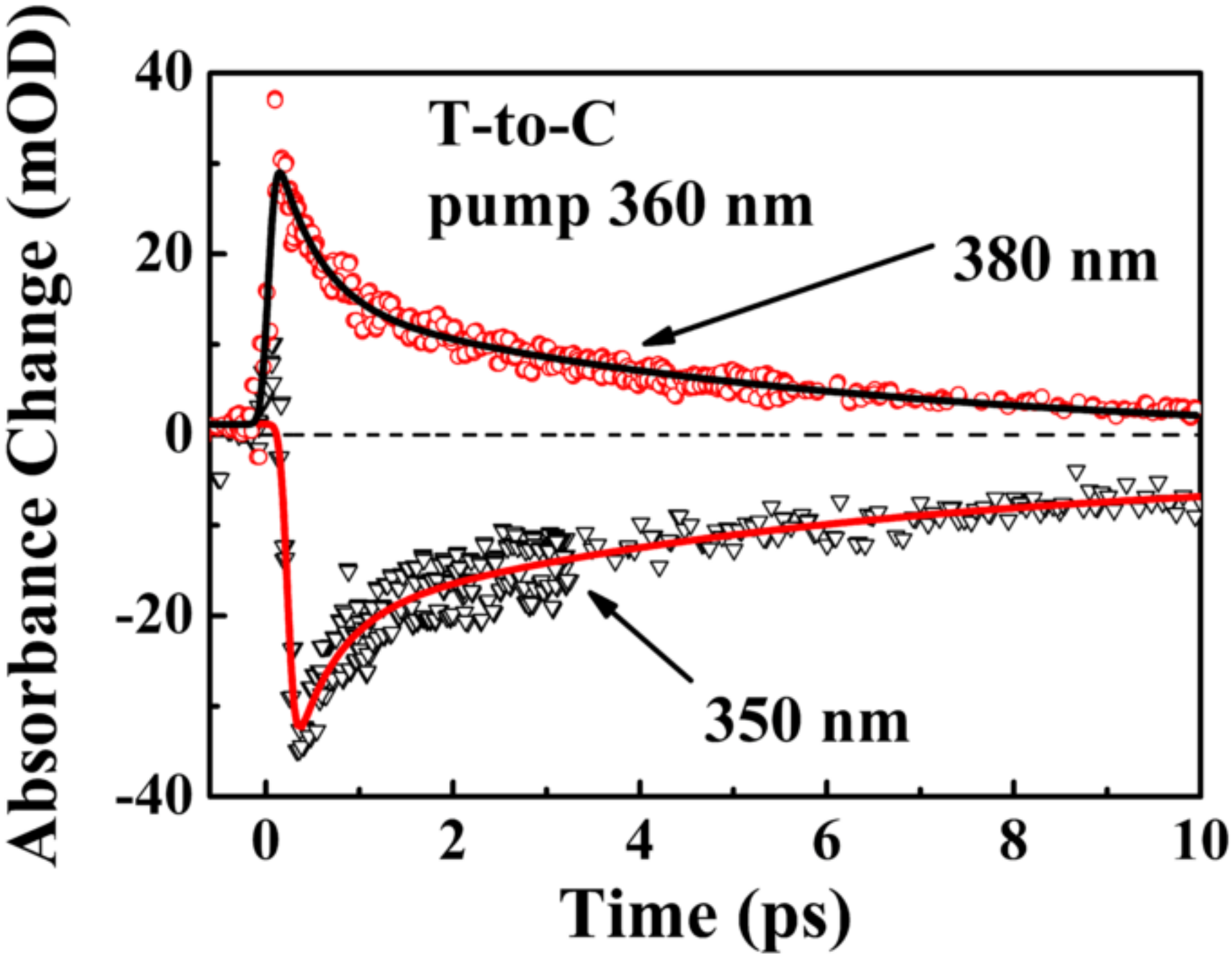
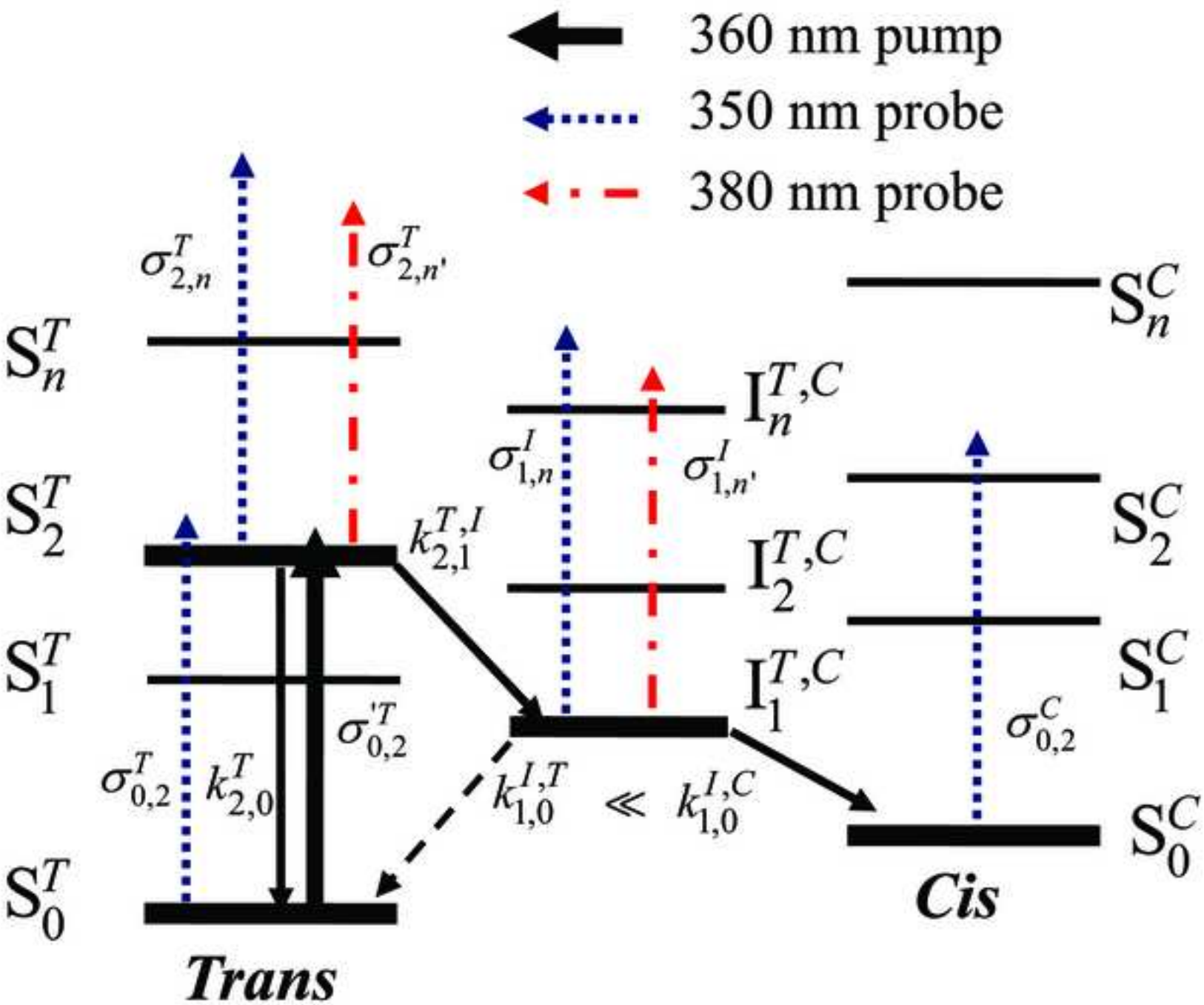
Figure3
Click here to download high resolution image