Monomeric DNA constituents studied by fluorescence upconversion

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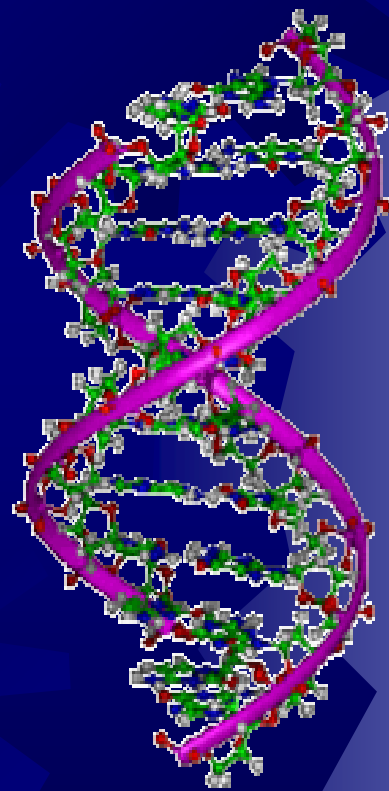

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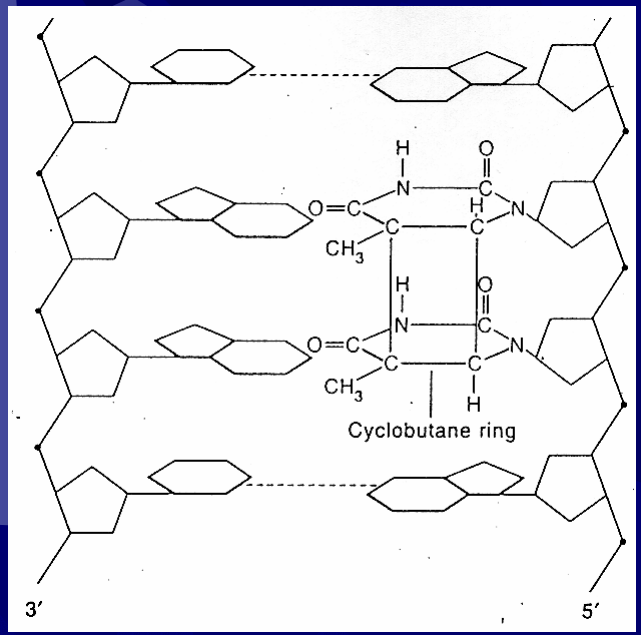
UV-induced DNA damage



block transcription and replication

⇒ cancerogenic mutations

Direct absorption of light

⇓

Cyclobutane Pyrimidine Dimers (CPD):

TT, CC, CT and TC

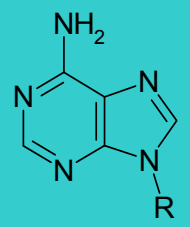
Energy migration ?

Underlying aim – understanding energy transfer in DNA

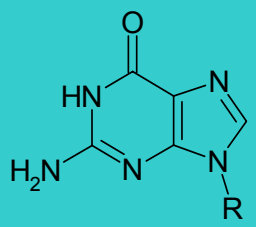
- Understanding DNA
  - first understand the monomers
  - then develop a model for the organised system
- Characterise the excited states – measure the lifetimes
- Time-resolved spectroscopy - absorption or fluorescence?
  - We have chosen fluorescence

Nucleosides/tides - Absorption Spectra

dA/dAMP

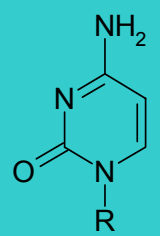


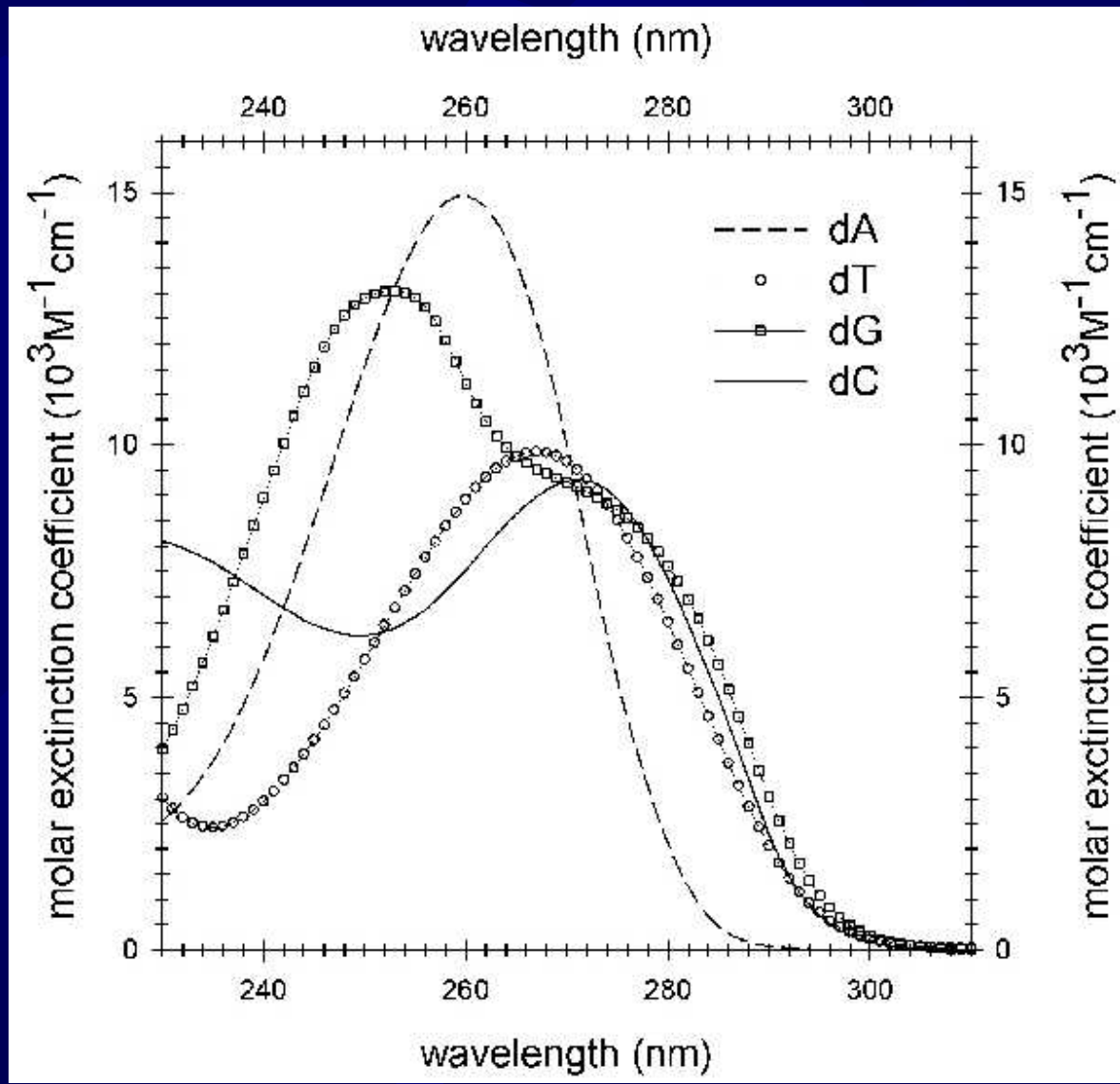
dG/dGMP



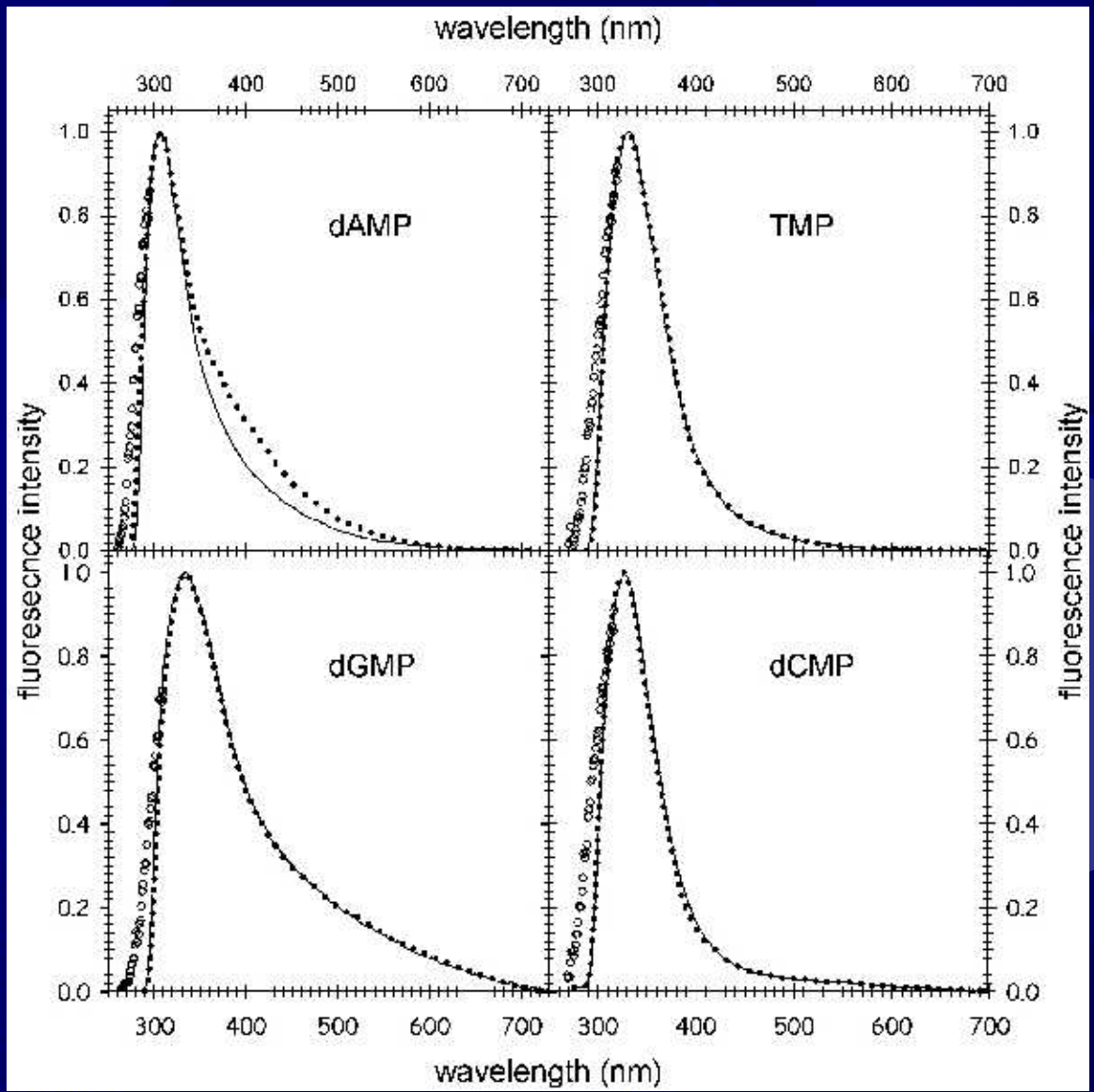
dT/TMP

dC/dCMP



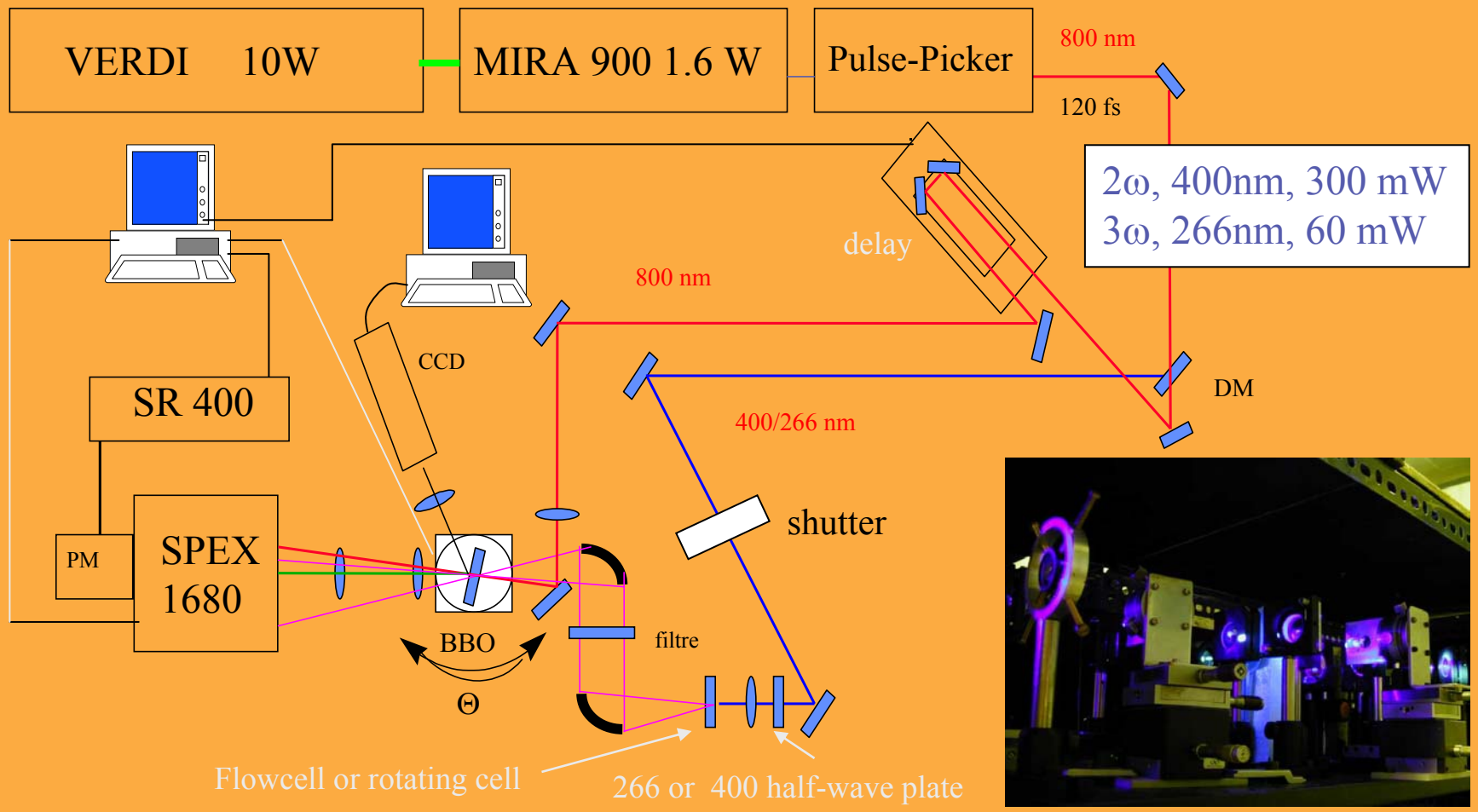


Fluorescence Spectra

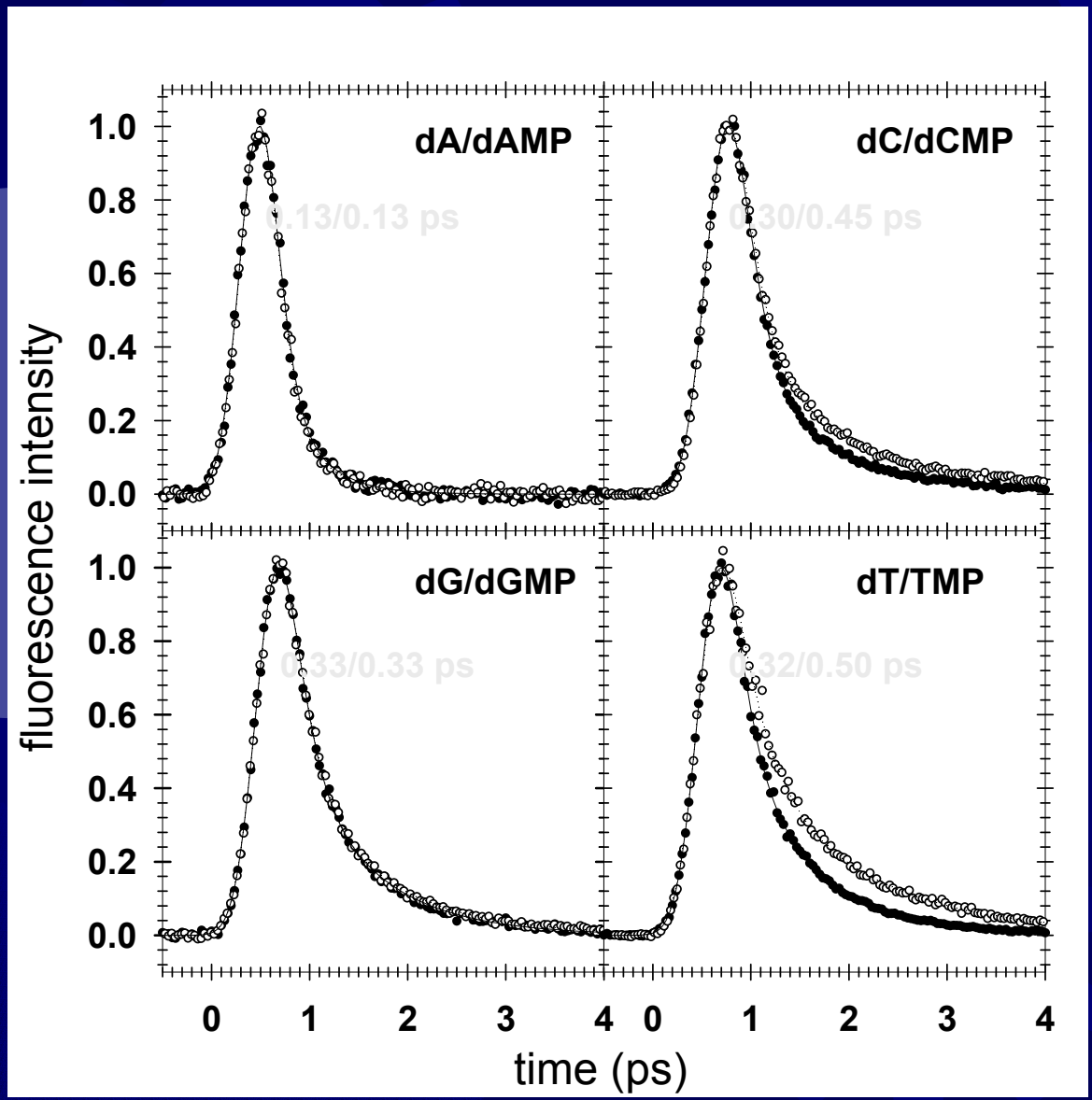


« Fluorescence upconversion » in the ultraviolet

Technically more difficult than in the visible
UV excitation (267 nm)
UV detection (300-400 nm)



Fluorescence decays of nucleosides and nucleotides

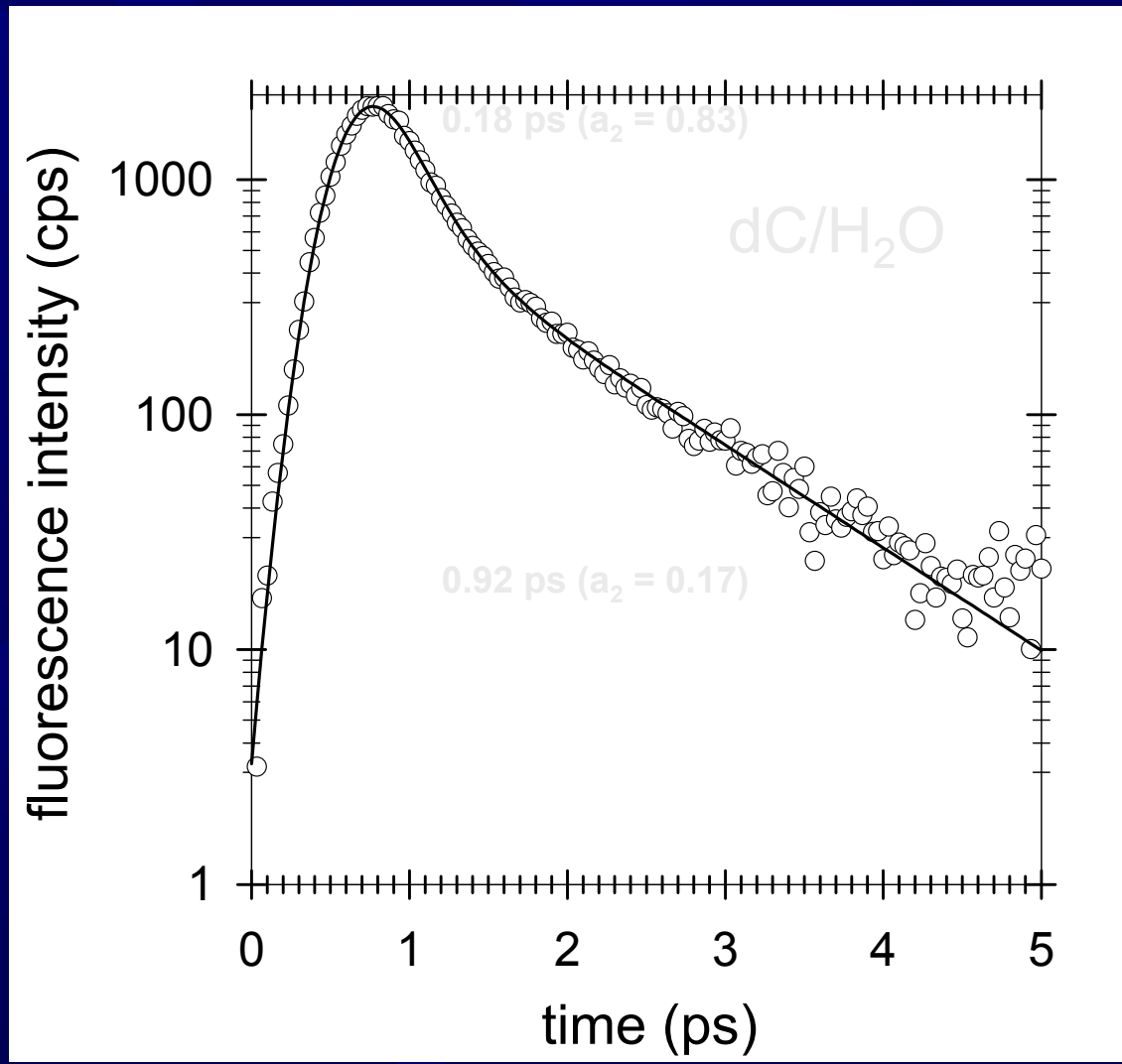


excitation 267 nm
fluorescence 330 nm

D. Onidas, D. Markovitsi, S. Marguet, T . Gustavsson, A. Sharonov, J. Phys. Chem. A (2002) 106, 11367-11374.

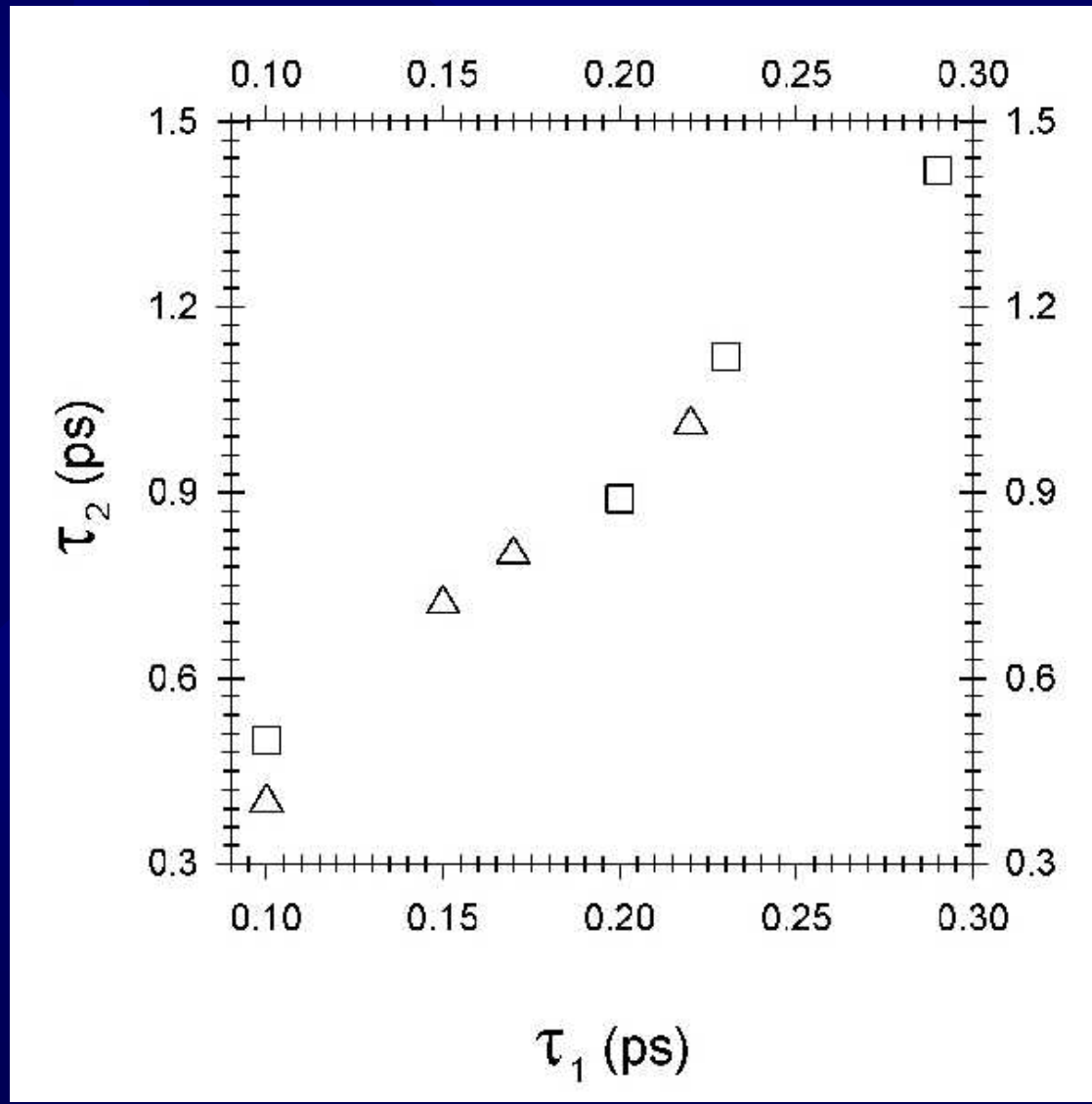
All fluorescence decays are non-exponential

The ultrafast component does not depend on concentration, excitation energy or laser pulse rate.



Bi-exponential times are correlated

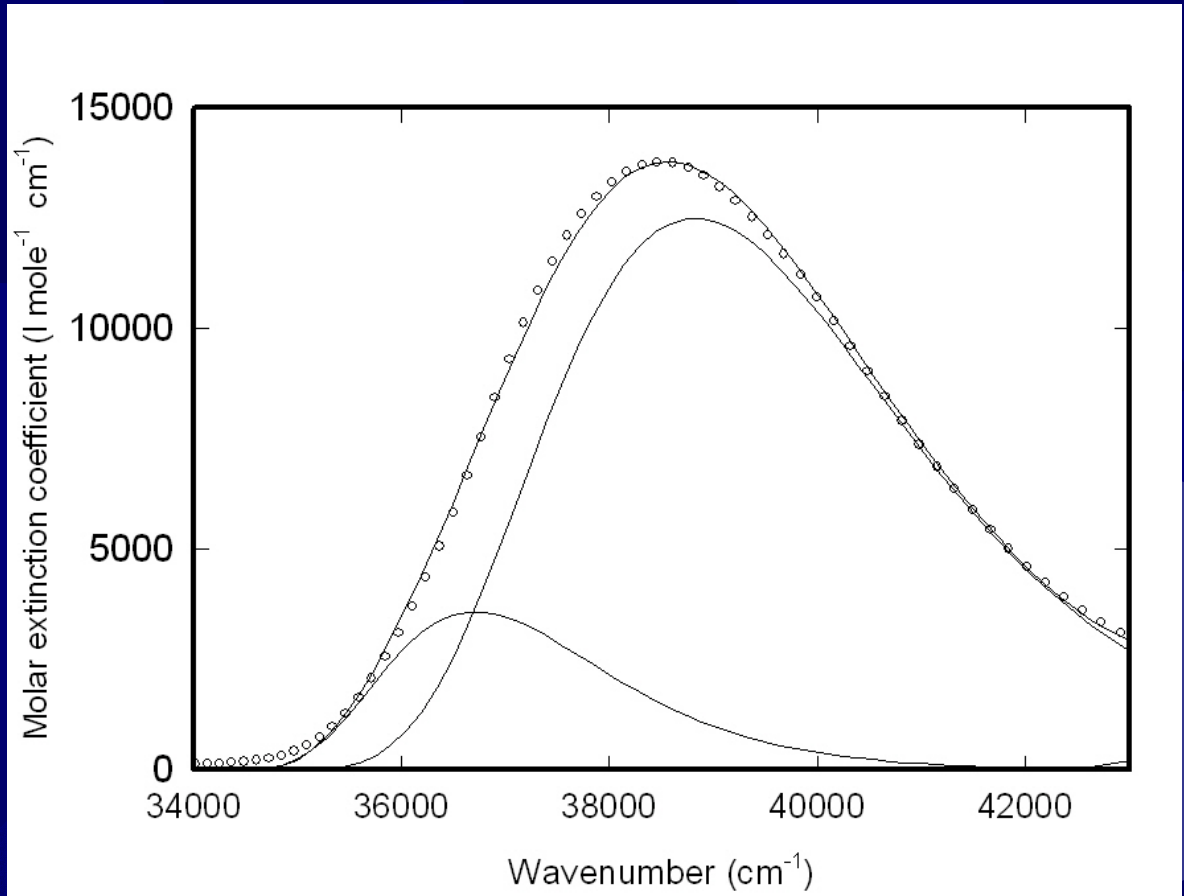
The ultrafast and the longer component are strongly correlated, implying a common underlying mechanism



The adenine chromophore

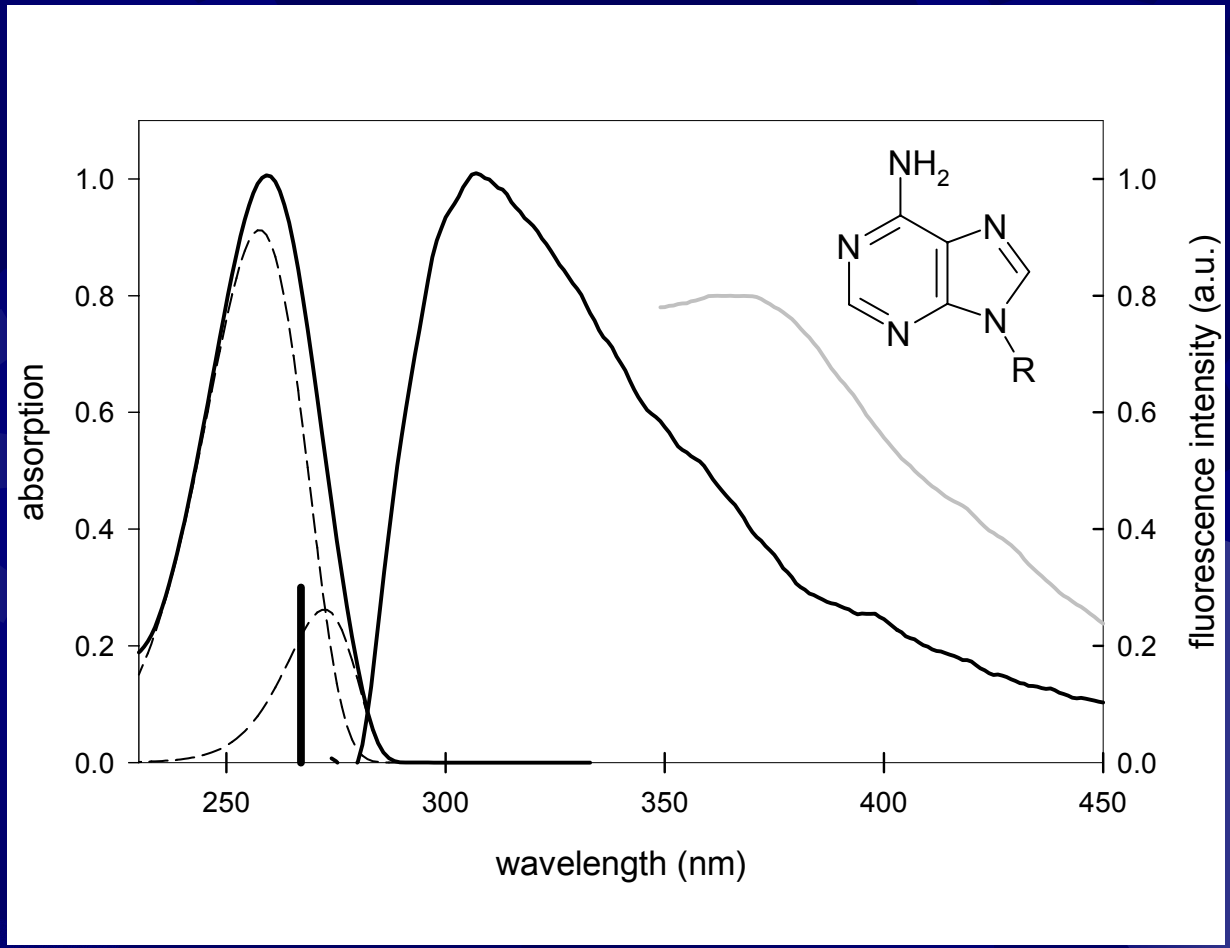
Adenine (R = H), dA (R = sugar) and dAMP (R = sugar + phosphate)

Steady-state absorption spectrum of dAMP



The first absorption band of dA/dAMP has been shown to consist of two overlapping electronic transistions, involving the two lowest $^1\pi\pi^*$ states. (Holmén et al., J. Am. Chem. Soc. 119 (1997) 12240). Exciting at 267 nm = 37000 cm_{-1} thus creates a mixture of roughly 1:2 proportions of S_1 and S_2.

Steady-state spectra of dAMP

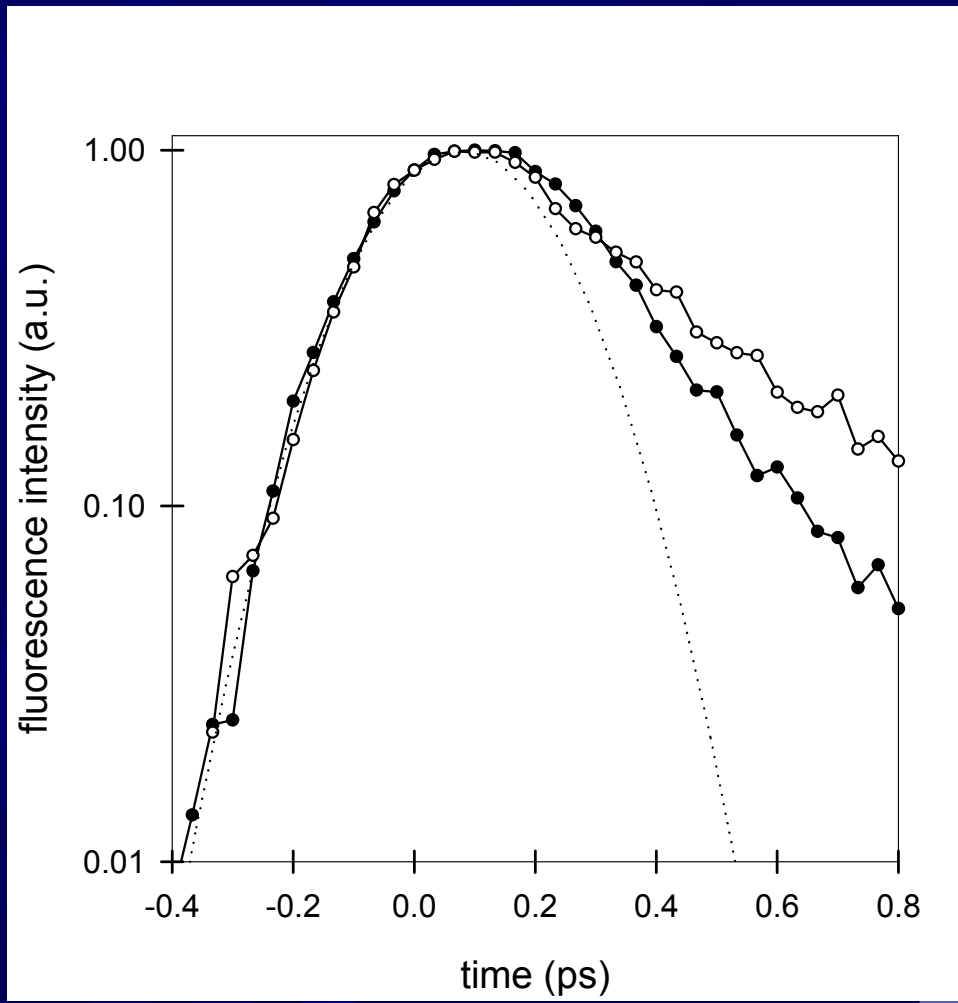


Normalized absorption and fluorescence spectra of dAMP in aqueous solution. The absorption spectrum is deconvoluted into the two separate electronic transitions. The fluorescence spectra were obtained for excitation at 255 nm (black) and 285 nm (grey). The excitation wavelength used for the time-resolved studies, 267 nm, is indicated by a short vertical bar.

Wavelength dependent fluorescence decays of dAMP

The fluorescence decays of dAMP are wavelength dependent. The decay is "slower" at 420 nm than at 310 nm.
Also shown is the Gaussian apparatus function (dots, 330 fs fwhm at 330 nm)

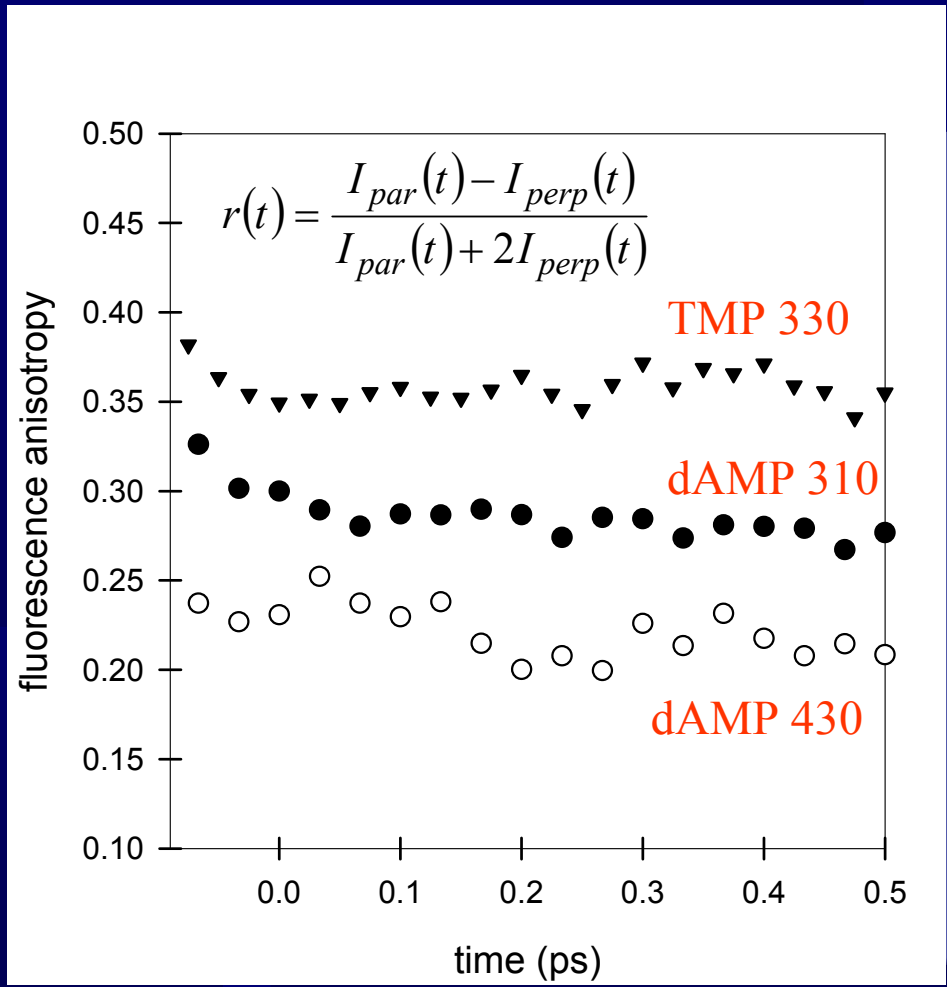
(ps)	310 nm	415 nm
α	0.89 ± 0.02	0.42 ± 0.08
τ_1	(0.10)	(0.10)
τ_1	0.38 ± 0.03	0.41 ± 0.02
r_0	0.31 ± 0.01	0.26 ± 0.01



Wavelength dependent fluorescence anisotropy decays of dAMP

The initial fluorescence anisotropy depends on the wavelength.

Fluorescence anisotropy decays of dAMP and TMP in aqueous solution. Excitation at 267 nm, TMP at 330 nm (triangles), AMP at 310 nm (filled circles) and AMP between 415-450 nm (open circles). Lines are smoothed interpolations.



Adenosine monophosphate (dAMP)

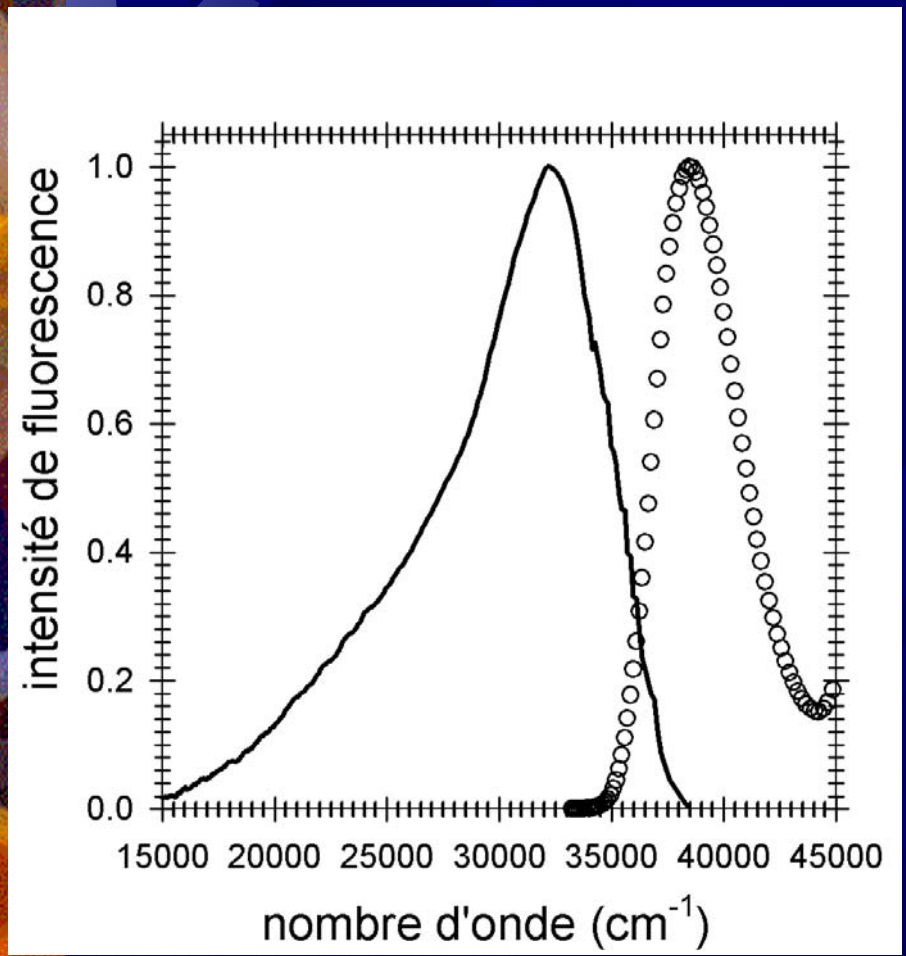
Comparing measured and calculated radiative lifetimes

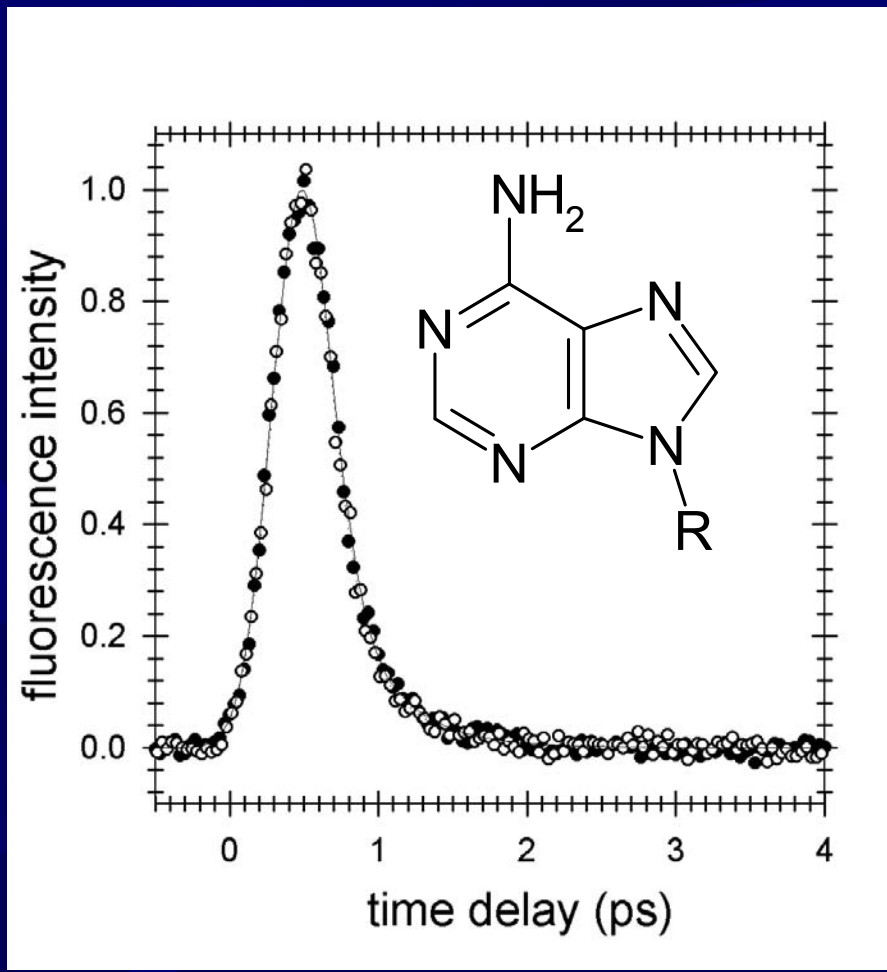
Strickler-Berg

using the total first absorption band or not ?

$$\tau_{rad} = \frac{\tau_F}{\Phi_F}$$

using τ_1, τ_2 or $<\tau>$?





Comparing measured and calculated radiative lifetimes for dAMP

$\tau_{rad} = 5.9\ ns$ ⟺ $\tau_{rad} = 5.1\ ns$

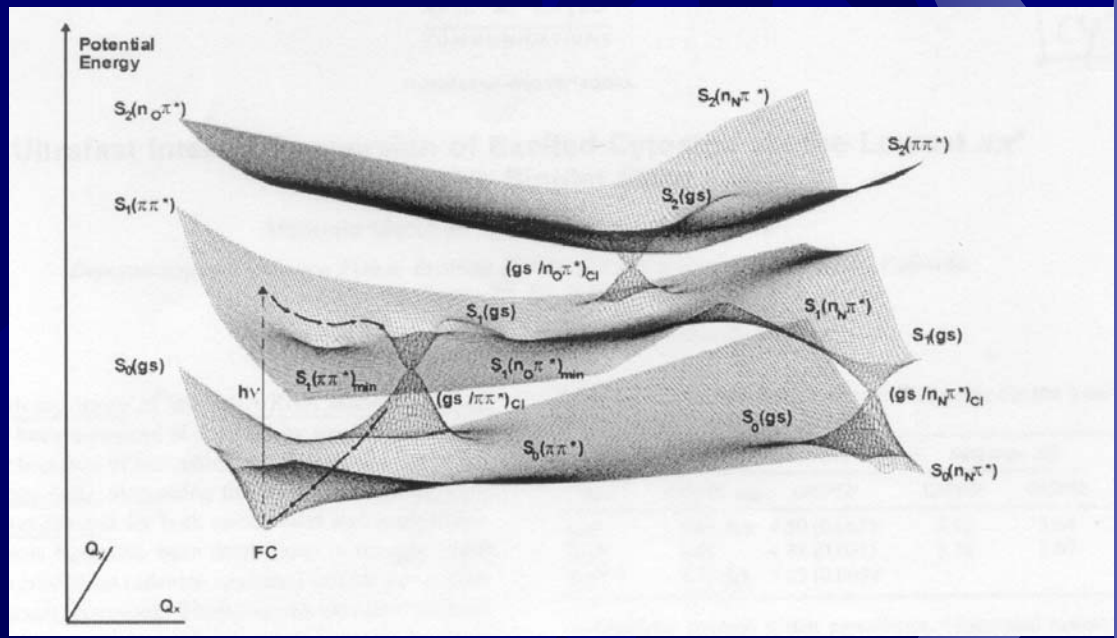
Works fine when using the whole first absorption band and the slow component τ_2

Monomers – internal conversion

Only a few theoretical studies in the literature

Example : *ab initio* study of cytosine on the CASPT2 level. Predicts the initially excited $^1\pi\pi^*$ state as the fluorescent state.

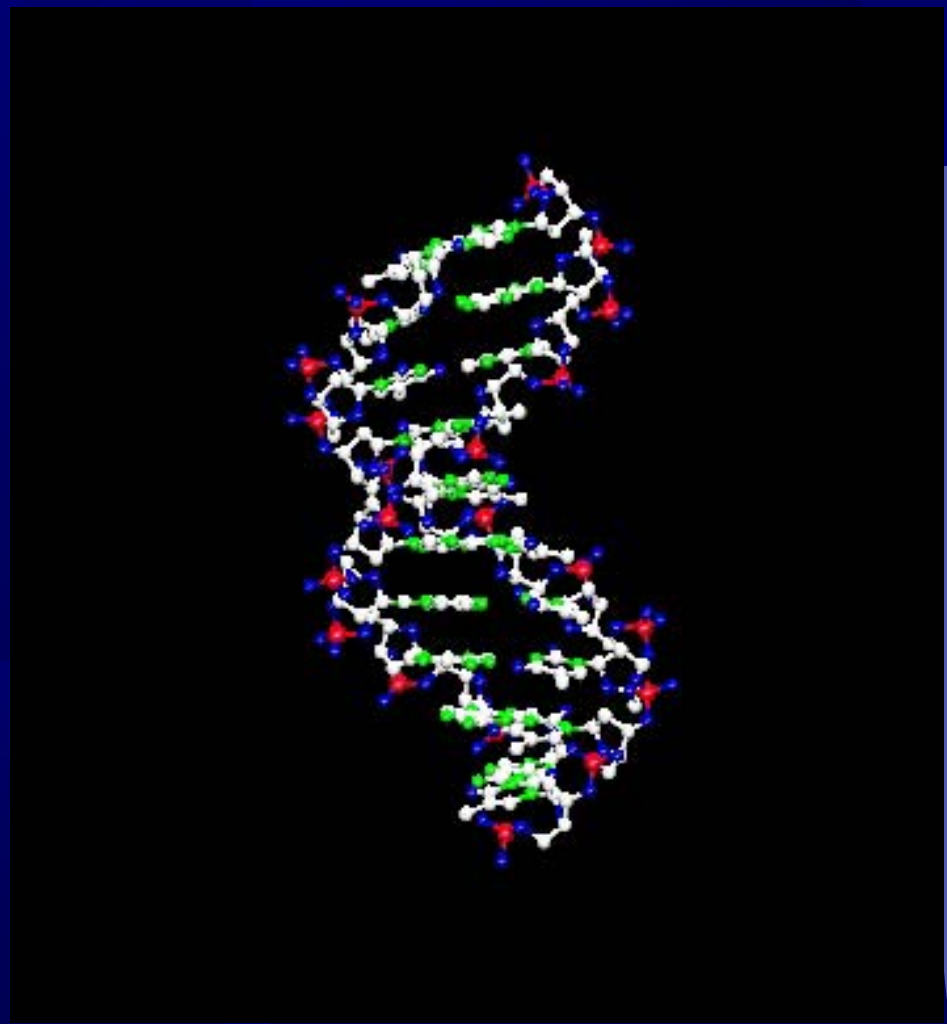
The $^1n_O\pi^*$ state is much higher. Direct internal S_1-S_0 conversion occurs from the $^1\pi\pi^*$ state to the ground state through a conical intersection



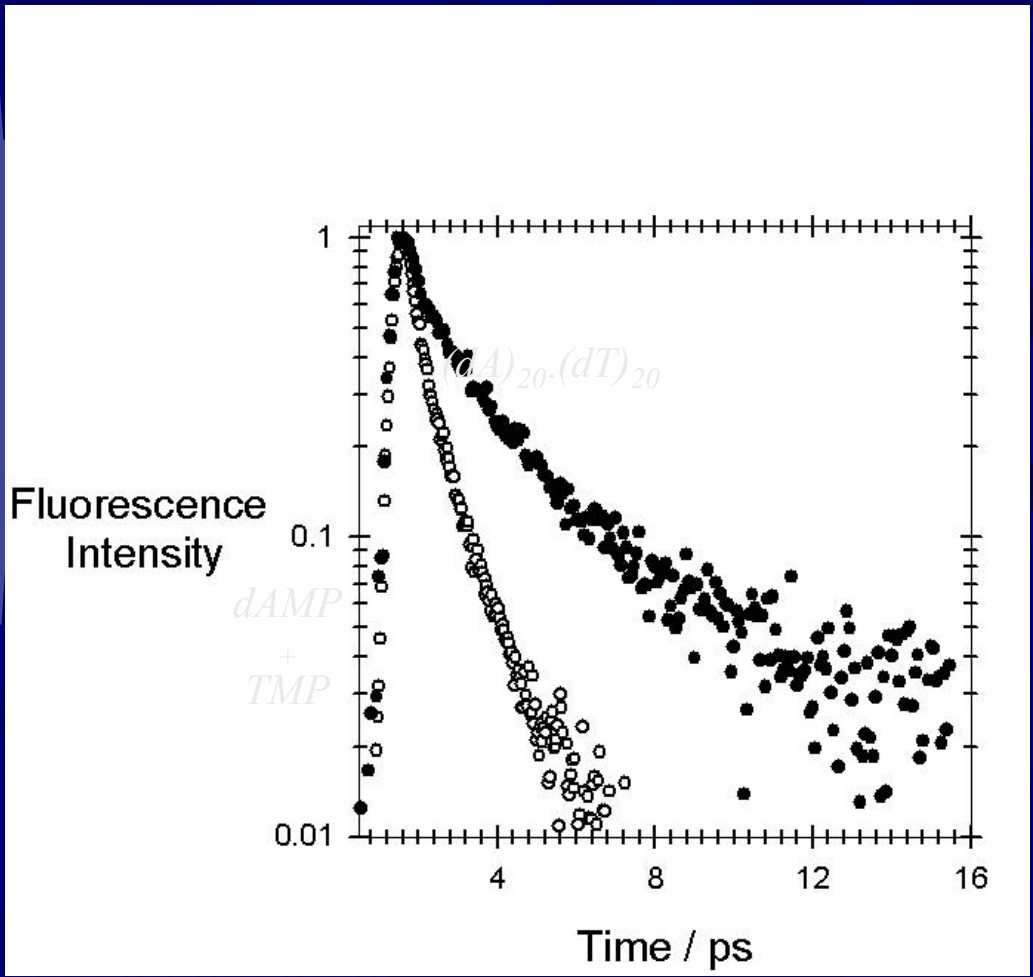
Merchan et al (2003)

Towards DNA: single and double 20-mer strands

How does the molecular organisation affect the excited state properties ?



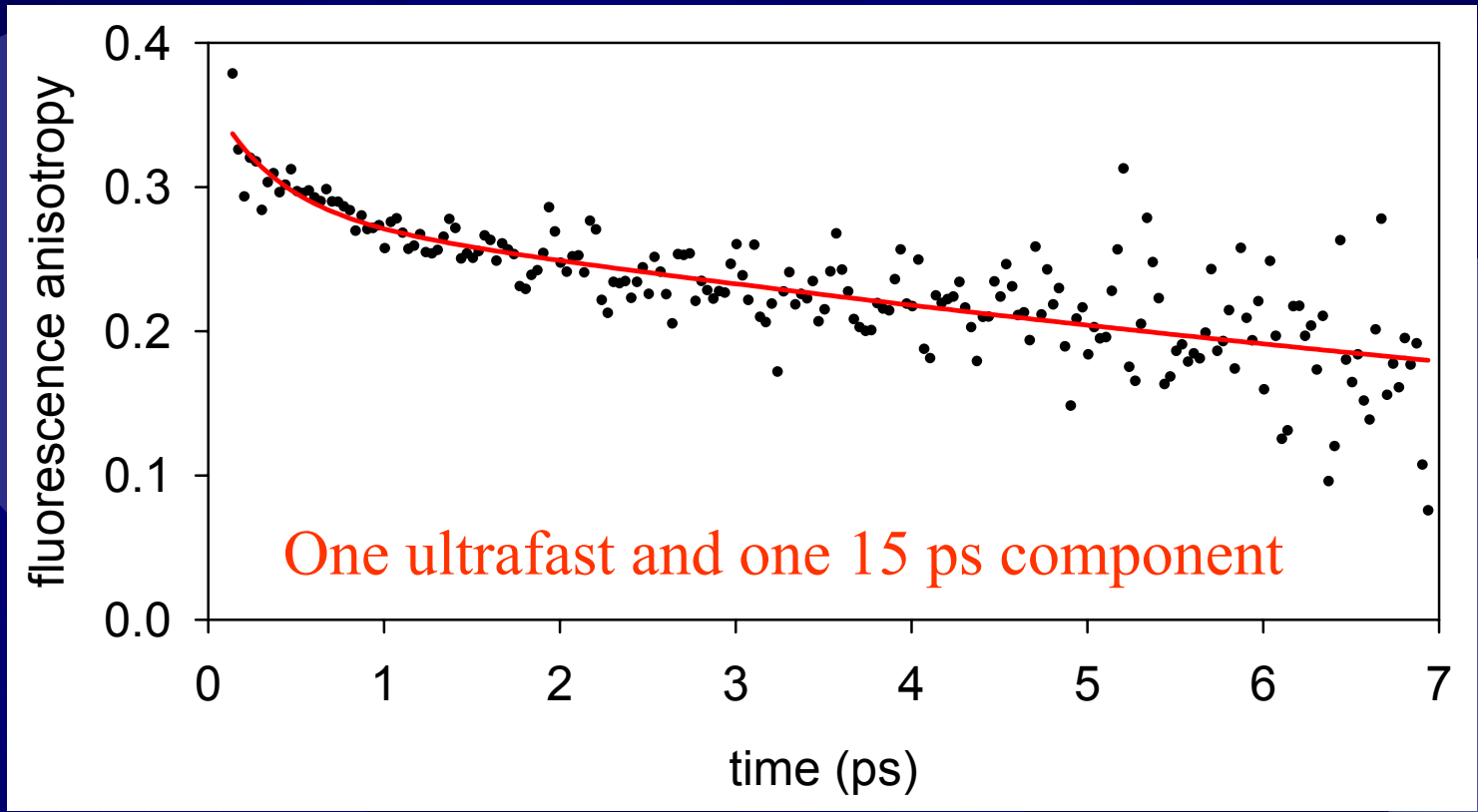
fluorescence decays of double strand $(dA)_{20}\cdot(dT)_{20}$ vs. monomers



excitation 267 nm
fluorescence 330 nm

D. Markovitsi, A. Sharonov, D. Onidas, T . Gustavsson, ChemPhysChem 3 (2003)

The fluorescence anisotropy decay of $A_{20}{\cdot}T_{20}$ observed at 330 nm



...too fast to [illegible] rotation

excitation 267 nm
fluorescence 330 nm

Thanks to

- Financial support from the Transnational European program CERC3 is gratefully acknowledged.