Electronic supplementary information

pH dependent ultrafast photodynamics of p-hydroxyphenacyl: Deprotonation accelerates the photo-uncaging reaction

Yannik Pfeifer,^a Till Stensitzki,^a Jakub Dostál, ^b Evgenii Titov,^c Miroslav Kloz,^b Peter Saalfrank^c and Henrike M. Müller-Werkmeister^{*a}

This supplementary information includes:

- Synthesis and analysis of pHP-FAc
- Supporting Computational Results: Table S1-S3
- Supporting Experimental Results: Figures S1-S9

Synthesis of pHP-FAc

Synthesis followed the protocol as published prior in Schulz, Mehrabi, Müller-Werkmeister et al., Nat. Meth. 2018 (doi.org/10.1038/s41592-018-0180-2).

A solution of sodium 2-fluoroacetate (1.02 g, 10.23 mmol) in dry CH_3CN /toluene 1:1 (100 mL) under a nitrogen atmosphere was treated with 18-crown-6 (2.70 g, 10.23 mmol) and 2-bromo-1-(4-hydroxyphenyl)ethanone (2.00 g, 9.30 mmol), stirred at 40 °C for 44 h, and the solvents were evaporated. Purification by flash chromatography (SiO₂; $CH_2CI_2/MeOH$ gradient 100:0 to 98:2) gave the product as white crystals. The procedure has been repeated numerous times to get the amount needed for flow-through measurements cycling/dumping the sample.

To confirm the purity of the product it was investigated via TLC, NMR; UV-Vis; mass spectrometry and elemental analysis:

Rf=0.24 (SiO₂; CH₂Cl₂/MeOH 99:1);

elemental analysis calcd (%) for C₁₀H₉FO₄: C 56.61, H 4.28, O 30.16; found: C 56.31, H 4.27, O 30.84,N 0.09;

HR-TOF-MS m/z(%): 213.0565 (100, [M - H]+, calcd for C₁₀H₉FO₄: 213.0563)

¹H-NMR (MeOH – d6, 300 MHz) δ [ppm]: 7.87 (d, J = 8.9 Hz, 2H; H-C(4,6)), 6.88 (d, J = 8.9 Hz, 2H; H-C(1,3)), 5.49 (s, 2H; CH₂O), 5.08 (d, J = 46.7 Hz, 1H; CH2F),

¹³C-NMR (MeOH – d6, 100 MHz) δ [ppm]: 192.07 OCH₂CO, 169.39 (d, 2J(C, F) = 22.4 Hz; CH₂F, 164.57 (C(2)), 131.54(C(4,6)), 126.97 (C(5)), 116.58 (c(1,3)), 78.62 (d, 1J(C, F) = 178.6 HZ; CH₂F), 67.48 (d, 4J(C, F), 1.2 Hz; CH₂O);

IR (cm⁻¹): 3205(br., s), 2933 (w), 1771 (s), 1660(m), 1576 (s), 1370 (m), 1209 (s), 1177 (s), 1079 (s), 963 (m), 841 (m), 703 (w).

^{a.} Institut für Chemie – Physikalische Chemie, Universität Potsdam Karl-Liebknecht-Strasse 24-25, 14476 Potsdam, Germany E-mail: henrike.mueller-werkmeister@uni-potsdam.de

b. ELI Beamlines Facility Extreme Light Infrastructure ERIC, 25241 Dolni Brezany, Czech Republic

^{c.} Institut für Chemie – Theoretische Chemie, Universität Potsdam

Karl-Liebknecht-Strasse 24-25, 14476 Potsdam, Germany

Supporting Table S1: Natural transition orbitals (NTOs) for the lowest two singlet transitions of the protonated and deprotonated reactants (for the latter, without and with explicit water molecules). The calculations are performed at the TD-B3LYP/def2-TZVP/PCM(water) level.

Transition	Hole NTO	Particle NTO	
	protonated		
$S_0 \rightarrow S_1$			
S₀→S₂			
	deprotonated		
$S_0 \rightarrow S_1$			
$S_0 \rightarrow S_2$			
	deprotonated with 3 explicit H ₂ O molecules		
S₀→S₁			
$S_0 \rightarrow S_2$			

Supporting Table S2: Calculated triplet transitions of the protonated reactant, TD-UB3LYP/def2-TZVP/PCM(water). The optimized T_1 geometry (UB3LYP/def2-TZVP/PCM(water)) is also shown.



Transition	<i>E</i> (eV)	λ (nm)	f
$T_1 \rightarrow T_2$	0.60	2070	0.00
$T_1 \rightarrow T_3$	1.38	896	0.00
$T_1 \rightarrow T_4$	1.92	644	0.01
$T_1 \rightarrow T_5$	2.29	541	0.01
$T_1 \rightarrow T_6$	2.69	460	0.00
$T_1 \rightarrow T_7$	3.17	391	0.04
$T_1 \rightarrow T_8$	3.18	389	0.00
$T_1 \rightarrow T_9$	3.27	380	0.00
$T_1 \rightarrow T_{10}$	3.34	371	0.48
$T_1 \rightarrow T_{11}$	3.83	324	0.00

Supporting Table S3: Top structure shows optimized geometry of p-quinone methide (PQM) at the B3LYP/def2-TZVP/PCM(Water) level (total charge of 0, singlet S_0) corresponding to the product state of Figure 7. Below are the calculated energies for the respective states, TD-B3LYP/def2-TZVP/PCM(water).



State	<i>E</i> (eV)	λ (nm)	f
T ₁	1.98	627	
T ₂	2.70	460	
S ₁	3.10	400	0.00
T ₃	3.21	386	
S ₂	4.20	295	0.01
S ₃	4.37	284	0.63
T ₄	4.56	272	
T ₅	4.99	248	
S ₄	5.49	226	0.00
S ₅	5.60	221	0.02



Supporting Figure 1: Absorbance change of pHP-FAc in H_2O and TRIS buffer at pH 9 after excitation at 340 nm at 275 nm. Orange line shows a mono-exponential fit for the data after 350 fs (circles). Since the fit describes the data quite well and reproduces the fast component of the SAS, we can rule out product generation from hot-ground state, which cools down with a time-constant of 1.6 ps.



Supporting Figure 2: Fit of the product spectrum of the SADS of pHP-FAc at pH 9 in H_2O with two gaussians and a constant background. The bleaching peak is 2.5 times larger than the product peak.



Supporting Figure 3. Top: Colormap of the transient spectra of pHP-FAc in TRIS buffer at pH 9 after excitation at 340 nm, black contours are separated by 5 mOD. Triangles indicate the position of the traces shown the bottom plot. Positions of stimulated emission (SE), excited state absorption (ESA), bleach (BL), product absorption (Prod) and the hot ground-state (hot-GS) are labeled. Black lines show a global two exponential fit of the data.



Supporting Figure 4. Top: Colormap of the transient spectra of pHP-FAc in ACN/TRIS 50:50 buffer at pH 9 after excitation at 340 nm, black contours are separated by 5 mOD. Triangles indicate the position of the traces shown the bottom plot. Positions of stimulated emission (SE), excited state absorption (ESA), bleach (BL), product absorption (Prod) and the hot ground-state (hot-GS) are labeled. Black lines show a global two exponential fit of the data. Signals after 10 ps are scaled by a factor of 5.



Supporting Figure 5. Species associated difference spectra (SADS) of pHP-FAc in ACN/TRIS 50:50 buffer at pH 9 after excitation at 340 nm. The kinetic model is shown in the bottom right. Components have the same colors in the kinetic scheme and the spectra.



Supporting Figure 6. Decay associated spectra (DAS) of pHP-FAc in PBS buffer at pH 6.8 after excitation at 266 nm.



Supporting Figure 7. Decay associated spectra (DAS) of pHP-FAc in TRIS buffer at pH 9 after excitation at 340 nm.



Supporting Figure 8. Decay associated spectra (DAS) of pHP-FAc in TRIS/ACN 50:50 buffer at pH 9 after excitation at 340 nm.



Supporting Figure 9: Reaction scheme for neutral pH (modified, after Givens et al., JACS 2008, DOI: 10.1021/ja7109579)



Supporting Figure 10. Schemes of potential energy surfaces for the deprotection of the deprotonated para-hydroxyphenacyl-fluoroacetate, for the case without explicit water molecules (left) and with 3 explicit H_2O molecules (right). Calculations are done at the (TD-)B3LYP/def2-TZVP/PCM(water) level (+D3(BJ) for the calculations with 3 explicit H_2O molecules).