

Excited State Proton Transfer Processes of DHICA Resolved – from Sub-Picoseconds to Nanoseconds

Alice Corani¹, Alessandro Pezzella², Torbjörn Pascher¹, Thomas Gustavsson³, Dimitra Markovitsi³, Annemarie Huijser^{1,4}, Marco d'Ischia² and Villy Sundström^{1*}

¹Department of Chemical Physics, Lund University, Box 124, 22100 Lund (Sweden)

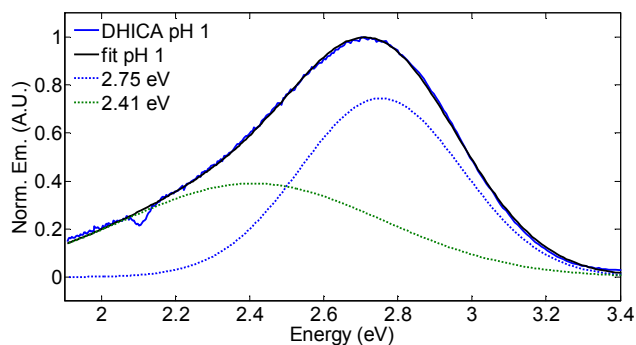
²Department of Chemical Sciences, University of Naples Federico II Via Cintia, 80126 Naples (Italy)

³Laboratoire Francis Perrin CEA/DSM/IRAMIS/SPAM-CNRS URA 2453, CEA/Saclay, F-91191 Gif-sur-Yvette (France)

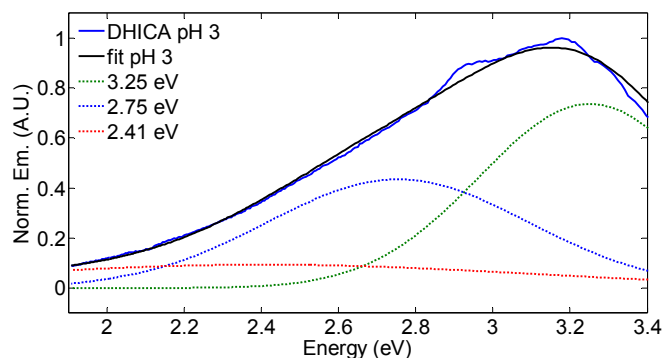
⁴Permanent address: Optical Sciences group, MESA+ Institute for Nanotechnology, University of Twente, P.O: Box 217 7500 AE, (The Netherlands)

*Villy.Sundstrom@chemphys.lu.se

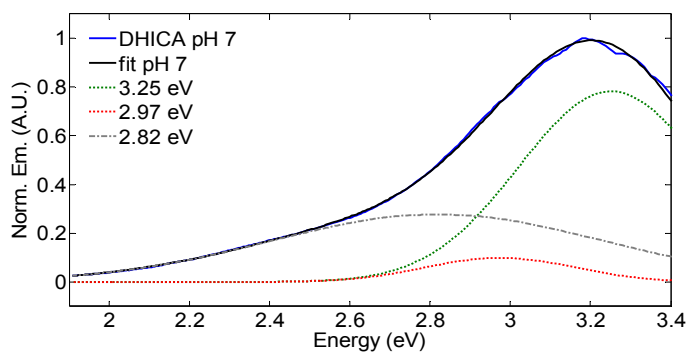
SI1-A: Fluorescence spectrum of DHICA at pH 1 fitted with two Gaussian bands, 2.75 eV (450 nm) and 2.41 eV (515 nm, to account for asymmetry of bands due to vibrational structure).



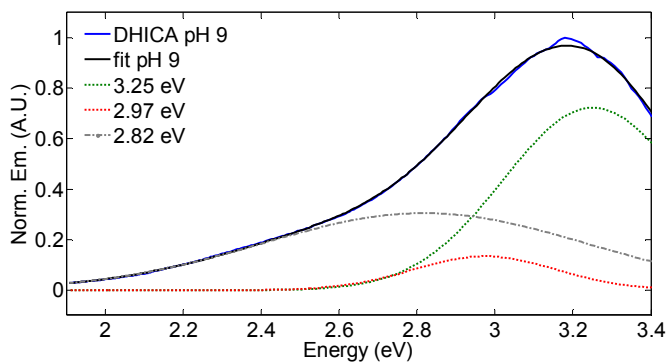
SI1-B: Fluorescence spectrum of DHICA at pH 3 fitted with three Gaussian bands, 3.25 eV (381 nm), 2.75 eV (450 nm) and 2.41 eV (515 nm, to account for asymmetry of bands due to vibrational structure).



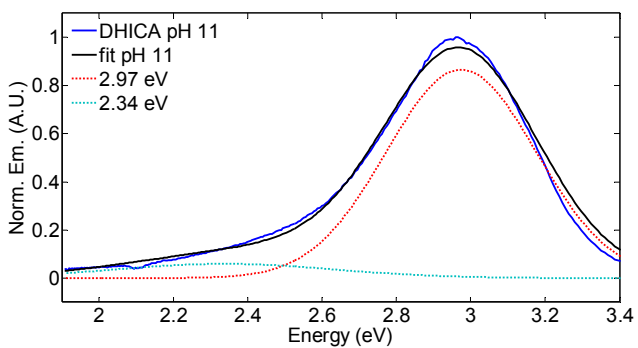
SI1-C: Fluorescence spectrum of DHICA at pH 7 fitted with three Gaussian bands, 3.25 eV (381 nm), 2.97 eV (416 nm) and 2.82 eV (440 nm), to account for asymmetry of bands due to vibrational structure).



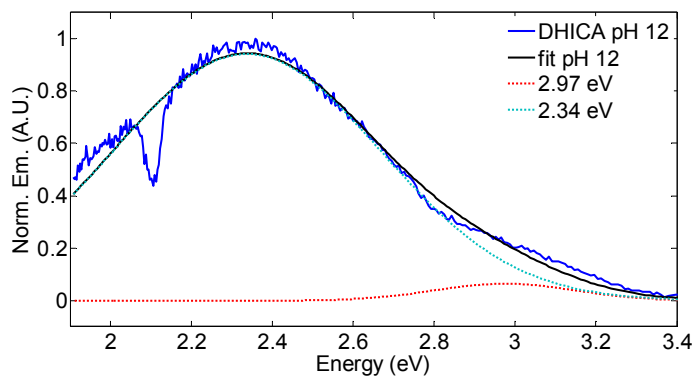
SI1-D: Fluorescence spectrum of DHICA at pH 9 fitted with three Gaussian bands 3.25 eV (381 nm), 2.97 eV (416 nm) and 2.82 eV (440 nm), to account for asymmetry of bands due to vibrational structure).



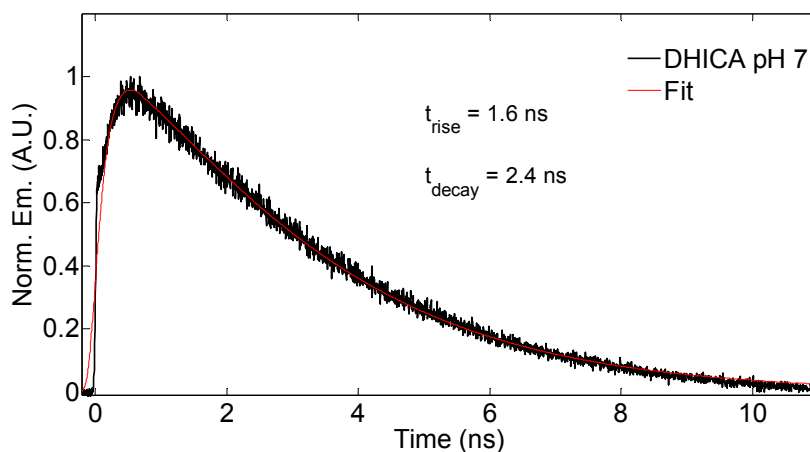
SI1-E: Fluorescence spectrum of DHICA at pH 11 fitted with two Gaussian bands 2.97 eV (416 nm) and 2.34 eV (530 nm).



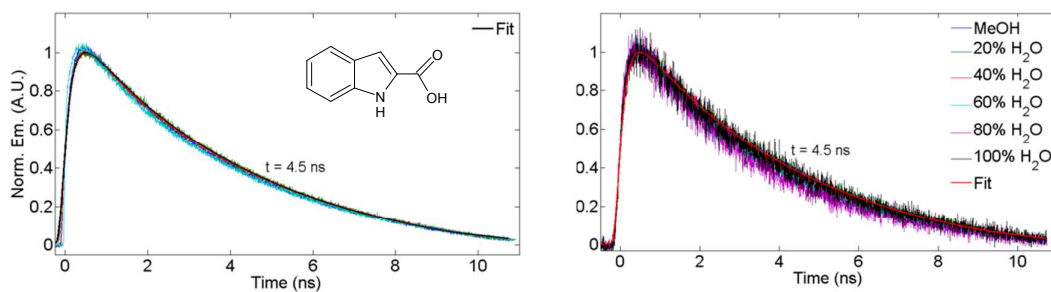
SI1-F: Fluorescence spectrum of DHICA at pH 12 fitted with two Gaussian bands 2.97 eV (416 nm) and 2.34 eV (530 nm).



SI2: TCSPC fluorescence decay of DHICA at pH 7 with excitation at 267 nm and emission 550 nm with fit yielding a 1.6 ns risetime and 2.4 ns decay (see also ref. 6).



SI3: Fluorescence decay kinetics of indole-2-carboxylic acid (ICA). Left panel: in methanol with fluorescence measured from 350 nm to 500 nm (50 nm step); right panel: in various water/methanol mixtures with fluorescence measured at 400 nm. All the fluorescence decays exhibit a decay of $\sim 4.5 \text{ ns}$



Experiment

Sample: DHICA was prepared as described elsewhere¹, ICA, methanol spectroscopic grade 99.5% and sodium phosphate buffer 0.1 M pH 7 and pH 3 were bought from Sigma Aldrich and used without further purification. The sample solutions at different pH were prepared by addition of NaOH 0.1 M and 0.1 M HCl to distilled water up to the desired pH. Absorption spectra were recorded with an Agilent spectrophotometer, and a SPEX Fluorolog was used to record fluorescence spectra with an excitation wavelength of 267 nm. Fluorescence decays were measured by either time correlated single photon counting (TCSPC), a Hamamatsu streak camera², or fluorescence up-conversion^{3,4}.

For the TCSPC measurements the detection system was a PicoHarp 300 (Picoquant) and frequency tripled (267 nm, 1-5 pJ) 150 fs, 800 nm, Ti:Sa pulses at a repetition rate of 82 MHz (Spectra-Physics, Tsunami) were used to excite the sample. The excitation beam was focused on the sample in a 1 mm quartz cuvette using a 100 mm focal length quartz lens. The fluorescence was collected through band pass filters at magic angle using two 1-inch diameter 50 mm focal length glass objective lenses and focused on an APD PDM series detector (Micro Photon Devices). The FWHM of the band pass filters was 10 nm and the temporal response function had a FWHM of ~350 ps.

Streak camera detection was performed as previously described² with a Hamamatsu C6860 device coupled to a Chromex spectrograph and using the same frequency tripled Ti:Sa fs pulses as for the TCSPC measurements. For these measurements the sample solution was kept under N₂ in a rotating quartz cuvette with an optical path length 2 mm.

The fluorescence up-conversion measurements were performed with a setup previously described in^{3,4}. Briefly, frequency-tripled pulses at 267 nm from a mode-locked Ti-sapphire laser (MIRA, Coherent) were used. The average excitation power was set to 25 mW. The fluorescence from the sample was collected with parabolic mirrors and mixed with the residual fundamental, serving as gating pulse, in a 0.5 mm type I BBO crystal to generate the sum-frequency light. This was spectrally filtered through a monochromator and detected by a photomultiplier in single-photon counting mode. The spectral resolution was approximately 5 nm. Fluorescence decays were measured by means of motorized delay-stage installed on the optical path of the gating pulse. Parallel and perpendicular excitation-detection polarization conditions were used by adjusting the polarization of the excitation beam with a zero-order half-wave plate. Total fluorescence kinetics were constructed from $I_{\text{par}}(t) + 2 \times I_{\text{perp}}(t)$. The solution was kept in a 1 mm rotating cell equipped with quartz windows.

All time-resolved fluorescence measurements were performed at room temperature ($20 \pm 1^\circ\text{C}$), under aerated conditions.

The measured fluorescence kinetics were fitted using Matlab R2011a software. Exponential modeling was performed to extract lifetimes. To fit the FU data Gaussian deconvolution was performed and the FWHM of the apparatus response was fixed to 346 fs after averaging the obtained FWHM after fitting of the experimental data. For the TCSPC measurement the measured response function of was used to deconvolute the decays.

Reference List

1. Edge, R.; d'Ischia, M.; Land, E. J.; Napolitano, A.; Navaratnam, S.; Panzella, L.; Pezzella, A.; Ramsden, C. A.; Riley, P. A. Dopaquinone Redox Exchange with Dihydroxyindole and Dihydroxyindole Carboxylic acid. *Pigment Cell Research* **2006**, *19*, 443-450.
2. Huijser, A.; Pezzella, A.; Hannestad, J. K.; Panzella, L.; Napolitano, A.; d'Ischia, M.; Sundstrom, V. UV-Dissipation Mechanisms in the Eumelanin Building Block DHICA. *Chemphyschem* **2010**, *11*, 2424-2431.
3. Gustavsson, T.; Sharonov, A.; Markovitsi, D. Thymine, Thymidine and Thymidine 5'-Monophosphate Studied by Femtosecond Fluorescence Upconversion Spectroscopy. *Chemical Physics Letters* **2002**, *351*, 195-200.
4. Gustavsson, T.; Improtà, R.; Banyasz, A.; Vaya, I.; Markovitsi, D. The Effect of Methylation on the Excited State Dynamics of Aminouracils. *Journal of Photochemistry and Photobiology A-Chemistry* **2012**, *234*, 37-43.