*Electronic Supplementary Information for*

# Time-resolved spectroscopy of the singlet excited state of betanin in aqueous and alcoholic solutions

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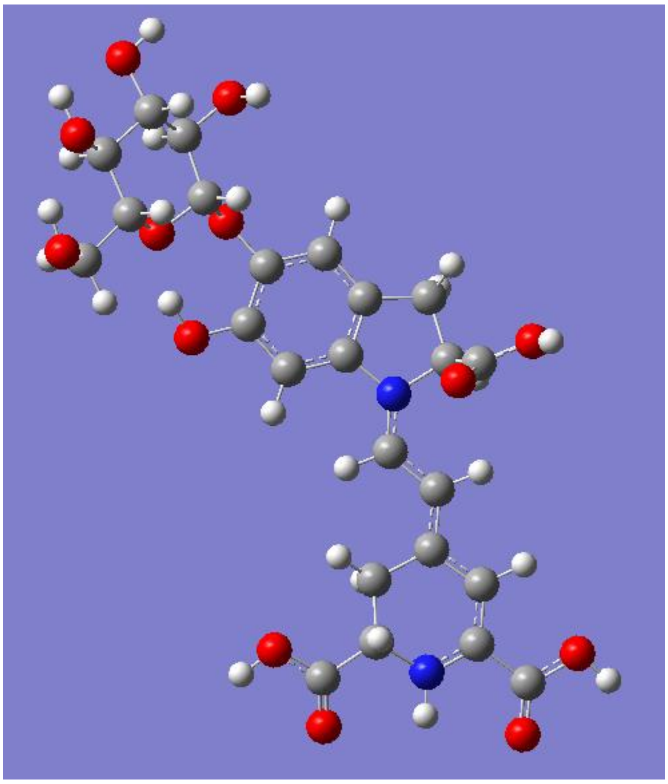
Table S1. TD-DFT of betanin at the B3LYP/6-311++G(d,p) level of theory with polarizable continuum model (PCM) for water.



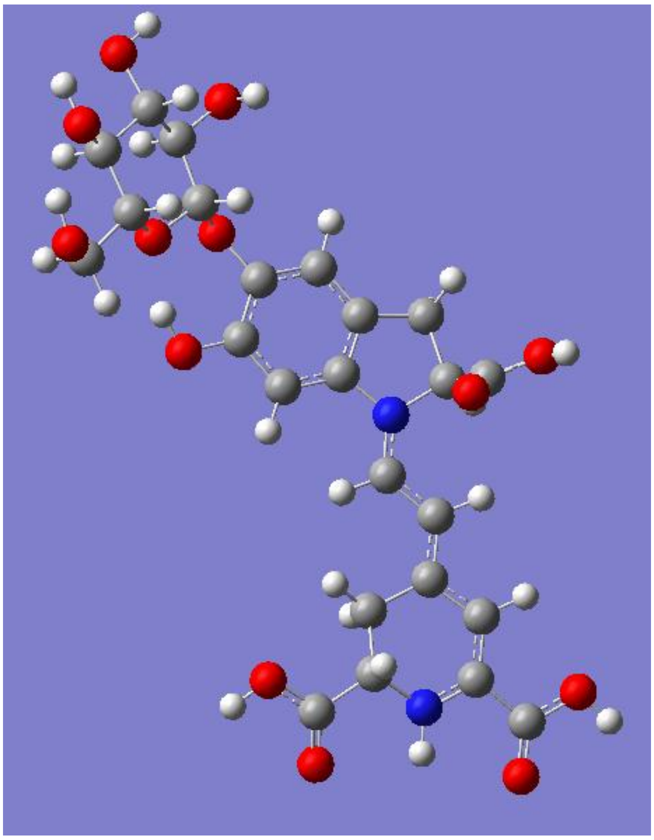
Energy = -2018.70586048 Hartree

| Wavelength (nm) | *f* |
|---|---|
| 509.64 | 0.9584 |
| 421.44 | 0.2365 |
| 349.85 | 0.0134 |
| 314.39 | 0.0000 |
| 303.51 | 0.0005 |
| 296.23 | 0.1263 |
| 290.06 | 0.0014 |
| 286.22 | 0.0192 |
| 284.34 | 0.0004 |
| 279.76 | 0.0056 |

| | | | |
|---|---|---|---|
| C | 0.031995000 | -1.063219000 | -0.527085000 |
| N | -1.306419000 | -1.466570000 | -0.280583000 |
| C | -2.364639000 | -0.661875000 | -0.253986000 |
| H | -2.141416000 | 0.374721000 | -0.468237000 |
| C | -3.672883000 | -1.051393000 | 0.010285000 |
| H | -3.882474000 | -2.085981000 | 0.253072000 |
| C | -4.764967000 | -0.182611000 | -0.023587000 |
| C | -6.061518000 | -0.675344000 | 0.221269000 |
| C | -4.648531000 | 1.271232000 | -0.442147000 |
| C | -7.163346000 | 0.161400000 | 0.135546000 |
| H | -6.220933000 | -1.727543000 | 0.404236000 |
| C | -5.759666000 | 2.117380000 | 0.198067000 |
| H | -4.723979000 | 1.312117000 | -1.535255000 |
| N | -7.051981000 | 1.484099000 | 0.011373000 |
| H | -7.886805000 | 2.056223000 | -0.052533000 |
| C | -8.583185000 | -0.337783000 | 0.167346000 |
| O | -9.529612000 | 0.408323000 | 0.073879000 |
| O | -8.663305000 | -1.661433000 | 0.302428000 |
| H | -9.598898000 | -1.921388000 | 0.319198000 |
| H | -3.691009000 | 1.706424000 | -0.166774000 |
| C | 0.836354000 | -2.186770000 | -0.703592000 |
| C | -1.354806000 | -2.930637000 | -0.080625000 |
| C | 0.015246000 | -3.446255000 | -0.612039000 |
| O | -1.814008000 | -4.569409000 | 1.543655000 |
| C | -1.538542000 | -3.266103000 | 1.402117000 |

| | | | |
|---|---|---|---|
| H | -1.888088000 | -4.778067000 | 2.489482000 |
| O | -1.432821000 | -2.485214000 | 2.313421000 |
| H | -2.184985000 | -3.355760000 | -0.643444000 |
| C | 2.187204000 | -2.017389000 | -0.967762000 |
| C | 0.527621000 | 0.232467000 | -0.591139000 |
| C | 2.709405000 | -0.729202000 | -1.044460000 |
| C | 1.888698000 | 0.402746000 | -0.853510000 |
| O | 2.365874000 | 1.668366000 | -0.930766000 |
| H | 3.336858000 | 1.662546000 | -0.824008000 |
| H | -0.083013000 | 1.110991000 | -0.430570000 |
| H | 2.845165000 | -2.862061000 | -1.132852000 |
| H | 0.448905000 | -4.194879000 | 0.051348000 |
| H | -0.116930000 | -3.910847000 | -1.591836000 |
| O | 4.038909000 | -0.565924000 | -1.416265000 |
| C | 6.362173000 | -0.755480000 | -0.893113000 |
| C | 4.980611000 | -0.297555000 | -0.412813000 |
| C | 7.450394000 | -0.259008000 | 0.069995000 |
| C | 7.320202000 | 1.239016000 | 0.325886000 |
| H | 7.348536000 | -0.784070000 | 1.030552000 |
| C | 5.899853000 | 1.580389000 | 0.791026000 |
| H | 7.519732000 | 1.775532000 | -0.610448000 |
| H | 6.531458000 | -0.321023000 | -1.881129000 |
| H | 5.685225000 | 1.073027000 | 1.740316000 |
| O | 4.985143000 | 1.122627000 | -0.220293000 |
| O | 8.229447000 | 1.676866000 | 1.337557000 |
| O | 8.754571000 | -0.478809000 | -0.459324000 |
| O | 6.438054000 | -2.163454000 | -1.075864000 |
| H | 6.268334000 | -2.610763000 | -0.236387000 |
| H | 8.885785000 | -1.428514000 | -0.568559000 |
| H | 9.126568000 | 1.465102000 | 1.050437000 |
| C | 5.666342000 | 3.082264000 | 0.970084000 |
| H | 5.975368000 | 3.612948000 | 0.060725000 |
| H | 4.600714000 | 3.258556000 | 1.124390000 |
| O | 6.339523000 | 3.589701000 | 2.117392000 |
| H | 7.261678000 | 3.297676000 | 2.063100000 |
| H | 4.690581000 | -0.788940000 | 0.525604000 |
| H | -5.558525000 | 2.192234000 | 1.277532000 |
| C | -5.828807000 | 3.546101000 | -0.324809000 |
| O | -6.846244000 | 4.077967000 | -0.696877000 |
| O | -4.632057000 | 4.137834000 | -0.281468000 |
| H | -4.718087000 | 5.055285000 | -0.589006000 |

Table S2. TD-DFT of betanin at the B3LYP/6-311++G(d,p) level of theory with PCM for methanol.



Energy = -2018.70360723 Hartree

| Wavelength (nm) | *f* |
|---|---|
| 510.47 | 0.9477 |
| 422.29 | 0.2466 |
| 350.22 | 0.0138 |
| 316.07 | 0.0000 |
| 305.50 | 0.0003 |
| 296.28 | 0.1246 |
| 290.58 | 0.0013 |
| 286.62 | 0.0167 |
| 285.83 | 0.0030 |
| 280.21 | 0.0055 |

| | | | |
|---|---|---|---|
| C | 0.032564000 | -1.064843000 | -0.527432000 |
| N | -1.306122000 | -1.468033000 | -0.281960000 |
| C | -2.364060000 | -0.663038000 | -0.255151000 |
| H | -2.140305000 | 0.373531000 | -0.469113000 |
| C | -3.672507000 | -1.051898000 | 0.009173000 |
| H | -3.882260000 | -2.086232000 | 0.252995000 |
| C | -4.764349000 | -0.182728000 | -0.024376000 |
| C | -6.060912000 | -0.674308000 | 0.222330000 |
| C | -4.647592000 | 1.270686000 | -0.444524000 |
| C | -7.162407000 | 0.163108000 | 0.137220000 |
| H | -6.221032000 | -1.726180000 | 0.406548000 |
| C | -5.757271000 | 2.118185000 | 0.196321000 |
| H | -4.724425000 | 1.310710000 | -1.537572000 |
| N | -7.050315000 | 1.485583000 | 0.012300000 |
| H | -7.884727000 | 2.058347000 | -0.052822000 |
| C | -8.582714000 | -0.334965000 | 0.170793000 |
| O | -9.528447000 | 0.411853000 | 0.078579000 |
| O | -8.663542000 | -1.658724000 | 0.305966000 |
| H | -9.599365000 | -1.917726000 | 0.323972000 |
| H | -3.689491000 | 1.705691000 | -0.170887000 |
| C | 0.836941000 | -2.188420000 | -0.703428000 |
| C | -1.355078000 | -2.932122000 | -0.082649000 |
| C | 0.015811000 | -3.447948000 | -0.611891000 |
| O | -1.817817000 | -4.571631000 | 1.539931000 |

| | | | |
|---|---|---|---|
| C | -1.541816000 | -3.268098000 | 1.399657000 |
| H | -1.893618000 | -4.780640000 | 2.485527000 |
| O | -1.437980000 | -2.487694000 | 2.311365000 |
| H | -2.184436000 | -3.356844000 | -0.647046000 |
| C | 2.187905000 | -2.018921000 | -0.967136000 |
| C | 0.528173000 | 0.230791000 | -0.591314000 |
| C | 2.710272000 | -0.730772000 | -1.043494000 |
| C | 1.889362000 | 0.401312000 | -0.853118000 |
| O | 2.365883000 | 1.666757000 | -0.930349000 |
| H | 3.337098000 | 1.661453000 | -0.825779000 |
| H | -0.082282000 | 1.109511000 | -0.431113000 |
| H | 2.845956000 | -2.863403000 | -1.132847000 |
| H | 0.448496000 | -4.195879000 | 0.052968000 |
| H | -0.114876000 | -3.913775000 | -1.591330000 |
| O | 4.039691000 | -0.567812000 | -1.414859000 |
| C | 6.363167000 | -0.754825000 | -0.892268000 |
| C | 4.981302000 | -0.298034000 | -0.411851000 |
| C | 7.451094000 | -0.256778000 | 0.070174000 |
| C | 7.319288000 | 1.241098000 | 0.326069000 |
| H | 7.349626000 | -0.781688000 | 1.031081000 |
| C | 5.898817000 | 1.580622000 | 0.792017000 |
| H | 7.517337000 | 1.777562000 | -0.610617000 |
| H | 6.531591000 | -0.321488000 | -1.880872000 |
| H | 5.685086000 | 1.072679000 | 1.741249000 |
| O | 4.984228000 | 1.121910000 | -0.219116000 |
| O | 8.228523000 | 1.680238000 | 1.336966000 |
| O | 8.755185000 | -0.475232000 | -0.459183000 |
| O | 6.440794000 | -2.163019000 | -1.073233000 |
| H | 6.274202000 | -2.609243000 | -0.232580000 |
| H | 8.881658000 | -1.423771000 | -0.583310000 |
| H | 9.125765000 | 1.469483000 | 1.049547000 |
| C | 5.663691000 | 3.082111000 | 0.972379000 |
| H | 5.974225000 | 3.614247000 | 0.064335000 |
| H | 4.597533000 | 3.257313000 | 1.124373000 |
| O | 6.333448000 | 3.588345000 | 2.121835000 |
| H | 7.256436000 | 3.299003000 | 2.068621000 |
| H | 4.692357000 | -0.789674000 | 0.526919000 |
| H | -5.554496000 | 2.194001000 | 1.275463000 |
| C | -5.826822000 | 3.546536000 | -0.327827000 |
| O | -6.845349000 | 4.079326000 | -0.694881000 |
| O | -4.628723000 | 4.136409000 | -0.291656000 |
| H | -4.715303000 | 5.053857000 | -0.599071000 |

Figure S1. Normalized absorption and fluorescence excitation spectra ($\lambda_f$ = 620 nm) of betanin in aqueous solution (pH = 7.0).

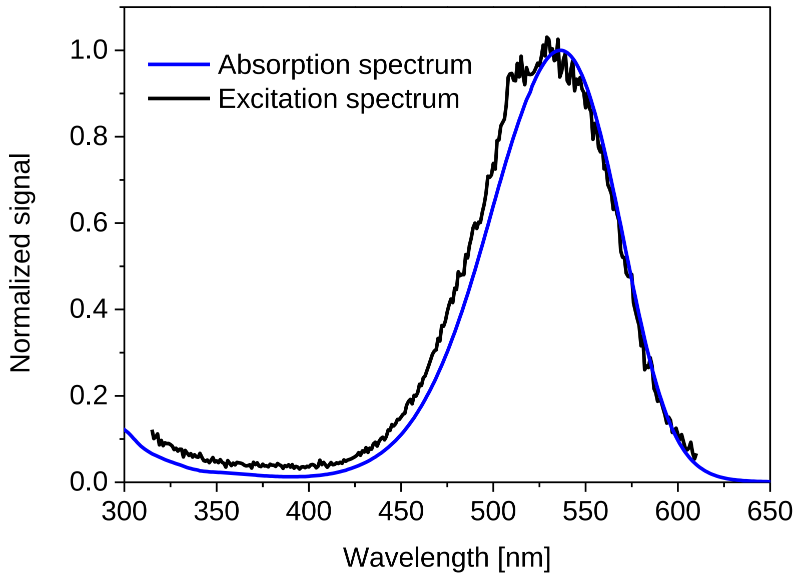
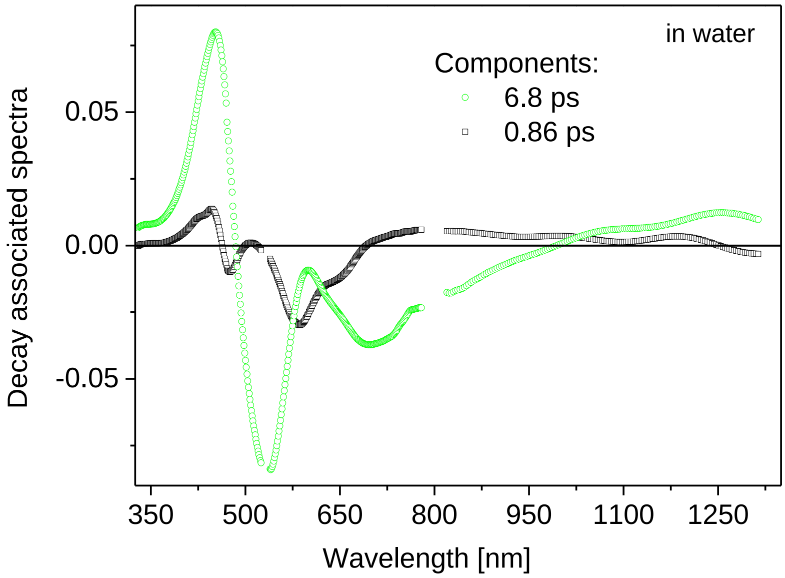
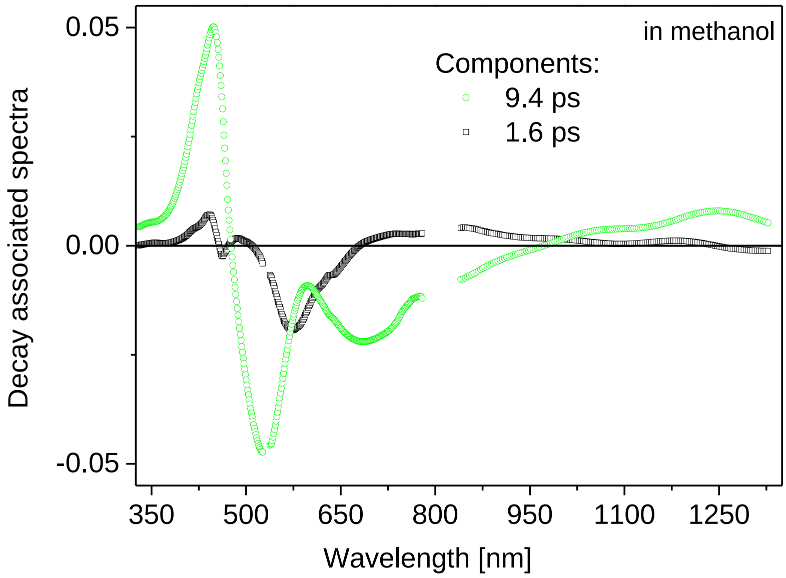


Figure S2. Determined decay-associated spectra - the wavelength-dependent amplitudes with their respective time constants (shown in the figures) obtained by double-exponential global fit for betanin excited at 535 nm in water (A), methanol (B) and ethylene glycol (C).

(A)




(B)




(C)

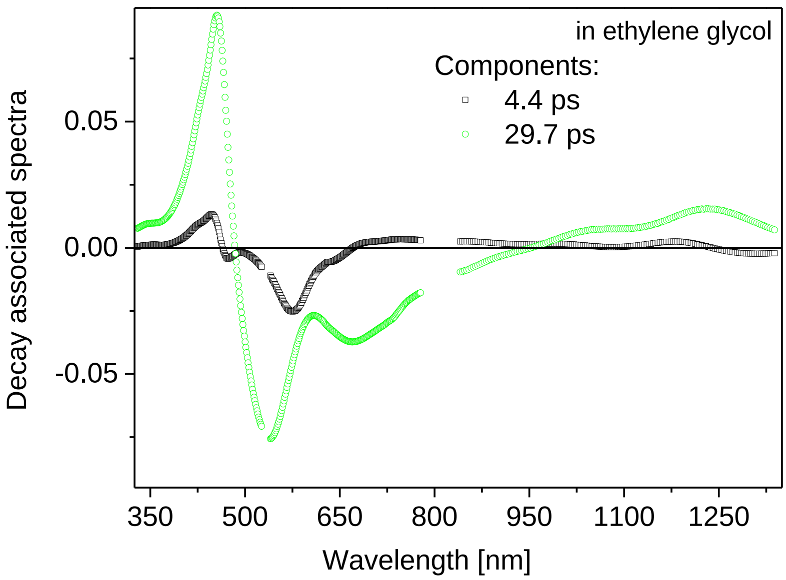


Figure S3. Transient absorption spectra recorded for betanin in aqueous solution ($4.8 \times 10^{-5}$ M) and the determined decay-associated spectra for data recorded with excitation at 475 nm.

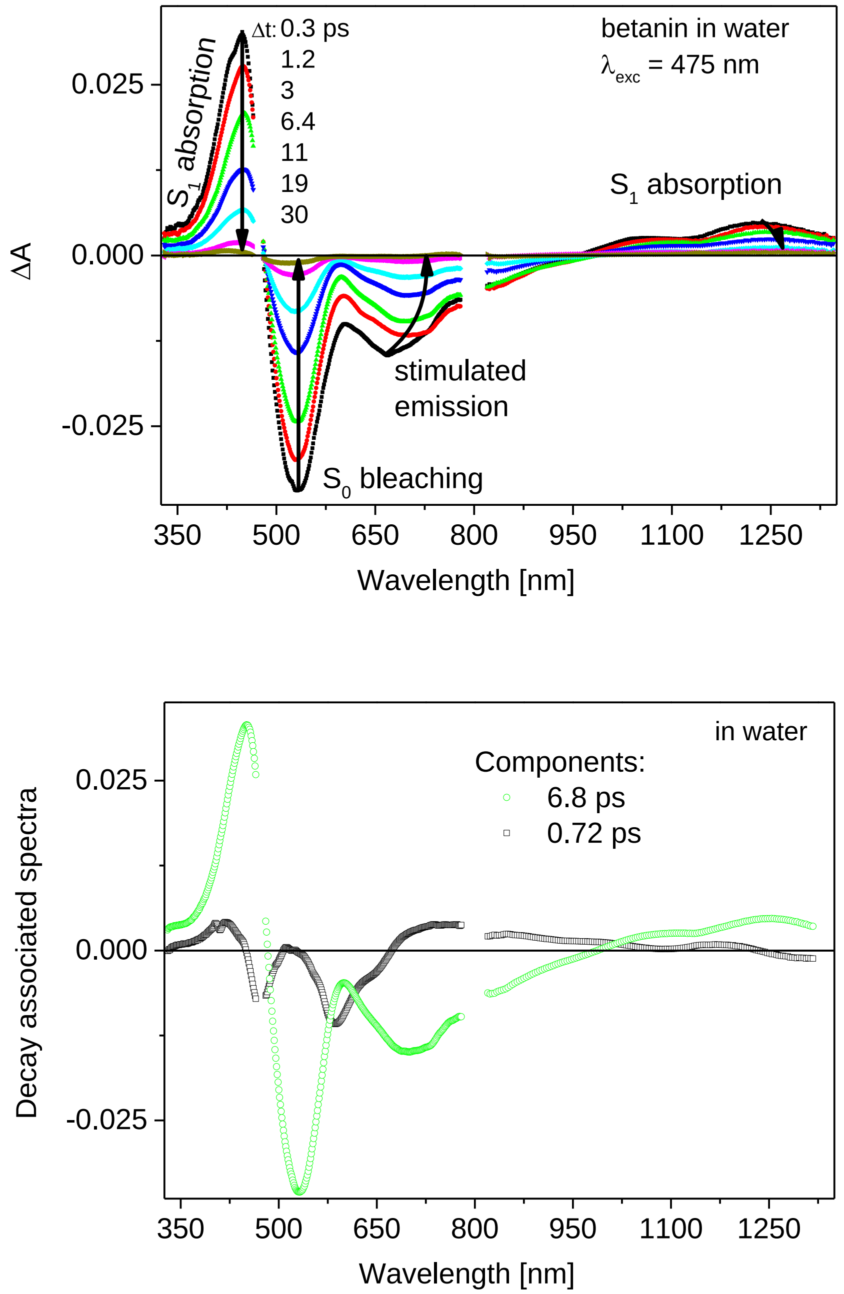
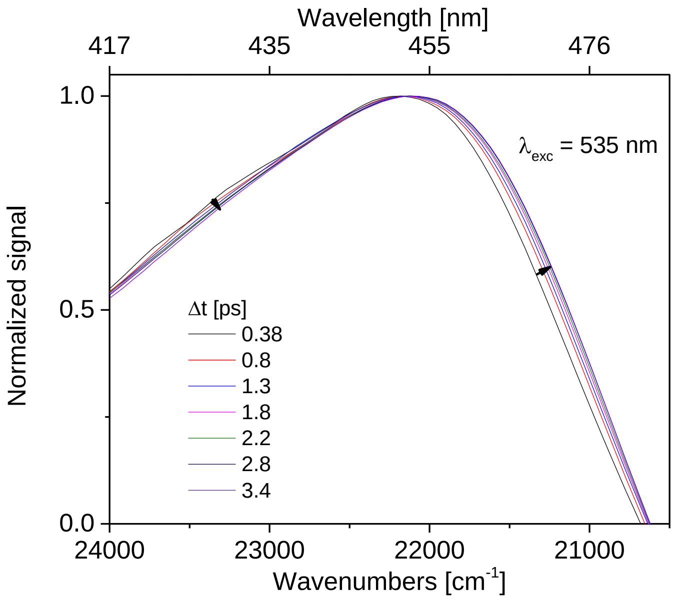


Figure S4. Comparison of transient absorption data recorded with excitations at 535 nm (A) and 287 nm (B) for betanin in water. Selected spectra were normalized at maximum of $S_1$ absorption located around 450 nm.

(A)




(B)

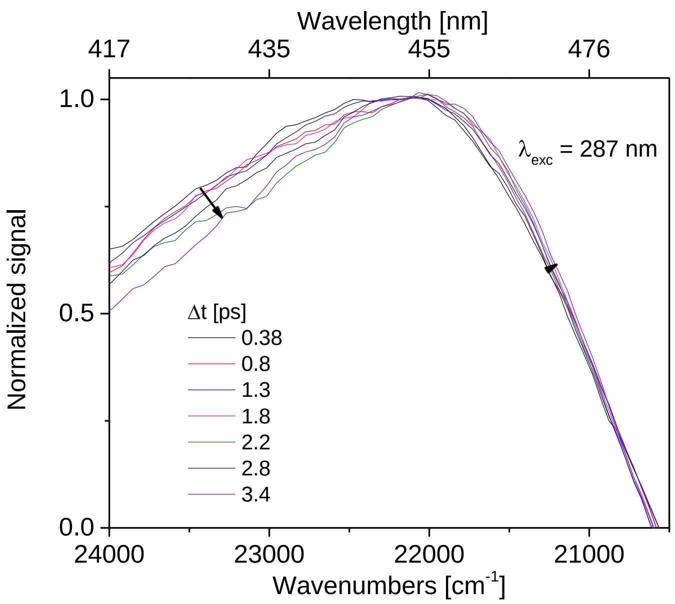


Figure S5. To estimate betanin differential molar absorption coefficient $\Delta\varepsilon(520nm) = \varepsilon_{S1}(520nm)-\varepsilon_{S0}(520nm)$ a comparative method is used.[1] The reference solution was $Ru(bpy)_3^{2+}$ in water prepared with the same absorbance A=0.25 at the excitation wavelength $\lambda_{exc}$ = 530 nm as the solution of **bn** in water, so that the same number of photons would be absorbed by the two solutions. Two samples were measured one after the other under identical experimental conditions, the obtained transient absorption spectra are given below. The spectrum for $Ru(bpy)_3^{2+}$ was multiplied by a factor of 5. The triplet formation quantum yield for $Ru(bpy)_3^{2+}$ is close to unity.[2] The following relation can be used in comparative actinometric measurements:

$\Delta\varepsilon(520nm)/\Delta A(520nm)=\Delta\varepsilon_{ref}(368nm)/\Delta A_{ref}(368nm)$, where the values $\Delta A(520nm)$ and $\Delta A_{ref}(368\ nm)$ are taken from graph below, $\Delta\varepsilon_{ref}(368\ nm) = 17500\ M^{-1}cm^{-1}$ is given in report.[3] The calculated $\Delta\varepsilon(520nm)$ is a substantial value of $-78000 \pm 20000\ M^{-1}cm^{-1}$.

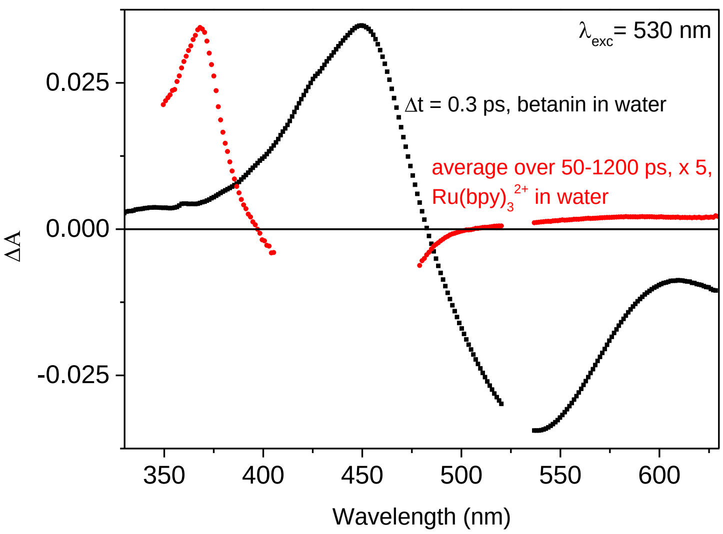
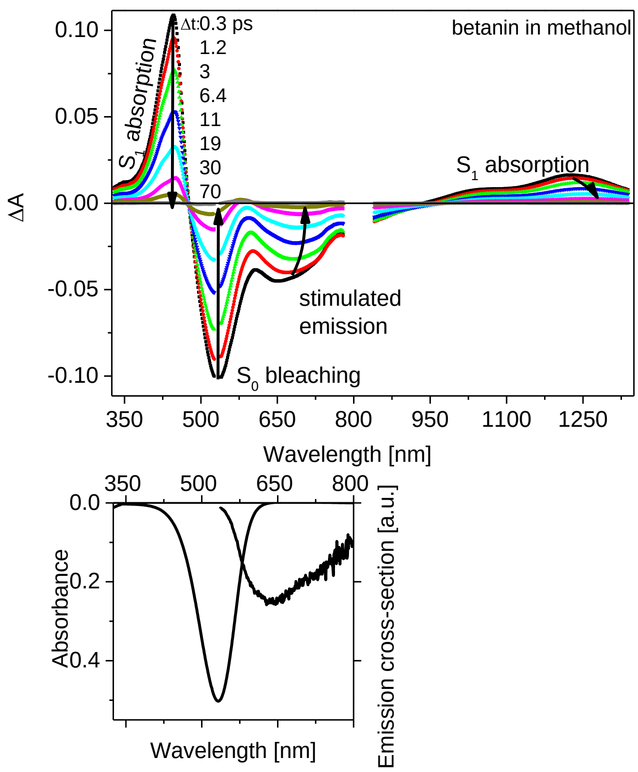


Figure S6. Transient absorption spectra recorded for betanin in methanol, $3.9 \times 10^{-5}$ M (A) and ethylene glycol, $4.5 \times 10^{-5}$ M (B) with photoexcitation at 535 nm. Sample stationary UV-vis absorption and emission cross-section spectra ($\lambda_{exc}$ = 530 nm) are also given for comparison.

(A)




(B)

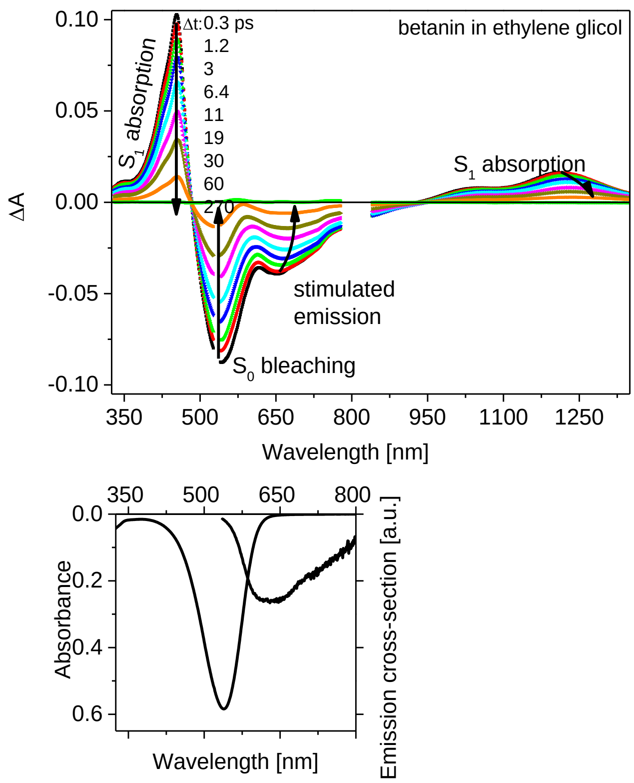
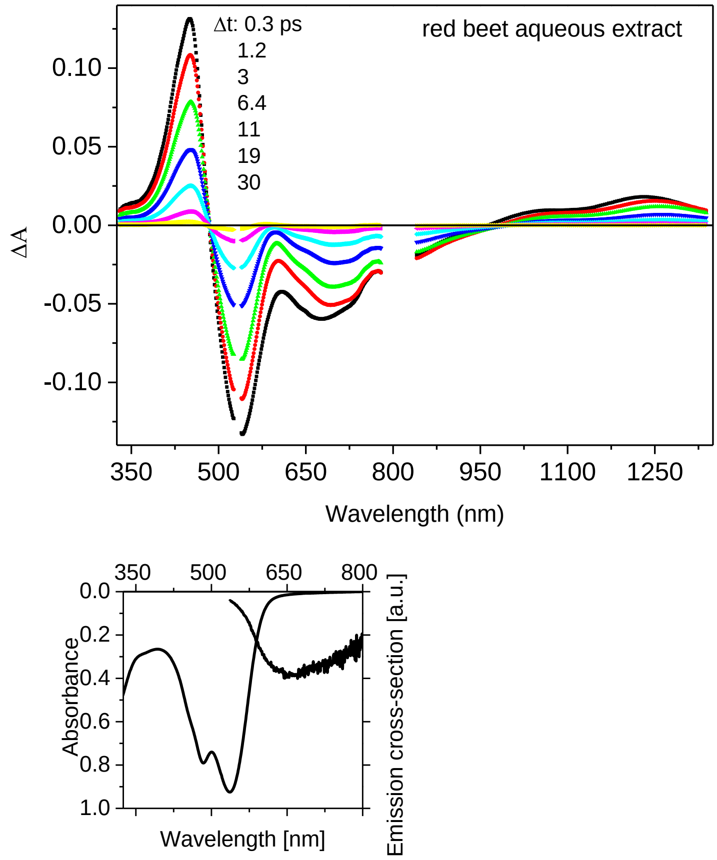


Figure S7. Transient absorption spectra recorded for aqueous extract from red beet (*Beta vulgaris* L.) with photoexcitation at 535 nm (A). Sample stationary UV-vis absorption and emission cross-section spectra ($\lambda_{exc}$=530 nm) determined for the extract are also given for comparison.

(A)




(B)

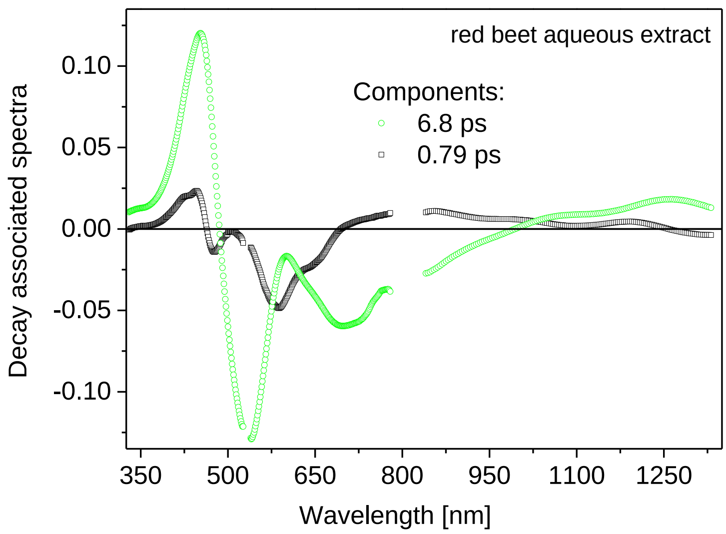
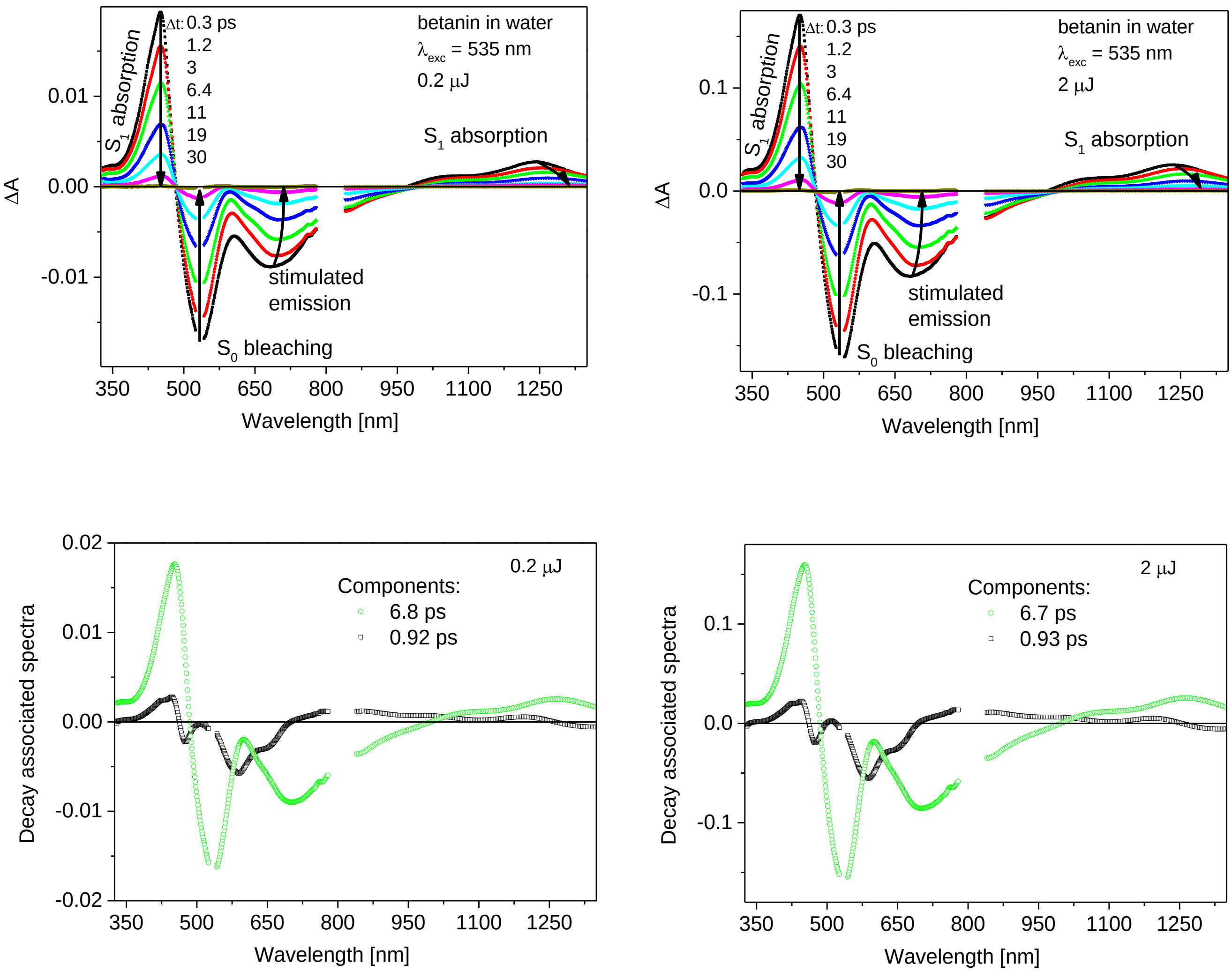


Figure S8. Control experiments with 0.2 and 2 μJ pulse excitation energy for betanin in aqueous solution (7.5 × 10$^{-5}$ M) with excitation at 535 nm (magic angle conditions).



## Experimental details

Betanin was obtained by purification of fruit extract of red *Opuntia ficus-indica*, L. Mill received from Italy. The following isolation procedure was applied, based on the method of indicaxanthin purification, accomplished by flash chromatography and preparative high-performance liquid chromatography.[4] Cactus pear fruits were squeezed to obtain fresh juice, which was fortified with ascorbic acid against pigment oxidation. The juice was filtered through a 0.2 μm i.d. pore size filter (Millipore, Bedford, MA). Before purification, hydrocolloids and proteins present in the juice were removed by adding 96% ethanol to the sample according to Stintzing et al.[5] Subsequent preconcentration of the solution under reduced pressure at 25 °C was performed after filtering off the originated mucilages through a layer of 0.063÷0.200 mm silica gel (J.T.Baker, Deventer, Holland) and a 0.2 μm i.d. pore size filter to obtain a clear solution of the pigments, which was flushed with argon before storage at -20 °C. In the next steps for pigment clean-up and isolation, the extract was chromatographically purified by open column chromatography on C18 sorbent (Merck, Darmstadt, Germany) following a modified procedure by Stintzing et al.[5] Typically, C18 sorbent was activated with 100% methanol and then rinsed with 1% formic acid. The samples were applied to the column, rinsed again with 1% formic acid and betanin was eluted with 1% formic acid solution in methanol. The eluates were pooled and preconcentrated under reduced pressure at 25 °C. Concentrated extract was submitted to semipreparative flash chromatography (preparative HPLC system with LC-20AP pumps, UV-Vis SPD-20AV detector and LabSolutions 5.51 operating software, Shimadzu Corp., Japan) on a C18 flash column (Interchim, France) for the separation of betanin. Further purification of the pigment was performed on an HPLC semipreparative column Luna C18(2) 250 x 30 mm i.d., 10 μm (Phenomenex, Torrance, CA, USA). The eluates were pooled and preconcentrated under reduced pressure at 25 °C and finally freeze-dried to obtain pure betanin. Fresh aqueous or alcoholic solutions of betanin were used in spectroscopic studies.

Red beet (*Beta vulgaris* L., cultivar *czerwona kula*) was collected at a local market in Poznan city. Red beet root was hand-peeled, and cut into small pieces. They were covered with water in a flask for 3 minutes, and the obtained extract was filtered with a Nylon syringe filter with 0.45 μm pores.

**Computational Methods.**

Density functional theory (DFT) and time-dependent DFT (TD-DFT) calculations were performed with use of the Gaussian 09 suite of programs[6] at the PL-Grid Infrastructure. The electronic spectra were computed with time-dependent DFT methods in Gaussian 09 for functional B3LYP with 6-311++G(d,p) basis set. 10 vertical excitation energies were calculated for the optimized ground-state geometries at B3LYP/6-311++G(d,p) level of theory. Vibrational frequency analyses at the B3LYP/6-31++G(d,p) level were used to verify that the resulting stationary points corresponded to energy minima (with zero imaginary vibrational frequencies).

**References:**


1. I. Carmichael and G. L. Hug, *J. Phys. Chem. Ref. Data*, 1986, **15**, 1.
2. A. N. Tarnovsky, W. Gawelda, M. Johnson, C. Bressler and M. Chergui, *J. Phys. Chem. B*, 2006, **110**, 26497.
3. A. Yoshimura, M. Z. Hoffman and H. Sun, *J. Photochem. Photobiol. A: Chem.*, 1993, **70**, 29.
4. M. Wendel, D. Szot, K. Starzak, D. Tuwalska, D. Prukala, T. Pedzinski, M. Sikorski, S. Wybraniec and G. Burdzinski, *Dyes Pigments*, 2015, **113**, 634.
5. F. C. Stintzing, A. Scheiber and R. Carle, *J. Agric. Food Chem.*, 2002, **50**, 2302.
6. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery Jr, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, *Gaussian 03, Revision B.01*.