Supplementary Data

**Dynamics of UV-induced DNA-lesions: Dewar-formation guided by pre-tension induced by the back-bone**

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**Figure S1.** Temperature dependent values of the fluorescence decay. Depicted is the natural logarithm of the rate constants for component 1 (red squares) and component 2 (black circles). The solid lines represent fits according to an Arrhenius law (see text).
Estimates on the error margins of the fluorescence measurements

Estimates on the errors for the relative populations as well as for the activation barriers can be derived as follows. Since the relative amplitudes are virtually independent of the temperature, the upper limit for the relative standard error (ratio of standard deviation to mean value) of the relative populations is about 10 % (the relative standard error of the fits are less). For the activation barriers the errors derived from the fits amount to 13 % for the fast and 7 % for the slow decay time constant, respectively. The fluorescence decay measurements were performed without polarisation selective detection, thus, rotational diffusion may contribute to the decay traces, more precisely it superimposes the fast decay component. The rotational diffusion time constant can be estimated by the length of the major axis of the van-der-Waals sphere of T(6-4)T. Including its temperature dependence a systematic error results, which underestimates the first time constant by – 5-7 % and consequentially the activation barrier $E_A$ by – 3 %. Concerning the relative populations, the contribution of the rotational diffusion causes a systematic error of + 11 %. It has to be noted that the estimated systematic errors should be lower due to a less pronounced anisotropy for the applied geometry [1]. In summary, the derived values are within an error margin on the order of 10 %.
Estimations on the triplet quantum yield of T(6-4)T

1. General definitions

The absorbance of any state \( i \) with an extinction coefficient \( \varepsilon_i \) and a time dependent concentration \( c_i(t) \) in a sample cell with thickness \( d \) is given by Beer-Lambert’s Law:

\[
A_i(t) = \varepsilon_i \cdot c_i(t) \cdot d
\]  

(1)

When a molecular system was originally in state \( S_0 \) and is converted to states \( i \) the absorption of the sample changes by \( \Delta A \):

\[
\Delta A(t) = \sum A_i(t) - A_{S_0}(t)
\]  

(2)

The quantum yield for the formation of a photoproduct \( i \) by absorption of light (prior to light absorption there was no molecule of type \( i \)) is defined by

\[
\Phi_i = \frac{\text{number of product } i \text{ molecules}}{\text{number of absorbed photons}}
\]  

(3)

When \( c_i \) is the concentration of newly formed molecules of intermediate \( i \) and \( c^* \) the concentration of initially excited sample molecules the quantum yield can be written as:

\[
\Phi_i = \frac{c_i}{c^*}
\]  

(4)

When light absorption leads exclusively to the formation of an intermediate \( i \), the change in absorption \( \Delta A_i \) becomes:

\[
\Delta A_i = \varepsilon_i c_id - \varepsilon_{S_0}c_id = c_i(\varepsilon_i - \varepsilon_{S_0})d = c_i \Delta \varepsilon_i d \quad \text{or} \quad c_i = \frac{\Delta A_i}{\Delta \varepsilon_i \cdot d}
\]  

(5)

The concentration of the photoproduct \( i \) can be calculated from the difference \( \Delta \varepsilon_i \) in the extinction coefficients between the ground state \( (S_0) \) and the reaction product \( i \) and the measured \( \Delta A_i \). \( \Delta \varepsilon_i \) can be derived from an experiment where the complete sample was converted to the product. Such an experiment was performed on T(6-4)T where the whole sample was converted to the Dewar isomer by extended illumination.

\[
\Delta A_i^0 = A_i^0 - A_{S_0}^0 = \Delta \varepsilon_i \cdot d \cdot c_0 \quad \text{or} \quad c_0 = \frac{\Delta A_i^0}{\Delta \varepsilon_i \cdot d}
\]  

(6)

Here, \( c_0 \) represents the original sample concentration. The observed transient absorption change for a certain intermediate or photoproduction can be written accordingly.

Combining equations (4)-(6) the quantum yield can be expressed by the absorption measurements:

\[
\Phi_i = \frac{c_i}{c^*} = \frac{c_i}{c_0} \cdot \frac{c_0}{c^*} = \frac{\Delta A_i}{\Delta A_i^0} \cdot \frac{c_0}{c^*}
\]  

(7)
2. Estimates on $\Phi_{\text{Triplet}}$

The transient data obtained on the ns and $\mu$s time scale after photo excitation of T(6-4)T (see Figure 6) can be used to obtain an estimate on the triplet state quantum yield. A global analysis of the transient absorption changes yields decay associated spectra (DAS) and related decay times ($\tau_i$). In Figure S2a the resulting DAS for late delay times (represented by DAS$_{\text{offset}}$, black line) and the decay on the 500 ns time scale (DAS$_{500\text{ns}}$, green line) are shown. The discussion in section 4 suggests that the 500 ns transient is related to the decay of a triplet state formed after excitation of the T(6-4)T.

We can assume that DAS$_{\text{offset}}$ only consists of two components:
(i) bleaching of the ground state absorption ($A_{S_0}$) due to Dewar formation
(ii) induced absorption by the Dewar isomer ($A_{\text{Dewar}}$).

The amplitude DAS$_{\text{offset}}$ can therefore be written as:

$$\text{DAS}_{\text{offset}} = A_{\text{Dewar}}(t = \infty) - A_{S_0}(t = \infty) = (\varepsilon_{\text{Dewar}} - \varepsilon_{S_0}) \cdot c_{\text{Dewar}} \cdot d = \Delta A_{\text{Dewar}} \quad (8)$$

For the decay associated spectrum of the 500 ns component we can write:

$$\text{DAS}_{500\text{ns}} = A_{500\text{ns}}(t = 10\text{ns}) - A_{S_0}(t = \infty) \quad (9)$$

If the 500 ns component can be assigned to the triplet decay we can write:

$$\text{DAS}_{500\text{ns}} = \text{DAS}_{\text{Triplet}} = (\varepsilon_{\text{Triplet}} - \varepsilon_{S_0}) \cdot c_{\text{Triplet}} \cdot d = \Delta A_{\text{Triplet}} \quad (10)$$

For the determination of the triplet concentration or the related quantum yield we have to determine at first the concentration $c^*$, afterwards we calculate $c_{\text{Triplet}}$ from equation (7) using realistic estimates for $\varepsilon_{\text{Triplet}}$.

**Determination of $c^*$, $c^*$ can be calculated from the known absorption properties of the Dewar lesion, the known quantum yield $\Phi_{\text{Dewar}}$ and the transient absorption data ($\Delta A_{\text{Dewar}}$). The quantum yield $\Phi_{\text{Dewar}}$ had been determined in an independent steady state experiment to be $\sim 8\%$. The absorption difference $\Delta A^0_{\text{Dewar}}$ can be obtained from $A^0_{\text{Dewar}}$ and $A^0_{S_0}$ as given in Figure S2b (black and red spectra). At 1781 cm$^{-1}$ (maximum of the Dewar marker band) $\Delta A^0_{\text{Dewar}}$ amounts to $\sim 24$ mOD. In the transient experiment a $\Delta A_{\text{Dewar}}$ of about 0.06 mOD was observed. Using equation (7) one can calculate

$$\frac{c_0}{c^*} = \Phi_{\text{Dewar}} \cdot \frac{\Delta A^0_{\text{Dewar}}}{\Delta A_{\text{Dewar}}} \approx 0.08 \cdot \frac{24\text{mOD}}{0.06\text{mOD}} = 32$$

or

$$\frac{c^*}{c_0} = 0.03.$$ 

The calculation shows that about 3% of the T(6-4)T molecules in the excitation volume were excited in the transient experiment. Using this value, the absorption spectrum of the Dewar valence isomer $A^0_{\text{Dewar}}$ has been calculated by adding the $S_0$ ground state spectra of the T(6-4)T lesion to the scaled DAS$_{\text{offset}}$. As expected the resulting spectra in Figure S2b (gray dotted line) is similar to the spectrum gained from FTIR measurements (black line) despite additional noise and less spectral resolution. This calculation gives an impression on the uncertainties of the method.
For the calculation of the triplet quantum yield using equation (7) the only missing value is $\Delta A^0_{\text{Triplet}}$. The DAS$_{500\text{ms}}$ of the triplet decay shows only negative signals. This indicates that the extinction coefficient of the triplet state is smaller than the extinction coefficient of the T(6-4)T ground state at all observed frequencies. At first we use the smallest possible value for $\varepsilon_{\text{Triplet}}$, i.e. $\varepsilon_{\text{Triplet}} = 0$. Assuming that the triplet state shows no extinction at 1663 cm$^{-1}$ and using a value of $\Delta A_{\text{triplet}}(1663 \text{ cm}^{-1}) \sim -0.037 \text{ mOD}$ from figure S2a, one obtains as the lower limit of the triplet quantum yield:

$$
\Phi_{\text{Triplet(min)}} = \frac{\Delta A_{\text{Triplet}}}{\Delta A^0_{\text{Triplet}}} \cdot \frac{c_0}{c^*} = \frac{\Delta A_{\text{Triplet}}}{-A^0_{S0}} \cdot \frac{c_0}{c^*} = \frac{-0.037 \text{ mOD}}{-48 \text{ mOD}} \cdot 32 \approx 0.025.
$$

An upper limit for the triplet yield requires information on the largest possible value for $A^0_{\text{Triplet}}$ or $\varepsilon_{\text{Triplet}}$. Here one can use the IR signature for the triplet state obtained from the frequency calculations (see Figure 7c) as reasonable estimate. The calculated spectra for the Dewar isomer and the T(6-4)T triplet state show similar peak extinction coefficients, with the triplet state being shifted to lower frequencies (by $\sim -5 \text{ cm}^{-1}$). Additionally, we expect only two absorption bands (due to the two C=O modes of the thymidilyl ring) for the triplet state in the observed spectral window. We now calculate the triplet spectrum $A^0_{\text{Triplet}}$ from the known spectra $A^0_{S0}$ and $\Delta A_{\text{Triplet}}$ for different values of the quantum yield $\Phi_{\text{Triplet}}$ and compare them with the requirements given by theory:

$$
A^0_{\text{Triplet}} = \frac{\Delta A_{\text{Triplet}}}{\Phi_{\text{Triplet}}} \cdot \frac{c_0}{c^*} + A^0_{S0}. \quad (11)
$$

The broken curves in figure S2c were calculated using quantum yields of 0.04 (black), 0.08 (blue) and 0.16 (green). With a quantum yield of 0.04 the black dotted line shows mainly two peaks at around 1650 cm$^{-1}$ and 1725 cm$^{-1}$ and gives a reasonable agreement with the theoretical expectations. Increasing the quantum yield to 0.08 and 0.16 produces spectra with an additional peak growing in at $\sim 1665 \text{ cm}^{-1}$ with rising $\Phi_{\text{Triplet}}$. The appearance of this peak in a spectrum suggests that the quantum yield was chosen too high.

To sum up: the estimates given above show that the triplet quantum yield is higher than 2.5 % and should be lower than 8 %.
Figure S2. Determination of the T(6-4)T triplet spectrum. (a) Decay associates spectra obtained from a global analysis of the transient absorption signals of T(6-4)T after excitation at 323 nm. The data representing the offset have been corrected for the contribution of heating of the solvent D$_2$O (by about 20 mK) as has been detailed in ref. [30]. (b) Ground state absorption spectra of T(6-4)T (red line) and the T(Dewar)T lesion (black line) derived from FTIR measurements. The broken line depicts the spectrum of T(Dewar)T calculated from DAS$_{offset}$ and a value of c$^* = 0.03$ representing the amount of initially excited molecules. (c) Spectrum of T(6-4)T (red) and spectra of the T(6-4)T triplet state calculated by using DAS$_{500\text{ns}}$ and different values for the triplet yield (see text above).
Table S1. Calculated absolute value of spin orbit coupling matrix elements (|SOCME|) between different singlet and the lowest triplet state at the geometry of the $S_1$ minimum.$^a$

|                  | (|SOCME|) / cm$^{-1}$ | (|SOCME|) / cm$^{-1}$ |
|------------------|----------------------|----------------------|
| $<S_0|\hat{H}_{SO}|T_1>$ | 29.9                 | 32.0                 |
| $<S_1|\hat{H}_{SO}|T_1>$ | 35.2                 | 34.4                 |
| $<S_2|\hat{H}_{SO}|T_1>$ | 18.7                 | 19.7                 |

$^a$ Calculated on ONIOM(CASSCF(12/9):HF) level of theory (basis: 6-31G*).
Figure S3. Structures of T(6-4)T (1 and 2 on the left) and potential T(Dewar)T photoproducts (right). By combinatorial means four different Dewar valence isomers (1a, 1b, 2a and 2b) are possible. The reactant structures 1 and 2 differ by the rotation of the pyrimidone unit around the 6-4 bond. The new Dewar σ-bond can be formed on either side of the π system of the former pyrimidone unit, leading to the respective Dewar photoproducts 1a, 1b, 2a and 2b. The T(6-4)T conformers 1 and 2 differ by 0.05 eV in their free energy ΔG. The Dewar isomers 1a and 1b as well as 2a and 2b differ by about 0.5 eV in ΔG.
Figure S4. Structures and simulated IR spectra of different isomers of the T(6-4)T and the T(Dewar)T lesion. The respective structures of the Dewar lesions are shown on the right (see also figure S3). The free reaction energies $\Delta G$ increase from 1a (1.66 eV) to 2a (1.77 eV) and 1b (2.16 eV) for the different T(Dewar)T isomers. The absorption spectra (left) of the different T(6-4)T lesions (red) show a difference in the frequency position of the lowest lying CO vibrational mode. While the CO absorption band is centered around 1665 cm$^{-1}$ in isomer 1a and 1b it shifts to 1655 cm$^{-1}$ in isomer 2. In the corresponding Dewar isomers (black) a similar red-shift of 10 cm$^{-1}$ is observed for the Dewar marker band. It shifts from 1780 cm$^{-1}$ for isomers 1a and 1b to 1760 cm$^{-1}$ in isomer 2a. Inspection of the difference spectra (blue) reveals a pronounced double peak bleach signature between 1650 cm$^{-1}$ and 1700 cm$^{-1}$ for isomer 1a. For isomer 1b this signature is less pronounced and in 2a the bleach consists of one peak only. The calculations give clear hints for expected absorption differences for each isomer. Nevertheless, the experimental data do not allow an unequivocal identification of the isomers formed. In the experiment we see a broad negative feature between 1625 cm$^{-1}$ and 1700 cm$^{-1}$ for the T(Dewar)T difference spectrum (see figure 6 and 7). It has strong similarities with the negative double peak signature seen for isomer 1a. However, small populations (on the order of 10 %) of the other isomers cannot be excluded. As the free energies differ only by small amount between the isomers it is likely that more than one isomer is present.
References