

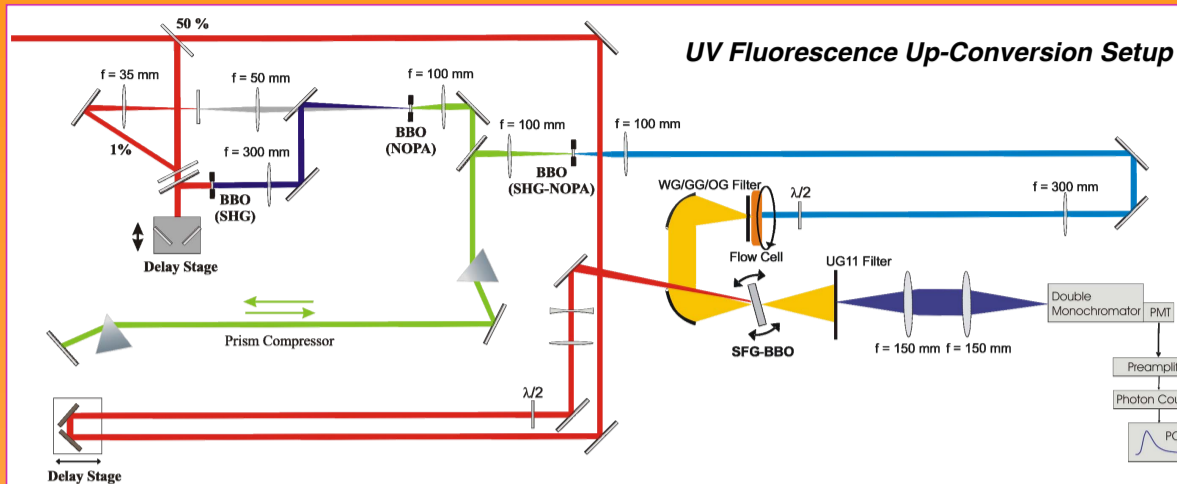
**Motivation & Aims**

Methylated DNA sequences control significant processes of gene recognition, regulation and genetic imprinting. Distinct methylation patterns allow cells to

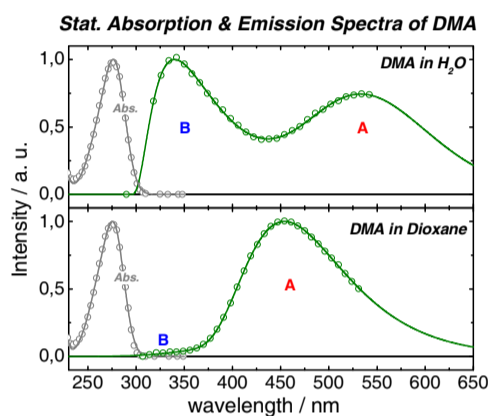
- identify foreign gene sequences.
- distinguish between the master copy and replicated strand during DNA synthesis.
- activate and inactivate certain DNA domains in order to regulate gene expression, which leads to crucial medical importance: methylated DNA bases are accumulated in tumor cells.

Environmental conditions can change the cell's methylation archetype dramatically, which consecutively leads to serious, for example photochemically induced, DNA damage and therefore, methylated nucleobases might show different response to photo-activation and the following relaxation mechanisms than unmethylated nucleobases do.

Here, we report on the adenine (A) derivative dimethyladenine (DMA), which differs from A only by the double methylation of the exocyclic group. A comprehensive investigation of the excited state lifetimes of DMA was performed, covering the excitation band of DMA, from the electronic origin ( $\lambda_{\text{pump}} = 294 \text{ nm}$ ) up to excess energies of more than  $6000 \text{ cm}^{-1}$  above the origin ( $\lambda_{\text{pump}} = 258 \text{ nm}$ ).



**Stationary Absorption and Fluorescence Measurements**

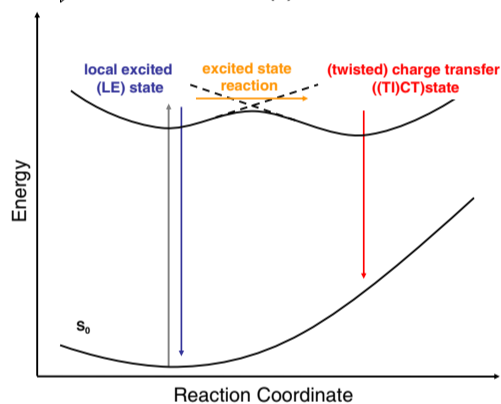


- A-like absorption.
- Double band structure in the emission spectra: band B in the near UV and a red-shifted band A.
- The emission band center of band A is sensitive to the polarity of the solvent.
- Fluorescence quantum yield  $\Phi_{\text{Fl}}$  is reduced in polar solvents, which is most pronounced for  $\Phi_{\text{Fl,A}}$ :

$$\Phi_{\text{Fl,A}} \approx \Phi_{\text{Fl,B}} \text{ in H}_2\text{O}$$

$$\Phi_{\text{Fl,A}} = 10 \times \Phi_{\text{Fl,B}} \text{ in dioxane}$$

**Dual Fluorescence(?)**

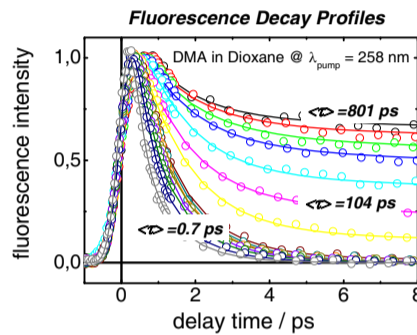


Literature<sup>[1-3]</sup> proposes a simple kinetic model:

- Photo-excitation populates the LE state, which emits in the near UV (band B).
- The initially prepared population is transformed via an excited state charge transfer reaction to the CT state that fluoresces red-shifted (band A, Stokes shift ~ 15 000  $\text{cm}^{-1}$ ).
- Charge transfer may be connected to a conformational isomerisation (e.g., twist) of the molecule.

[1] Albinsson et al. 1997; [2] Andreasson et al. 1999; [3] Parusel et al. 2002.

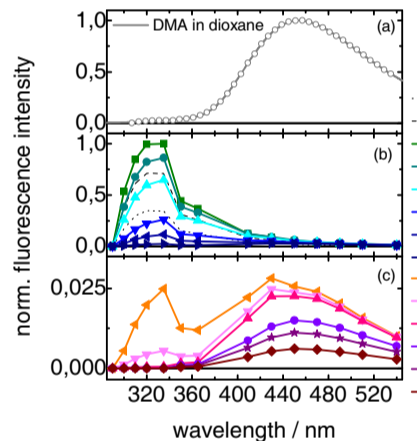
**Time-Resolved Fluorescence Decay Measurements in Dioxane**



Dynamical processes take place on five distinct time scales:

- $\tau_1 = 0.19 \text{ ps}$ , which is only observable for  $\lambda_{\text{pump}} = 258 \text{ nm}$  and  $\lambda_{\text{Fl}} = 290 \text{ nm}$ .
- $\tau_2 = 0.63 \text{ ps}$  obtained for the UV band emission at  $\lambda_{\text{pump}} = 258 \text{ nm}$  and  $285 \text{ nm}$ .
- $\tau_3 = 1.3\text{-}2.1 \text{ ps}$ , which is a minor, secondary component for the blue fluorescence but the initial component for profiles at  $\lambda_{\text{pump}} = 294 \text{ nm}$ .
- $\tau_4 = 7\text{-}10 \text{ ps}$ , which becomes important for slightly red-shifted fluorescence decay profiles.
- $\tau_5 = 1.3 \text{ ns}$  is shared by all profiles, independent on  $\lambda_{\text{pump}}$ , but its amplitude is strongly dependent on  $\lambda_{\text{Fl}}$ .

**Stat. & Transient Fluorescence Spectra**



Reconstruction of transient fluorescence spectra:

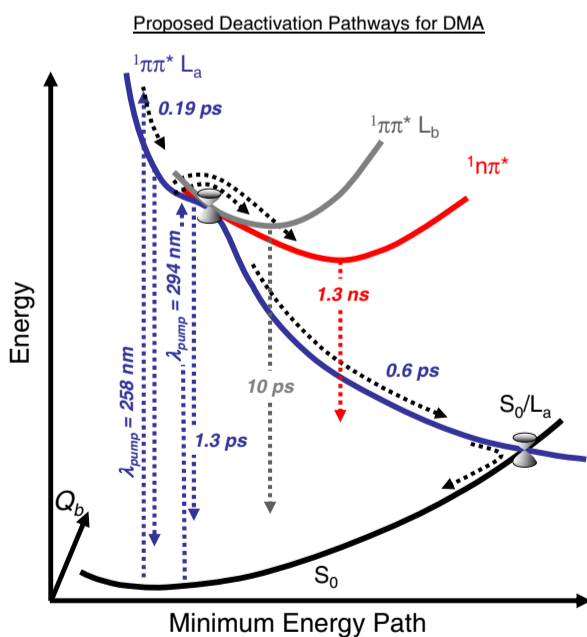
The transient fluorescence spectra  $S(\lambda, t)$  were reconstructed from the static emission spectra  $S_{\text{st}}(\lambda)$  and the fluorescence decay curves  $I(t)$ :

$$S(\lambda, t) = \frac{S_{\text{st}}(\lambda) \times I(t)}{\int_{-\infty}^{\infty} I(t) dt}$$

- The two main bands B and A and their corresponding different time behaviours were observed.
- Band A starts to gain intensity at the cost of band B after  $\approx 1\text{-}2 \text{ ps}$ , has an equal contribution after  $\approx 5 \text{ ps}$  and is the main component afterwards.
- The low intensity of band A gives evidence for a forbidden transition.

**Discussion and Conclusion**

- Simple kinetic models for the so-called “dual fluorescence” of DMA needs to be revised.
- As adequate high-level calculations on the PES of DMA are missing, we explain our observations on the basis of recent findings for A<sup>[4,5]</sup>.
- We suggest, in accordance to theory, at least three close lying excited states, which are  ${}^1\pi\pi^*(L_a)$ ,  ${}^1\pi\pi^*(L_b)$  and  $n\pi^*$  states.
- The  ${}^1\pi\pi^*(L_a)$  state has a flat plateau, which is isoenergetic with the  ${}^1\pi\pi^*(L_b)$  and  $n\pi^*$  states.
- Moreover, the  ${}^1\pi\pi^*(L_a)$  state has a direct conical intersection (CI) with  $S_0$ .
- Both the  ${}^1\pi\pi^*(L_b)$  and  $n\pi^*$  states have a PES minimum and further direct/indirect CIs with  $S_0$  are discussed.
- The minimum structure of the  $n\pi^*$  state has a twisted exocyclic amino group.
- The polarity of the solvent changes the relative position of the states with high dipole moment.



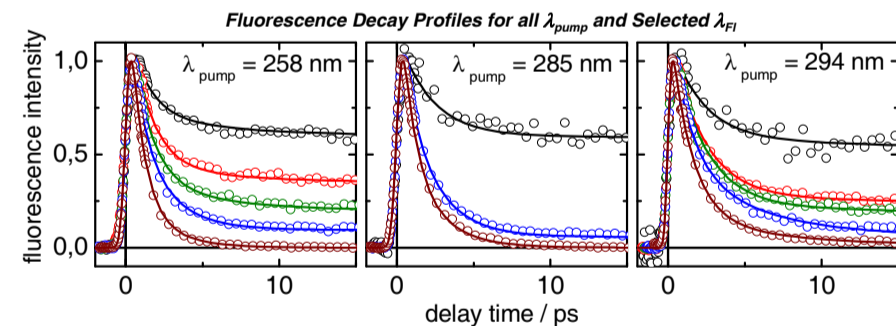
**Short wavelength excitation ( $\lambda_{\text{pump}} = 258 \text{ nm}$ )**

- Prepares the optically bright  $\pi\pi^*(L_a)$  state.
- Leaving the Franck-Condon region takes  $< 0.19 \text{ ps}$ .
- Following the  $\pi\pi^*(L_a)$  pathway, the dynamics slow down to  $\approx 0.6 \text{ ps}$  before the  $S_0/L_a$  CI is reached.
- Some population might be trapped in the plateau/minimum region, giving lifetimes of  $\approx 1\text{-}2 \text{ ps}$ .
- Population is transferred through a CI to the  ${}^1\pi\pi^*(L_b)$  and  $n\pi^*$  states.
- Fluorescence from those minima is on a  $\approx 7\text{-}10 \text{ ps}$  ( $\pi\pi^*(L_b)$ ) and  $> 1000 \text{ ps}$  ( $n\pi^*$ ) time scale, respectively.

**Long wavelength excitation ( $\lambda_{\text{pump}} = 294 \text{ nm}$ )**

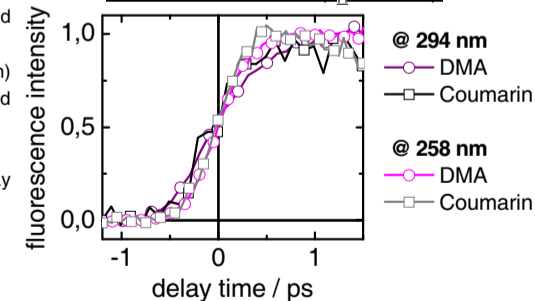
- Direct population of the  $\pi\pi^*(L_b)$  state close to/at the isoenergetic, flat PES region.
- The fastest observable time scale now is leaving the plateau region within  $\approx 1\text{-}2 \text{ ps}$ .
- Possibly no  $\pi\pi^*(L_a)$  deactivation pathway.
- Part of the population evolves to the  ${}^1\pi\pi^*(L_b)$  minimum (7-10 ps) or switches to the  $n\pi^*$  state.
- Deactivation from the  $n\pi^*$  minimum occurs radiative with  $\tau > 1000 \text{ ps}$ .

[4] Serrano-Andrés et al. 2006; [5] Marian et al. 2007.

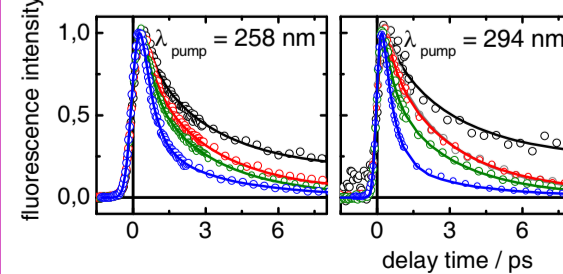


- The fluorescence decay profiles recorded at  $\lambda_{\text{pump}} = 258$  and  $285 \text{ nm}$ , respectively, were virtually identical.
- Profiles monitored at the electronic origin ( $\lambda_{\text{pump}} = 294 \text{ nm}$ ) slow down slightly and did not show sub-picosecond components at all.
- No significant delay of the red-shifted fluorescence decay profiles was observed (within our time-resolution).

**Rise time measurements ( $\lambda_{\text{Fl}} = 510 \text{ nm}$ ):**



**Time-Resolved Fluorescence Decay Measurements in Water**



- The fluorescence decay profiles recorded in water at  $\lambda_{\text{pump}} = 258$  and  $294 \text{ nm}$ , respectively, are much faster than in dioxane and gave completely identical fluorescence lifetimes with  $\tau_1 = 0.46(2)$ ,  $\tau_2 = 2.8(1)$  and  $\tau_3 = 62(5)$ .
- No significant delay of the red-shifted fluorescence decay profiles was observed (within our time-resolution).