The role of water in the (indirect) solvent effects governing the excited state dynamics of DNA bases

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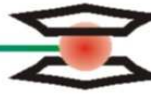


Outline

- Introduction - motivation
- Something about methods and techniques
  - fluorescence
- Excited state dynamics of DNA bases
  - Current status
  - Solvent effects ? Water ?

No Man’s Land ?

30 minutes – better hurry ..

Biomolecules and Water

Interaction of UV with DNA

Biomolecules and Water

Interaction of UV with DNA

Photoproducts -> Mutations -> Cancer

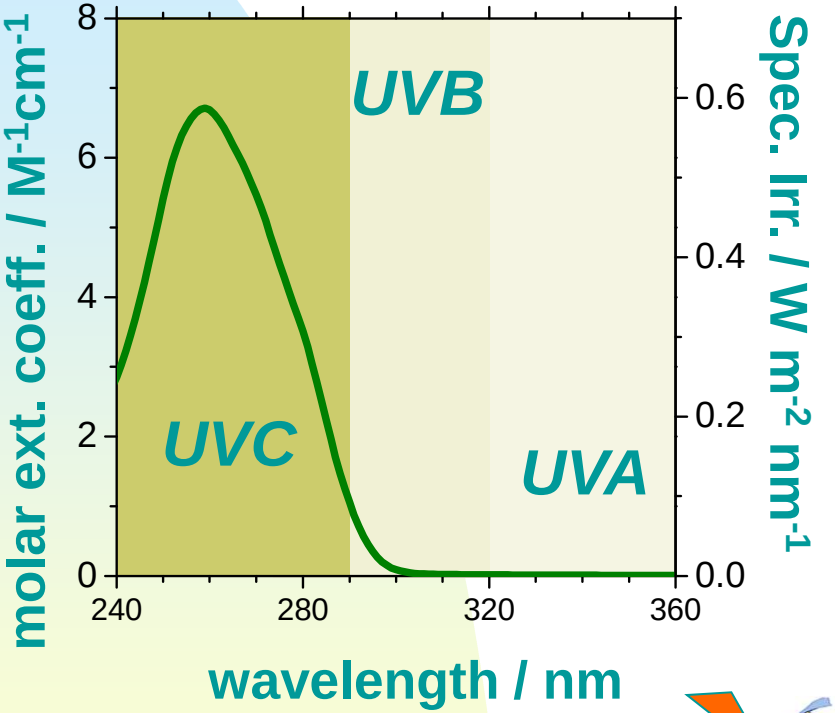
This is known as is much of the the photochemistry taking place on longer timescales (ms ….)

But at early times :

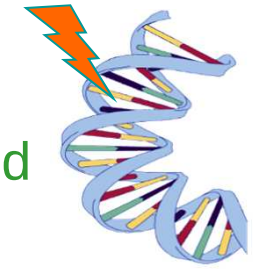
- What is the nature of the directly excited states ???
- How are the photoproducts formed ???

Background - DNA photoproducts

Absorption spectrum of calf thymus DNA

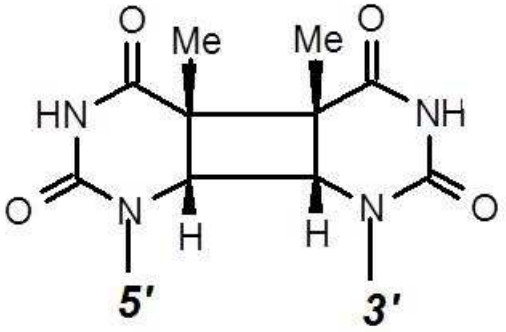


Absorption of UV light - homogeneously distributed over the double strand



Main photoproduct :

cyclobutane dimer (about 80%)



but also 6-4 dimer, photohydrates etc.

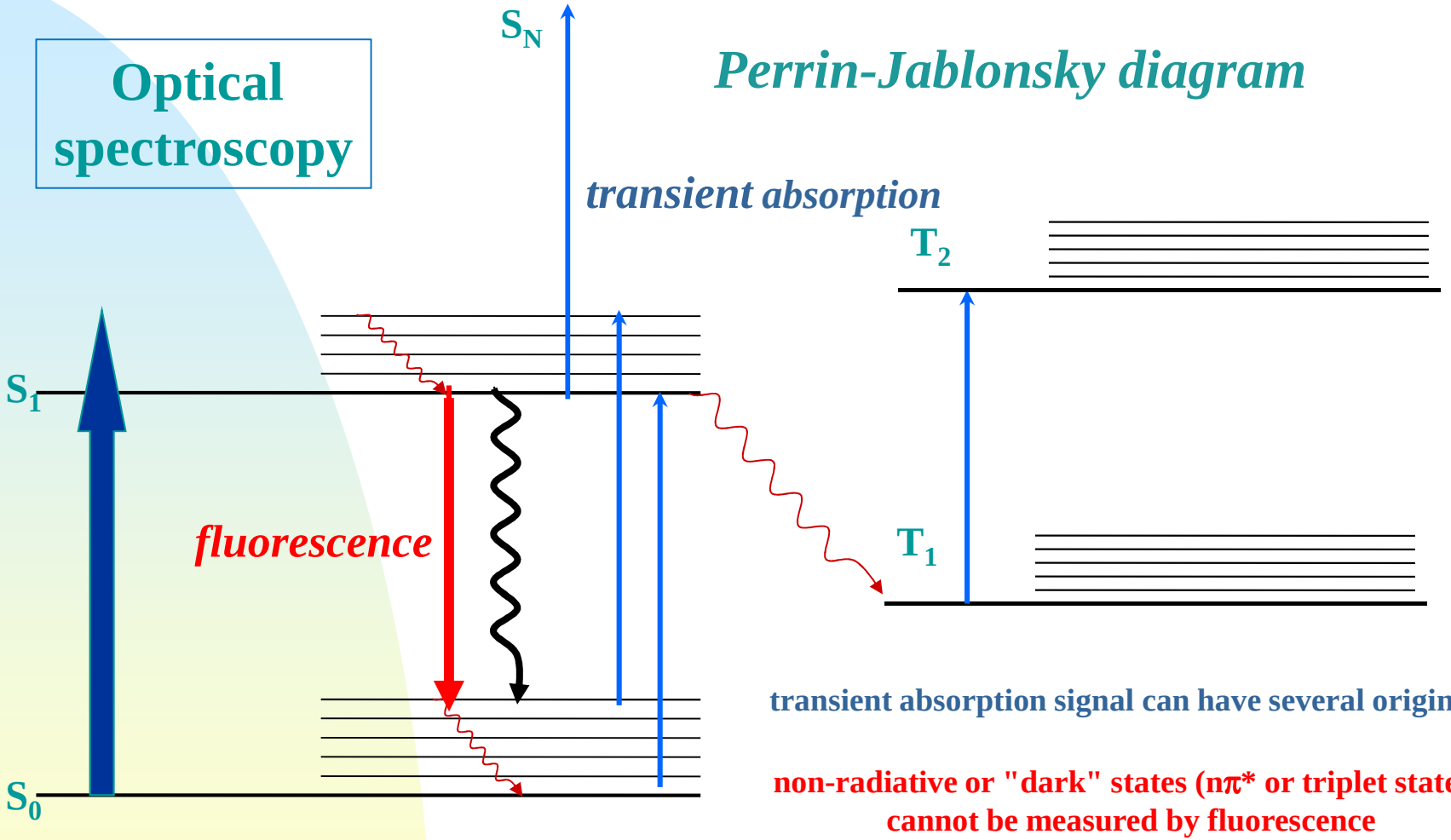
Photoproduct formation is localized

Understand the excited states

Monomers and Helices

To probe the excited state population

Optical spectroscopy



transient absorption signal can have several origins ☹

non-radiative or "dark" states (nπ* or triplet states) cannot be measured by fluorescence ☹

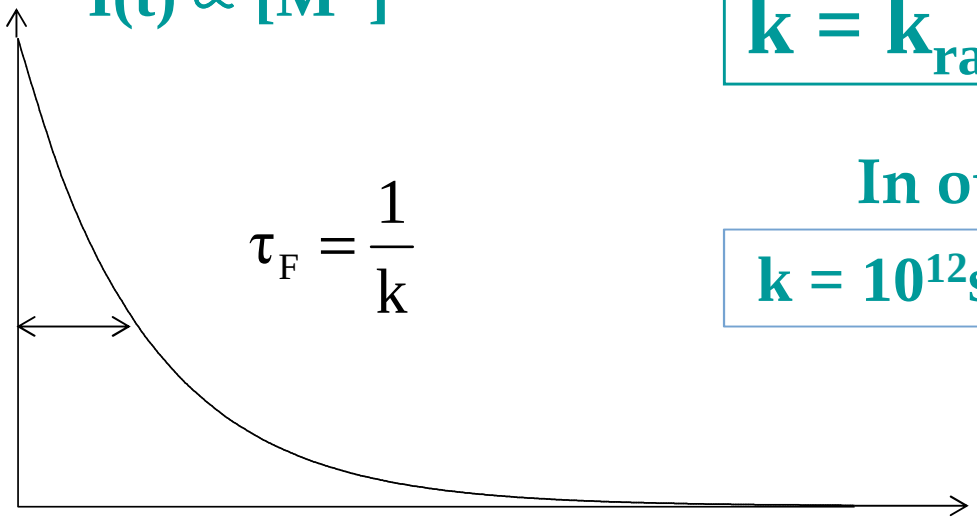
Best is a combination of both techniques !

Time-resolved fluorescence

$$\frac{d[M^*]}{dt} = -k[M^*] \qquad [M^*] = [M^*]_0 e^{-t/\tau_F}$$

[M*] is the concentration of excited molecules and k the deactivation rate.

I(t) ∝ [M*]



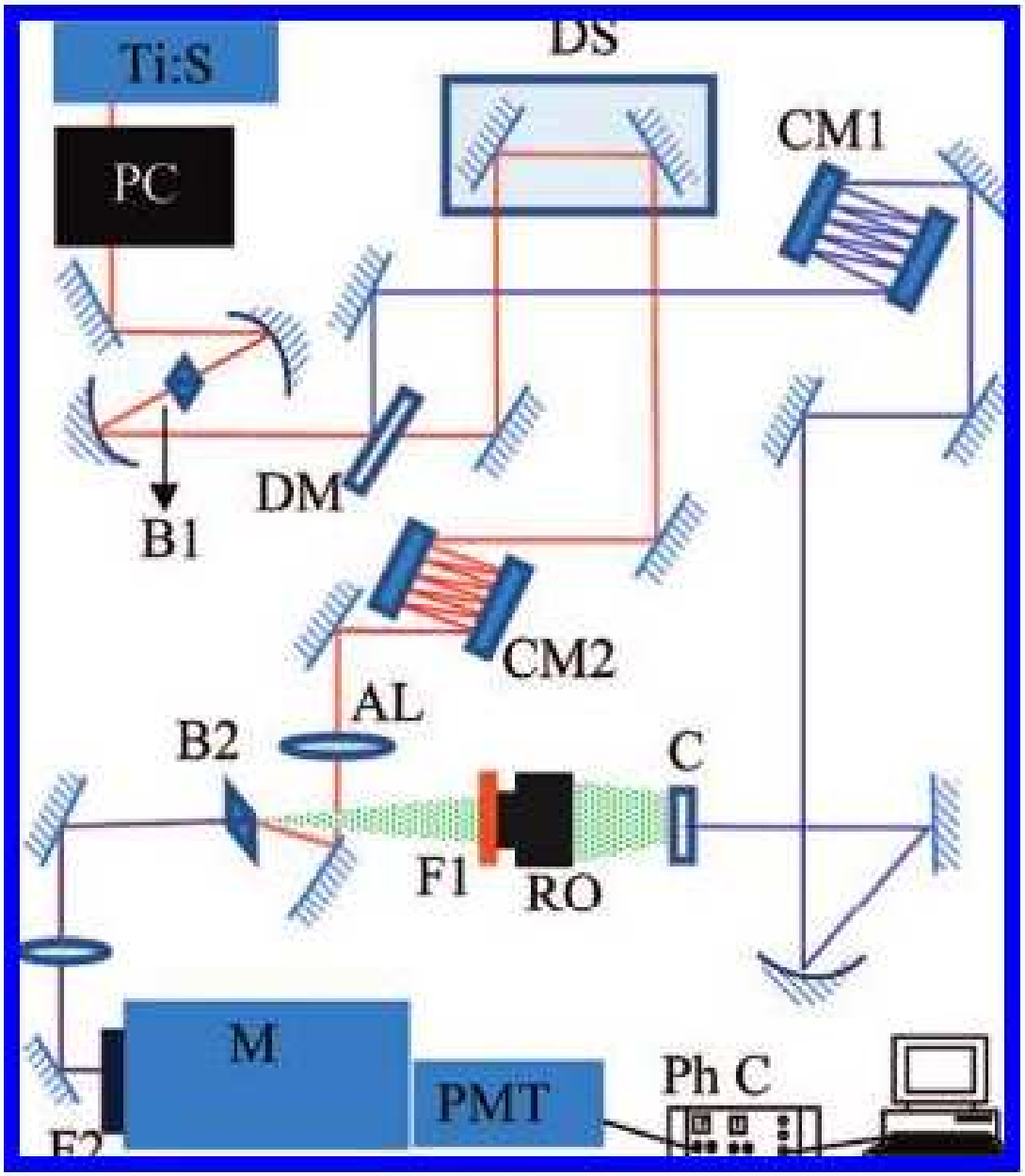
$$\tau_F = \frac{1}{k}$$

$$k = k_{rad} + k_{nonrad}$$

In our case

$k = 10^{12} s^{-1}$ -> τ = 1 ps

To measure ultrafast fluorescence

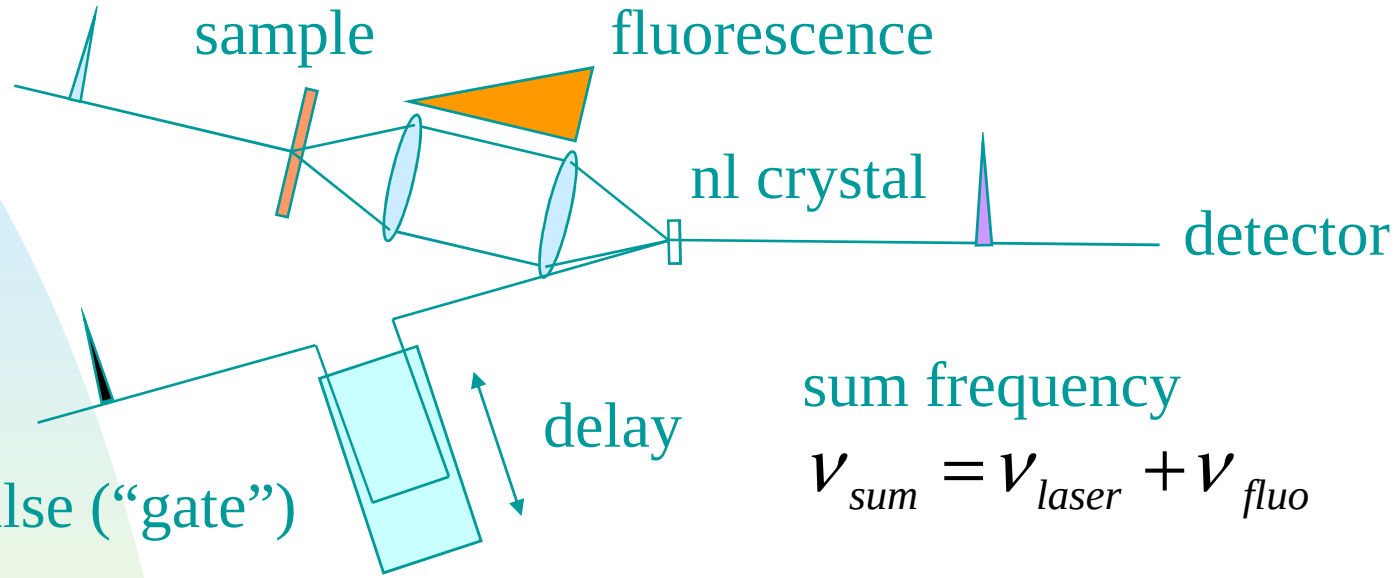


Fluorescence Upconversion

We heard Steve Meech telling us about This technique last Monday

Heisler, I. A.; Kondo, M.; Meech, S. R., *J. Phys. Chem. B* 2009, *113* (6), 1623–1631.

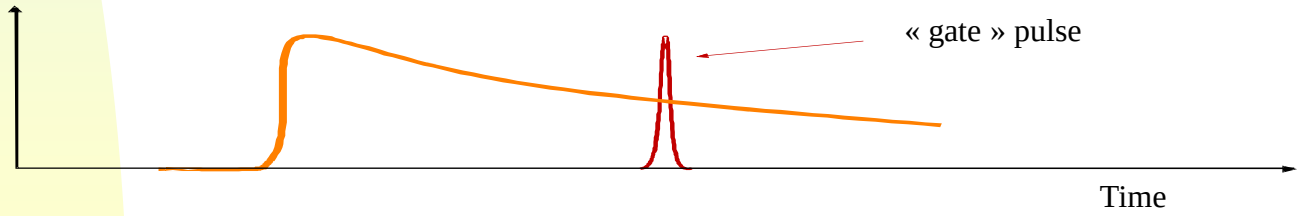
To measure ultrafast fluorescence



sum frequency

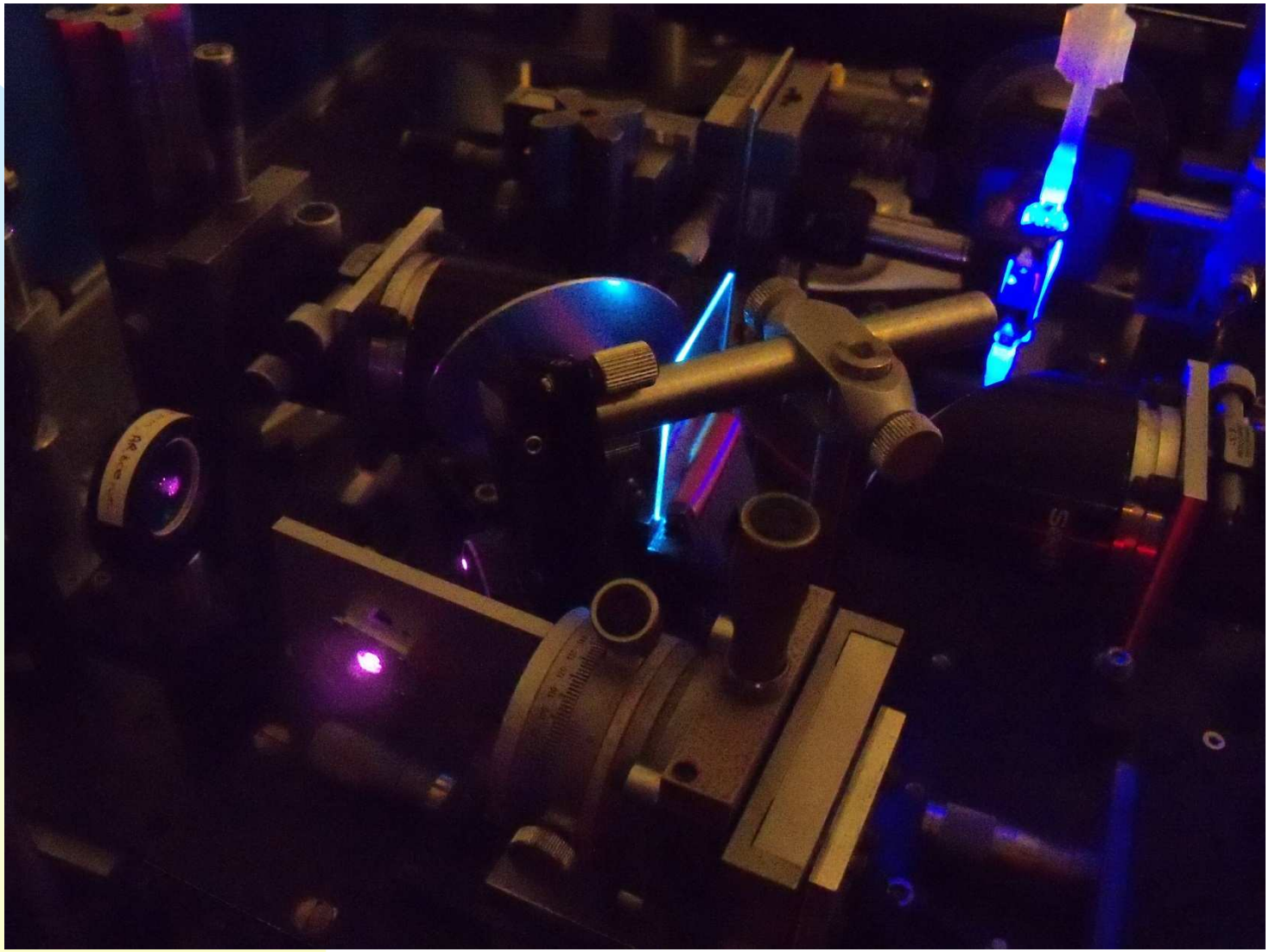
$$\nu_{sum} = \nu_{laser} + \nu_{fluo}$$

Kinetics at fixed wavelength



150 µm *2 = 1 ps = 10^{-12} s

Fluorescence upconversion

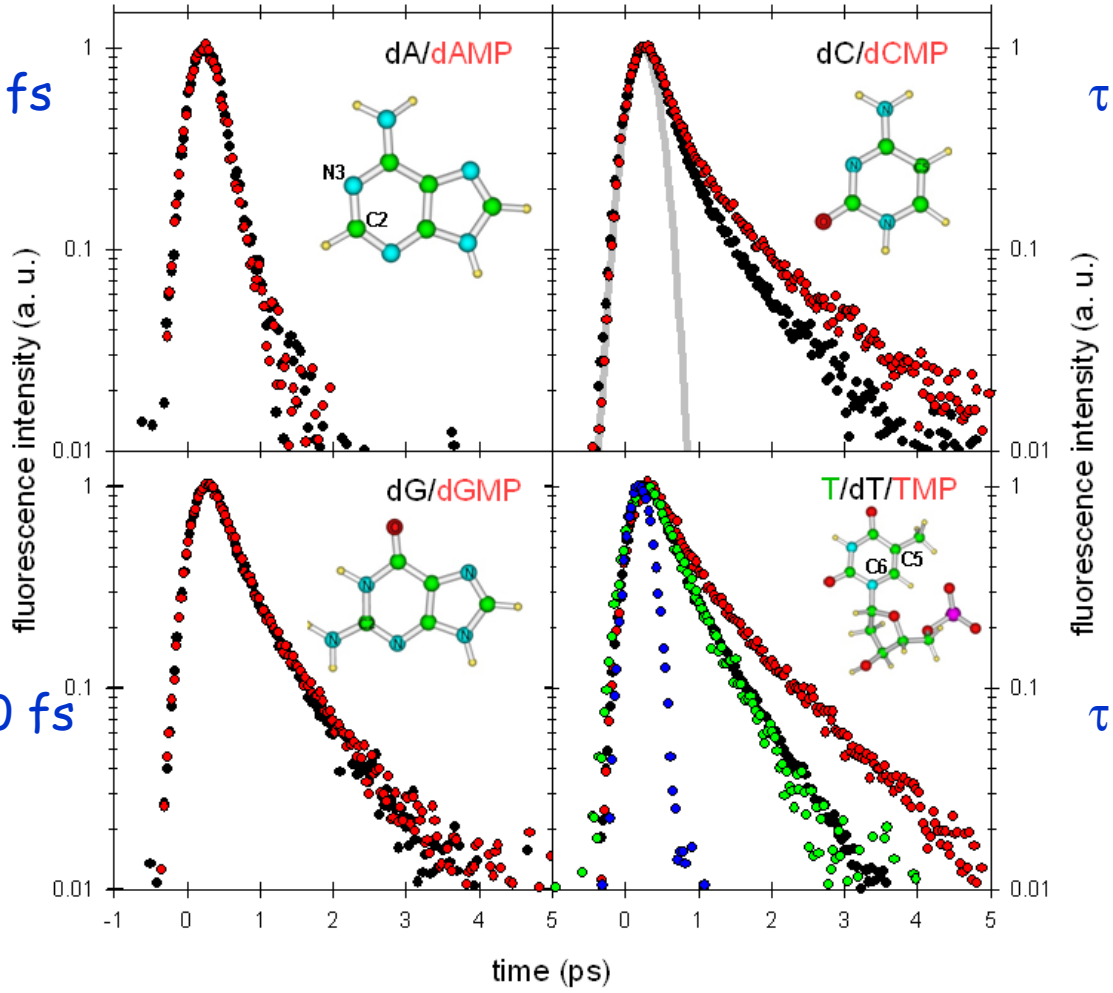


this is what it looks like … but what about DNA ?

Monomeric chromophores

Fluorescence decays at 330 nm after 267 nm excitation

τ ≈130 fs



τ ≈300-450 fs

τ ≈330 fs

τ ≈300-500 fs

$\Phi \approx 10^{-4}$

all lifetimes much faster than 1 ps for all of the monomers

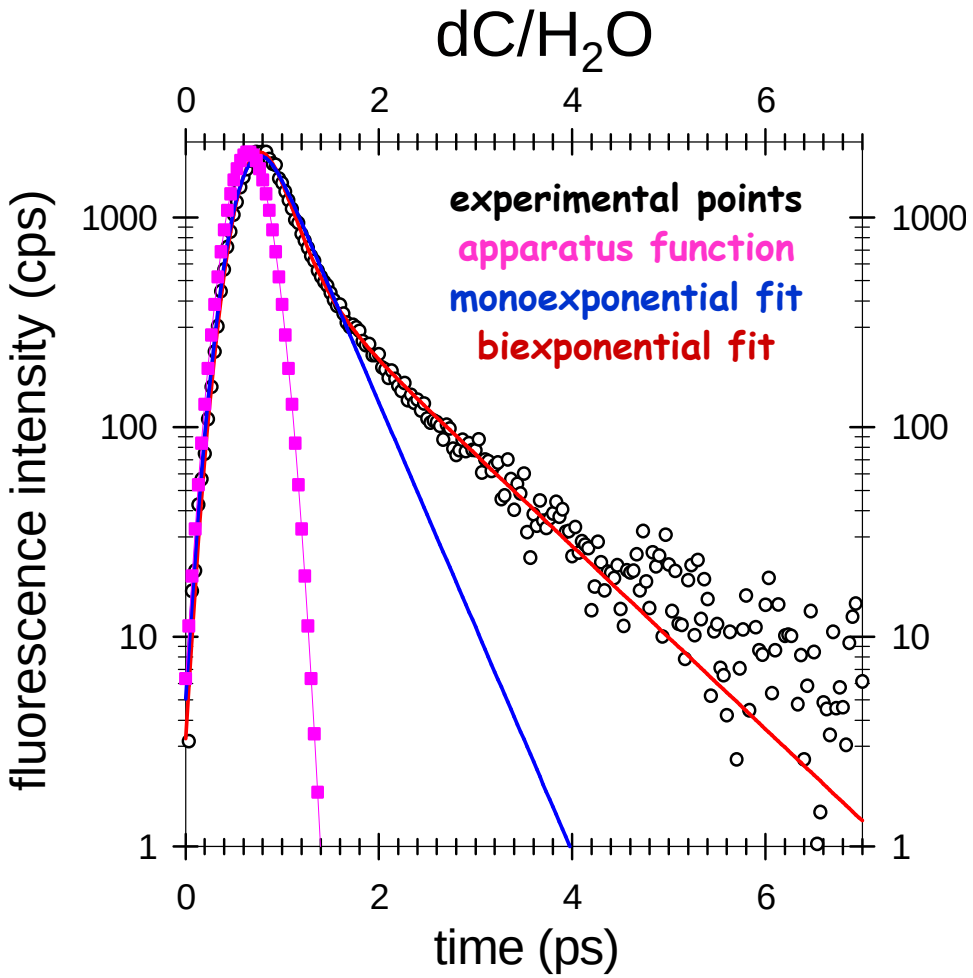
Onidas et al. JPCB **2002**

All fluorescence decays are non-exponential

ultrafast component independent of :

- concentration
- excitation power
- repetition rate

=> Complex excited state deactivation



Note : there are no long-lived (> a few ps) emitting states.

Complex excited state deactivation ?

Experimentalist's approach :

- What are the effects of different substitutional groups ?
- What is the effect of the environment (solvent) ?

Gather sufficient amount of data,

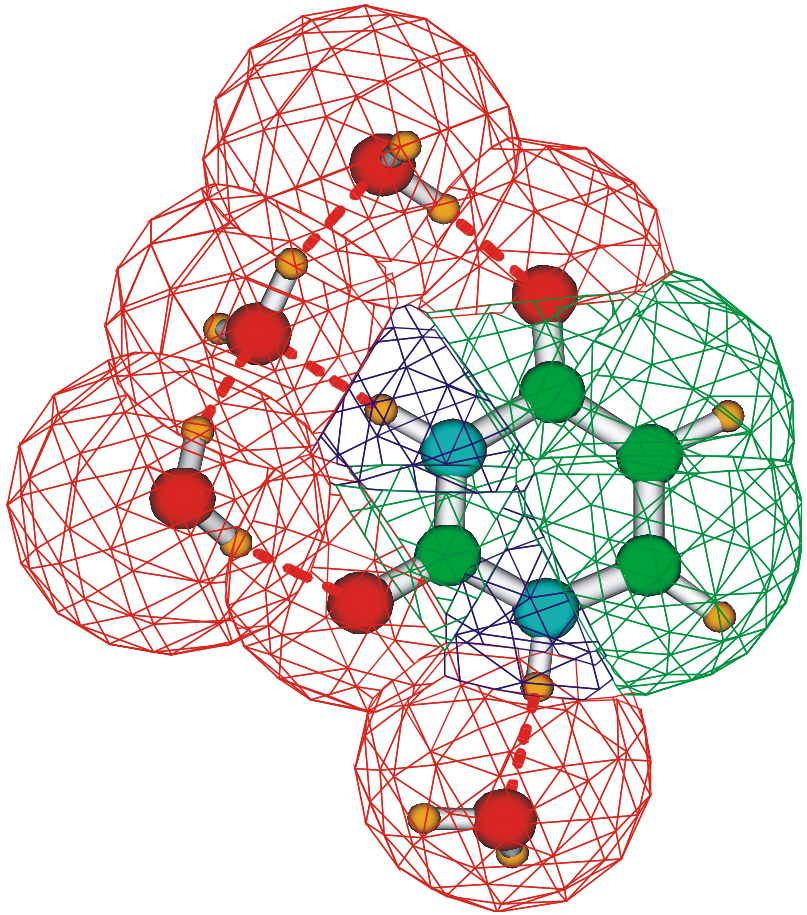
then ask the theoreticians !

TD-DFT quantum chemistry calculations

Hybrid model for the solvent

Polarizable Continuum Model (PCM) for the bulk

n water molecules in the first solvent shell treated on the quantum level



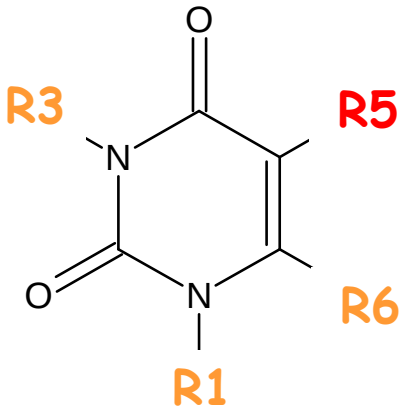
Collaboration with
Roberto Improta, Naples

Gustavsson et al.JACS **2006**

Santoro et al.JACS **2006**

Substituent effects - uracils

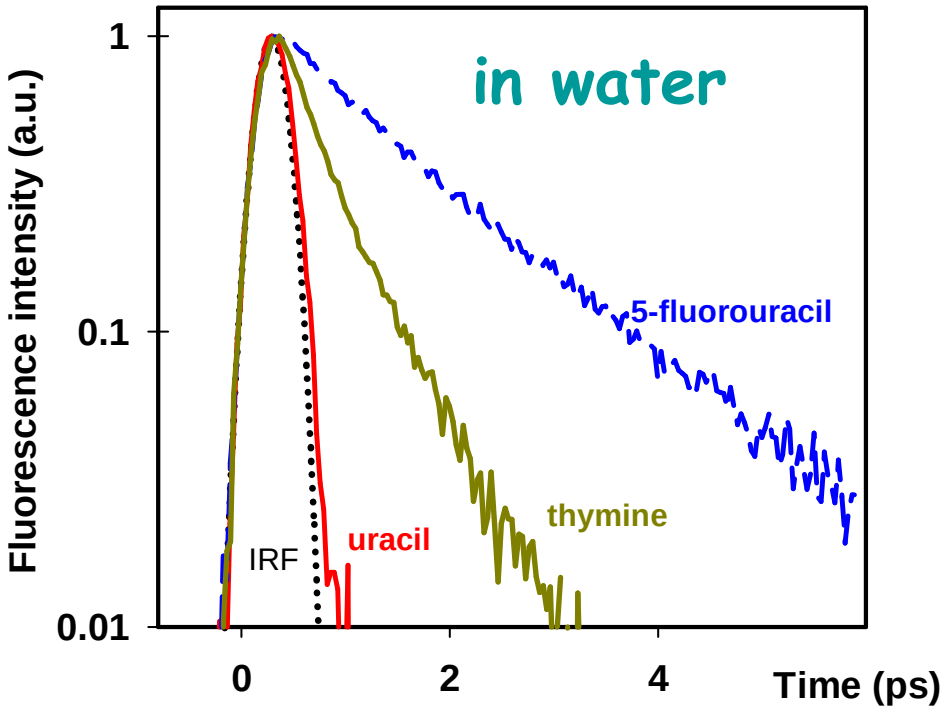
Example : uracils



- Methylation at different positions

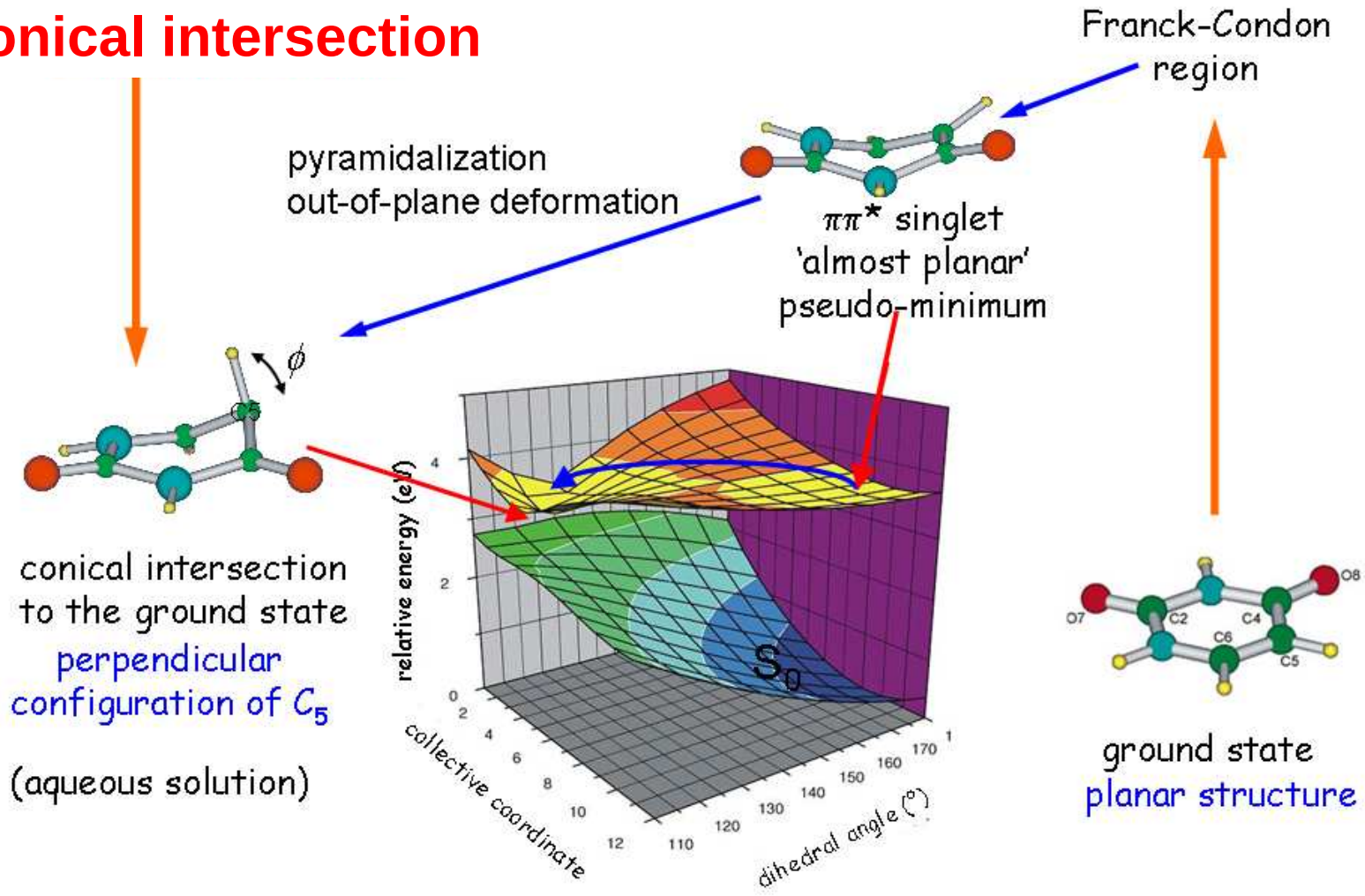
- 1, 3 and 6 positions
  no effect

- 5 position:
  strong effect !!!



Gustavsson et al.JACS **2006**

Excited state dynamics in uracil

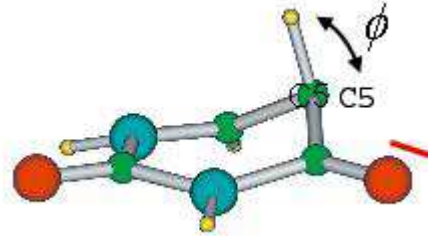


Gustavsson et al. JACS **2006**

Internal conversion mechanism

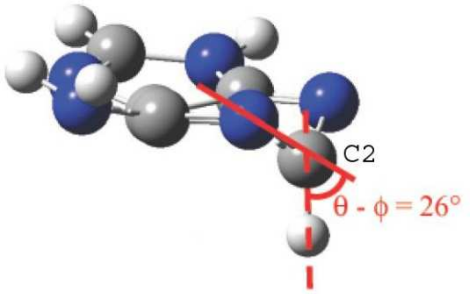
Pyrimidines
Out-of-plane flip of 5-substituent

Purines
Out-of-plane flip of 2-substituent

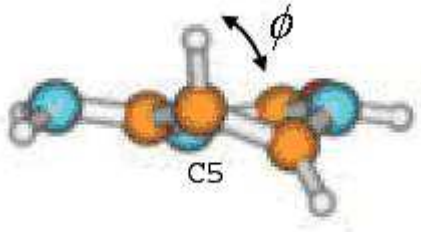


Uracil / Thymine
Improta et al. JACS 2006

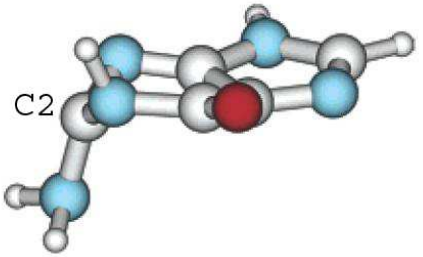
Conical intersections between S_1 and S_0



Adenine
Conti et al JACS 2009



Cytosine
Kistler and Matsika JCP 2008

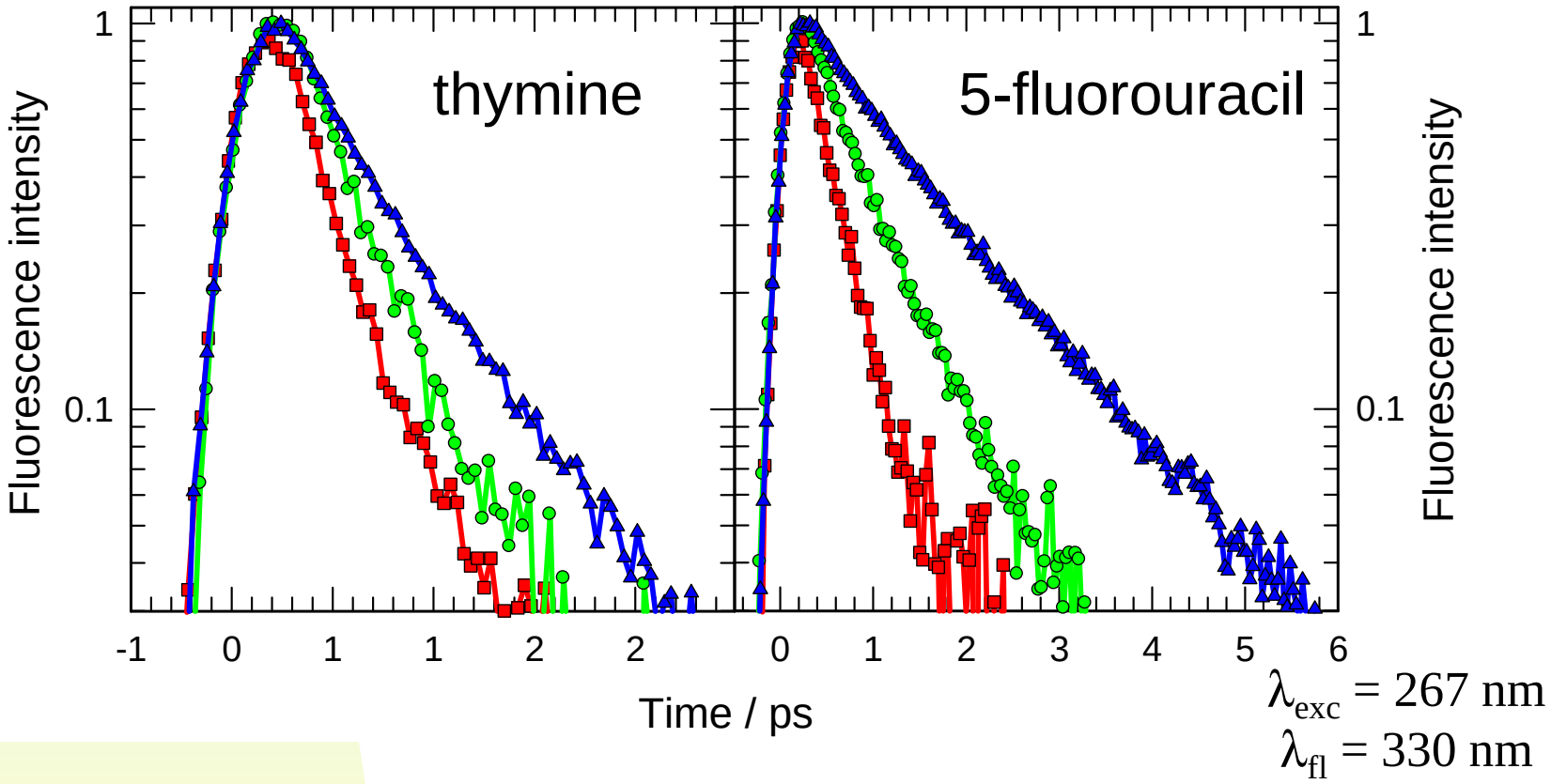


Guanine
Marion JPCA 2007

Detailed studies indicate there are subtle differences and also several possible CI's

Gustavsson JPCL **2010**

Solvent effects - uracils



In acetonitrile (red squares), methanol (green circles) and water (blue triangles

Decay faster in aprotic acetonitrile than in water

Gustavsson et al. JPC B 2006, CPL 2006

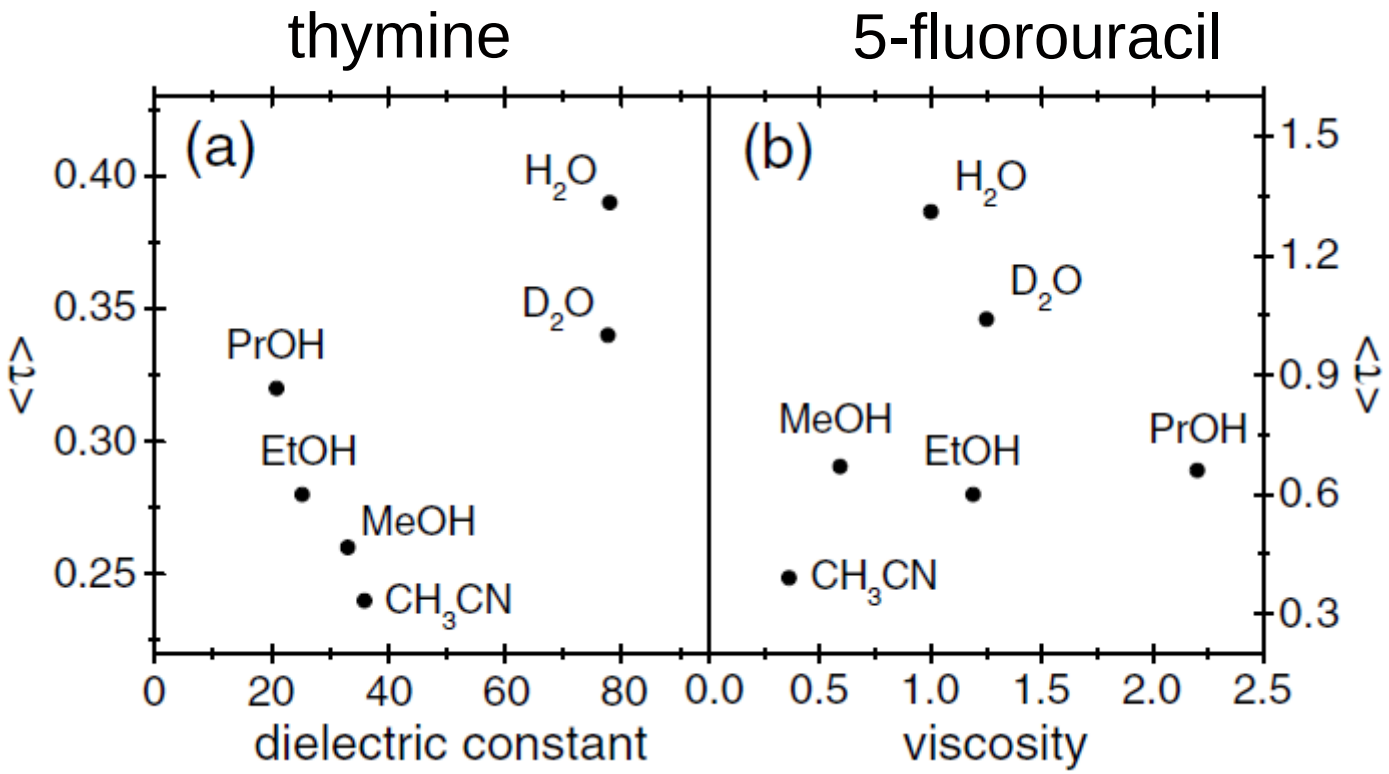
Solvent effects - uracils

What solvent effects can be expected ?

Change solvent viscosity – large amplitude motions, such as isomerization, scale with viscosity (some power law)
cf. Kramer's equation

Change solvent polarity – charge transfer processes, scale with polarity (some power law)
cf. Smolochowski equation (Meech, Monday talk)

Solvent effects - uracils

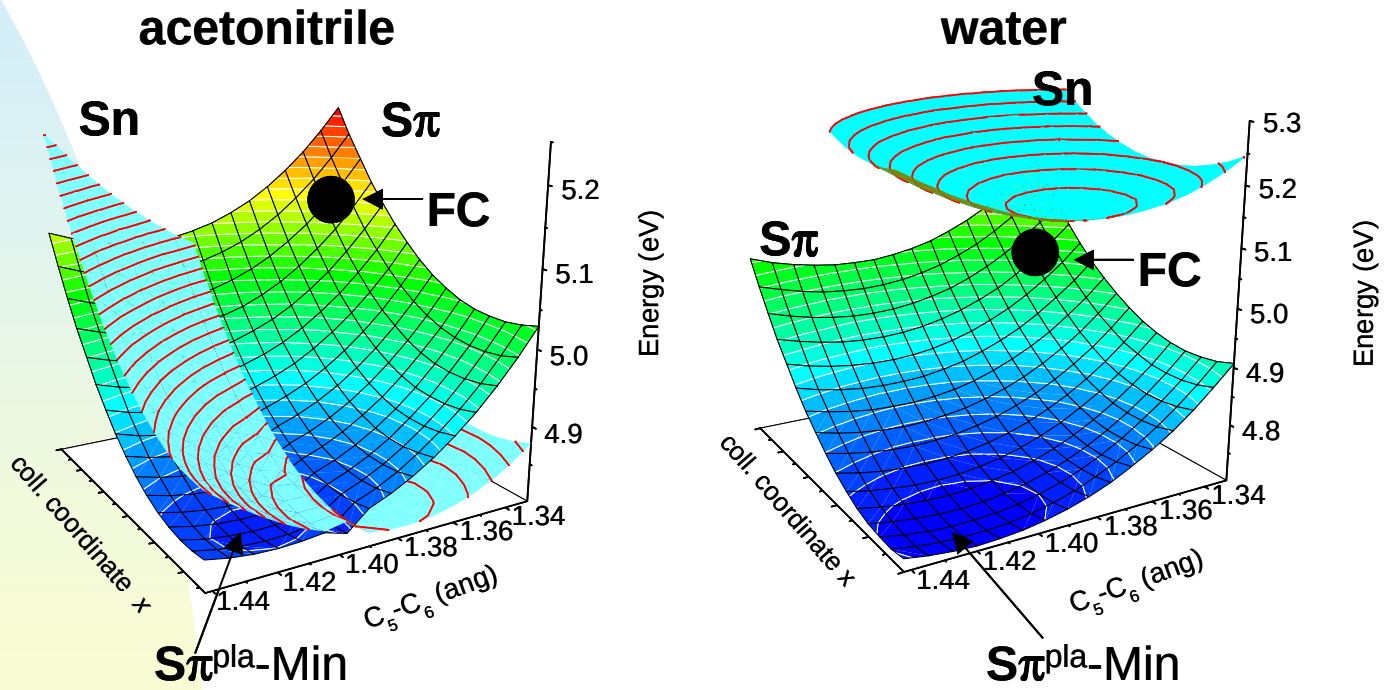


The mean fluorescence lifetimes <τ> of
(a) thymine as a function of solvent polarity and of
(b) 5-fluorouracil as a function of solvent viscosity.
No obvious correlations !

T. Gustavsson, et al.; Chem. Phys. 350 (2008) 186-192.

Relaxation mechanisms for 5F-uracil in acetonitrile and water solution

Energies of the ππ* and the dark nπ* states in the region connecting the FC point and the minimum on ππ*.

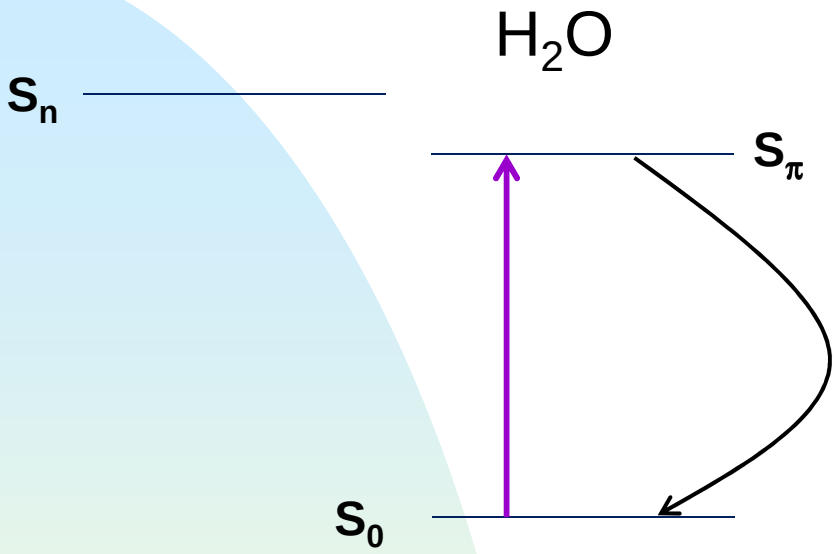


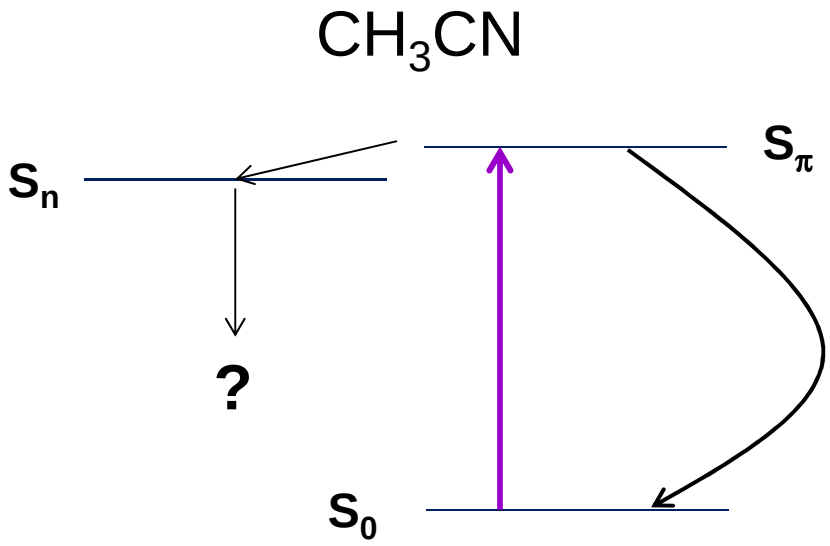
TD-DFT calculations

3D representation in two coordinates : C_5-C_6 and a collective coordinate

F. Santoro, V. Barone, T. Gustavsson, R. Improta, J. Am. Chem. Soc. 128 (2006) 16312.

5F-uracil in water and acetonitrile





One decay channel:

- CI: ππ*/ground state

nπ* state is not accessible

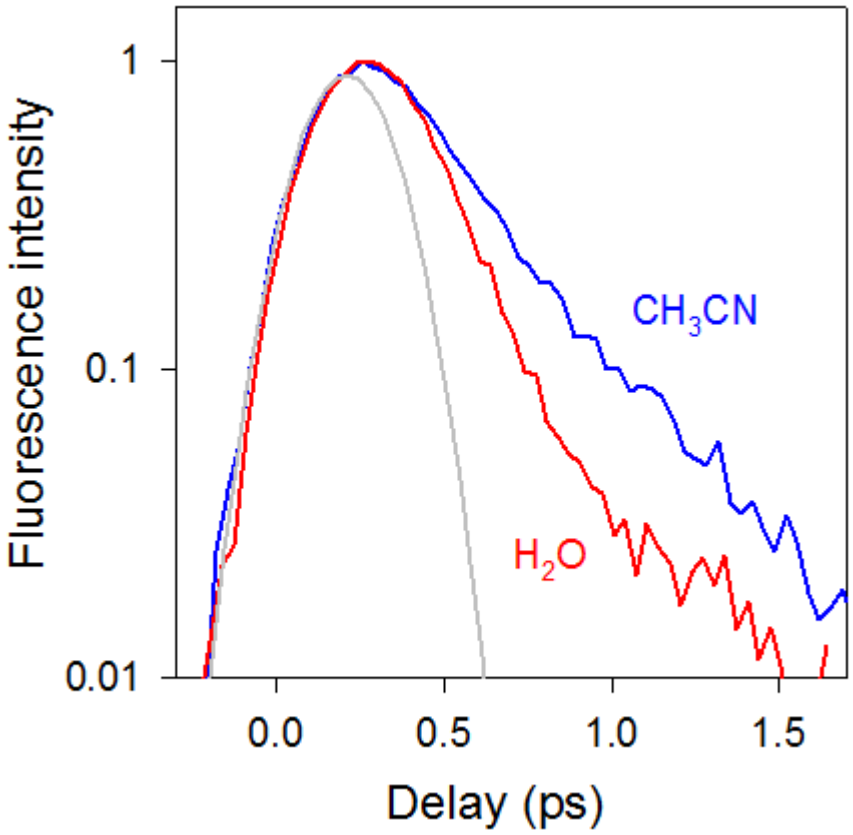
Two decay channels:

- CI: ππ*/ground state
- additional channel: CI ππ*/nπ*

Decay faster in aprotic acetonitrile than in water

Is this a general phenomena?

Solvent effects - adenine



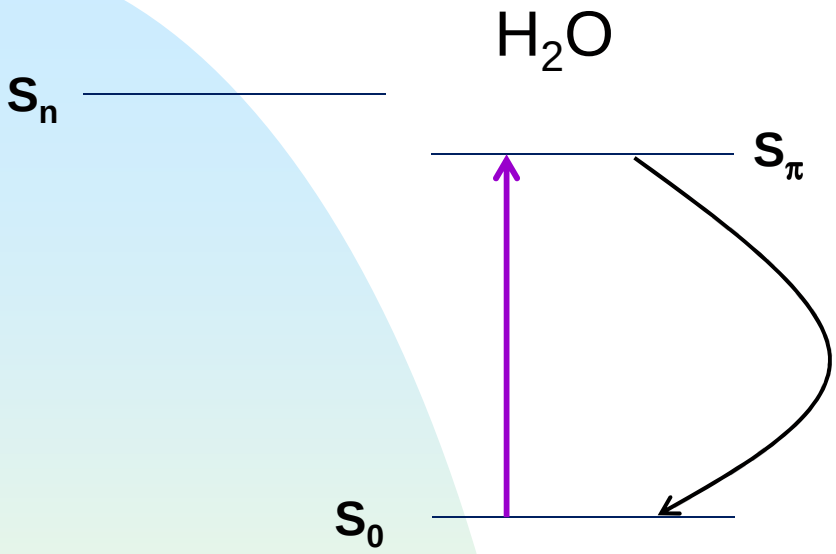
λ_{exc} = 267 nm
λ_{fl} = 330 nm

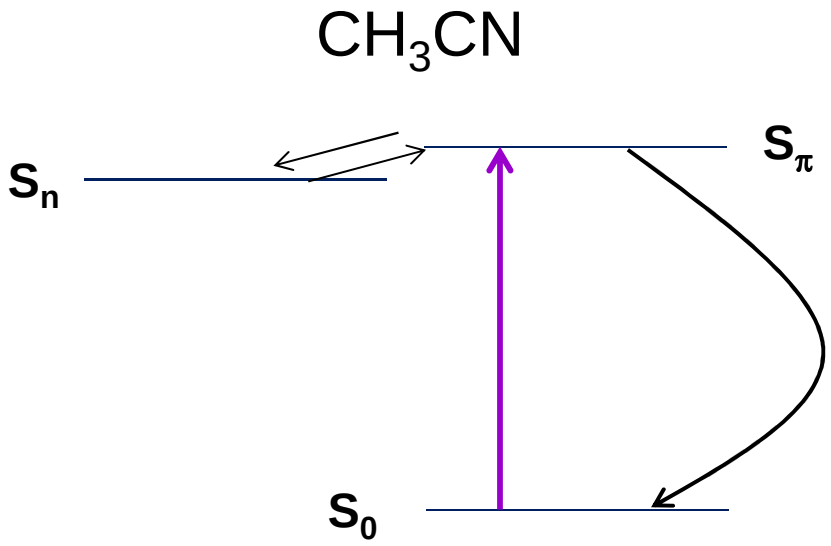
This is **contrary** to what was observed for uracil.

Decay slower in aprotic acetonitrile than in water

T. Gustavsson, et al.; PPS (2013) in press

Adenosine in water and acetonitrile





One decay channel:

- CI: ππ*/ground state

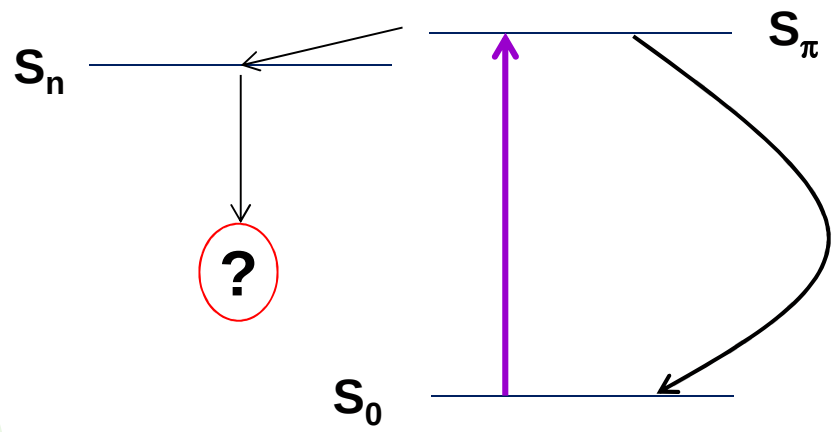
nπ* state is not accessible

Two decay channels:

- CI: ππ*/ground state
- additional channel: CI ππ*/nπ*
- back transfer nπ* -> ππ*

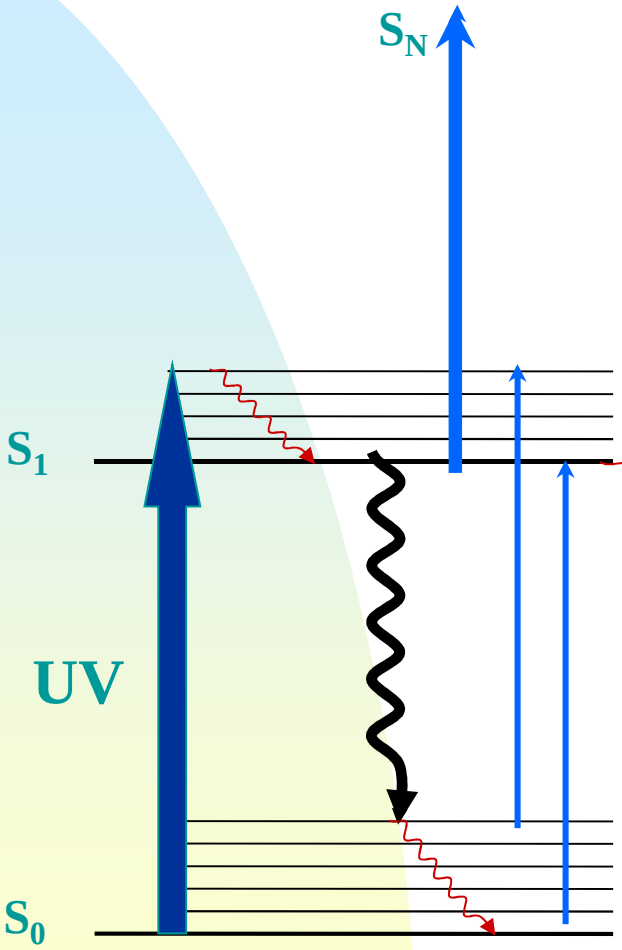
Smaller nπ-L_a energy gap in acetonitrile -> higher mixing of the two states.
But, no nπ decay! The dark nπ state acts as a “reservoir” state

5F-uracil in acetonitrile

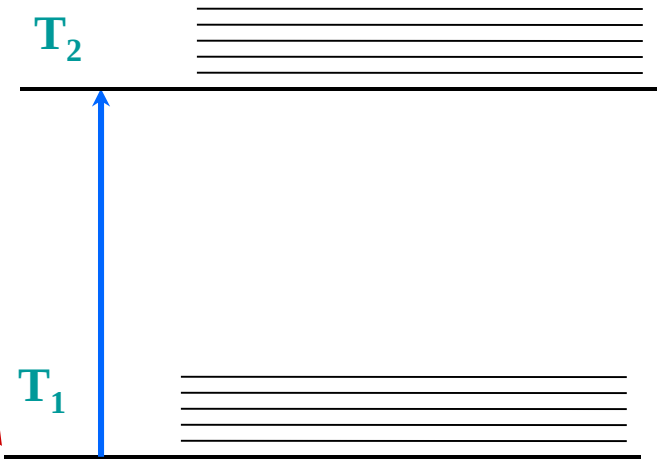


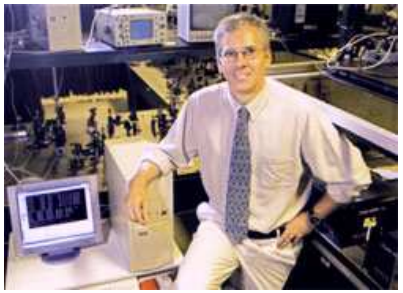
What is this unknown relaxation channel?
Are there any experimental hints?

Transient absorption spectroscopy of DNA bases



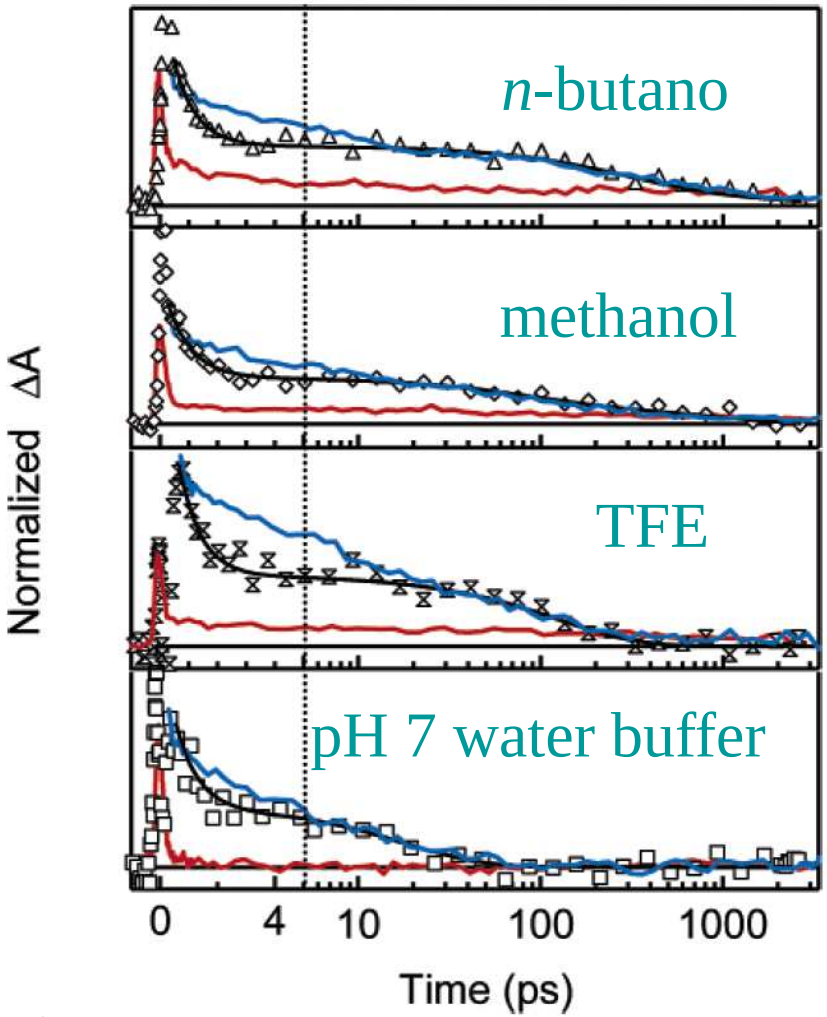
The transient signal can have many origins – potentially very rich information!





Bern Kohler, Montana University, has studied DNA using transient absorption

Solvent effects - uracils



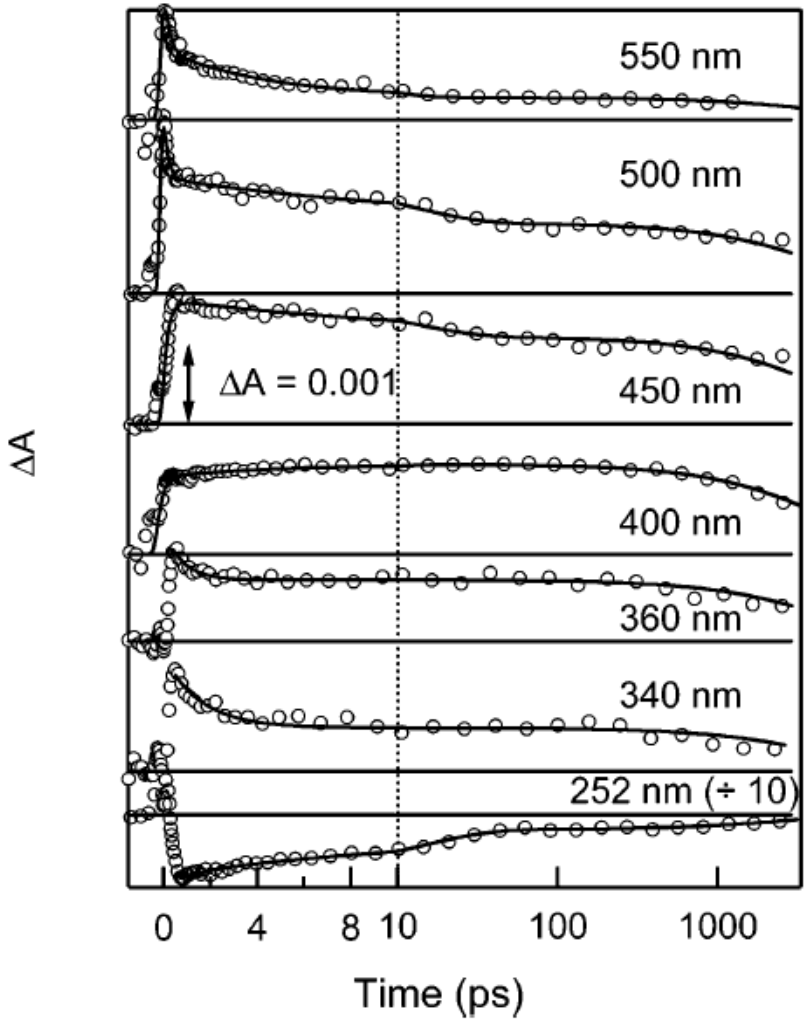
λ_{exc} = 267 nm
λ_{probe} = 340 nm

Transient absorption measurements of 1-cyclohexyluracil in various protic solvents.

A long-lived component appears when the polarity decreases

Middleton et al., J. Phys. Chem. B 110 (2006) 18645

Solvent effects - uracils



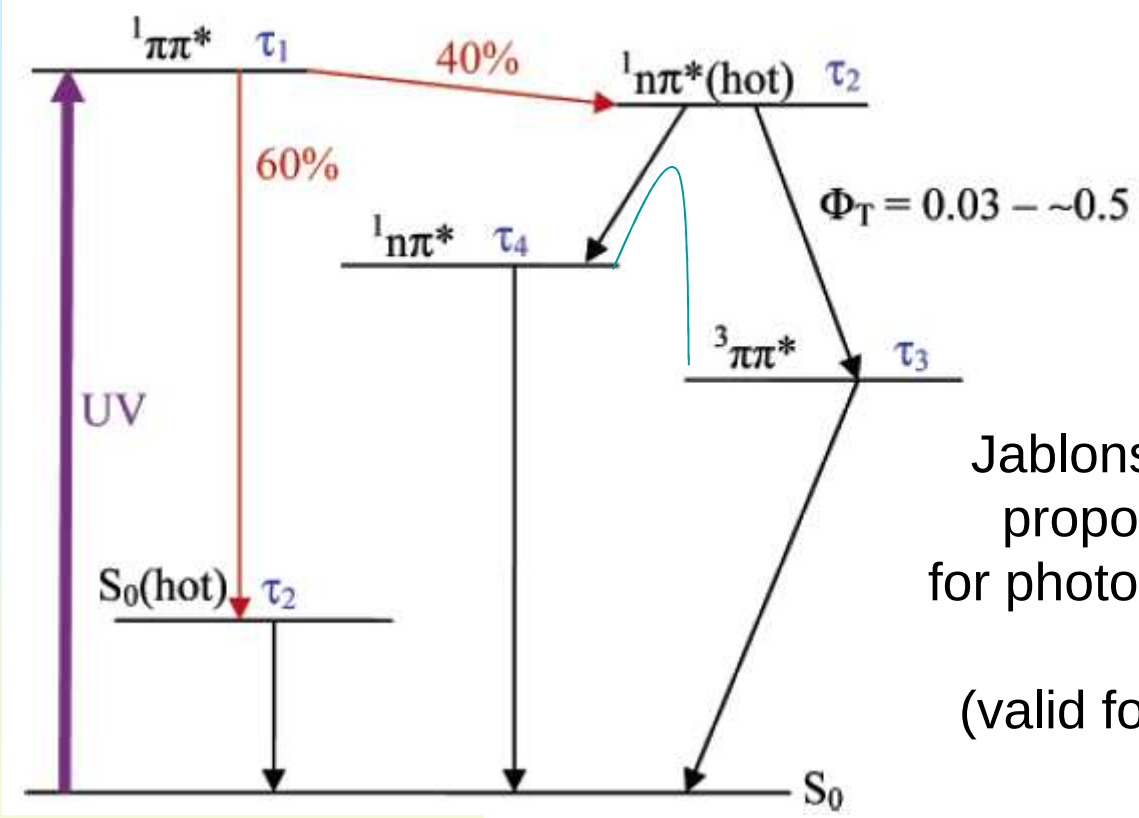
Transient absorption measurements of 1-cyclohexyluracil in aprotic acetonitrile

A nanosecond component
-> triplet formation

λ_{exc} = 267 nm
λ_{probe} = 250-550 nm

Middleton et al., J. Phys. Chem. B 110 (2006) 18645

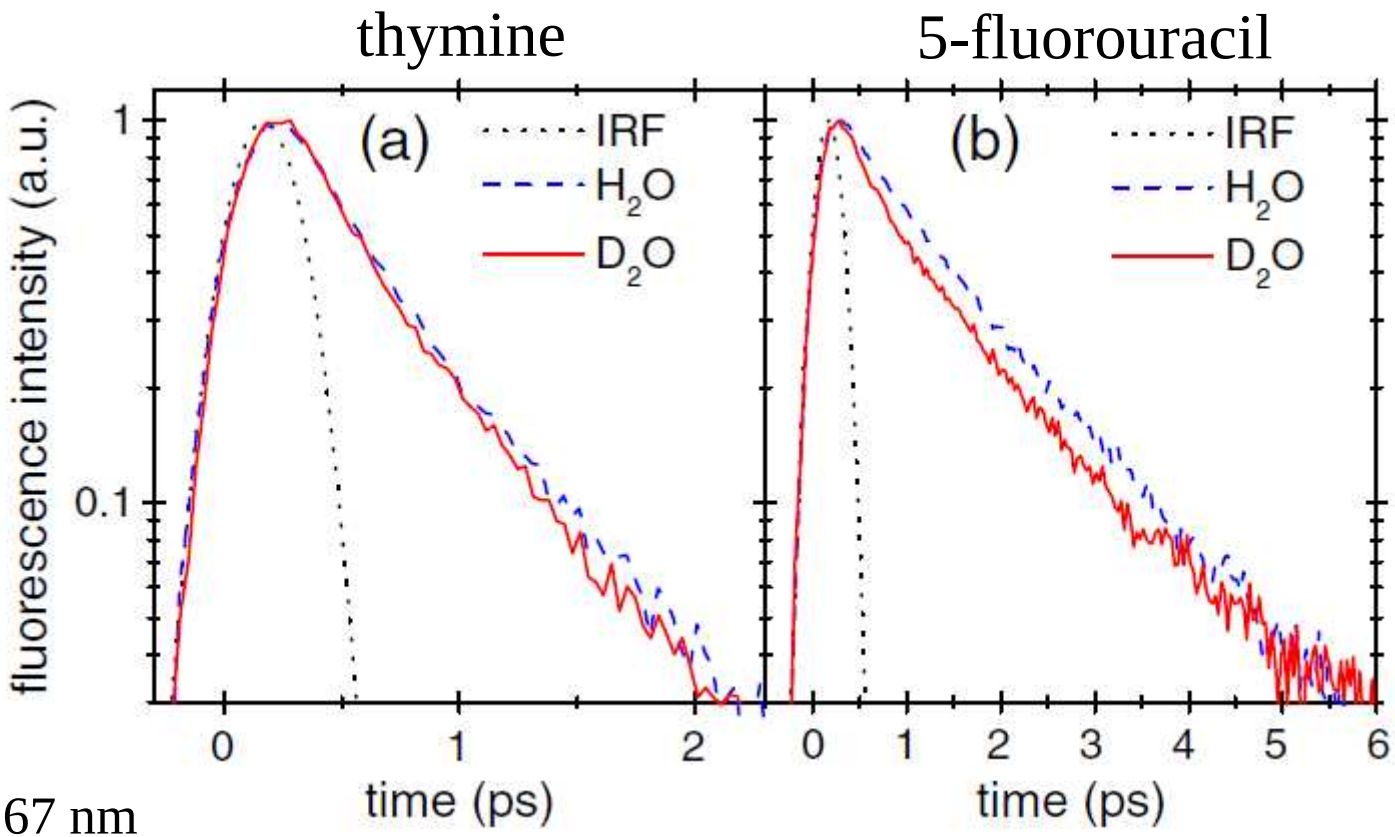
Solvent effects - uracils



Jablonski diagram showing the proposed decay mechanism for photoexcited 1-cyclohexyluracil in acetonitrile
(valid for pyrimidines in general, not purines)

The triplet formation occurs via hot vibrational levels in the $n\pi^*$ state.
The more efficient the vibrational cooling, the less efficient the triplet formation. Water wins!

Deuterium isotope effect



λ_{exc} = 267 nm

λ_{fl} = 330 nm

The fluorescence decays are faster in D_2O than in H_2O !

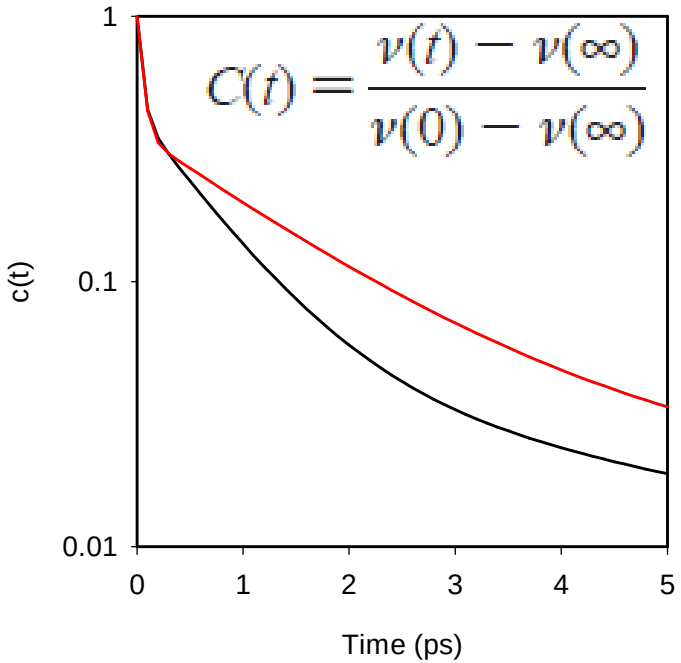
T. Gustavsson, et al.; Chem. Phys. 350 (2008) 186-192.

Deuterium isotope effect in solvation dynamics

Even though literature data on D_2O solvation dynamics are scarce, the average solvation times in bulk D_2O are longer than that in bulk H_2O

Ex. Fluorescence time-correlation function of coumarin 1 in H_2O and D_2O

$$c(t) = \alpha e^{-\frac{1}{2}\omega_0^2 t^2} + (1-\alpha)\beta e^{-t/\tau_1} + (1-\alpha)(1-\beta)e^{-t/\tau_2}$$



H_2O : τ_1 = 0.75 ps
D_2O : τ_1 = 1.5 ps

!

Can be interpreted as due to:

- stronger hydrogen bonding in D_2O compared to H_2O
- slows down the reorientation of the excited state dipoles in the bulk D_2O.

Mialocq et al. J. Physique IV: Proc. 1999, 9 (Pr5), 101.

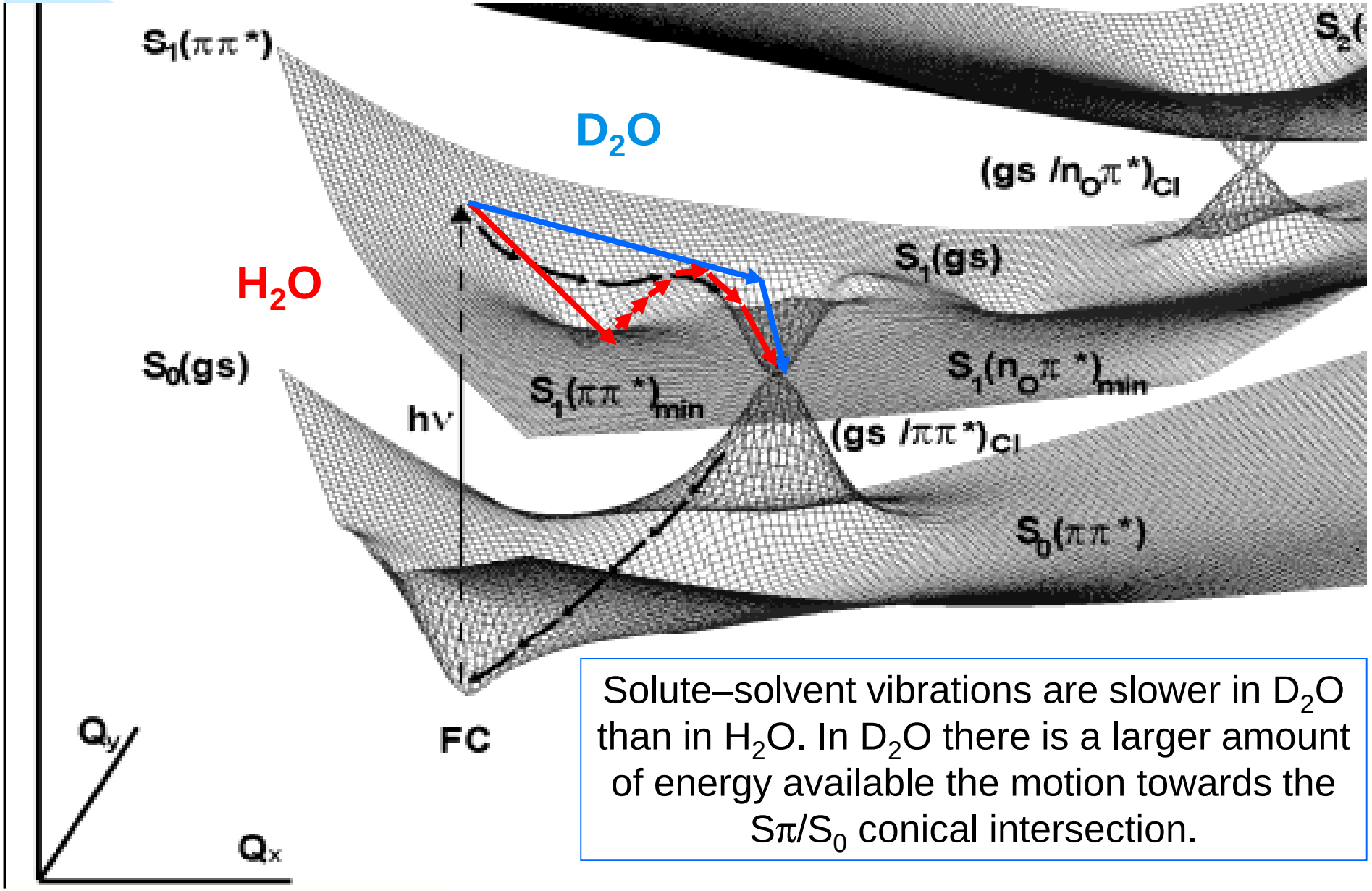
Pant and Levinger;J. Phys. Chem. B 103 (1999) 7846-7852

Deuterium isotope effect

If the solvation dynamics are faster H_2O than in D_2O,

Why are the uracil fluorescence decays faster in D_2O than in H_2O ?

Deuterium isotope effect - uracils



Solute–solvent vibrations are slower in D_2O than in H_2O. In D_2O there is a larger amount of energy available the motion towards the $S\pi/S_0$ conical intersection.

Conclusion

- Solvent effects, and in particular water hydrogen bonding, are important for excited state processes.
- Indirect, not direct, but still important
- Both static and dynamical effects.
- Water’s ultrafast dynamical properties make it very special.
- Deuteration effect far from well understood!

Excited Biomolecules Group

Ying Hua
Akos Banyasz

Dimitra Markovitsi
Marion Perron
Pascale Changenet-Barret

(Ignacio Vaya)
Johanna Brazard