Supporting information for

DNA Intercalated Psoralen Undergoes Efficient Photoinduced Electron Transfer

Sascha Fröbel, Anna Reiffers, Christian Torres Ziegenbein and Peter Gilch

Contents

1. Materials

i) 4'-aminomethyl-4,5',8-trimethylpsoralen (AMT) was synthesized from trioxsalen (*TCI Europe*, >98%) according to a procedure described by Heindel et al. (*J.Heterocycl.Chem.*, 1985, 22, 73-76). In contrast to the description there, the final purification was performed by flash chromatography on silica gel (60M, *Macherey Nagel*). Residual trioxsalen was first eluted with ethyl acetate whereupon AMT remained on the column. Subsequently, AMT (blue fluorescing spot) was eluted with pure acetone and dried *in vacuo*. As a final step AMT hydrochloride was prepared as described in Ref. 8 (main text). The hydrochloride was used for all measurements except for the nanosecond experiment in acetonitrile. There, a commercial sample of the amine form (*Santa Cruz Biotechnology*) was used for solubility reasons.

ii) Lyophilized DNA oligomers (HPLC purified) were purchased from *Thermo Fisher Scientific GmbH* (Ulm, Germany). Fluorescence, absorption and HPLC experiments in our lab indicate excellent purity. Annealing of the strands was performed after dissolving in a phosphate buffer solution (*Fluka*, *BioUltra*, pH = 7.2, 66 mM Na_2HPO_4/NaH_2PO4 with 100 mM NaCl and 3 mM KCl added) by heating the strands to ~90°C in a water bath. The bath was then allowed to cool down to room temperature over several hours. The annealing procedure was performed within ~24 h before each measurement.

iii) Other chemicals used were pure water (*VWR Chemicals*, HiPerSolv Chromanorm), spectroscopic grade acetonitrile (*AppliChem*, UV-IR-HPLC) and *N,N*-dimethylaniline (*Aldrich*, ≥99.5%, purified by redistillation).

2. Femtosecond Transient Absorption – Experimental Details

A portion of the output of a Ti:Sapphire laser/amplifier system (*Coherent*, Libra) was used to generate a single filament white light continuum in CaF_2 as probe pulse. The 340 nm pump

pulses were generated by frequency doubling the output of a NOPA system (*Light conversion,* TOPAS-white) and were attenuated to 0.8 µJ. The pulses were focused into the sample (with relative polarization set to the magic angle of 54.7°) with diameters of ~25 (probe, FWHM) and ~120 µm (pump). 139 time steps were measured within the ~3.3 ns time window (linear steps up to 1 ps, logarithmic steps after 1 ps). At each step, signal was accumulated for 3 s per scan (1500 actinic pump pulses) and 2 scans of the delay stage were averaged per sample. The time resolution was roughly 200 fs. Signals of the neat solvent were recorded in separate measurements and subtracted after proper scaling (*cf.* Lorenc et al., *Appl. Phys. B: Lasers Opt.* **2002**, *74*, 19–27.). The instrumental time zero shift was determined as a function of the detection wavelength via the optical Kerr effect and was corrected for. During the measurements the sample solutions were circulated through a 1 mm path length fused silica flow cell (*Hellma*, custom fabrication). The sample volume was large enough to render contributions of photoproducts during an experiment negligible.

3. Scaling of Free AMT Signal for Subtraction from AMT/DNA Data

A scaling factor S is required to remove the contribution of free AMT molecules from the AMT/DNA data (Equation S1). Although the fs-experiments were performed with identical AMT concentrations, the absorption A of the mixture at the excitation wavelength is lower than the one of free AMT. This is due to the hypochromic effect upon intercalation. So, to make the signal strengths directly comparable, both data sets first have to be normalized to the number of excitation photons they absorbed. This is achieved by dividing both data sets by $1\text{-}10^{-A}$. Then, to determine the fraction of free excited AMT molecules relative to all excited ones, the concentrations c (as deduced from K_d) and absorption coefficients ε of free and intercalated AMT in the mixture have to be accounted for.

$$S = \frac{\varepsilon_{free} c_{free}}{\varepsilon_{free} c_{free} + \varepsilon_{int.} c_{int.}} \qquad \text{(S1)}$$

With absorption coefficients (340 nm) for free and intercalated AMT in aqueous solution of 5800 $M^{-1}cm^{-1}$ and 4520 $M^{-1}cm^{-1}$, respectively, a scaling factor S of 0.14 is obtained for the subtraction.

4. Decay Associated Spectra of the Global Fits

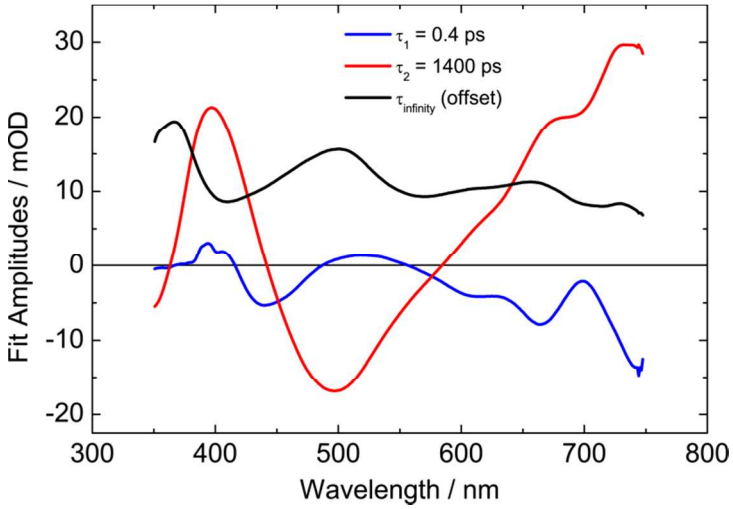

Figure S1: Decay associated spectra of a global multiexponential fit of the *unscaled* fs-data set on free AMT (Figure 2, left panel, main text). Note that a second time constant of 0.4 ps is required to properly fit the data set. This minor component is necessary due to slight shifting and narrowing of the bands which is caused by dielectric relaxation (as expected after photoexcitation in a polar solvent).

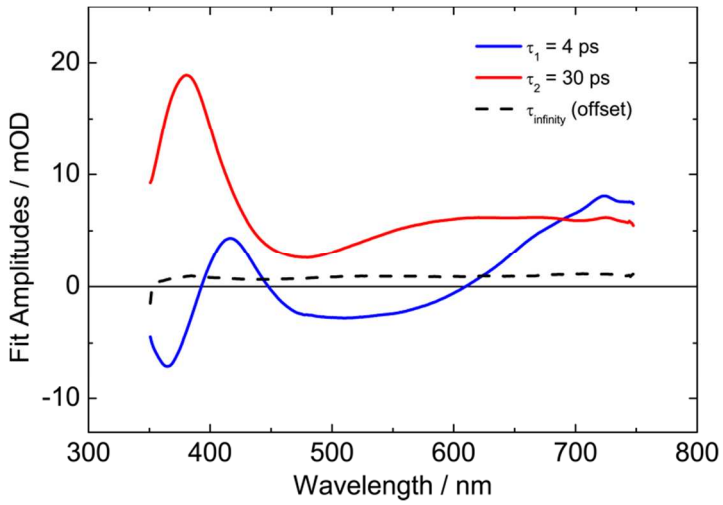

Figure S2: Decay associated spectra of a global multiexponential fit of the fs-data set on intercalated AMT (Figure 3, main text).

5. Nanosecond Transient Absorption – Experimental Details

The nanosecond transient absorption setup (*Applied Photophysics*, LSK 6.0) uses the output of a frequency tripled (355 nm) pulsed Nd:YAG laser (*Innolas*, Spitlight 600) for photoexcitation. The sample was excited with ~7 ns laser pulses and 1 Hz repetition rate. The excitation energy was attenuated to ~4 mJ. The pump beam of about 8 mm diameter was directed into the sample (3.5 ml fused silica cuvette, pathlength 1 cm) in a ~90° crossed beam configuration to the probe beam (ca. 8 mm × 2 mm). A high-intensity pulsed xenon lamp (*Hamamatsu,* Mod.nr. 2273, 150 W) was used as probe source. The pulsed output of the lamp remains flat in terms of intensity for ~400 µs.

Measurements were conducted within this temporal window. Behind the sample the probe light was dispersed by a grating monochromator for wavelength selection. The output signal was detected by a photomultiplier (*Hamamatsu*, 1P28), digitized by an oscilloscope (*Agilent*, Infiniium) and transferred to a workstation (*Iyonix*). 10 laser pulses were averaged to record a kinetic trace at a selected wavelength. The spectral resolution was 4 nm and the sample was purged with nitrogen to remove dissolved oxygen.

6. Photoreduction of AMT - Nanosecond Transient Absorption Data

Before each photo reduction experiment with *N,N*-dimethylaniline (DMA), an analogous experiment without DMA was conducted to observe the triplet signature and its decay in the respective solvent. However, since the triplet signature is also present at the beginning of the photo reduction experiments, only these measurements are shown here (Figures S3&S4).

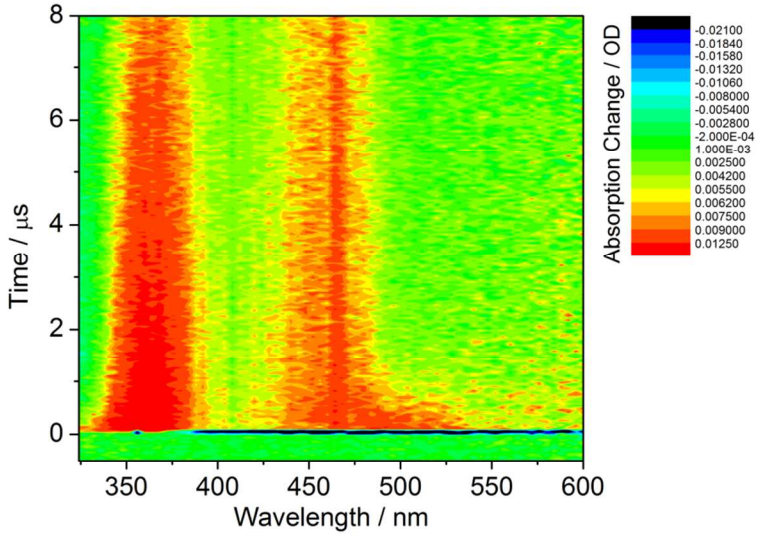

Figure S3. Contour plot of nanosecond transient absorption data on the photoreduction of 0.1 mM AMT by 1.2 mM DMA in water. The triplet signature of AMT is visible up to ~0.5 µs. After that the triplet is quenched by DMA to yield $AMT^{\bullet-}$ (maximum around 365 nm) and $DMA^{\bullet+}$ (maximum around 460 nm). A global multiexponential fit gives a time constant of 0.7 µs for the quenching, which translates into a quenching constant k_q of 1.2×10^9 $M^{-1}s^{-1}$.

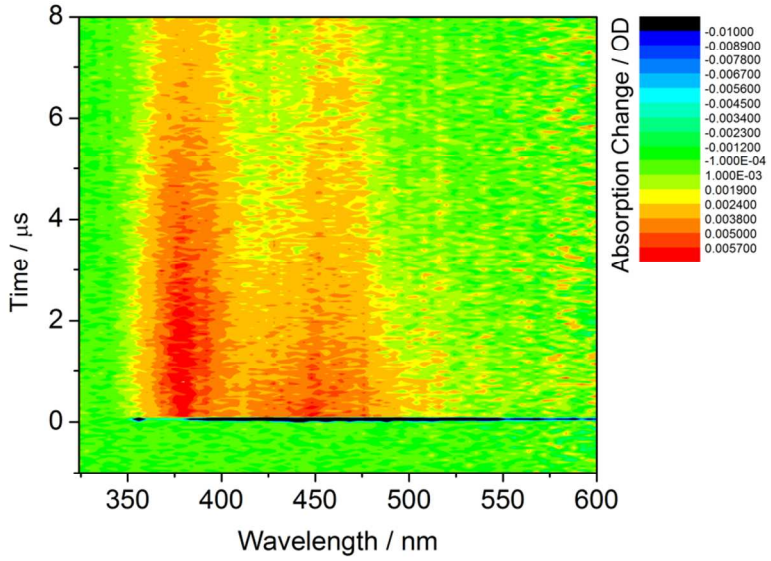

Figure S4. Contour plot of nanosecond transient absorption data on the photoreduction of 20 µM AMT by 1.2 mM DMA in acetonitrile. The triplet signature of AMT is discernible up to ~1 µs. It is hard to distinguish from the radical signal by visual inspection since the absorption maxima are close to each other. However, the exact positions and band shapes differ. After that the triplet is quenched by DMA to yield $AMT^{\bullet-}$ (maximum around 380 nm) and $DMA^{\bullet+}$ (maximum around 470 nm). A global multiexponential fit gives a time constant of ~2 µs for the quenching, which translates into a quenching constant k_q of ~4 × 10^8 $M^{-1}s^{-1}$.

7. Calculation of Radical Pair Spectra from ns-Data

As a first step, the $AMT^{\bullet-}$ spectra have to be extracted from the photo reduction experiments with DMA. In these experiments, one $DMA^{\bullet+}$ is formed per $AMT^{\bullet-}$.

A $DMA^{\bullet+}$ spectrum in aqueous solution was published by Ito et al. (Ito, T. et al. *J. Am. Chem. Soc.* **2006**, *128*, 10934-10942.), one in acetonitrile by Sumalekshmy et al. (Sumalekshmy, S.; Gopidas, K. R. *Chem. Phys. Lett.* **2005**, *413*, 294-299.). In both cases the absorption peaks around 460-470 nm, in agreement with our ns-data (Figures S3&S4). The spectra from the literature were scaled to the peak height measured in our experiments and subtracted to yield the $AMT^{\bullet-}$ spectra (dashed lines in Figures S5&S6 and in Figure 4 in the main text). The $AMT^{\bullet-}$ spectra from 4-7 µs were averaged in each case to reduce the noise level.

As a second step, the radical pair spectra were computed as sums of the $AMT^{\bullet-}$ and purine radical cation ($P^{\bullet+}$) spectra.

For that, the absorption coefficients $\varepsilon(\lambda)$ of $AMT^{\bullet-}$ have to be estimated. Exact values for $AMT^{\bullet-}$ are unknown, but values for the psoralen, 5-methoxypsoralen and 8-methoxypsoralen radical anions in water have been published by Bensasson et al. (Ref. 26 in the main text). The corresponding spectra have substantial similarity with the $AMT^{\bullet-}$ spectra recorded here, so that we assume that the absorption coefficients will also be similar. Based on their values we assigned an ε_{max} of 11.000 $M^{-1}cm^{-1}$ to our $AMT^{\bullet-}$ spectra. The absorption spectra and corresponding $\varepsilon_{(\lambda)}$ of the guanosine and adenosine monophosphate radical cations in aqueous solution were published by Candeias et al. (Ref. 29 in the main text).

$P^{\bullet+}$ spectra in acetonitrile could not be found. However, a spectrum of a guanosine radical within a DNA double helix was published by Stemp et al. (Ref. 30 in the main text). It is virtually identical to the one in water. Additionally, compared to the $AMT^{\bullet-}$ absorption, the $P^{\bullet+}$ absorption contributes only weakly to the radical pair spectrum in the spectral region of interest. For these two reasons we use the $P^{\bullet+}$ spectra in water for the generation of radical pair spectra for both solvents and assume that only a minor error is introduced this way. Shortly, the $AMT^{\bullet-}$ spectra in water and acetonitrile were both summed up with the $P^{\bullet+}$ spectra to yield the radical pair spectra shown in the main text (Figure 4) and Figures S5&S6.

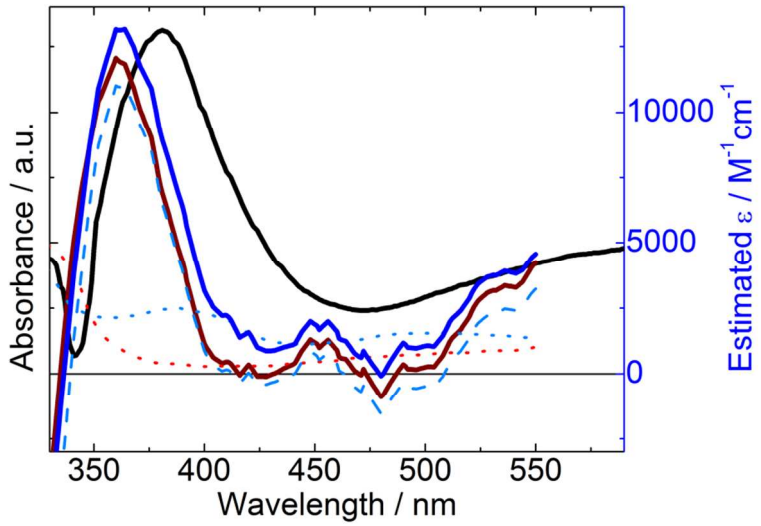

Figure S5. Spectrum of the intermediate found for intercalated AMT (black line, fs-data at 16 ps) compared to radical pair spectra in water. The sum (blue line) of $AMT^{\bullet-}$ in water (blue, dashed line) and oxidized guanosine radical (blue, dotted line) and the sum (red line) of $AMT^{\bullet-}$ in water and oxidized adenosine radical (red, dotted line) are shown. The fs-spectrum is scaled arbitrarily, estimated/published absorption coefficients ε for the other spectra are given on the right.

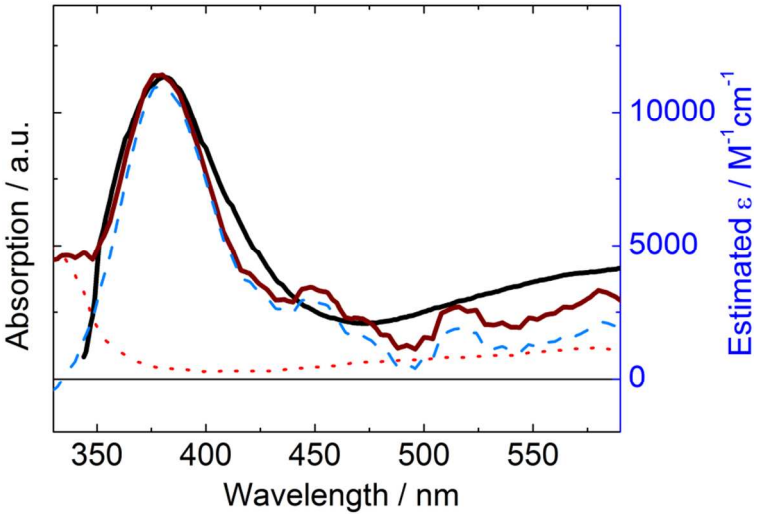

Figure S6. Spectrum of the intermediate found for intercalated AMT (black line, fs-data at 16 ps) compared to a radical pair spectrum containing adenosine in acetonitrile. The sum (red line) of $AMT^{\bullet-}$ in acetonitrile (blue, dashed line) and oxidized adenosine radical (red, dotted line) are

shown. The fs-spectrum is scaled arbitrarily, estimated/published absorption coefficients ε for the other spectra are given on the right.

8. Estimate of Gibbs Energy for Photoinduced DNA-AMT Electron Transfer

Calculation of the Gibbs energy for photoinduced charge separation $\Delta G_{CS}(\varepsilon_r)$ within DNA is performed with the following parameters. Relying on Weller's empirical equation (see main text), the standard electrode potentials of the reactants in acetonitrile are required. These values amount to $E^o(D^{\cdot+}/D)$ = 1.96 eV vs standard hydrogen electrode (SHE) for adenine and $E^o(D^{\cdot+}/D)$ = 1.49 eV vs SHE for guanine (see Ref. 27 in the main text). The standard electrode potential for reduction of AMT has not been published. Therefore we insert a value of $E^o(A/A^{\cdot-})$ = -1.55 eV vs SHE of the related 8-methoxypsoralen in acetonitrile as a stand-in (Ref. 33 main text). A zero-zero transition energy of E_{oo} = 3.25 eV is derived from steady-state spectra for AMT in water. The dipole moment $\mu = 5.5 \times 10^{-29}$ Cm is estimated assuming that one unit charge is transferred and separated by roughly one base pair distance (~3.4 Å) in the CIP. The CIP radius a is set to 4 Å.

An uncertainty arises from the relative permittivity of the surrounding medium. For the DNA core, no guide value exists. However, the interior of DNA is regarded as only moderately polar so that we assume an ε_r in the range of ~10-40 within DNA. The values given in the main text were computed using ε_r = 20. However, the statement that the electron transfer is exergonic is valid for a broad range of ε_r (for any ε_r in the case of guanine and for $\varepsilon_r > 10$ for adenine as a donor).