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Supporting Information (SI)

Direct Observation of the Oxidation of DNA Bases by Phosphate Radical Formed under Radiation: A Model of Backbone-to-base Hole Transfer

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1. *Spectroscopic evaluation of DNA bases dissolved in 6 M H_3PO_4*

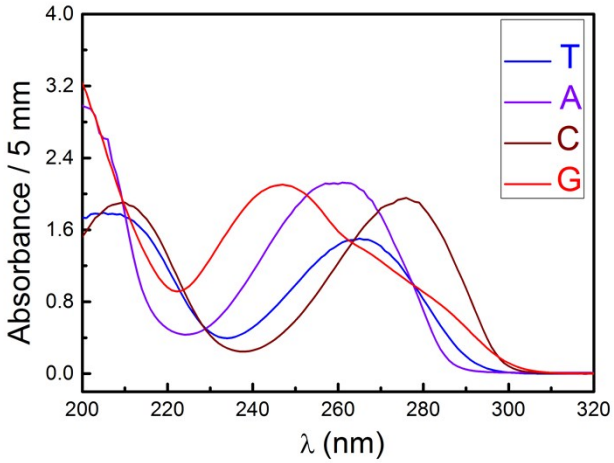


Figure SI1. Steady state UV-Visible spectrophotometric measurement of DNA base (T, G, A and C) in 6 M H_3PO_4 aqueous solutions.

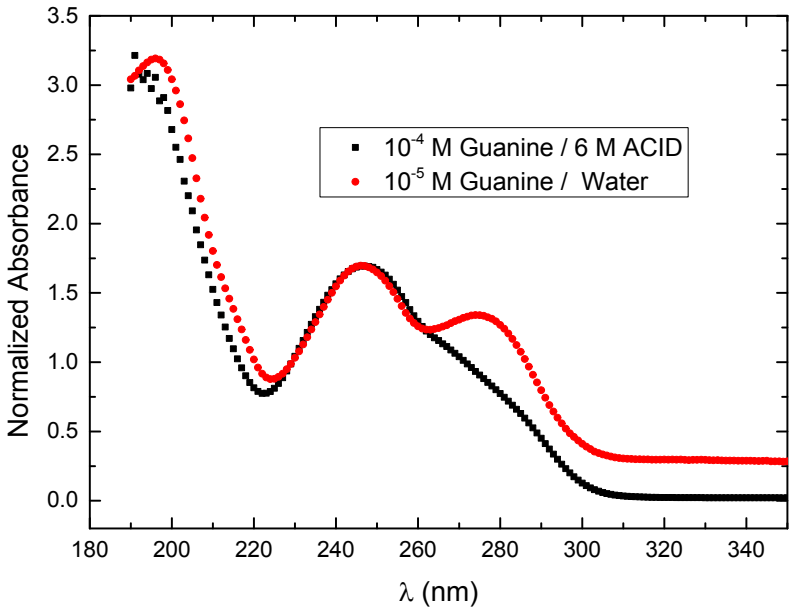


Figure SI2. Normalized absorption spectrum of guanine in neat water (black) comparing to that in 6 mol L^{-1} H_3PO_4 solution (red). The absorbance was normalized according to the peak at 247 nm.

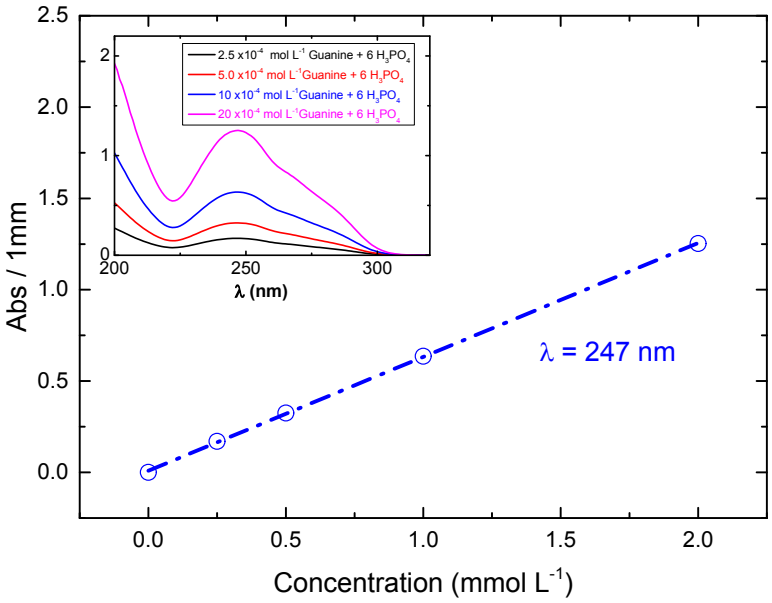


Figure SI3. The absorbance at a fixed wavelength of 247 nm plotted as a function of guanine concentration dissolved in 6 M H_3PO_4 solutions. UV-Visible absorption spectra of guanine in different guanine concentrations (insert).

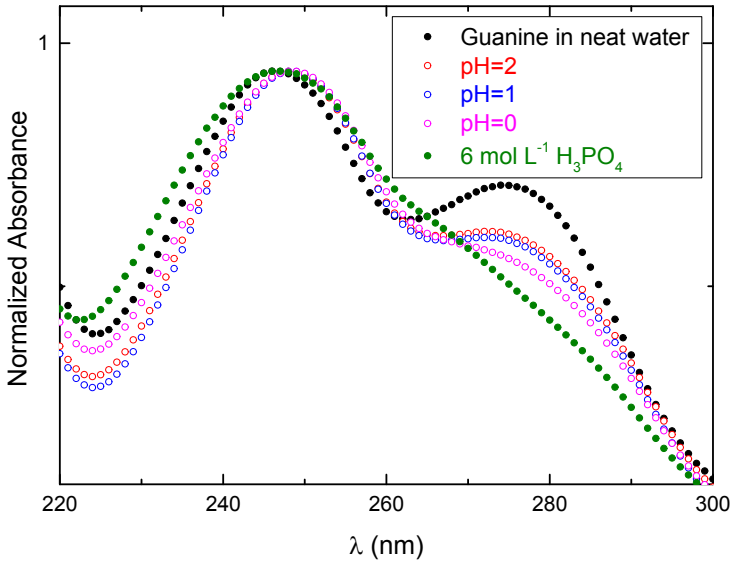


Figure SI4. Normalized spectrum of guanine dissolved in aqueous solutions at different pH comparing with that in neat water and 6 M H_3PO_4.

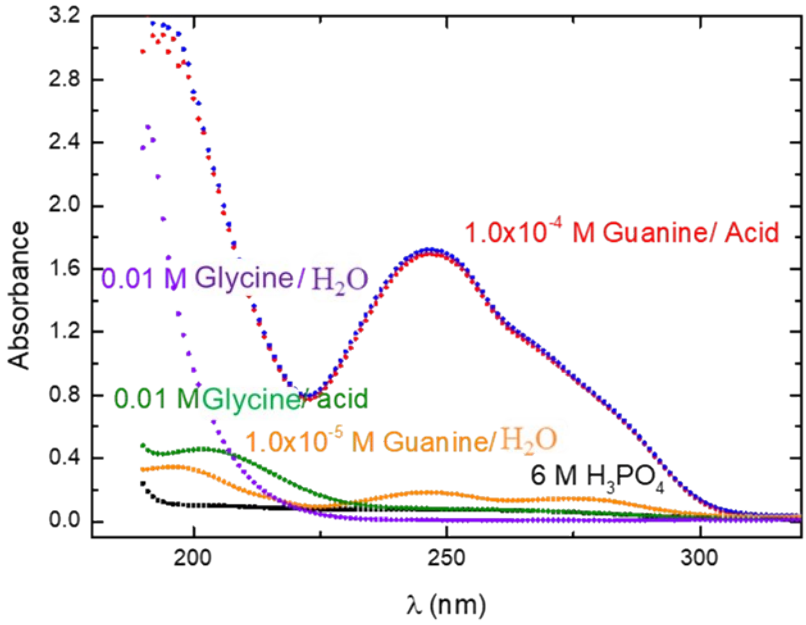


Figure SI5. UV-Visible absorption spectrum of guanine molecules in fresh acid (red) and the one placed after 48 hours (blue). The spectrum of the solvent of 6 M H_3PO_4 (black) and glycine solutions are measured as the reference.

2. Guanine radical cation transformation

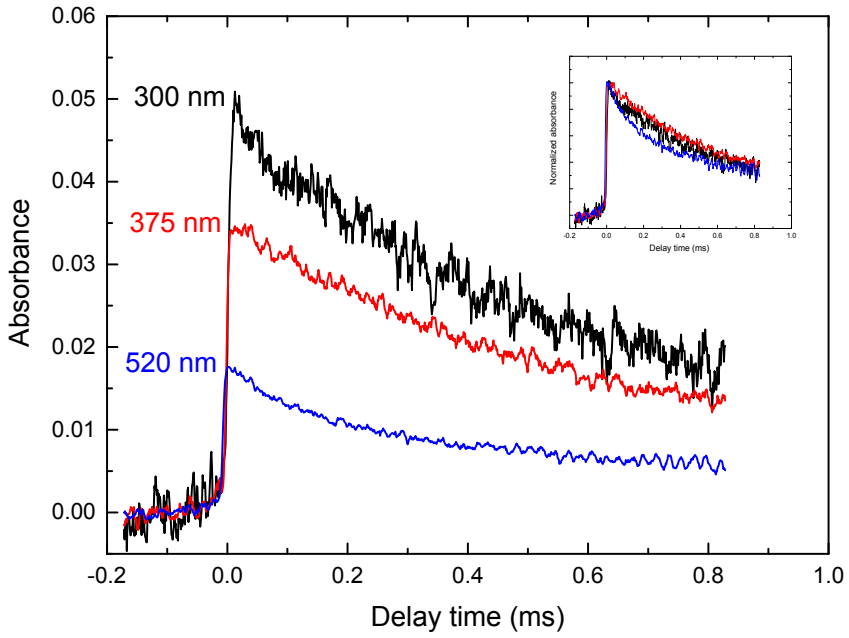


Figure SI6 Kinetics in 2mM Guanine / 6 M H_3PO_4 solutions at longer delay time (1 ms).

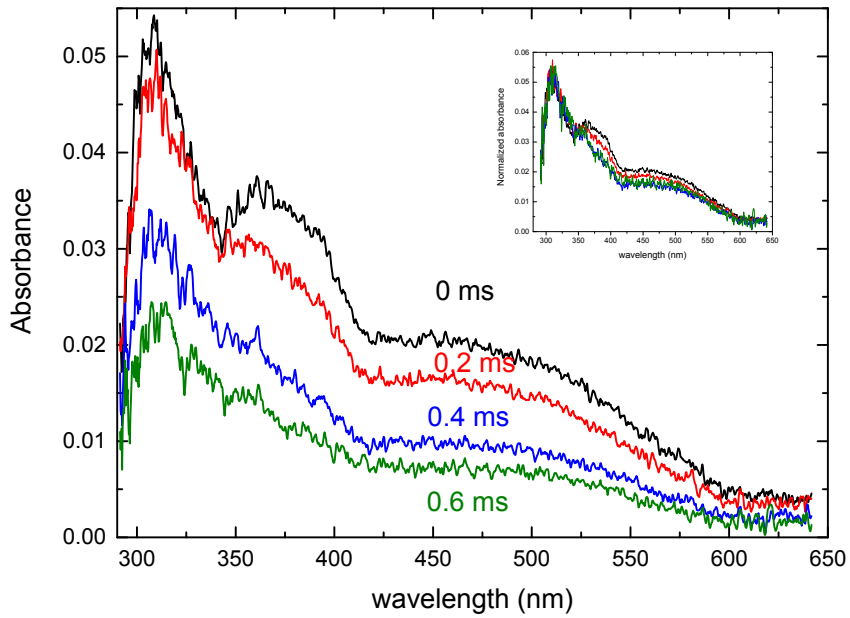
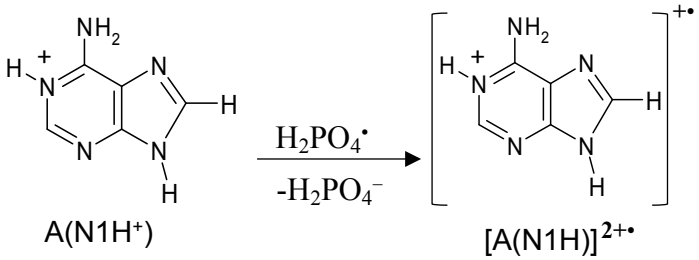


Figure SI7 Transition absorption spectrum of 2mM Guanine / 6 M H_3PO_4 solutions at different delay on the time scale of millisecond.

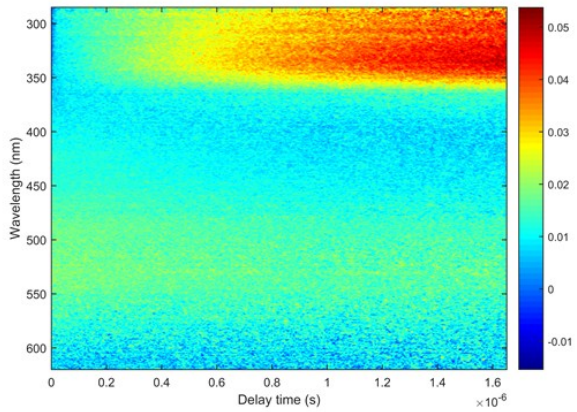
3. Adenine Oxidization by $H_2PO_4^{\bullet}$

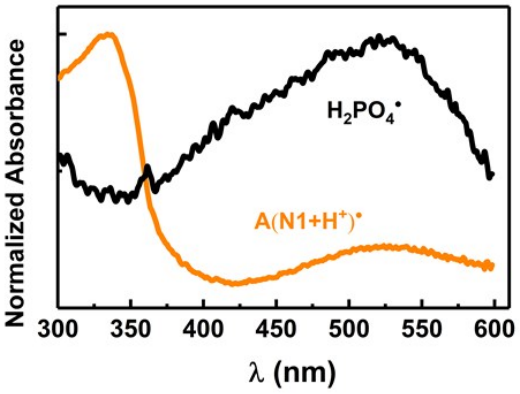
Pulse radiolysis, flash photolysis, ESR, and theoretical studies of one-electron oxidized adenine in its cation radical state ($A^{+\bullet}$) and the conjugate base ($A(N6-H)^{\bullet}$) have been reported in monomers as well as in single stranded and in duplex DNA.[1,2]Adenine with the *pKa* value of 4.15 is protonated at N1 sites,[3] $A(N1+H)^+$ in our system (6 M H_3PO_4). In analogy to the guanine case, the reaction of $H_2PO_4^{\bullet}$ with adenine is evident; the results are presented in Figure 5. The two-dimensional color image analysis clearly shows only two absorbing species which co-exist within the observing time window (0-5 μs). The decay of $H_2PO_4^{\bullet}$ and the concomitant formation of new species are correlated with the adenine concentration. This leads us to conclude that $H_2PO_4^{\bullet}$ causes the oxidation of N1-protonated adenine, [$A(N1H^+)$]. As shown in Figure SI6 (middle), adenyl radicals produced via reaction of $H_2PO_4^{\bullet}$ with [$A(N1H^+)$] in 6 M H_3PO_4 exhibit one sharp maximum at 330 nm and an additional broad band at 525 nm. Though the spectral shape of the resulting adenyl radical intermediate and the position of the sharp maximum match with the reported spectra of one-electron oxidized adenine, the position of the broad maximum (525 nm, Figure SI6 (middle)) appears to be blue-shifted from the reported one (*ca*. 600 nm) in the literature.[1,2] In the recent work on spectral characterization of adenyl radicals, it has been shown experimentally and theoretically that the broad band around 600 nm exhibits the blue shift in the UV-vis spectrum due to cation radical in comparison to the spectrum of the conjugate base, i.e., $A(-H^+)^{\bullet}$. Therefore, based on this blue shift, and on the recent studies regarding *pKa* of adenine and $A^{\bullet+}$, and on our theoretical studies (vide infra), the spectrum in Figure SI6 is assigned to $[A(N1H)]^{2+\bullet}$ (see scheme SI1 and Table 3).

Our results obtained from pulse radiolysis of adenine / 6 M H_3PO_4 established that $H_2PO_4^{\bullet}$ acts as a strong one-electron oxidant towards adenine. By varying the adenine concentration, the rate constant of this oxidation reaction is determined to be 2.4×10^8 L mol^{-1} s^{-1} (Figure 4 and Table 2, in main text), two times less than that for guanine (Figure 4 in main text); this result points out that guanine is easier to be oxidized than adenine and is in agreement with the redox potential values of G and A. Similar to our results obtained employing the guanine solutions, we find that •OH-mediated formation of $[A(N1H^+)]^{\bullet}$ (here the N6-proton is lost owing to •OH-addition at C4=C5 in the adenine base followed by water elimination) has to be faster than that of the $[A(N1H)]^{2+\bullet}$ production via one-electron of A by $H_2PO_4^{\bullet}$.



Scheme SI1. Formation of $A(N1H^+)^{+\bullet}$ via one-electron oxidation of $A(N1H^+)$ by $H_2PO_4^\bullet$ in 6 M H_3PO_4.





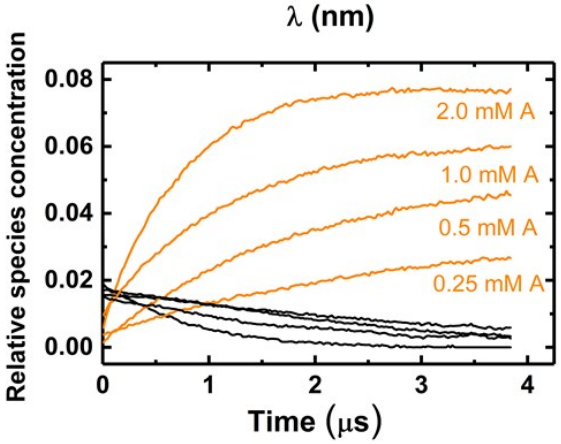


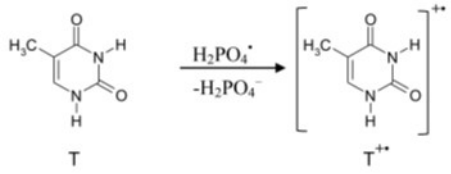
Figure SI 8. Experimental two-dimensional image, showing the evolution of the absorbance at every wavelength vs. time after one electron pulse applied in an aqueous 6 M H_3PO_4/1.0 mM adenine solution (top). The intensity profile of absorption spectra of absorbing species over the wavelengths (middle) and kinetics of each species (bottom) are obtained from the analysis via a MCRALS approach of above images recorded at different adenine concentrations.

4. Thymine oxidation / adducting by $H_2PO_4^{\bullet}$

Based on above-mentioned evidences regarding oxidation of purine bases (G and A) by $H_2PO_4^{\bullet}$, the decay of $H_2PO_4^{\bullet}$ observed on the 5 μs timescale has presented the evidence of its reaction with thymine in 6 M H_3PO_4 (see Figure SI9). The transient spectrum of resulting intermediate is similar with previous work by Steenken *et al*.[4] and O'Neill *et al*.[1] in which $SO_4^{\bullet-}$ was used as the one-electron oxidant and thus, the spectrum of the thymyl radical generated in our system is due to the one-electron oxidized thymine. We assign the spectrum of the thymyl radical in Figure SI9 (middle) to the thymine cation radical (vide infra). However, some striking differences are found in thymine / 6 M H_3PO_4 system compared to those in purine bases.

Our results in sections 2 and 3 have shown that in the cases of Guanine and Adenine, formation of OH• adducts to the electron rich C4=C5 double bond of protonated G and of protonated A happen very rapidly (i.e., within 100 ns). Therefore, we expect similar rapid formation of OH• adduct (T(C5-OH)$^{\bullet}$ or T(C6-OH)$^{\bullet}$) via electrophilic addition of OH• to the C5=C6 double bond of T. We note that the formation of either T(C5-OH)$^{\bullet}$ or T(C6-OH)$^{\bullet}$ is also a diffusion-controlled reaction (4.6×10^9 L mol^{-1} s^{-1}) and these adduct radicals have been well-studied by pulse radiolysis and ESR. Similar to our results in samples containing G and A (sections 2 and 3),[5,6] we do observe thymine concentration-dependent increase in the $H_2PO_4^{\bullet}$ mediated formation of thymyl radicals (Figure 6 (bottom)). From Figure SI9 (middle) and Figure SI9 (bottom), it is evident that after the fast processes that lead to generation of $H_2PO_4^{\bullet}$, the thymyl radicals are subsequently formed via reactions of thymine with $H_2PO_4^{\bullet}$. The spectrum of the thymyl radical in Figure SI9 (middle) matches well with that of the authentic thymine cation radical ($T^{\bullet+}$) formed via $SO_4^{\bullet-}$ induced one-electron oxidation of 1,3-dimethylthymine and 1,3,6-trimethylthymine45. On this basis, we assign this spectrum in Figure SI9 (middle) to $T^{\bullet+}$ (see scheme 3 and Table 3). However, our theoretical calculations predict that the spectrum of $H_2PO_4^{\bullet}$ mediated thymyl radical could be due to O4-protonated thymine cation radical ($T(O4H^+)^{\bullet+}$).

The *pKa* of thymine cation radical in thymidine (Thd) has been reported as 3.6.[3,4] Therefore, in our system (6 M H_3PO_4), the $H_2PO_4^{\bullet}$ induced thymyl radical exists as $T^{\bullet+}$. Owing to the electropositivities and redox potential values of the bases, the rates of oxidation of pyrimidine bases by $SO_4^{\bullet-}$ have been reported to be lower than those of purine bases. In view of this, $H_2PO_4^{\bullet}$ mediated oxidation of the purine bases is considered to be more thermodynamically and kinetically favorable. However, it is evident from Figure 4 and Table 2 that the rate constant of the reaction of thymine in 6 M H_3PO_4 with $H_2PO_4^{\bullet}$ is 1×10^9 L mol^{-1} s^{-1}; this rate constant value is ca. 1.5 times higher than the corresponding reaction of guanine and is ca. 4 times higher than adenine under identical conditions. The high rate constant of T oxidation by $H_2PO_4^{\bullet}$ indicates that oxidation potential values of the DNA bases are not the only factor involved in the reaction between a DNA base and $H_2PO_4^{\bullet}$.Moreover, in the case of thymine, immediately after the formation of $T^{\bullet+}$, a pseudo-first-order decay of the cation radicals has been observed. This decay depends on the concentration of thymine (see Figure SI9). On the basis of similar type of concentration-dependent decay of N-centered Hoechst 33258 (N-centered H-258) radicals[7] which has been attributed to the aggregation of an N-centered H-258 radical with a parent H-258 molecule, this concentration-dependent decay is attributed to the formation of thymine cation radical dimer ($T_2^{\bullet+}$).



Scheme SI2. Formation of $T^{+\bullet}$ via one-electron oxidation of T by $H_2PO_4^{\bullet}$ in 6 M H_3PO_4.

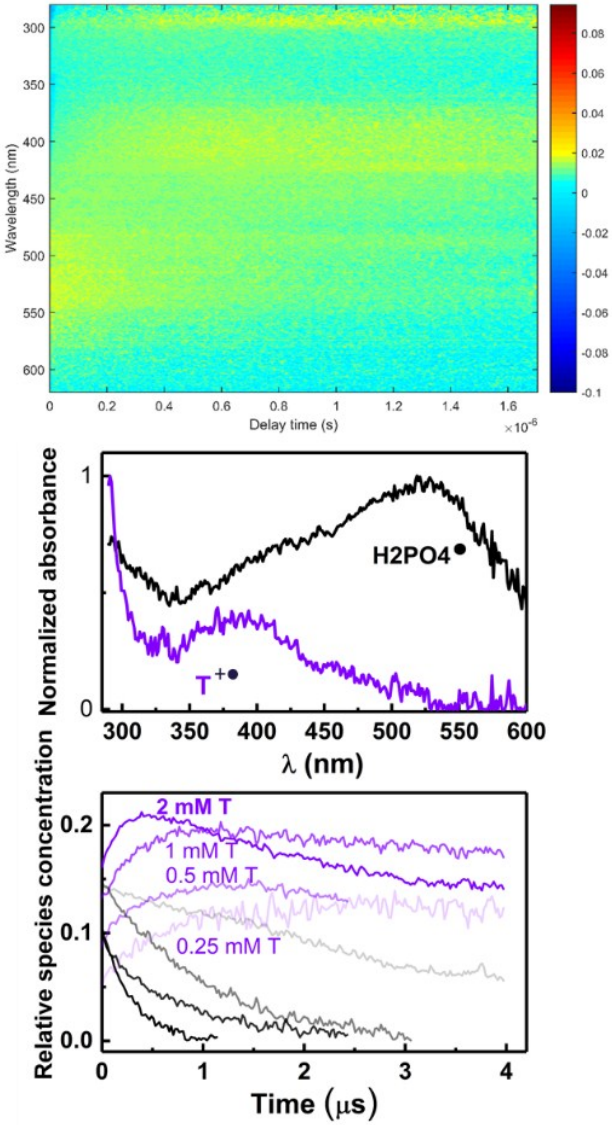


Figure SI9. Experimental two-dimensional image, showing the evolution of the absorbance at every wavelength versus time after one electron pulse applied in an aqueous 6 M H_3PO_4/1.0 mM thymine solution (top). The intensity profile of absorption spectra of absorbing species over the wavelengths (middle) and kinetics of each species (bottom) are obtained from the analysis via a MCRALS approach of above images recorded at different thymine concentrations.

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[1] O'Neill, P.; Davies, S. E. Pulse Radiolytic Study of the Interaction of $SO_4^{\bullet-}$ with Deoxynucleosides. Possible Implications for Direct Energy Deposition. *Int. J. Radiat. Biol.* **1987**, *52*, 577 – 587.

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[7] Adhikary, A.; Bothe, E.; Jain, V.; von Sonntag, C. Pulse radiolysis of the DNA-binding bisbenzimidazole derivatives Hoechst 33258 and 33342 in aqueous solutions. *Int. J. Radiat. Biol.* **2000**, *76*, 1157 – 1166.

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