

# Solvent effects on the steady-state absorption and fluorescence spectra of uracil, thymine and 5-fluorouracil

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**Abstract**

We report a comparison of the steady-state absorption and fluorescence spectra of three representative uracil derivatives (uracil, thymine and 5-fluorouracil) in alcoholic solutions. The present results are compared with those from our previous experimental and computational studies of the same compounds in water and acetonitrile. The effects of solvent polarity and hydrogen bonding on the spectra are discussed in the light of theoretical predictions. This comparative analysis provides a more complete picture of the solvent effects on the absorption and fluorescence properties of pyrimidine nucleobases, with special emphasis on the mechanism of the excited state deactivation.

## **Introduction**

Recent advances in ultrafast spectroscopy and computational chemistry have together contributed to unveil some of the mystery that surrounds the extremely rapid ( $< 1$  ps) internal conversion of DNA and RNA bases in solution (1). Several theoretical studies point towards the existence of near barrierless paths, which imply important ring deformation, leading from the excited state through a conical intersection to the ground state in uracil (2-5), cytosine (6-9) and adenine (10,11,4,12-14).

Up to now, nearly all spectroscopic studies, steady-state or time-resolved, have been performed in aqueous solution and the few exceptions provide somewhat contradictory results (15-18). Consequently, it is not surprising that the solvent effect on the excited state relaxation of nucleobases has been considered 'modest' and that the ultrafast deactivation has been ascribed to be of purely intramolecular origin, little or not affected by intermolecular effects, such as solute-solvent interactions (1).

However, there are indications that environmental effects have a sizable influence on the excited state behavior. For example, gas phase studies have shown that much longer lived states, having nanosecond lifetimes, exist in vacuum (19).

Very recently we have started a thorough study of solvent effects on the excited state behavior of uracil derivatives and the first results depict a very different scenario (20-23), suggesting that the solvent can significantly modify the mechanism of internal conversion. In fact, time-resolved fluorescence upconversion experiments show that excited state lifetimes of thymine and 5-fluorouracil in acetonitrile is significantly shorter than in water. Quantum mechanical calculations suggest that an additional decay channel can exist for the bright state (hereafter  $S_\pi$ ), involving a dark  $n\pi^*$  state (hereafter  $S_n$ ) almost isoenergetic to the  $S_\pi$  state in the Franck-Condon (FC) region. The efficiency of this additional decay channel is modulated by the interplay between solvent and substituent effects, since the relative energy of the  $S_\pi$  and  $S_n$  states in the gas phase depends on the substituent of the pyrimidine ring. The results of another recent study of the excited state dynamics of a uracil derivative confirm the relevance of the solvent effect (24).

It is thus interesting to ascertain what are the microscopic mechanisms underlying solvent effect on the  $S_\pi$  behavior, discriminating, for example, between the role played by bulk solvent effect (such as solvent dielectric constant) and that of explicit interactions, such as solute-solvent hydrogen bonds.

Moreover, the dependence of the excited state lifetimes of the bases on the local environment (solvation) is of utter importance in order to characterize the nature of the

excited states of DNA double helices. As a matter of fact, it has now been clearly shown, by both time-resolved fluorescence (25,26) and transient absorption (27) with femtosecond time-resolution, that the excited states of double helices are substantially longer-lived than those of the monomeric chromophores. The origin of this effect (excimers, excitonic states...) is under discussion (28,29). However, it cannot be excluded that the local organization in the double helix around a photoexcited base acts as a "solvation" inhibiting non-radiative deactivation channels.

(Chart 1)

A definitive assessment of the interplay between solvent and substituent effects requires comparative studies of different substituted nucleobases in different solvents. We have thus chosen to investigate uracil, thymine (5-methyluracil) and 5-fluorouracil in the polar and protic solvent methanol as well as in the two linear alcohols, ethanol and 1-propanol, and compare the findings with our recent results obtained for these compounds in aqueous solution (20) and the polar but aprotic solvent acetonitrile (21,22). This paper is mainly devoted to the analysis of steady-state absorption and fluorescence measurements at room temperature, providing a firmer basis for the interpretation of the time-resolved experiments that will be reported in a forthcoming study.

## ***Experiments and methods***

Uracil, thymine and 5-fluorouracil were purchased from Sigma Aldrich. Methanol, ethanol and 1-propanol (Merck UV spectroscopic grade), were used without further purification.

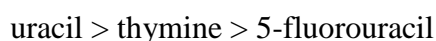
Absorption spectra were recorded with a Perkin Lamda 900 spectrophotometer using 1 mm, 2 mm and 1 cm quartz cells (QZS). Fluorescence spectra were recorded with a SPEX Fluorolog-2 spectrofluorometer, corrected for the spectral response of the detection system and smoothed by adjacent averaging. For the fluorescence measurements, 1 cm × 1 cm and 0.2 cm × 1 cm quartz cells (QZS) were used for dilute ( $10^{-5}$  -  $10^{-4}$  mol/dm<sup>3</sup>) and concentrated ( $\sim 10^{-3}$  mol/dm<sup>3</sup>) solutions, respectively. For dilute solutions the neat alcohol "signal" (Raman line, weak fluorescence from impurities) was not negligible compared to the solute fluorescence, wherefore, its contribution was subtracted from the fluorescence spectra. This was sufficient in the case of methanol, but in the case of ethanol and 1-propanol, the fluorescence of uracil and thymine turned out to be too weak to be corrected. Both absorption and fluorescence spectra were fitted with a simplified lognormal function (30) to obtain their characteristic parameters, peak position, width.

## Results

Absorption spectra of uracil, thymine and 5-fluorouracil in methanol are shown in Figure 1. Similar spectra were obtained for the other alcohols. The absorption maxima of the three studied compounds in different solvents are shown in Table 1.

(Figure 1)

The first noticeable feature concerns the relative energy of the first absorption band ( $S_0 \rightarrow S_1$ ) which corresponds to a  $\pi \rightarrow \pi^*$  transition. Confirming the results obtained in aqueous and acetonitrile solutions, in all three solvents the energy of the absorption maximum decreases in the order:



The absorption maximum of thymine is red-shifted about  $700\text{-}800\text{ cm}^{-1}$  compared to uracil in the three alcohols. The same trend is maintained in case of 5-fluorouracil, where the absorption maxima are shifted about  $1000\text{-}1100\text{ cm}^{-1}$  compared to uracil. Similar red shifts were observed in aqueous solution, and slightly larger shifts in acetonitrile.

This energy trend can be rationalized on the ground of the shape of the molecular orbitals involved in the  $S_0 \rightarrow S_1$  electronic transition (see Figure 2). The methyl group and the fluoro substituent in C5 position give an antibonding contribution to the frontier orbitals, thus making them less stable. Due to the different weights of the atomic orbitals of C5 and its substituents in the frontier orbitals, the antibonding contribution is more pronounced for the Highest Occupied Molecular Orbital (HOMO) than for the Lowest Unoccupied Molecular Orbital (LUMO). As a consequence, when substituents able to give rise to a hyperconjugative effect are present in C5 position the HOMO-LUMO energy gap decreases and the  $S_0 \rightarrow S_1$  transition is shifted towards lower energy with respect to uracil.

For a given compound, the position and the shape of the absorption spectrum in the examined alcohols varies very little ( $< 100\text{ cm}^{-1}$ ), see Table 1. Only for 5-fluorouracil could any visible, though very small, solvent effects be observed in the absorption spectrum. On the other hand, the absorption maxima in alcohols and water are red-shifted by  $\sim 500\text{ cm}^{-1}$  with respect to  $\text{CH}_3\text{CN}$ , and they are all strongly red-shifted with respect to the gas phase. We recall that the gas phase absorption maximum for uracil was reported by Clark et al. to be  $244\text{ nm}$  (31).

(Figure 2)

Our calculations show that both an increase in the solvent polarity and the formation of hydrogen bonds with solvent molecules slightly decreases the HOMO-LUMO energy gap, explaining why the absorption maximum in alcohols is red-shifted with respect to the gas

phase. The effect of the bulk solvent dielectric constant on the energy of the  $S_0 \rightarrow S_1$  transition can be explained by the larger dipole moment of the  $S_\pi$  state than the ground state ( $\Delta\mu \approx 1$  D according to the PCM/TD-PBE0/6-31G(d) calculations, see also ref. (23)). Thus, an increase of solvent polarity stabilizes the  $S_\pi$  state more than the ground state. The increase of the electron density on the carbonyl  $C_4O_8$  group is likely responsible of the  $S_\pi$  stabilization due to solute-solvent hydrogen bonds. Indeed PCM/PBE0/6-31G(d) and PCM/TD-PBE0/6-31G(d) geometry optimizations predict that the length of the hydrogen bond between the  $C_4O_8$  carbonyl group and the closest water molecule in the first solvation shell decreases upon photoexcitation (23).

The analysis of our experimental findings gives interesting insights on the relative importance of bulk properties and hydrogen bonding effects of the solvent on the absorption maxima. It indicates that, at least for polar solvents as those examined in the present study, the measured shift depends more on the hydrogen bonding ability of the solvent than on its bulk dielectric constant. As a matter of fact, the absorption maximum in acetonitrile, that cannot form hydrogen bond with the solute, is blue-shifted with respect to that found in alcoholic solution, even if the dielectric constant of acetonitrile (36.6) is larger than that of methanol (33), ethanol (24.3), and propanol (20.1). Analogously, the absorption maxima measured in hydrogen bonding solvents (water and alcohols) are extremely similar, despite the large difference of their dielectric constants.

Steady-state fluorescence spectra of uracil, thymine and 5-fluorouracil in methanol solution obtained for excitation at 255 nm are shown in Fig. 3. The spectra were analyzed as described above and the resulting spectral parameters are reported in Table 1. Unfortunately, the much weaker fluorescence of uracil and thymine was perturbed by fluorescent impurities in ethanol and propanol, rendering the analysis impossible. Also given in Table 1 are the Stokes shifts, i.e. the difference in wavenumbers between the absorption and the fluorescence maxima.

While the relative shifts of the absorption maxima of thymine and 5-fluorouracil with respect to uracil differ little from one solvent to another, the relative shifts of the fluorescence maxima vary more. In methanol, the relative red shifts of the fluorescence maxima with respect to uracil are  $\sim 1500$   $\text{cm}^{-1}$  for thymine and  $\sim 2500$   $\text{cm}^{-1}$  for 5-fluorouracil, while in water they are  $\sim 1400$  and  $\sim 1800$   $\text{cm}^{-1}$ , respectively. In acetonitrile, on the other hand, the relative shifts with respect to uracil are only  $\sim 300$  and  $\sim 900$   $\text{cm}^{-1}$ .

(Figure 3)

It is interesting to note that the Stokes shifts are not uniform from one base to another and depend strongly on the solvent. For example, in acetonitrile, uracil exhibits the largest Stokes

shift among the nucleobases investigated,  $\sim 8200 \text{ cm}^{-1}$ , whereas in methanol it has the smallest one,  $\sim 5700 \text{ cm}^{-1}$ . Although the behavior of thymine and 5FU are more uniform, they exhibit smaller Stokes shifts in methanol than in acetonitrile and in water.

This puzzling behavior is not surprising when dealing with ultrafast excited state decays and broad fluorescence spectra. The main part of the observed fluorescence spectrum could originate from the Franck-Condon region and the excited state population could be quenched before it reaches minimum of the potential energy surface of the  $S_\pi$  state. More precisely, the compound with the longest excited state lifetime exhibits a fully developed fluorescence redshift, since the wave packet on  $S_\pi$  surface remains in the minimum long enough for the emission from this region to dominate the steady-state fluorescence. Analogously, an underlying dark excited state in the FC region could provide an additional decay route, decreasing the part of the wave packet on  $S_\pi$  reaching the excited state minimum. Consequently, the relative weight of emission from the FC region in the fluorescence spectra would increase. Moreover, in the FC region only the fast (electronic) solvent degrees of freedom are at equilibrium with the excited state electron density ( $\rho_{S_\pi}$ ), while during the motion on the excited state surface also the slow solvent degrees of freedom start to be fully equilibrated with  $\rho_{S_\pi}$  (32). It is clear that those processes can modulate the fluorescence energy, since solvent equilibration stabilizes the excited state and increases the Stokes shift. The presence of strong solute-solvent interactions (such as hydrogen bonds) can slow down solvent equilibration, since a rearrangement of the solvation shell is required (23). This could explain the experimental finding that uracil exhibits a larger Stokes shift in acetonitrile than in water, because solvent equilibration is expected to be faster in the former solvent.

To resume, all the studied compounds exhibit rather large Stokes shifts in methanol ( $\sim 1 \text{ eV}$ ,  $\sim 8 \times 10^3 \text{ cm}^{-1}$ ), as found previously in water (20) and acetonitrile (21). This suggests significant changes of the excited state geometry in all three solvents. Indeed, PCM/TD-PBE0/6-31G(d) excited state geometry optimizations (20,21), show that the main features of the  $S_\pi$  minimum do not depend on the solvent. At this minimum, the pyrimidine ring exhibits a "boat-like" conformation, while some bond lengths undergo relevant changes with respect to the ground state equilibrium geometry. In line with the bonding/antibonding character of HOMO and LUMO with respect to these bonds, calculations on uracil and 5-fluorouracil indicate that the  $C_4C_5$  and  $C_4O_8$  bond lengths decreases and increases by  $\sim 0.03 \text{ \AA}$  respectively (23). Most importantly, the  $C_5C_6$  bond is significantly elongated by  $\sim 0.10 \text{ \AA}$ .

## Concluding remarks

In this paper we have reported a comparison of the steady-state absorption and fluorescence spectra of three representative uracil derivatives (uracil, thymine and 5-fluorouracil) in different solvents (water, acetonitrile, methanol, ethanol and 1-propanol), differing in polarity and hydrogen bonding ability. On the basis of the present results as well as previous experimental and computational studies performed for these compounds in water (20) and acetonitrile (21), we have provided a more complete picture of the solvent effects on the absorption and fluorescence properties of pyrimidine nucleobases and additional hints on the mechanism of the excited state deactivation.

For all the compounds examined, the absorption spectra are significantly red-shifted in solution as compared to gas phase. This trend can be rationalized on the ground of the shape of the frontier orbitals of uracils and on the increase of the dipole moment upon electronic excitation. It is important to highlight that the dependence of the absorption spectra on the solvent dielectric constant seems to have reached a "plateau" already for  $\epsilon \geq 15$ , as shown by the very similar spectra obtained for propanol and water solutions. On the other hand, the possibility of forming solute-solvent hydrogen bonds appears to be a key factor, as shown by the blue shift of the absorption maximum observed for acetonitrile solution with respect to the alcohols and water. These results are in line with our previous considerations, based on theoretical calculations (21), about the role of intermolecular hydrogen bonds in modulating the interplay between dark and bright states and, consequently, the excited state lifetime of uracils. Given the prominent role of hydrogen bonding interactions, we could expect that the excited state lifetime of uracils in alcohols is similar to that found in water, and intermediate between that found in this latter solvent and in acetonitrile.

Significant Stokes shifts are observed for all the compounds in all the solvents examined, in line with the remarkable geometry changes associated with the electronic transition to the  $S_\pi$  state. On the other hand, no clear trend can be recognized for what concerns the relative value of the Stokes shifts exhibited by the uracils in different solvents. This result, obviously related to the complexity of the excited state decay mechanism, suggests that different effects are operative in the fluorescence quenching and confirms that solvent can affect this process. We have previously shown that the interplay between solvent and substituent effect modulates the accessibility of an additional decay channel through the  $S_n$  dark state. The comparative analysis of the fluorescence spectra of uracils, presented here, provide additional, though indirect, support for this hypothesis.



## ***Acknowledgements***

The authors express their gratitude to CNRS for financial support within the framework of the European CERC3 (Chairmen of the European Research Councils) program "Photochemistry of Nucleic Acids"

**Table 1.** Characteristic parameters of the first absorption and fluorescence bands of uracil and its derivatives in water, acetonitrile and different alcohols. The peak frequency  $\nu_{\max}$ , the peak wavelength  $\lambda_{\max}$ , and the Stokes shift  $\Delta\nu$  (absorption maximum minus fluorescence maximum)

solute	solvent	absorption		fluorescence		Stokes shift
		$\nu_{\max}$ ( $10^3 \text{ cm}^{-1}$ )	$\lambda_{\max}$ (nm)	$\nu_{\max}$ ( $10^3 \text{ cm}^{-1}$ )	$\lambda_{\max}$ (nm)	$\Delta\nu$ ( $10^3 \text{ cm}^{-1}$ )
uracil	water <sup>a</sup>	38.6	259	31.3	312	7.3
	methanol	38.6	259	32.9	304	5.7
	ethanol	38.6	259	- <sup>c</sup>	- <sup>c</sup>	- <sup>c</sup>
	1-propanol	38.6	259	- <sup>c</sup>	- <sup>c</sup>	- <sup>c</sup>
	acetonitrile <sup>b</sup>	39.1	256	30.9	311	8.2
thymine	water <sup>a</sup>	37.8	265	29.9	329	7.9
	methanol	37.9	264	31.4	318	6.5
	ethanol	37.8	264	- <sup>c</sup>	- <sup>c</sup>	- <sup>c</sup>
	1-propanol	37.8	265	- <sup>c</sup>	- <sup>c</sup>	- <sup>c</sup>
	acetonitrile <sup>b</sup>	38.2	261	30.6	315	7.6
5-fluorouracil	water <sup>a</sup>	37.6	266	29.5	335	8.1
	methanol	37.6	266	30.4	328	7.2
	ethanol	37.6	266	30.2	331	7.4
	1-propanol	37.5	266	30.1	332	7.4
	acetonitrile <sup>b</sup>	37.9	264	30.0	322	7.9

a) From reference (20), b) from reference (21), c) the fluorescence of uracil and thymine was perturbed by fluorescent impurities in ethanol and propanol

## ***Figure captions***

Chart 1. The schematic structure of the substituted uracils studied in the present work, where R denotes the different substituents on the 5-position.

Figure 1. Absorption spectra of uracil, thymine and 5-fluorouracil in methanol solution.

Figure 2. Schematic drawing of the HOMO (a) and LUMO (b) of uracil, and of the HOMO (c) and LUMO (d) of 5-fluorouracil, according to PCM-PBE0/6-31G(d) calculations in aqueous solution.

Figure 3. Fluorescence spectra of uracil, thymine and 5-fluorouracil in methanol solution.

## References

1. Crespo-Hernandez, C. E., B. Cohen, P. M. Hare and B. Kohler (2004) Ultrafast Excited-State Dynamics in Nucleic Acids. *Chem. Rev.* **104**, 1977-2020.
2. Improta, R. and V. Barone (2004) Absorption and Fluorescence Spectra of Uracil in the Gas Phase and in Aqueous Solution: A TD-DFT Quantum Mechanical Study. *J. Am. Chem. Soc.* **126**, 14320-14321.
3. Matsika, S. (2004) Radiationless Decay of Excited States of Uracil through Conical Intersections. *J. Phys. Chem. A* **108**, 7584-7590.
4. Matsika, S. (2005) Three-state conical intersections in nucleic acid bases. *J. Phys. Chem. A* **109**, 7538-7545.
5. Zgierski, M. Z., S. Patchkovskii, T. Fujiwara and E. C. Lim (2005) On the Origin of the Ultrafast Internal Conversion of Electronically Excited Pyrimidine Bases. *J. Phys. Chem. A* **109**, 9384 - 9387.
6. Ismail, N., L. Blancafort, M. Olivucci, B. Kohler and M. A. Robb (2002) Ultrafast decay of electronically excited singlet cytosine via a  $\pi, \pi^*$  to  $n_o, \pi^*$  state switch. *J. Am. Chem. Soc.* **124**, 6818-6819.
7. Blancafort, L. and M. A. Robb (2004) Key Role of a Threefold State Crossing in the Ultrafast Decay of Electronically Excited Cytosine. *J. Phys. Chem. A* **108**, 10609-10614.
8. Zgierski, M. Z., S. Patchkovskii and E. C. Lim (2005) Ab initio study of a biradical radiationless decay channel of the lowest excited electronic state of cytosine and its derivatives. *J. Chem. Phys.* **123**, 081101-081104.
9. Blancafort, L., B. Cohen, P. M. Hare, B. Kohler and M. A. Robb (2005) Singlet excited-state dynamics of 5-fluorocytosine and cytosine: an experimental and computational study. *J. Phys. Chem. A* **109**, 4431-4436.
10. Marian, C. M. (2005) A new pathway for the rapid decay of electronically excited adenine. *J. Chem. Phys.* **122**, 104314.
11. Marian, C., D. Nolting and R. Weinkauff (2005) The electronic spectrum of protonated adenine: Theory and experiment. *Phys. Chem. Chem. Phys.* **7**, 3306 - 3316.
12. Nielsen, S. B. and Theis I. Sølling (2005) Are Conical Intersections Responsible for the Ultrafast Processes of Adenine, Protonated Adenine, and the Corresponding Nucleosides? *ChemPhysChem* **6**, 1276-1281.
13. Perun, S., A. L. Sobolewski and W. Domcke (2005) Photostability of 9H-adenine: mechanisms of the radiationless deactivation of the lowest excited singlet states. *Chem. Phys.* **313**, 107-112.
14. Perun, S., A. L. Sobolewski and W. Domcke (2005) Ab Initio Studies on the Radiationless Decay Mechanisms of the Lowest Excited Singlet States of 9H-Adenine. *J. Am. Chem. Soc.* **127**, 6257 -6265.
15. Clark, L. B. and I. Tinoco Jr. (1965) Correlations in the ultraviolet spectra of the purine and pyrimidine bases. *J. Am. Chem. Soc.* **87**, 11-15.
16. Becker, R. S. and G. Kogan (1980) Photophysical properties of nucleic acid components. 1. The pyrimidines: thymine, uracil, N,N-dimethyl derivatives, and thymidine. *Photochem. Photobiol.* **31**, 5-13.
17. Georghiou, S. and L. S. Gerke (1999) Excited-state properties of thymidine and their relevance to its heterogeneous emission in double-stranded DNA. *Photochem. Photobiol.* **69**, 646-652.
18. Häupl, T., C. Windolph, T. Jochum, O. Brede and R. Hermann (1997) Picosecond fluorescence of nucleic acid bases. *Chem. Phys. Lett.* **280**, 520-524.

19. Canuel, C., M. Mons, F. Piuzzi, B. Tardivel, I. Dimicoli and M. Elhanine (2005) Excited states dynamics of DNA and RNA bases: Characterization of a stepwise deactivation pathway in the gas phase. *J. Chem. Phys.* **122**, 0743161-0743166.
20. Gustavsson, T., A. Banyasz, E. Lazzarotto, D. Markovitsi, G. Scalmani, M. J. Frisch, V. Barone and R. Improta (2006) Singlet excited-state behavior of uracil and thymine in aqueous solution: a combined experimental and computational study of 11 uracil derivatives. *J. Am. Chem. Soc.* **128**, 607-619.
21. Gustavsson, T., N. Sarkar, E. Lazzarotto, D. Markovitsi, V. Barone and R. Improta (2006) Solvent effect on the singlet excited state dynamics of 5-fluorouracil in acetonitrile as compared to water. *J. Phys. Chem. B* **110**, 12843 - 12847.
22. Gustavsson, T., N. Sarkar, E. Lazzarotto, D. Markovitsi and R. Improta (2006) Singlet excited state dynamics of uracil and thymine derivatives. A femtosecond fluorescence upconversion study in acetonitrile. *Chem. Phys. Lett.* **429**, 551-557.
23. Santoro, F., V. Barone, T. Gustavsson and R. Improta (2006) Solvent effect on the singlet excited state lifetimes of nucleic acid bases: a computational study of 5-fluorouracil and uracil in acetonitrile and water. *J. Am. Chem. Soc.* **128**, 16312-16322.
24. Hare, P. M., C. E. Crespo-Hernandez and B. Kohler (2006) Solvent-Dependent Photophysics of 1-Cyclohexyluracil: Ultrafast Branching in the Initial Bright State Leads Nonradiatively to the Electronic Ground State and a Long-Lived  $^1n\pi^*$  State. *J. Phys. Chem. B* **110**, 18641-18650.
25. Markovitsi, D., A. Sharonov, D. Onidas and T. Gustavsson (2003) The effect of molecular organisation in DNA oligomers studied by femtosecond fluorescence spectroscopy. *ChemPhysChem* **3**, 303-305.
26. Markovitsi, D., D. Onidas, T. Gustavsson, F. Talbot and E. Lazzarotto (2005) UV interaction with DNA bases: collective behaviour of excited states in double helices. *J. Am. Chem. Soc.* **127**, 17130 -17131.
27. Crespo-Hernández, C. E., B. Cohen and B. Kohler (2005) Base stacking controls excited-state dynamics in AT DNA. *Nature* **436**, 1141-1144.
28. Markovitsi, D., F. Talbot, T. Gustavsson, D. Onidas, E. Lazzarotto and S. Marguet (2006) Complexity of excited state dynamics in DNA. *Nature* **441**, E7.
29. Crespo-Hernandez, C. E., B. Cohen and B. Kohler (2006) Molecular spectroscopy: Complexity of excited-state dynamics in DNA - Reply. *Nature* **441**, E8-E8.
30. Siano, D. B. and D. E. Metzler (1969) Band shapes of the electronic spectra of complex molecules. *J. Chem. Phys.* **51**, 1856-1861.
31. Clark, L. B., G. G. Peschel and I. Tinoco (1965) Vapor Spectra and Heats of Vaporization of Some Purine and Pyrimidine Bases. *J. Phys. Chem.* **69**, 3615 - 3618.
32. Horng, M. L., J. A. Gardecki, A. Papazyan and M. Maroncelli (1995) Subpicosecond measurements of polar solvation dynamics: Coumarin 153 revisited. *J. Phys. Chem.* **99**, 17311-17337.