Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Excited state deactivation mechanisms in Shikonin rationalized from its naphthoquinone parent structures

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Synthesis of 5,8 – diacetoxy-1,4-naphthoquinone (DiAc)

Characterization of the new synthetized compound, Diac, is presented. The H, C, HSQC spectra are shown in Figures SI 1 – 3 and the and ESI-MS in Figure SI4.

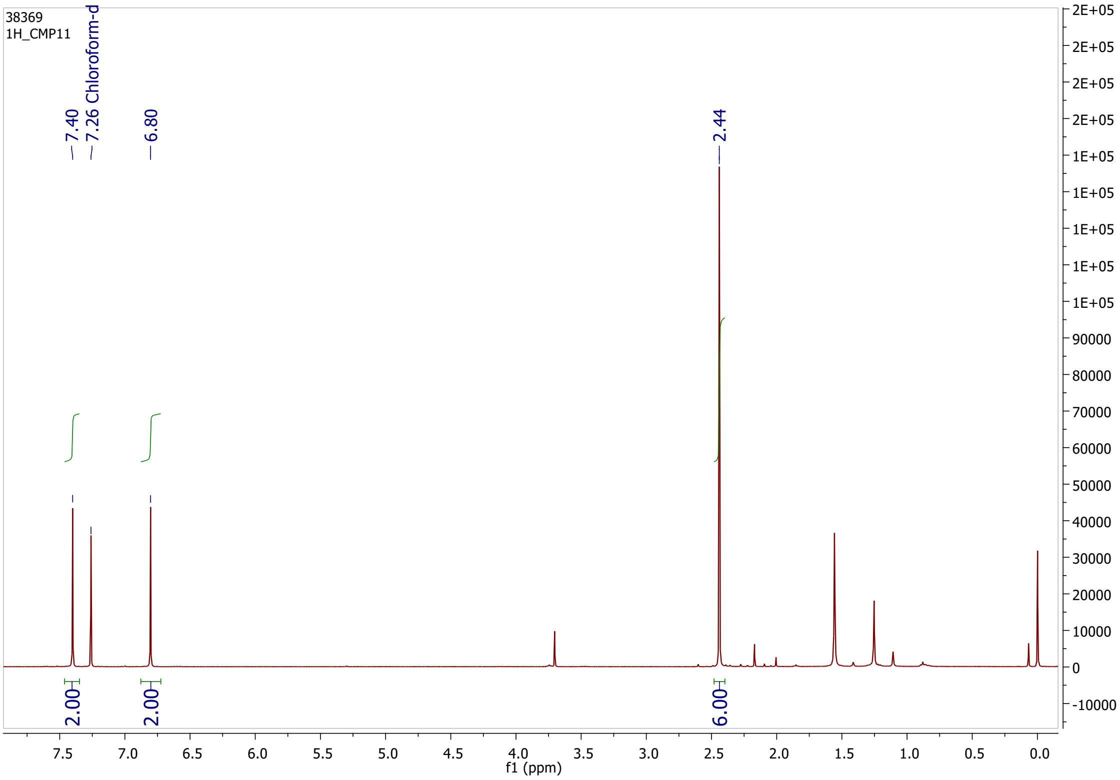

Figure SI 1. ^{1}H NMR spectrum of the DiAc.

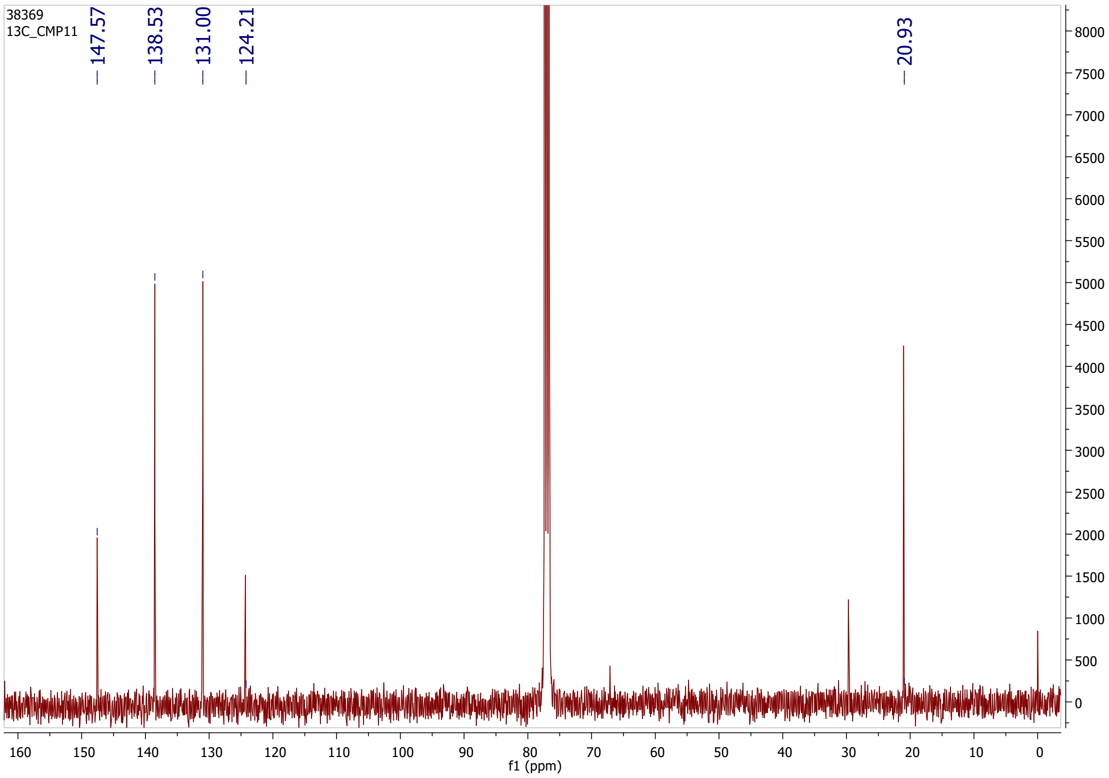

Figure SI 2. ^{13}C spectrum of the DiAc.

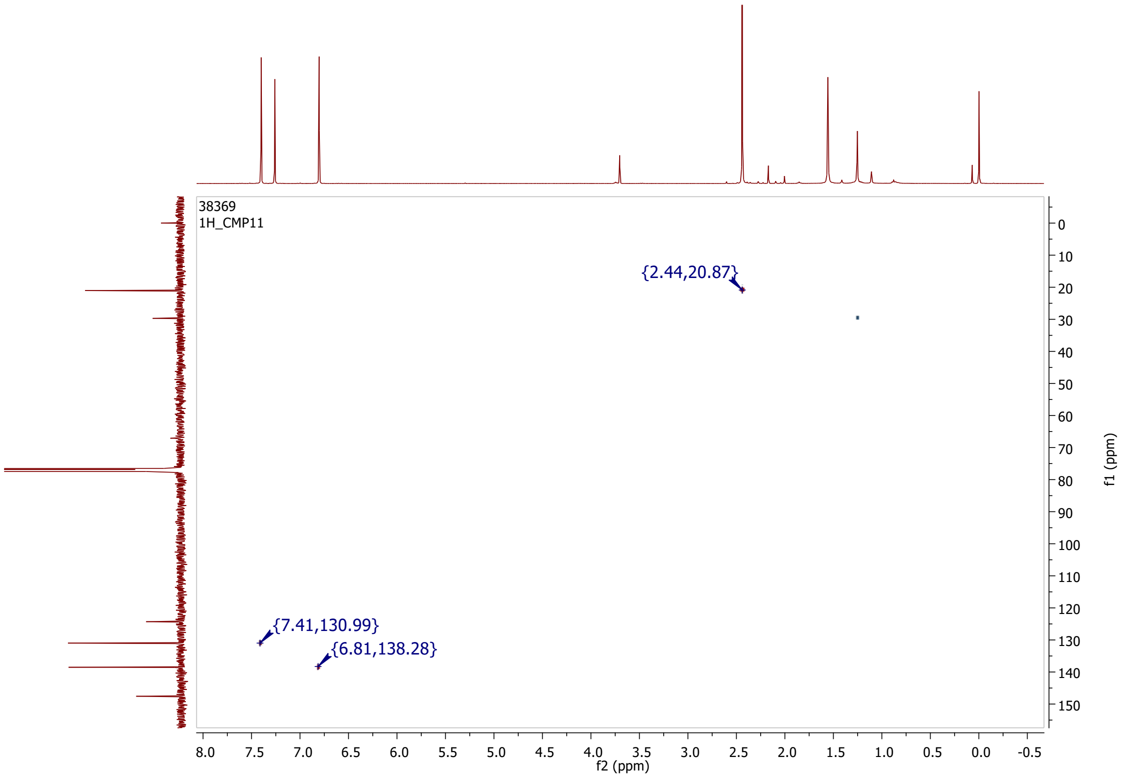

Figure SI 3. HSQC spectrum of the DiAc.

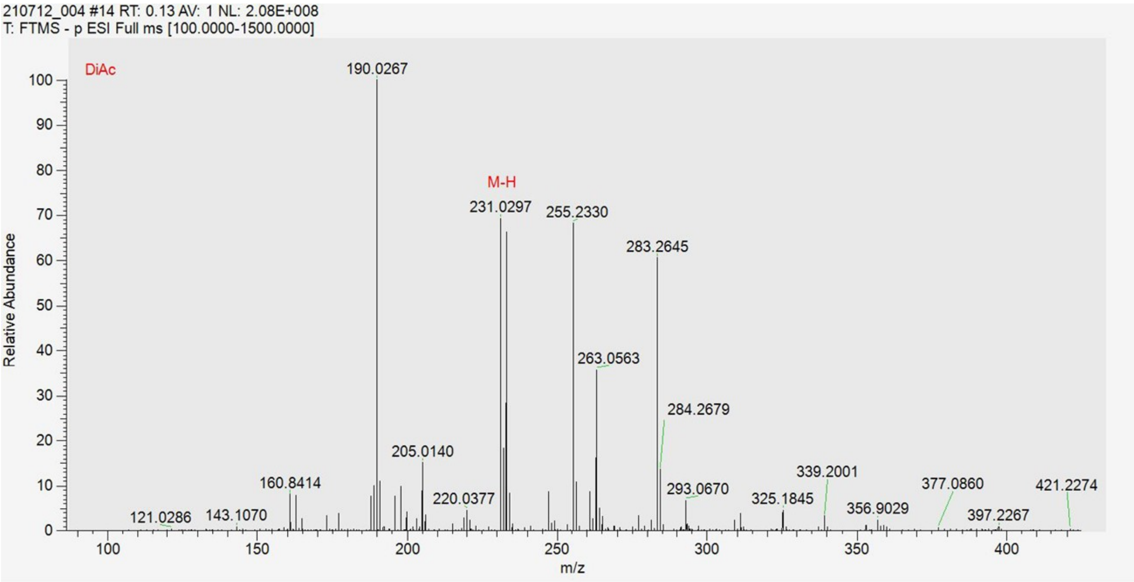

Figure SI 4. ESI-MS spectra of the DiAc.

Absorption and fluorescence spectra

The absorption, fluorescence emission and excitation spectra of the naphthoquinone derivatives in acetonitrile (ACN), together with the dependence of the fluorescence emission intensity with the excitation wavelength is presented in Figure SI5.

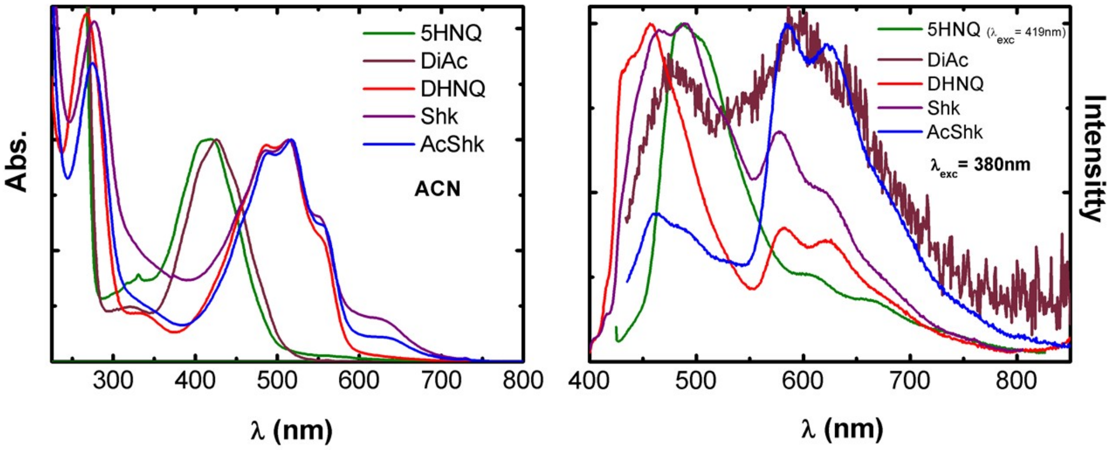

Figure SI 5. Normalized room temperature absorption and fluorescence emission spectra for the naphthoquinone derivatives in acetonitrile solutions collected with excitation at 380 nm (except for 5HNQ with λ_{exc}= 419 nm) with concentrations in the $1.5\text{x}10^{-6}$ M – $2.3\text{x}10^{-6}$ M range.

The fluorescence emission spectra of Shk in cyclohexane, obtained with different excitation wavelengths is presented in Figure SI 6, clearly showing the nature of the two band emission. For all the naphthoquinone derivatives the absorption and the fluorescence excitation and emission spectra in acetonitrile are given in Figure SI 7, and the emission spectra in cyclohexane in Figures SI 8 – 10. Figure SI 11 depicts the room temperature absorption spectra of 5HNQ and DiAc together with the fluorescence excitation spectra of Shk. Spectroscopic data in ACN is summarized in Table SI 1.

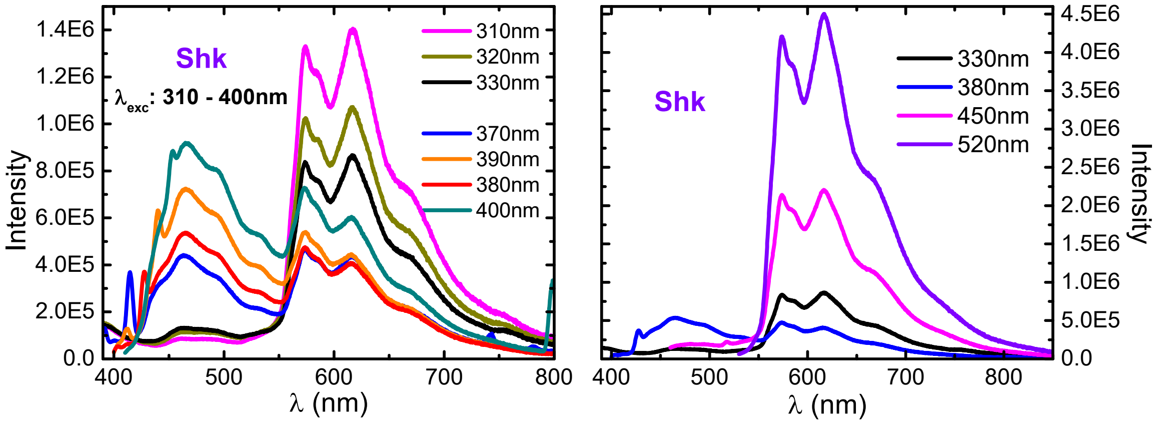

Figure SI 6. Fluorescence emission spectra of **Shk** at different excitation wavelengths in cyclohexane.

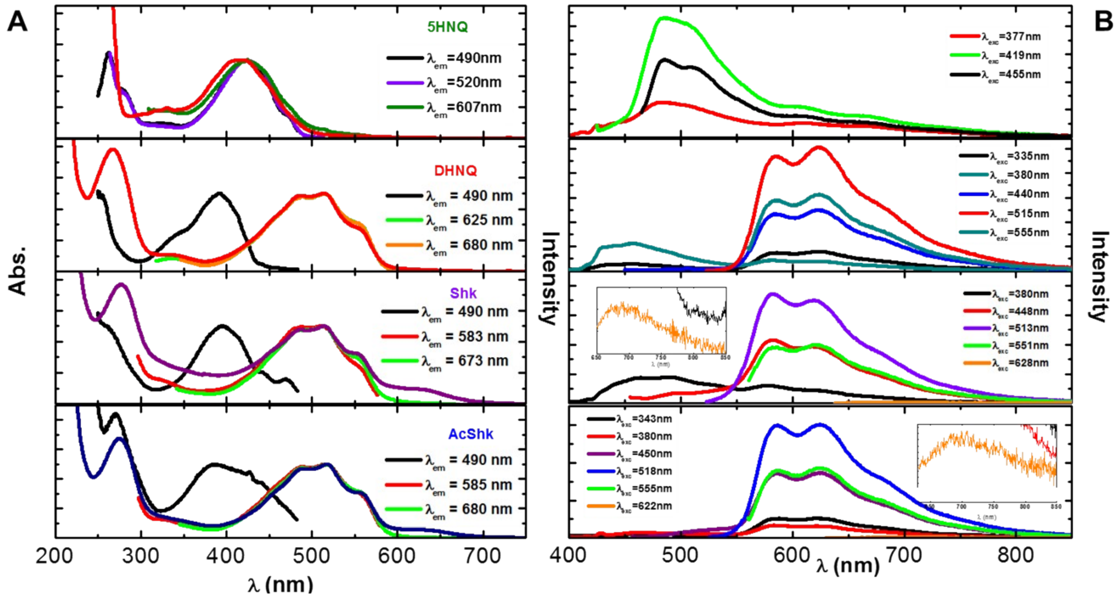

Figure SI 7. (A) Normalized room temperature absorption and fluorescence excitation spectra and (B) Fluorescence emission spectra of the naphthoquinone derivatives (**5HNQ**, **DHNQ**, **Shk** and **ACShk**) at different excitation and emission wavelengths in acetonitrile.

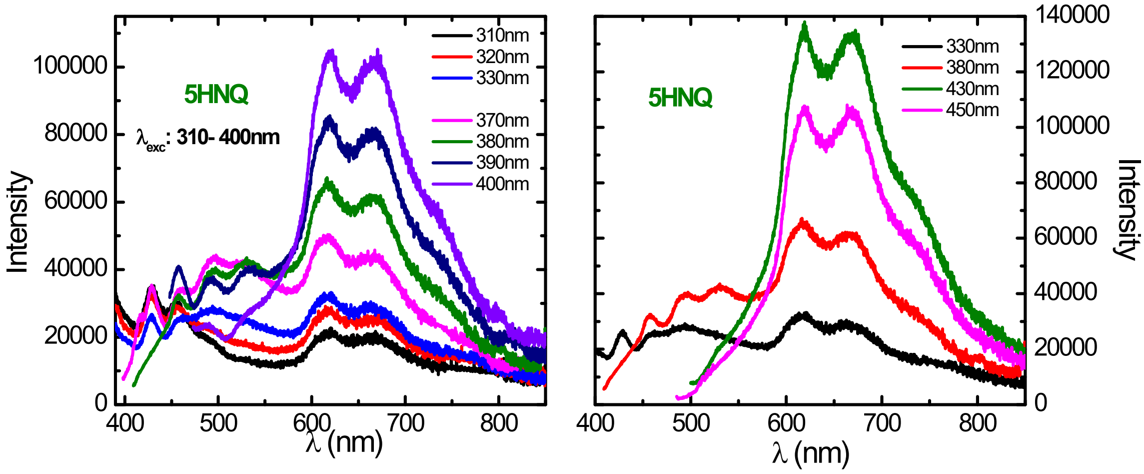

Figure SI 8. Fluorescence emission spectra of **5HNQ** at different excitation wavelengths in cyclohexane.

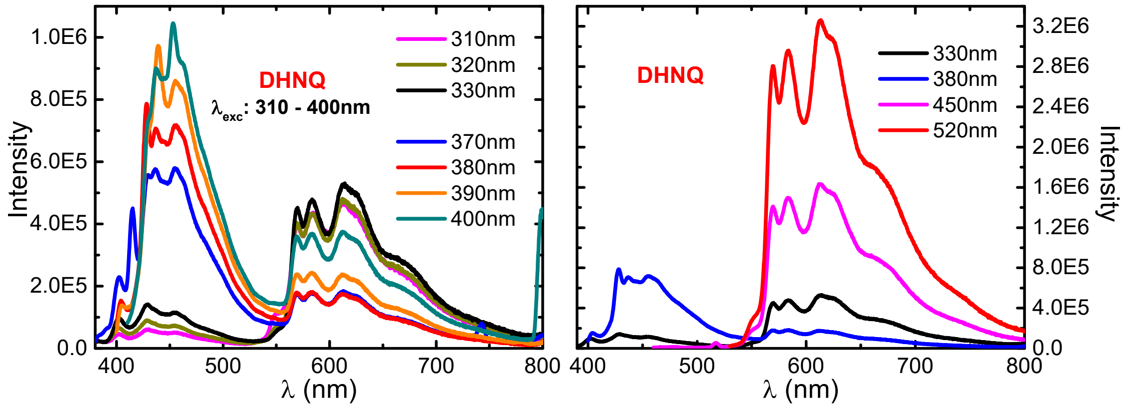

Figure SI 9. Fluorescence emission spectra of **DHNQ** at different excitation wavelengths in cyclohexane.

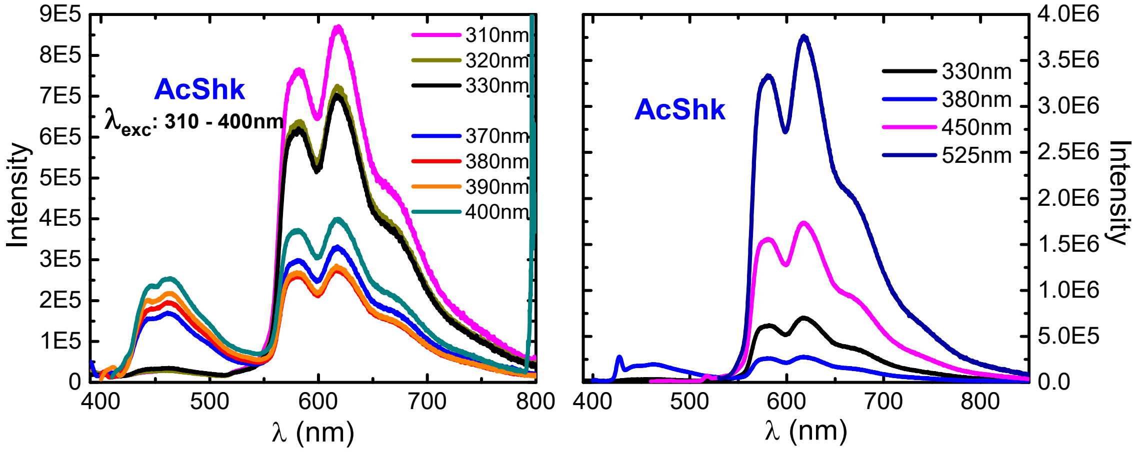

Figure SI 10. Fluorescence emission spectra of **AcShk** at different excitation wavelengths in cyclohexane.

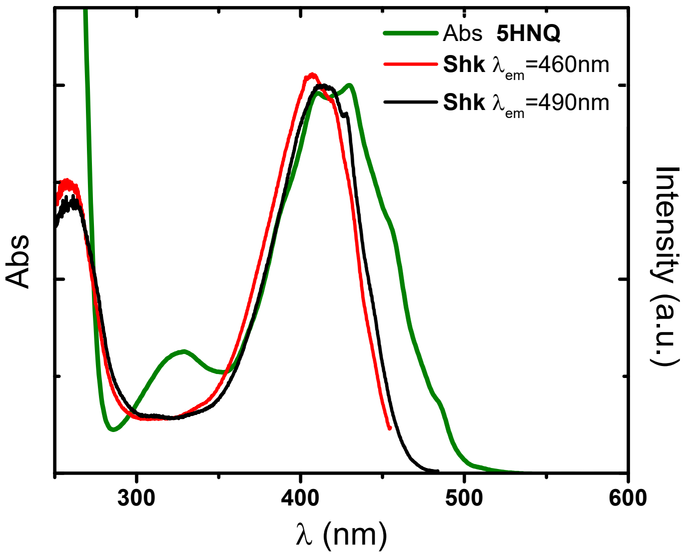

Figure SI 11. Normalized room temperature absorption spectra of **5HNQ**, together with the fluorescence excitation spectra of **Shk** (collected at λ_{em}= 460 and 490 nm) in cyclohexane.

Table SI 1. Spectral data (including absorption, λ_{abs} and emission λ_{em} wavelength maximum together with Stokes Shift, ΔSS) for 5-hidroxy-1,4-naphtoquinone (**5HNQ**), 5,8-dioxyacetyl-1,4-naphthoquinone (**DiAc**), 5,8-dihydroxy-1,4-naphthoquinone (**DHNQ**), shikonin (**Shk**) and acetylshikonin (**AcShk**) in acetonitrile at room temperature.

Solvent	**Compound**	λ_{max}^{Abs} **(nm)**	λ_{max}^{Fluo} **(nm)**	Δ_{SS} **(cm^{-1})**
ACN	**5HNQ**	415	486	3520
	DiAc	426	596	6696
	DHNQ	514	582	2273
	Shk	514	577	2124
	AcShk	517	585	2248

T-T Absorption

Figures SI12 – 15 show the transient triplet-triplet absorption spectra, together with the decays at the indicated wavelength for the naphthoquinones **5HNQ**, **DHNQ**, **Shk** and **AcShk** in cyclohexane and at room temperature.

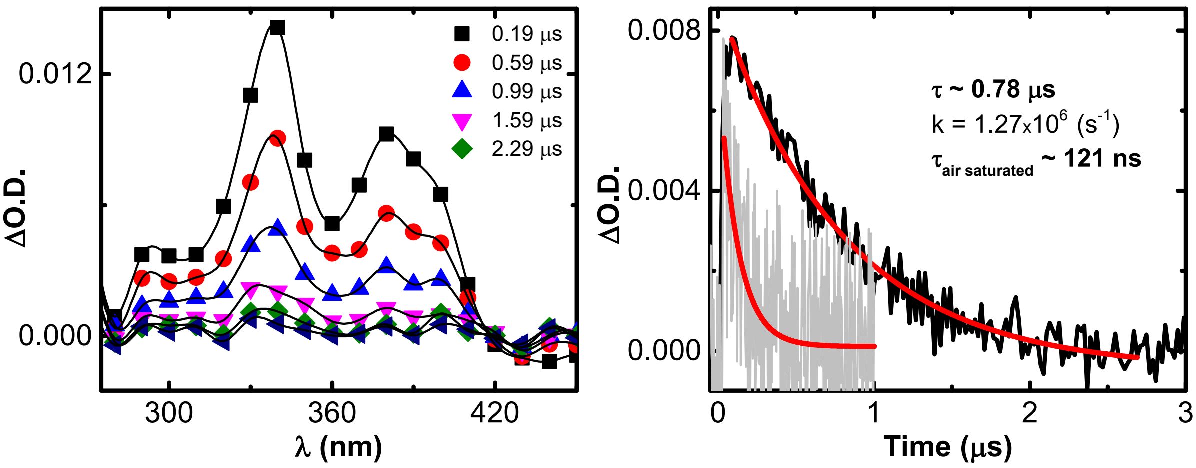

Figure SI 12. Singlet-triplet difference absorption spectra and kinetic behavior at 390 nm for **5HNQ**, collected with excitation at λ_{exc}= 355 nm, in N_2 degassed cyclohexane solution (20 min), at room temperature. Also presented the kinetic in air saturated cyclohexane solution to demonstrate the oxygen quenching of the triplet lifetime.

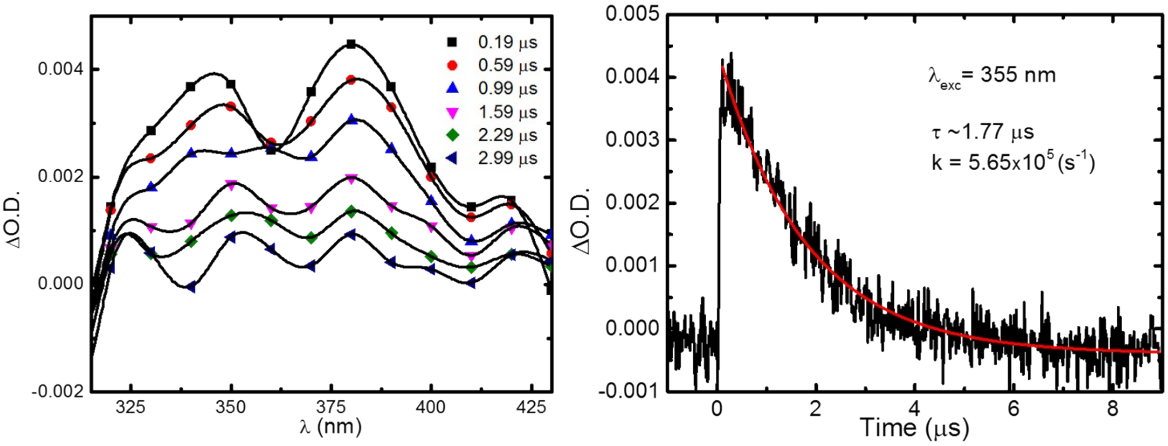

Figure SI 13. Singlet-triplet difference absorption spectra and kinetic behavior at 380 nm for **DHNQ**, collected with excitation at λ_{exc}= 355 nm, in N_2 degassed cyclohexane solution (20 min), at room temperature.

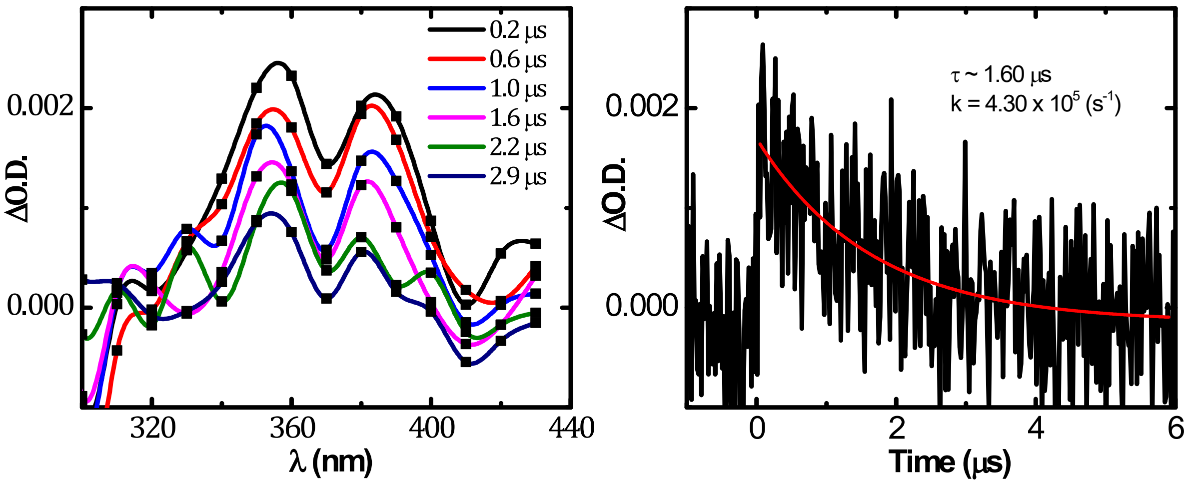

Figure SI 14. Singlet-triplet difference absorption spectra and kinetic behavior at 380 nm for **Shk**, collected with excitation at λ_{exc}= 532 nm, in N_2 degassed cyclohexane solution (20 min), at room temperature.

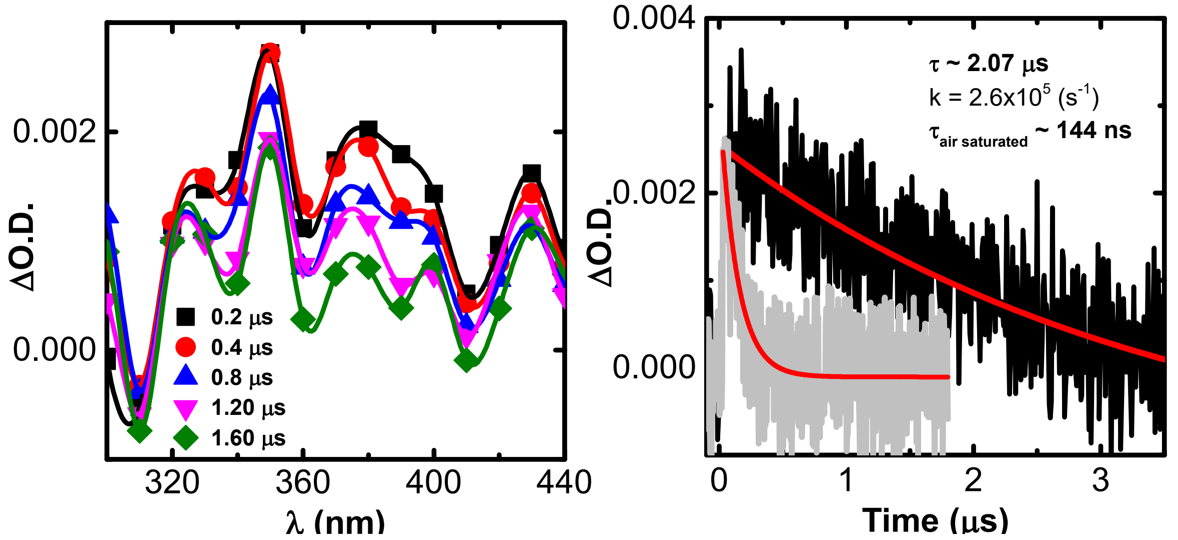

Figure SI 15. Singlet-triplet difference absorption spectra and kinetic behavior at 380 nm for **AcShk**, collected with excitation at λ_{exc}=532nm, in N_2 degassed cyclohexane solution (20 min), at room temperature. Also presented the kinetic in air saturated cyclohexane solution to demonstrate the oxygen quenching of the triplet lifetime.

DFT Calculations

The relative energies for the tautomers **Shk A – D** and the conformer **Shk A'** and the corresponding predicted absorption wavelengths are depicted in Table SI 2.

Table SI 2. Relative energies for the tautomers **Shk A – D** and the conformer **Shk A'**, obtained through the DFT calculations at the BPBE/6-31G** level.

Compound	**Structures**	**E**	**ΔE [a] (kJ/mol)**	**λ_{abs} Theoretical (nm)**
Shk	Shk **A**	-993.341	-	505
	Shk **B**	-993.338	6.481	507
	Shk **C**	-993.331	24.658	521
	Shk **D**	-993.312	75.605	520
	Shk **A'** [b]	-993.308	85.373	429

[a] energy difference relative to the energy of ShkA.
[b] calculated taking into account the solvent cyclohexane.

Ultrafast Time Resolved Spectroscopy

fs-UC

Femtosecond fluorescence up-conversion (fs-UC) decays for the **5HNQ**, **DiAc**, **DHNQ** and **AcShk**, in Cx with at excitation wavelengths λ_{exc}=450nm and 530nm are shown in Figures SI 16 – 24. The fs-UC decays for **5HNQ**, **DiAc**, **DHNQ**, **Shk** and **AcShk** in ACN obtained with λ_{exc}=450nm and 530nm are shown in figures SI 21 – 25. Table SI 3, is summarizes the lifetime values for the studied compounds obtained in ACN.

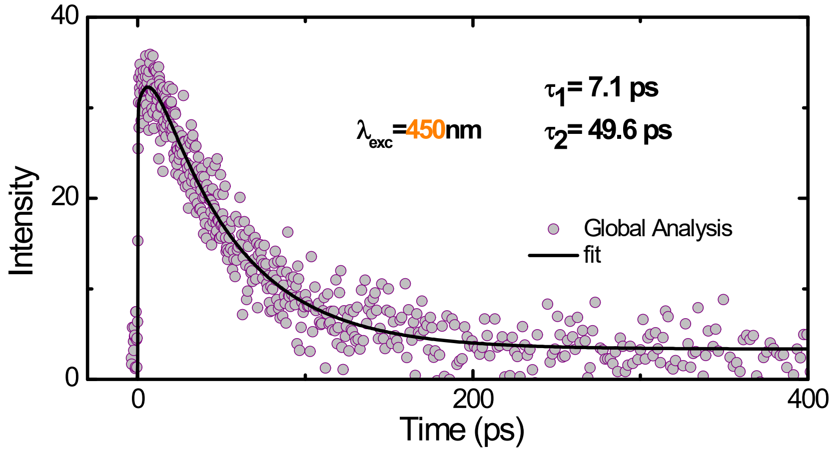

Figure SI 16 Time resolved fluorescence decays of **5HNQ** collected by fluorescence up conversion with excitation at 450nm in cyclohexane, at T= 293 K, together with the global analysis of the data.

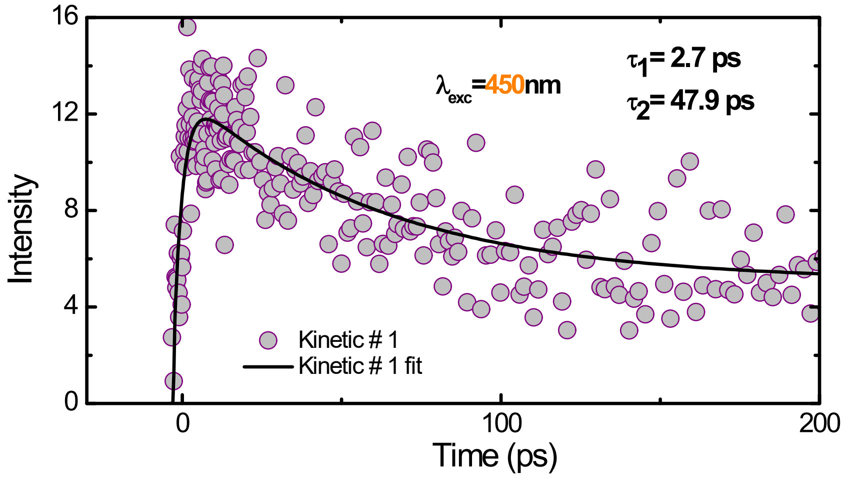

Figure SI 17. Time resolved fluorescence decays of **DiAc** collected by fluorescence up conversion with excitation at 450nm in cyclohexane, at T= 293 K, together with the global analysis of the data.

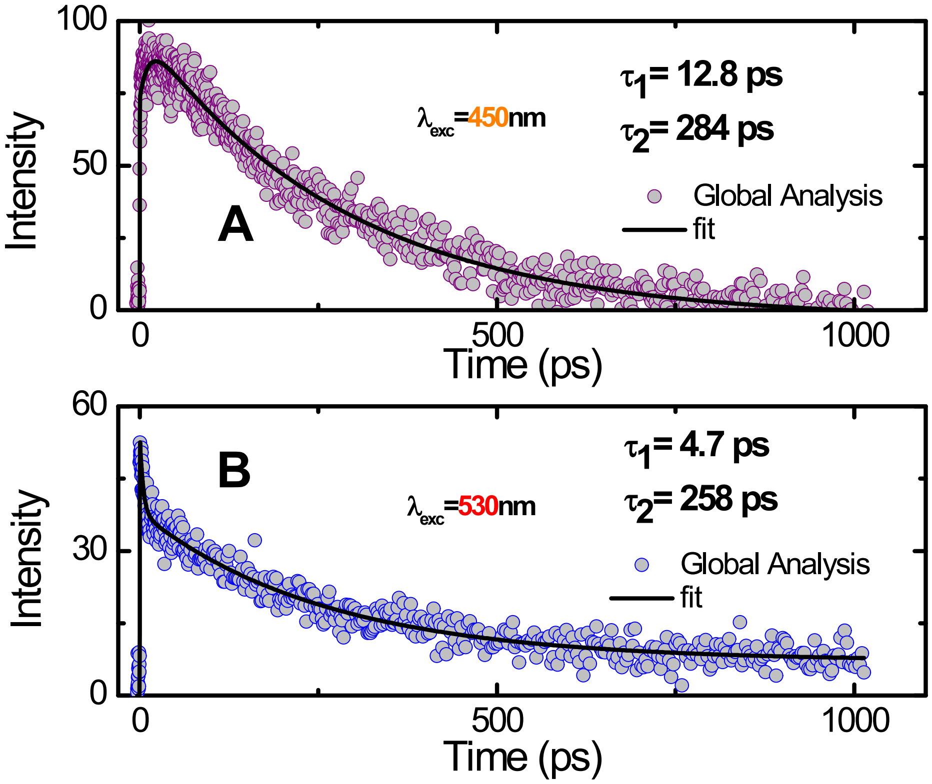

Figure SI 18. Time resolved fluorescence decays of **DHNQ** collected by fluorescence up conversion with excitation at 450nm (A) and 530nm (B) in cyclohexane, at T= 293 K, together with the global analysis of the data.

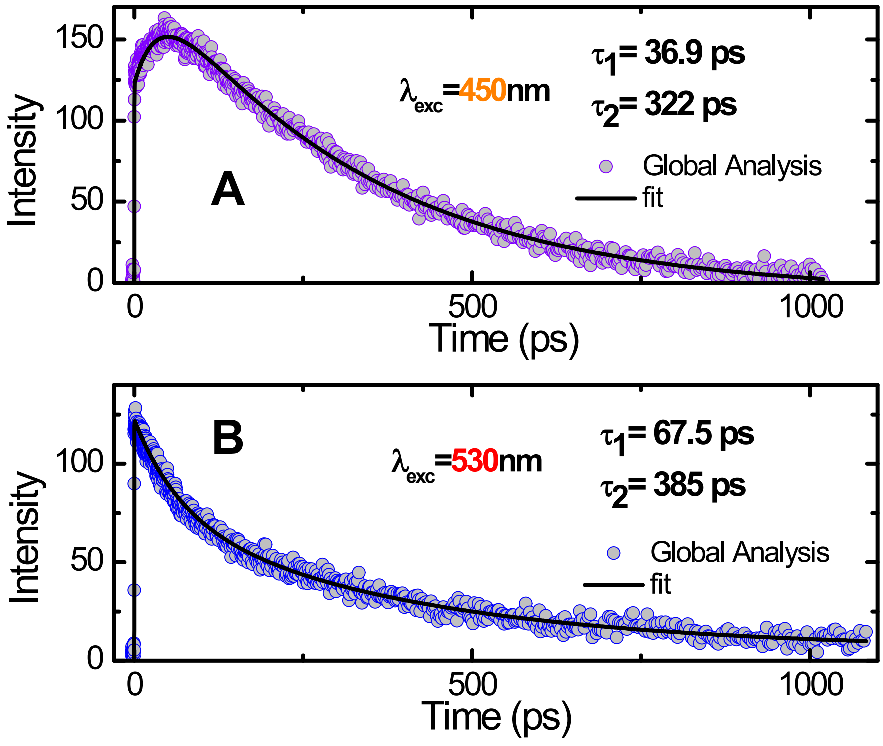

Figure SI 19. Time resolved fluorescence decays of **AcShk** collected by fluorescence up conversion with excitation at 450nm (A) and 530nm (B) in cyclohexane, at T= 293 K, together with the global analysis of the data.

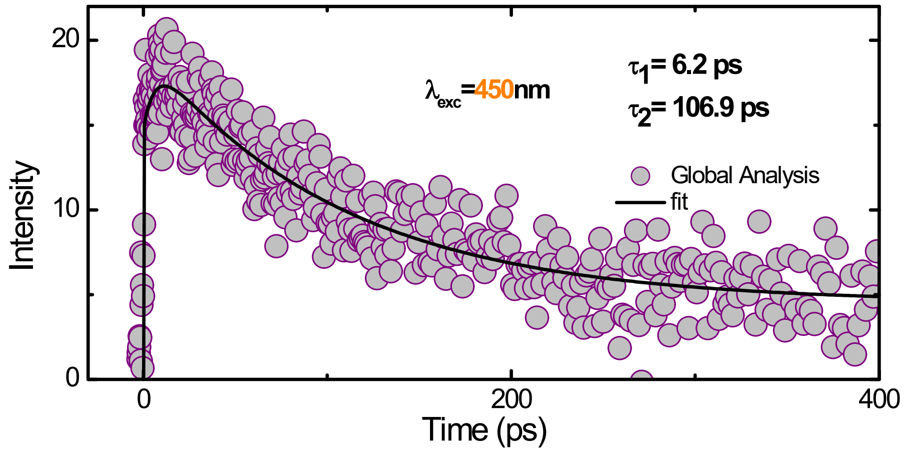

Figure SI 20. Time resolved fluorescence decays of **5HNQ** collected by fluorescence up conversion with excitation at excitation at 450nm in acetonitrile, at T= 293 K, together with the global analysis of the data.

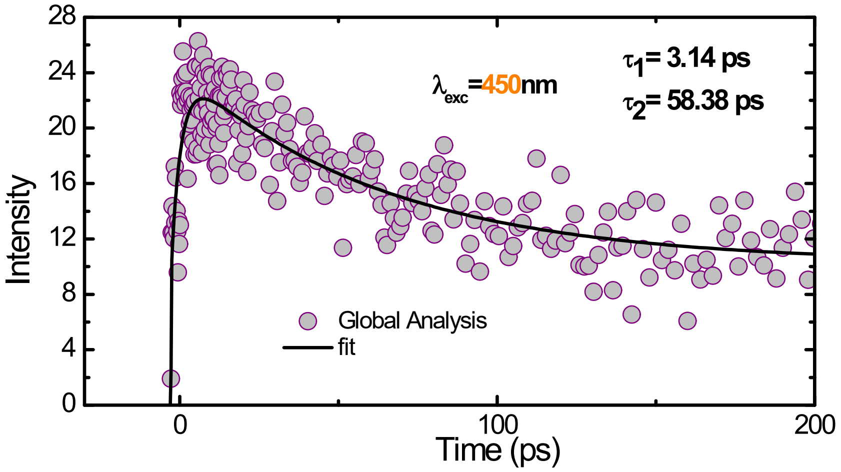

Figure SI 21. Time resolved fluorescence decays of **DiAc** collected by fluorescence up conversion with excitation at excitation at 450nm in acetonitrile, at T= 293 K, together with the global analysis of the data.

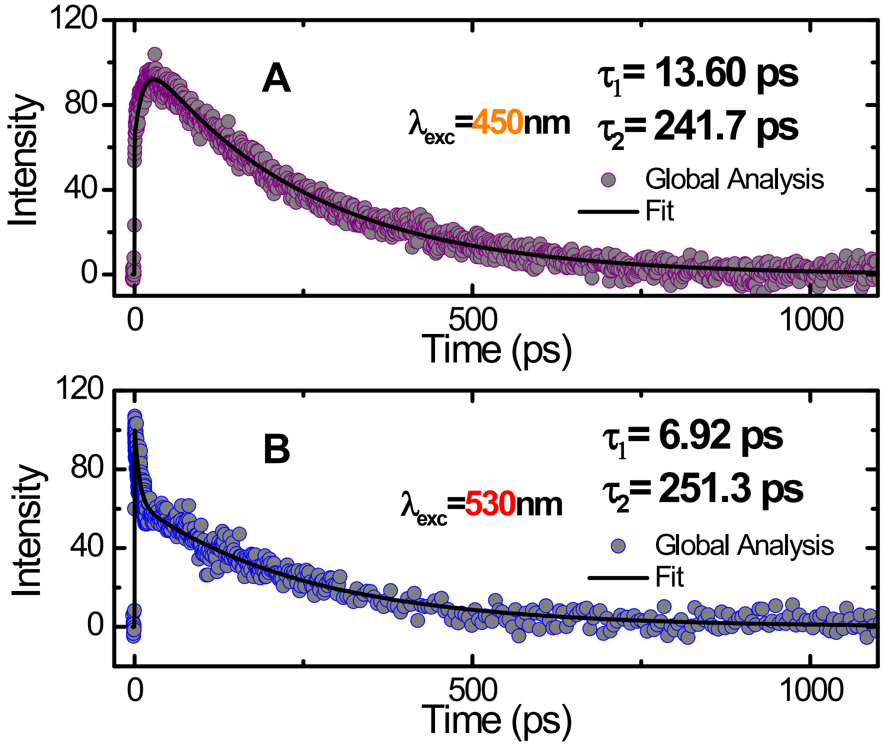

Figure SI 22. Time resolved fluorescence decays of **DHNQ** collected by fluorescence up conversion with excitation at 450nm (A) and 530nm (B) in acetonitrile, at T= 293 K, together with the global analysis of the data.

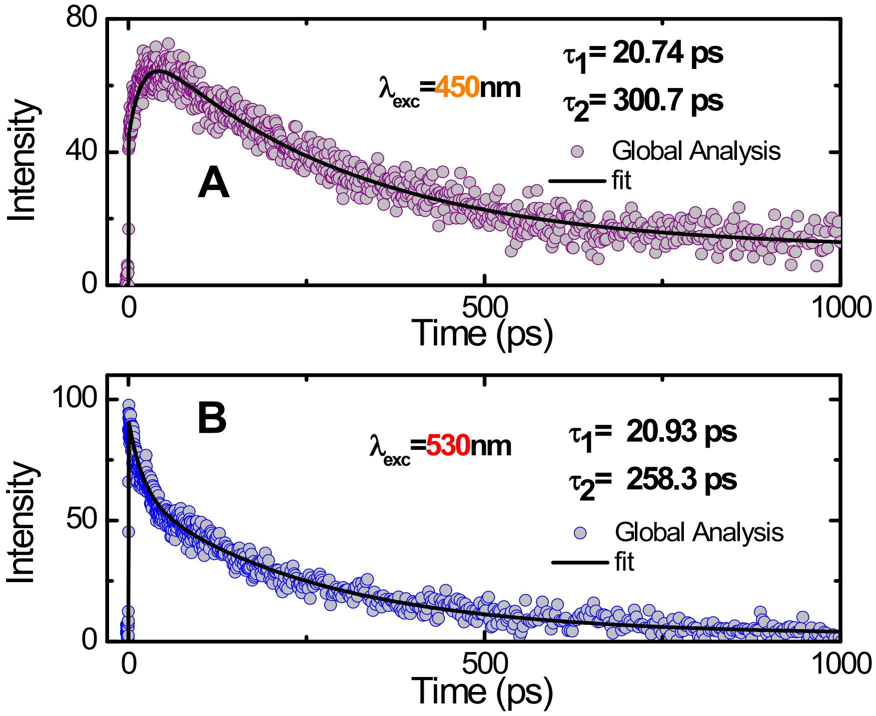

Figure SI 23. Time resolved fluorescence decays of **Shk** collected by fluorescence up conversion with excitation at 450nm (A) and 530nm (B) in acetonitrile, at T= 293 K, together with the global analysis of the data.

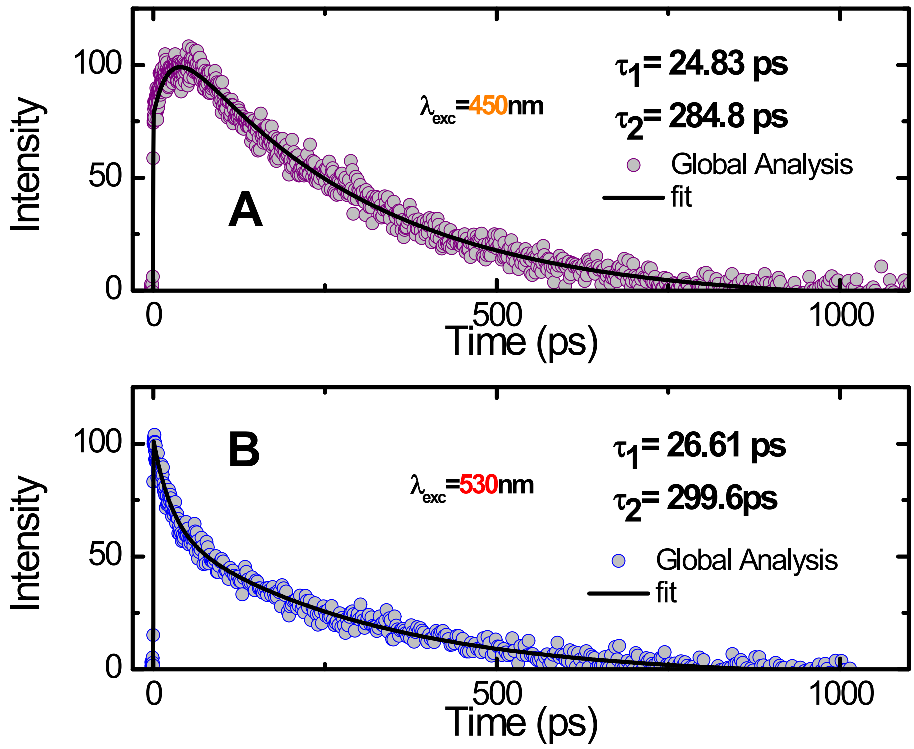

Figure SI 24. Time resolved fluorescence decays of **AcShk** collected by fluorescence up conversion with excitation at 450nm (A) and 530nm (B) in acetonitrile, at T= 293 K, together with the global analysis of the data.

Table SI 3. Room temperature fluorescence decay times (**τ_i**) obtained by fluorescence–up conversion (collected with excitation at 450 nm and 530 nm) for **5HNQ**, **DiAc**, **DHNQ**, **Shk**, **AcShk** in acetonitrile.

Compound	**λ_{exc} (nm)**	**τ_1 (ps)**	**τ_2 (ps)**
5HNQ	450	6.2[a]	106.9
DiAc	450	3.14[a]	58.38
DHNQ	450	13.60[a]	241.7
	530	6.92	251.3
Shk	450	20.74[a]	300.7
	530	20.93	258.3
AcShk	450	24.83[a]	284.8
	530	26.61	299.6

[a] associated to a negative pre-exponential value (rise time).

femtosecond transient absorption (fs-TA)

Figures SI 25 – 27 will show the fs-TA spectrum and decays for the **5HNQ**, **DHNQ** and **AcShk** obtained in cyclohexane at T=293 K and with excitation wavelengths λ_{exc}=460, 470 and 565nm.

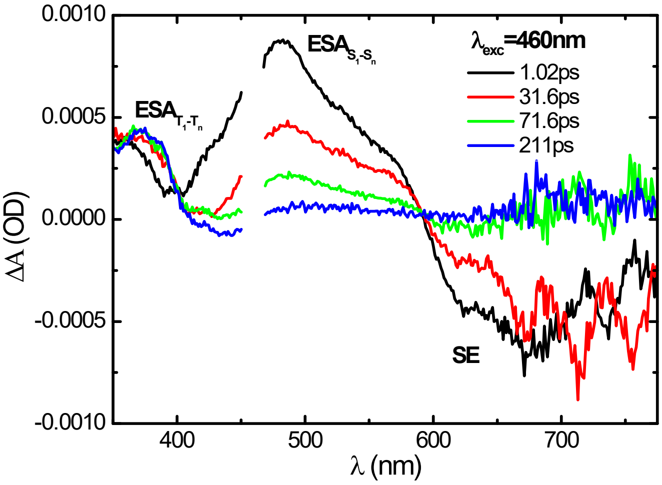

Figure SI 25. Room temperature time resolved transient absorption spectra for **5HNQ** in aerated cyclohexane solution after 250 fs laser pulse (IRF) at excitation wavelength of with λ_{exc}=460nm. As inset is highlighted the positive excited state absorption band (ESA) that results from the contribution of ESA_{S1-Sn} at shorter delays and ESA_{T1-Tn} at longer delay times, the stimulated emission (SE) is also highlighted.

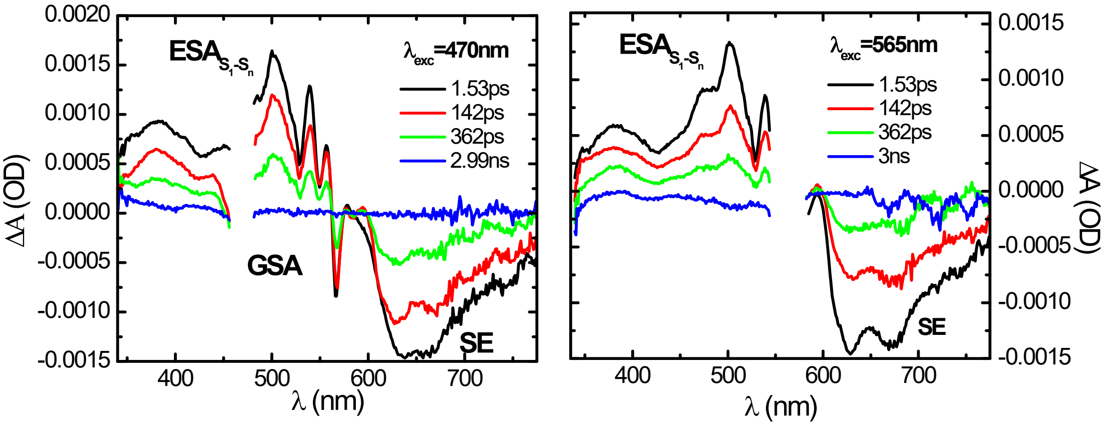

Figure SI 26. Room temperature time resolved transient absorption spectra for **DHNQ** in aerated cyclohexane solution after 250 fs laser pulse (IRF) at excitation wavelength of with λ_{exc}=470 and 565nm. As inset is highlighted the positive excited state absorption band (ESA) that results from the contribution of ESA$_{S1-Sn}$ at shorter delays, the ground state absorption (GSA) and the stimulated emission (SE) is also highlighted.

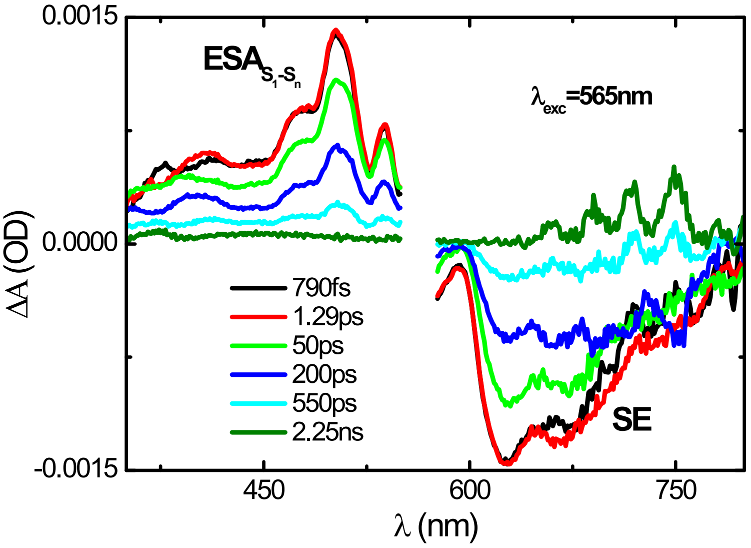

Figure SI 27. Room temperature time resolved transient absorption spectra for AcShk in aerated cyclohexane solution after 250 fs laser pulse (IRF) at excitation wavelength of with λ_{exc}=565nm. As inset is highlighted the positive excited state absorption band (ESA) that results from the contribution of ESA$_{S1-Sn}$ at shorter delays and the stimulated emission (SE) is also highlighted.