

Intersystem crossing in thiobases proceeds by a dark intermediate state

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Abstract. 4-thiouracil (4TU) is studied by transient absorption spectroscopy employing sub-20 fs UV-pulses and hybrid QM(CASPT2) / MM(AMBER) computations (static and dynamic), evidencing that, along the photoexcited relaxation pathway, intersystem crossing originates from a dark intermediate state.

Thiobases are DNA or RNA nucleobases where an exocyclic carbonyl oxygen is replaced by a sulfur atom [1]. Thiation induces a red-shift in the absorption spectrum of the thiobases to the 280–400 nm range and causes also a dramatic change in the photophysics with respect to the canonical nucleobases [2,3]: while in DNA/RNA monomers repopulation of the ground state occurs on an ultrafast timescale through a high rate of internal conversion $S_1 \rightarrow S_0$ mediated by a conical intersection (CI), the major relaxation pathway in thiobases is via the population of triplet states via an ultrafast intersystem crossing (ISC) [4]. Thiobases have attracted much interest in the last few years due to their biological relevance and also the many possibilities of photo-therapeutic applications [1]. In spite of that, their ultrafast deactivation mechanisms are not yet fully understood. In this work we combine UV transient absorption spectroscopy featuring sub-20-fs time resolution in the UV with hybrid quantum mechanics/molecular mechanics (QM/MM) state-of-the-art computational tools to analyse the ultrafast photoinduced dynamics in 4TU. We provide the first experimental evidence that ISC occurs from a dark state of $n\pi^*$ character, that is intermediately populated from the bright $\pi\pi^*$ state excited by near-UV light [5].

Pump-probe measurements were carried out starting from a Ti:Sapphire laser producing 100-fs pulses at 800 nm wavelength and 1-kHz repetition rate. Sub-20 fs UV pump pulses (320–360 nm) were generated by frequency up-conversion of a visible NOPA with the fundamental beam (Figure 2b) [6]. The probe pulses (250–700nm) were obtained by white

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light generation in a CaF_2 plate [7]. The 4TU solution was prepared in a PBS solution at pH 7.4 with concentration of 4 mM.

The QM/MM scheme couples a state-of-the-art ab-initio multireference dynamically correlated description (CASPT2) of the 4TU with an explicit classical atomistic model (AMBER force field) of the solvent [8]. A topological analysis of the populated excited states (stationary points, CIs and ISCs) is supplemented by mixed-quantum classical molecular dynamics simulations realized through a limited ensemble of 30 room temperature trajectories employing Tully's fewest switches surface hopping algorithm run for 200 fs with a time step of 1.0 fs at the SS-RASPT2/SA-3-RASSCF(12,9|2,4) level of theory comprising all valence π -orbitals, the lone pair of the sulfur and four extra-valence virtual orbitals with up to double excitations. The state averaging covers the ground, the lowest (dark) $\pi\pi^*$ (S_1) and the lowest (bright) $\pi\pi^*$ states (S_2), the dynamics was initiated in S_2 .

A 2D map of the differential transmission ($\Delta T/T$) is displayed in Figure 1a. Panel 1b shows the dynamics for selected wavelengths. At early times we observe three signatures that we attribute to the bright photoexcited $\pi\pi^*$ state: the ground state bleaching (GSB, below 360 nm) a stimulated emission (SE) signal peaking at 400 nm and a photo-induced absorption (PA) band above 500 nm. The SE band displays a first ultrafast decay on the sub-100-fs timescale and subsequently a second PA band peaking at 550 nm grows in on a ≈ 500 -fs timescale. This band has been observed in previous studies with lower, 200-fs time resolution [4] and has been assigned to triplet-triplet absorption, thus providing a fingerprint of the ISC process. Our data clearly show that ISC does not occur directly from the photoexcited bright state but is mediated by an intermediate dark state. A global analysis of the data confirms this scenario and reveals time constants of 78 fs and 201 fs for the $\pi\pi^*$ SE decay and triplet PA formation. Furthermore, in the 2D map we can also clearly observe the ultrafast impulsively excited vibrational motions with two frequencies: 400 cm^{-1} and 680 cm^{-1} which are in good agreement with the calculated: a breathing mode with frequency of 457 cm^{-1} and in two hydrogen-out-of-plane bending modes with frequencies 697 cm^{-1} and 784 cm^{-1} .

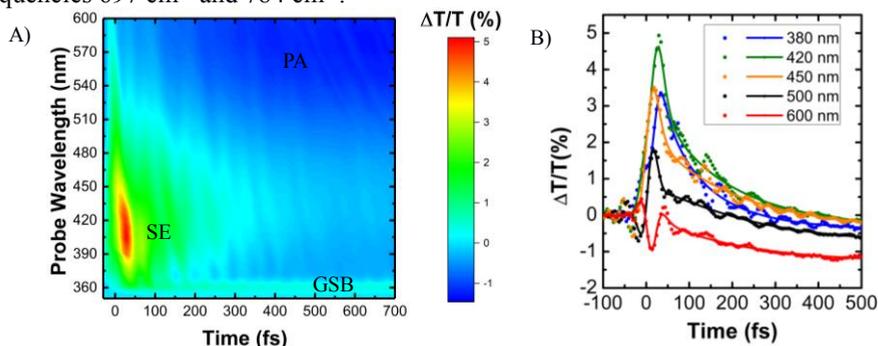


Fig 1. Transient absorption measurements: a) 2D map, b) Transient dynamics at selected wavelengths, experimental data in circles and fittings in solid lines.

By supplementing the experiments with topology analysis and molecular dynamics simulations we provide a complete picture of the photo-induced events. Figure 2b shows the experimental (black) and calculated (green, blue and magenta) linear absorption spectra [9] together with the pump pulse spectrum (yellow-dashed area). The good agreement allows to assign the underlying transitions to the lowest bright $\pi\pi^*$ states (bands A, B and C in Figures 2a and 2b). The pump pulse populates the lowest $\pi\pi^*$ state (S_2 in the Franck-

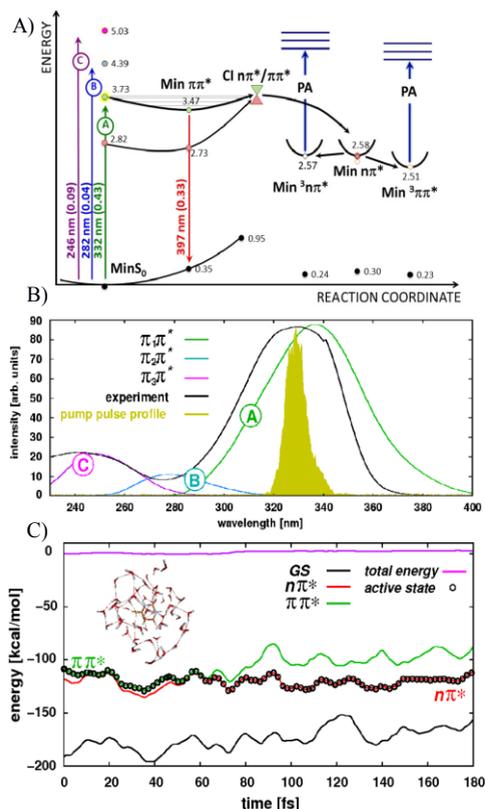


Fig 2. Computational results: a) Schematic picture of the ES deactivation processes; b) linear absorption spectrum of the lowest three bright $\pi\pi^*$ transitions (green, blue and magenta), experimental spectrum (black), pump pulse spectrum (yellow dashed); c) a representative molecular dynamics trajectory.

Condon (FC) region, green circles in Figure 2a). From there the decay path leads to a sloped CI seam with the dark $n\pi^*$ state populated upon thermal fluctuations. We find that the $n\pi^*$ state minimum ($\text{Min } n\pi^*$) is isoenergetic with two triplet states, of $n\pi^*$ and of $\pi\pi^*$ nature, respectively, allowing for the effective population of the triplet manifold. The limited ensemble of room temperature trajectories (example trajectory shown in Figure 2c) shows that ultrafast $\pi\pi^* \rightarrow n\pi^*$ transition is facilitated when thermal energy is inserted in the system despite the pronounced $\pi\pi^*/n\pi^*$ energy gap of 1 eV at equilibrium (Figure 2a). $\pi\pi^* \rightarrow n\pi^*$ population decay is found to occur with a time constant of 67.5 fs, in agreement with the 78 fs time constant extracted from the global analysis. The longer time constant of 201 fs is assigned to the build up of PA from the ${}^3\pi\pi^*$ triplet state, supported by our computations revealing higher lying states in the triplet manifold absorbing around 550 nm (see PA label in Figure 1a and in Figure 2b). We note that the ${}^1n\pi^*$ state is completely dark in the probed spectral window as it exhibits no SE or PA.

In conclusion, the combination of high time resolution transient spectroscopy in the UV range with ab initio numerical modelling allowed us to capture the dark intermediate state in the relaxation path to the triplet state in the 4TU.

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