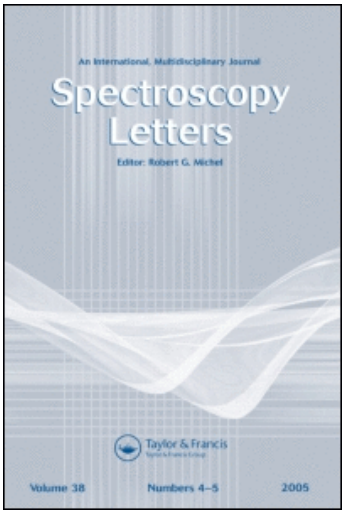


Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Investigation of Photoinduced Electron Transfer Between ZnPcLTs and Guanine: The Role of Type I Mechanism in Photodamage of Calf Thymus DNA

Shaohua Wei[ab]; Jiahong Zhou[a]; Yuying Feng[a]; Xuesong Wang[c]; Baowen Zhang[c]; Deyin Huang[b]; Jian Shen[a]
[a] Jiangsu Engineering Research Center for Bio-medical Function Material, Nanjing Normal University, Nanjing, People's Republic of China [b] School of Chemistry and Chemical Technology, Shanghai JiaoTong University, Shanghai, People's Republic of China [c] Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing, People's Republic of China

To cite this Article Wei, Shaohua , Zhou, Jiahong , Feng, Yuying , Wang, Xuesong , Zhang, Baowen , Huang, Deyin and Shen, Jian(2007) 'Investigation of Photoinduced Electron Transfer Between ZnPcLTs and Guanine: The Role of Type I Mechanism in Photodamage of Calf Thymus DNA', Spectroscopy Letters, 40: 1, 129 — 137
To link to this Article: DOI: 10.1080/00387010600934477
URL: http://dx.doi.org/10.1080/00387010600934477

Spectroscopy Letters, 40: 129–137, 2007

ISSN 0038-7010 print/1532-2289 online
DOI: 10.1080/00387010600934477

Investigation of Photoinduced Electron Transfer Between ZnPcLTs and Guanine: The Role of Type I Mechanism in Photodamage of Calf Thymus DNA

Shaohua Wei
Jiangsu Engineering Research Center for Bio-medical Function Material, Nanjing Normal University, Nanjing, People's Republic of China and School of Chemistry and Chemical Technology, Shanghai JiaoTong University, Shanghai, People's Republic of China

Jiahong Zhou and Yuying Feng
Jiangsu Engineering Research Center for Bio-medical Function Material, Nanjing Normal University, Nanjing, People's Republic of China

Xuesong Wang and Baowen Zhang
Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing, People's Republic of China

Deyin Huang
School of Chemistry and Chemical Technology, Shanghai JiaoTong University, Shanghai, People's Republic of China

Jian Shen
Jiangsu Engineering Research Center for Bio-medical Function Material, Nanjing Normal University, Nanjing, People's Republic of China

Abstract: The photoinduced electron transfer between ZnPcLTs and guanine was studied by steady-state emission and electron spin resonance as well as

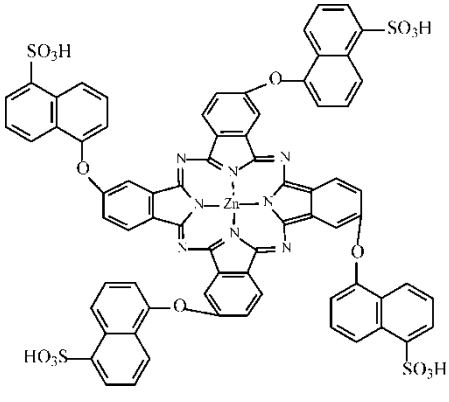
Received 14 February 2006, Accepted 31 July 2006
Address correspondence to Shaohua Wei, Jiangsu Engineering Research Center for Bio-medical Function Material, Nanjing Normal University, Nanjing 210097, People's Republic of China. Fax: 86-25-8359-8359; E-mail: shwei@njnu.edu.cn

time-resolved absorption and emission, indicating the key role of type I (electron transfer) mechanism in photodynamic damage of calf thymus DNA by ZnPcLTs.

Keywords: Guanine, mechanism, photoinduced electron transfer, ZnPcLTs

INTRODUCTION

The derivatives of sulfonated zinc and aluminum phthalocyanines have attracted much attention as second-generation photosensitizers for treatment of malignant tumors using photodynamic therapy (PDT) in the past decade.[1–3] Their PDT efficiencies depend not only on the formation of the cytotoxic species ($^1O_2$, $O_2^{\bullet -}$, and $OH^{\bullet}$) but also on the amphiphilicity of the photosensitizer.[4,5] Because the hydrophilic character benefits the transport of the drug in the body, while the lipophilic character benefits the uptake of the drug by tumor cells, the amphiphilicity is considered to be an important factor in the design of a new photosensitizer[6–8] However, their large π conjugation leads to a strong stacking tendency in solution, which usually decreases their luminescence quantum yields, shortens their triplet state lifetime, and reduces their photosensitizing efficiencies because the more active form of the dye is monomeric.[9,10] Recently, we synthesized a novel zinc phthalocyanine (ZnPcLTs) containing sulfonic naphthoxy substituents (Scheme 1). The naphthoxy groups not only enhanced the lipophilic character of compound but also prevented molecular aggregation in solution by providing steric hindrance. The results of our work indicated that ZnPcLTs possessed stronger photodamage capability toward calf thymus DNA (CT DNA) than the Zn-phthalocyanine without naphthoxyl groups.[11] Though the photodynamic drug can photodamage the tumor cell via either a type I (electron transfer) mechanism or a Type II (energy transfer) mechanism,[12] singlet oxygen generation has generally been the main



Scheme 1. The structure of ZnPcLTs.

concern when evaluating photodynamic properties of photosensitizers, and the type II mechanism was often invoked. We recently found that an efficient photoinduced electron transfer (PET) can occur between ZnPcLTs and guanine (G), the base being most readily oxidized among the natural bases due to its lowest oxidation potential. The presence of O_2 promoted this PET further, implying a type I mechanism may play an important role in the photodamage of CT DNA by ZnPcLTs even in anaerobic conditions.

Here, the PET between ZnPcLTs and G was determined by steady-state or time-resolved absorption, emission, and ESR spectroscopy. To the best of our knowledge, this is the first report on PET between metal phthalocyanine and G, providing a better understanding of the photodynamic mechanism associated with such types of photosensitizers.

MATERIALS AND METHODS

Materials

Zinc phthalocyanine (ZnPcLTs) was prepared according to Ref. 11. *N-tert*-butyl-*R*-phenylnitrone (PBN) and guanine (G) were all purchased from Aldrich Chemical Company (St. Louis, USA). Dimethylsulfoxide (DMSO) and other reagents of analytical grade were obtained from Beijing Chemical Plant (Beijing, China). All experimental solutions were buffered at pH 6.5 by 1.0 mmol/L PBS, and water was freshly distilled before use.

Electron Spin Resonance Measurements

ESR spectra were obtained using a Bruker ESP-300E spectrometer (Wissembourg, France) operating at room temperature, and the operating conditions were as follows: microwave bridge, X-band with 100 Hz field modulation; sweep width, 100 G; modulation amplitude, 1.0 G; modulation frequency, 100 kHz; receiver gain, 1×10^5; microwave power, 5 mW. Samples were injected into the specially made quartz capillaries for ESR analyses and purged with argon, air, or oxygen for 30 min in the dark, respectively, according to the experimental requirements and illuminated directly in the cavity of the ESR spectrometer with an Nd: YAG laser (532 nm, 5–6 ns of pulse width; repetition frequency, 10 Hz, 10 mJ/puse energy).

Nanosecond Laser Flash Photolysis Experiments

In nanosecond flash photolysis studies, the second harmonic of a YAG laser ($\lambda_{ex} = 532$ nm, 8 mJ/pulse) was used as pump source and a pulsed xenon lamp as the probe source traveling in small-angle geometry relative to the

excitation light. The transmitted light passing through the sample was fractioned through a multichromator and was detected by an OMA-III system (EG & G Princeton Applied Research, USA).

Time-Resolved Fluorescence Spectroscopy Experiments

The time-resolved emission spectra were measured as follows: a regenerative amplifier Ti:sapphire laser (Tsunami, Spectra Physics Ltd, Newbury, UK) pumped by a mode-locked argon ion laser (Beamlock, Spectra Physics Ltd, Newbury, UK) was used as a light source. The wavelength of the system was tuned from 800 to 513 nm by OPA (OPA-800C, Spectra Physics Ltd, Newbury, UK) (FWHM = 120 fs, 1 kHz, 0.5 mJ). The resulting fluorescence was collected into a multichromator (Hamamatsu C5094, Hamamatsu Corp., Bridgewater, USA) and detected with a streak camera (Hamamatsu C2909, Hamamatsu Corp., Bridgewater, USA). The instrumental response time was about 30 ps.

RESULTS AND DISCUSSION

ZnPcLTs has a fluorescence emission peak at 728 nm in the aqueous buffer solution. When G was added, a gradual decrease of the fluorescence intensity of ZnPcLTs was observed (Fig. 1). According to the classical Stern–Volmer equation:

$$F_0/F = 1 + K_s[Q] \tag{1}$$

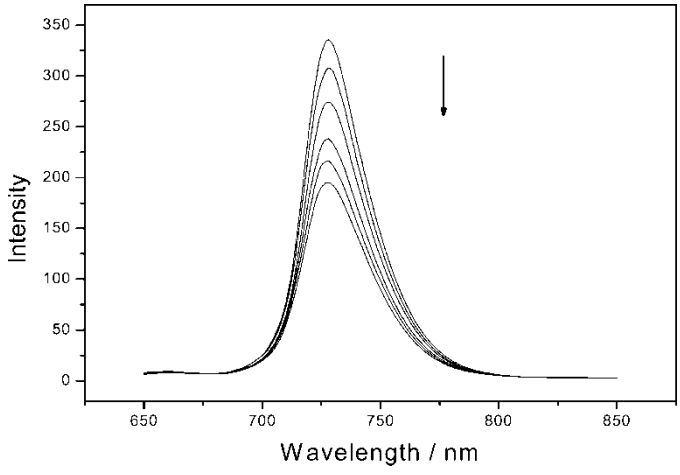


Figure 1. The changes of the fluorescence spectra of ZnPcLTs (1.2×10^{-3} mol/L) in aqueous buffer solution upon addition of G (0, 7.5×10^{-6}, 15×10^{-6}, 22.5×10^{-6}, 30×10^{-6}, 37.5×10^{-6} mol/L, respectively, ex = 613 nm).

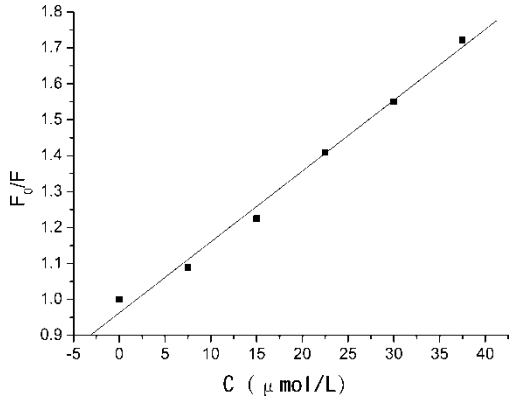


Figure 2. The curve of Stern–Volmer (fluorescence quenching of LSPcZn by G).

where F_0/F represents the ratio of emission intensity in the absence and presence of quencher, K_s is the quenching constant, $[Q]$ is the concentration of quencher; the Stern–Volmer curve of this process can be obtained (Fig. 2). From Fig. 2, it can be found that the curve is a straight line with linear correlation coefficient of 0.9956, and the value of K_s is 1.97×10^4 L/mol. In addition, the UV experiments indicated that the addition of G cannot affect the UV spectra of ZnPcLTs, so the quenching mechanism between ZnPcLTs and G is dynamic quenching.

The oxidation potential of G is 1.3 V, the reduction potential of ZnPcLTs is 0.48 V versus NHE, in addition, based on the absorption edges of ZnPcLTs (750 nm), the reduction potentials of the excited ZnPcLTs is estimated to be 1.65 eV, so the electron transfer from the ground state G to the excited state ZnPcLTs is a thermodynamically favorable process ($\Delta G = -19.14$ kcal/mol for PET occurs in the excited singlet state), estimated by the Rehm–Weller equation [Eq. (2)].[13] As a result, the electron transfer may account for the fluorescence quenching between ZnPcLTs and G:

$$\Delta G = 23.06[E^{ox} - E^{red} - E_{0,0}\,(\text{excited state energy})] \qquad (2)$$

The time-resolved fluorescence spectroscopy also supported this conclusion. Upon exciting the air-saturated aqueous buffer solution of ZnPcLTs with the wavelength of 678 nm, the fluorescence decay curve of ZnPcLTs could be obtained (Fig. 3). The fluorescence decay of ZnPcLTs in the aqueous buffer solution has a best fit to monoexponential function with a decay time of 4.84 ns (the relative value is 0.9943) (Fig. 3, spectrum a). When 20 μM G was added by 100 μL, the singlet excited state lifetime will decrease to 1.64 ns (the relative value is 0.9617) (Fig. 3, spectrum b). This phenomenon was consistent with the results of the fluorescence spectroscopy. Furthermore, the results also suggested that ZnPcLTs can photodamage CT DNA via type I mechanism.

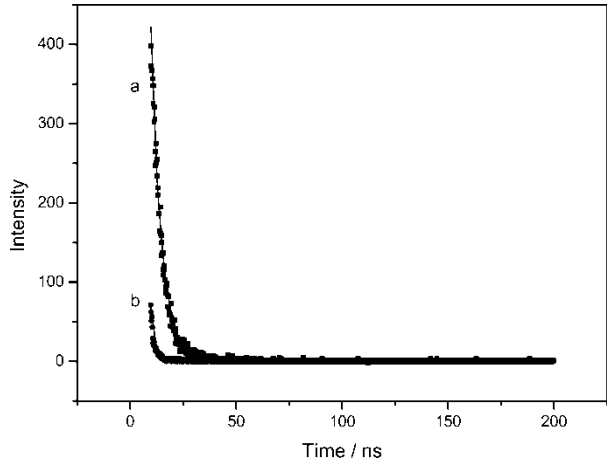


Figure 3. Time-resolved fluorescence spectroscopy of ZnPcLTs (1 μM) in deaerated aqueous buffer solution probed at 503 nm in absence of G (a) or presence of G (25 μM) (b). Dotted, experimental data; line, simulation data based on monoexponential decay.

Our previous studies have indicated that ZnPcLTs possessed higher singlet oxygen generation quantum yield (1.26, assuming that of hematoporphyrin was unity) and can photodamage CT DNA through type II mechanism.[11] Because the generation efficiency of $^1O_2$ depends on the photophysical properties of sensitizers, including the intersystem crossing quantum yield, the triplet-state lifetime, and the triplet-state energy level, time-resolved absorption spectra have been widely applied to study the triplet excited state of phthalocyanines.[14] The time-resolved absorption spectra of the transients produced after 532-nm laser excitation of a deaerated aqueous buffer solution of about 1 μM of ZnPcLTs are shown in Fig. 4. There are one positive absorption band with maximum at 503 nm and two ground state bleaching bands with maxima at 612 nm and 681 nm. Air saturation of the solution led to more rapid decay of all three bands. So the complete spectrum can be attributed to triplet–triplet (T-T) absorption from the excited triplet state of ZnPcLTs. The triplet excited state lifetime of ZnPcLTs was determined to be 376 μs by monitoring the transient absorption at 503 nm, which decays monoexponentially. When 1 mM G was added by 20 μL (the concentration of G in the solution is 5 μM), the triplet excited state lifetime of ZnPcLTs decreased to 0.1 μs markedly. The triplet quenching of ZnPcLTs by G may also be the result of electron transfer between them.

Electron spin resonance is a very useful method to detect and characterize the radicals of the individual nucleic acid bases; but most of those experiments have focused on the generation of radicals using thermal chemistry[15] few works are related to photochemistry. In our experiments, an ESR signal with two hyperfine coupling constants, $\alpha^N = 15$ G and $\alpha_\beta^H = 3$ G, was observed

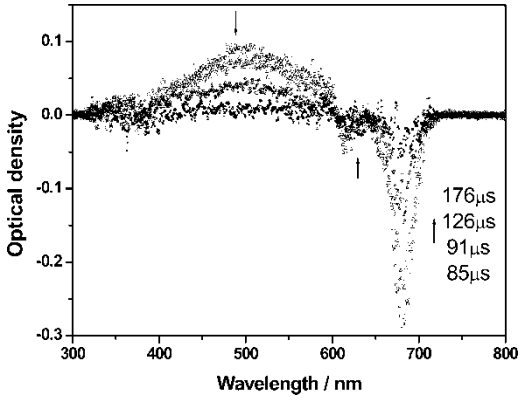


Figure 4. Time-resolved absorption spectra of ZnPcLTs (1 μM) in deaerated aqueous buffer solution (pH = 7.0) measured at 85 μs, 91 μs, 126 μs, and 176 μs after laser pulse at 532 nm. Arrows indicate increasing time delay.

upon irradiation of a deaerated aqueous buffer solution containing 1 μM guanine, 1 μM ZnPcLTs, and 1 mM PBN for 1 min (Fig. 5, spectrum a). The coupling of the electron spin to the nuclear spin of the nitrogen atom split the signal into a triplet, while the β-H split the triplet further into doublets. The signal was consistent with the ESR spectrum for PBN–G adduct, previously reported by Schiemann et al.[16] In the absence of any component (ZnPcLTs, G, PBN, or irradiation), PBN–G. formation did not occur (Fig. 5, spectrum c). From the above results, the G. can be produced by the following mechanism (Scheme 2). First, G was oxidized to guanine radical cation ($G^{\bullet+}$) by the excited state (both singlet and triplet) of ZnPcLTs, and the guanine radical cation was then deprotonated, yielding the

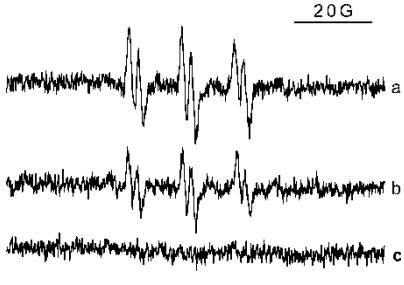
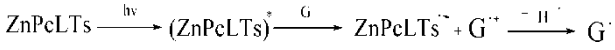


Figure 5. Spectrum a: Photoinduced ESR spectrum from the air-saturated aqueous buffer solution of ZnPcLTs (1 μM), G (1 μM), and PBN (1 mM) on illumination was with 532-nm pulsed laser for 1 min. Spectrum b: Similar to spectrum a, but oxygen was bubbled. Spectrum c: Similar to spectrum a, but ZnPcLTs or G or PBN was omitted.



Scheme 2. The generative mechanism of G radical.

neutral guanine radical (G.). The photoinduced electron transfer between a photosensitizer and biologically related molecules such as nucleic acid bases is of importance for PDT because many cancerous cells grow in anaerobic circumstance, where the generation of reactive oxygen species are restricted severely. When the buffer solution was air-saturated, the signal of PBN-G. adduct could be observed as well, and interestingly, the intensity was increased (Fig. 4, spectrum b). Oxygen may accept electron from the radical anion of ZnPcLTs (generating $O_2^{\bullet -}$) and therefore refrain the electron recombination process between radical cation $G^{\bullet +}$ and radical anion $ZnPcLTs^{\bullet -}$, favoring the formation of $G^{\bullet +}$. The improved generation of $O_2^{\bullet -}$ upon adding G into irradiated air-saturated aqueous solution of ZnPcLTs supports this explanation.

In addition, to confirm Scheme 2 further, the pH dependence of the fluorescence/triplet quenching is being carryed out.

CONCLUSIONS

In summary, the results of the steady-state fluorescence and the time-resolved absorption and fluorescence show that the PET between ZnPcLTs and G can proceed efficiently. The ESR signal of G radical was also observed. The enhanced ESR signal of $G^{\bullet}$ in the presence of O_2 suggest that type I mechanism can not only dominate the photodamage of CT DNA under anaerobic conditions but also may be involved in the photodamage under aerobic conditions, where type II mechanism is generally invoked.

ACKNOWLEDGMENTS

We would like to thank the financial support of the Natural Science Foundation of Jiangsu Education Department and Jiangsu Science Department (grant no. 04KJB150068 and no. BK2006216, respectively).

REFERENCES

1. Foley, M. S. C.; Beeby, A.; Parker, A. W.; Bishop, S. M.; Phillips, D. Photophysics of disulphonated aluminium phthalocyanine in reverse micelles of aerosol-OT. *J. Photochem. Photobiol. B Biol.* **1997**, *38*, 18.

2. Owens, J. W.; Robins, M. Phthalocyanine photophysics and photosensitizer efficiency on human embryonic lung fibroblasts. *J. Porphyrins Phthalocyanines* **2001**, *5*, 460.
3. Vrouenraets, M. B.; Visser, G. W. M.; Stigter, M.; Oppelaar, H.; Snow, G. B.; Vandongen, A. M. S. Comparison of aluminium phthalocyanine tetrasulfonate- and meta-tetrahydroxyphenylchlorin-monoclonal antibody conjugates for their efficacy in photodynamic therapy in vitro. *Int. J. Cancer* **2002**, *98*, 793.
4. Decreau, R.; Richard, M. J.; Julliard, M. Photodynamic therapy against achromic M6 melanocytes: phototoxicity of lipophilic axially substituted aluminum phthalocyanines and hexadecahalogenated zinc phthalocyanines. *J. Porphyrins Phthalocyanines* **2001**, *5*, 390.
5. Foley, M. S. C.; Beeby, A.; Parker, A. W.; Bishop, S. M.; Phillips, D. Excited triplet state photophysics of the sulphonated aluminium phthalocyanines bound to human serum albumin. *J. Photochem. Photobiol. B Biol.* **1997**, *38*, 10.
6. Huang, J. L.; Chen, N. S.; Huang, J. D.; Liu, E. S.; Xue, J. P.; Yang, S. L.; Huang, Z. Q.; Sun, J. C. Metal phthalocyanine as photosensitizer for photodynamic therapy (PDT)—preparation, characterization and anticancer activities of an amphiphilic phthalocyanine ZnPcS2P2. *Sci. China Ser. B* **2001**, *44*, 113.
7. András, G.; Péter, B.; István, B.; Viktor, C.; Mikós, K.; Klára, S.; Janka, T.; Tamás, V. Triple state properties of tetrasubstituted zinc phthalocyanine derivatives. *J. Mol. Struct.* **2004**, *704*, 11.
8. Sakamoto, K.; Kato, T.; Ohno-Okumura, E.; Watanabe, M.; Cook, M. J. Synthesis of novel cationic amphiphilic phthalocyanine derivatives for next generation photosensitizer using photodynamic therapy of cancer. *Dyes Pigments* **2005**, *64*, 63.
9. Drechsler, U.; Pfaff, M.; Hanack, M. Synthesis of novel functionalised zinc phthalocyanines applicable in photodynamic therapy. *Eur. J. Org. Chem.* **1999**, 3441.
10. Fernández, D.; Awruch, A. J.; Dicelio, L. E. 6,4,4-Trimethylangelicin photoadduct immunodetection in DNA: induction and repair in Fanconi's anemia and normal human fibroblasts. *J. Photochem. Photobiol. B Biol.* **1997**, *38*, 227.
11. Wei, S. H.; Zhou, J. H.; Feng, Y. Y.; Wang, X. S.; Zhang, B. W.; Huang, D. Y. Synthesis and type I/type II photosensitizing properties of a novel amphiphilic zinc phthalocyanine. *Dyes Pigments* **2006**, *71* (1), 61.
12. Foote, C. F. Definition of type I and type II photosensitized oxidation. *Photochem. Photobiol.* **1991**, *54*, 659.
13. Kavarnos, G. J.; Turro, N. J. Photosensitization by reversible electron transfer: theories, experimental evidence, and examples. *Chem. Rev.* **1986**, *86*, 401.
14. Oldham, T. C.; Phillips, D. Triplet-state photophysics of aluminium phthalocyanine sensitizer in murine cancer cells. *J. Photochem. Photobiol. B Biol.* **2000**, *55*, 16.
15. Novais, H. M.; Steenken, S. ESR studies of electron and hydrogen adducts of thymine and uracil and their derivatives and of 4,6-dihydroxypyrimidines in aqueous solution. Comparison with data from solid state. The protonation at carbon of the electron adducts. *J. Am. Chem. Soc.* **1986**, *108*, 1.
16. Schiemann, O.; Turro, N. J.; Barton, J. K. EPR Detection of guanine radicals in a DNA duplex under biological conditions: selective base oxidation by Ru(phen)2dppz3+ using the Flash–Quench technique. *J. Phys. Chem. B* **2000**, *104*, 7214.