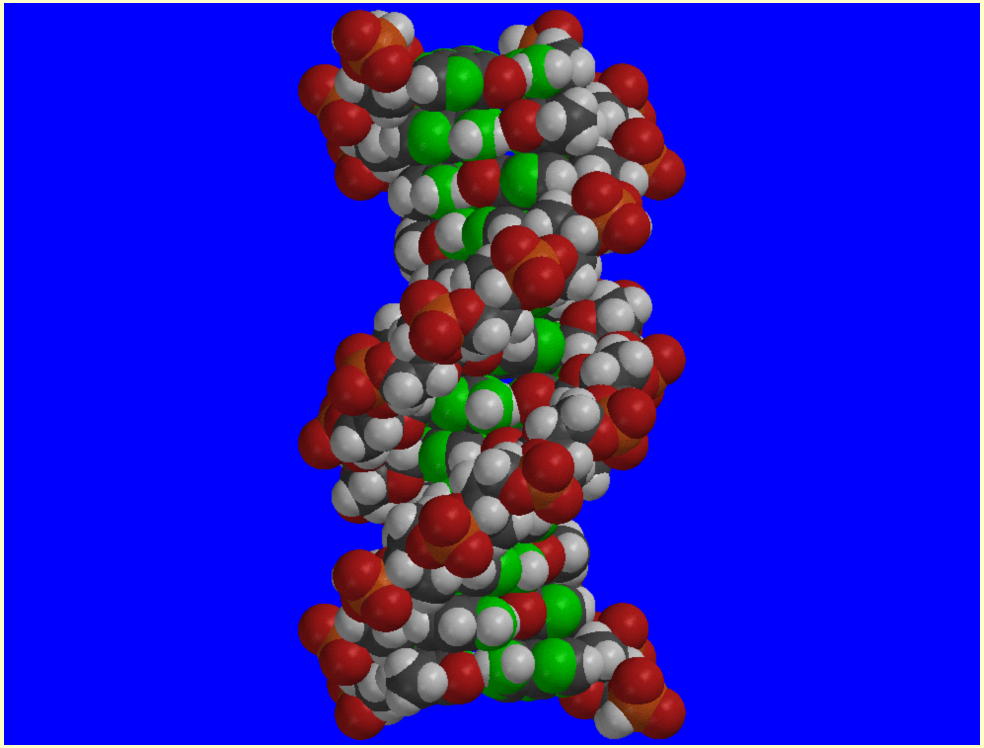
Radiation Chemical Studies of γ- and Ion-beam Irradiated DNA



Amitava Adhikary

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Oakland University

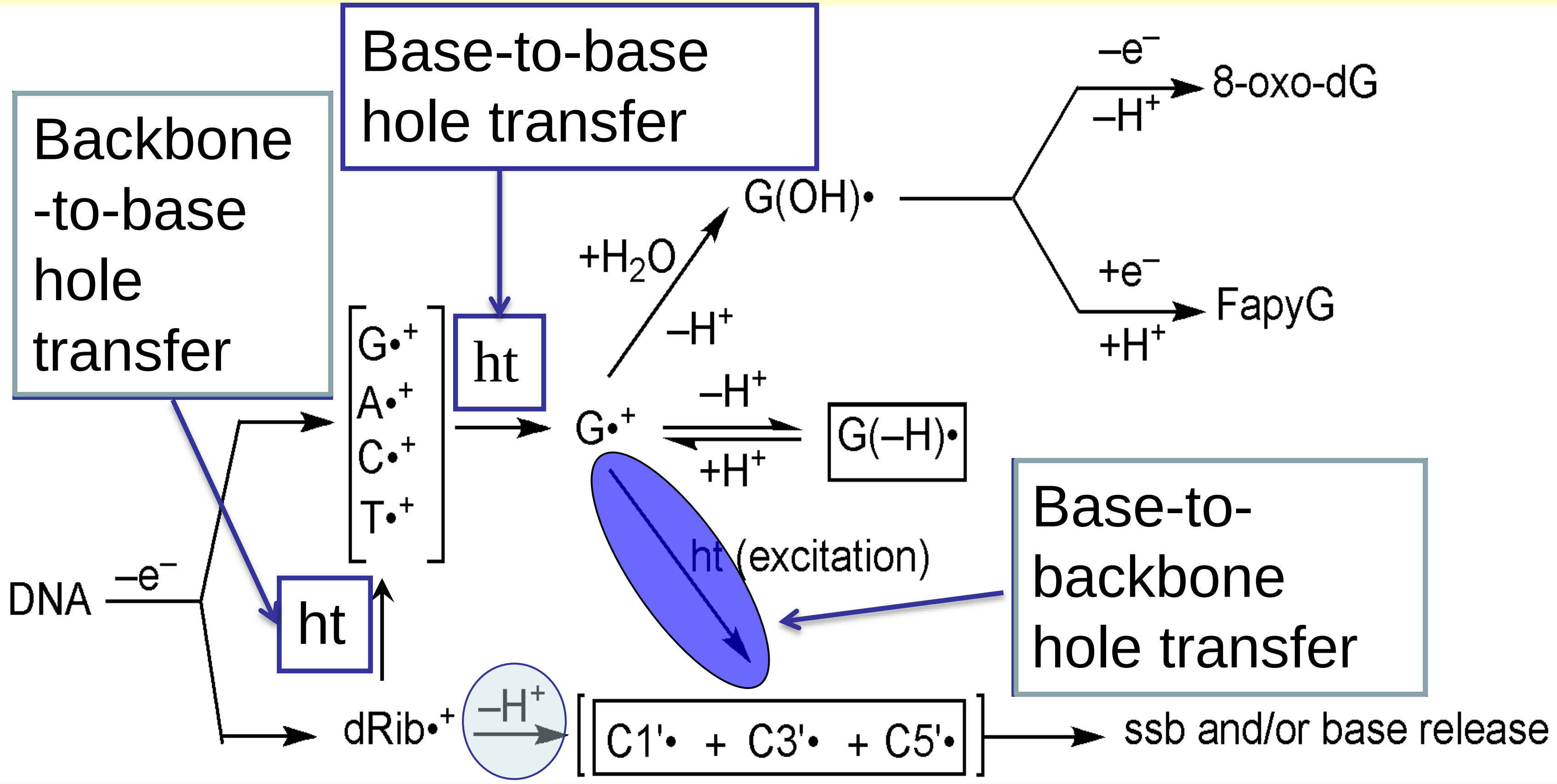
$$\mathbf{DNA} \xrightarrow[\text{Radiation}]{\text{Ionizing}} DNA^{\bullet +} + DNA^{\bullet -}$$

Oxidative (hole)

Reductive path

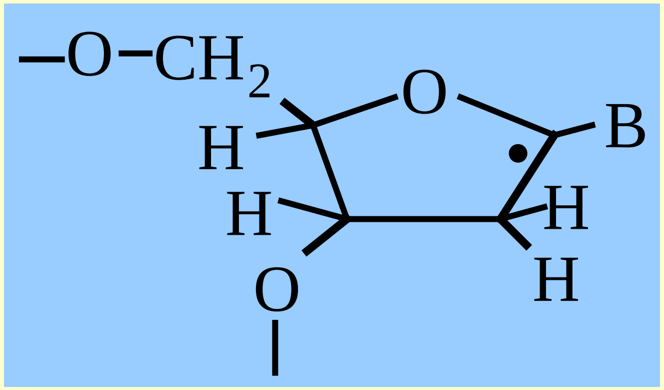
Our model includes contributions of excitations and Low energy electrons (LEE)

DNA-radicals formed via oxidative (i.e., electron-loss) pathway

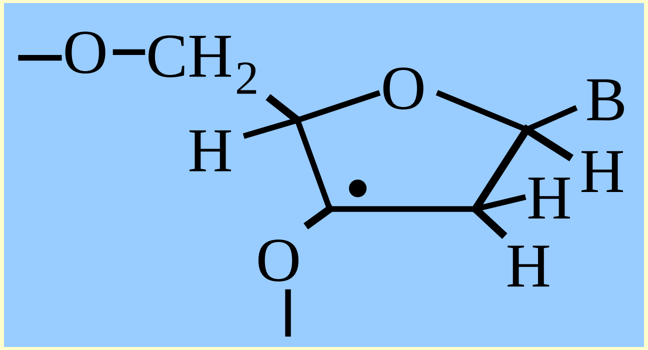


Adhikary, A., Kumar, A., Becker, D., and Sevilla, M. D. (2012) Theory and ESR spectral studies of DNA-radicals. In **Encyclopedia of Radicals in Chemistry, Biology and Materials**. (C. Chatgilialoglu, A. Struder (Eds.)), John Wiley & Sons Ltd, Chichester, UK, pp 1371-1396.

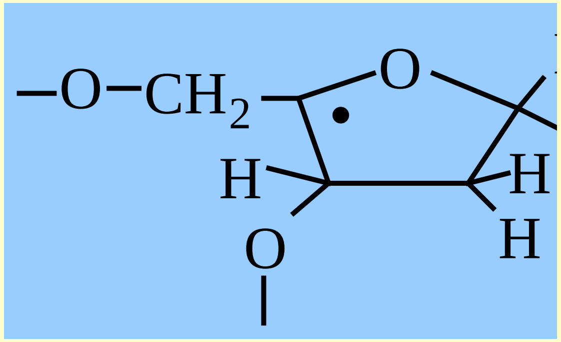
deoxyribose-backbone radicals via hole excitation and/or direct ionization (Oxidative pathway)



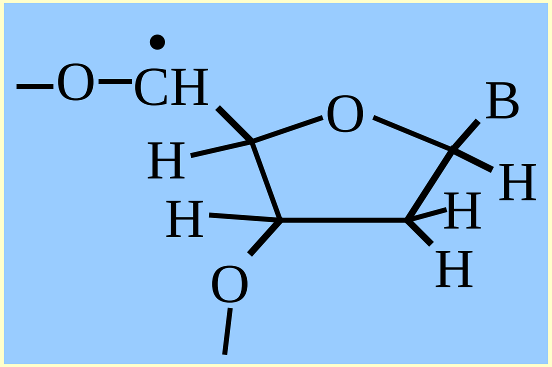
C1′•



C3′•



C4′• (?)



C5′•

Intra-base pair proton transfer is expected from the *p*K$_a$ values of the respective species

$$G^{\bullet +} \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} G(-H)^{\bullet} \qquad K_a = 10^{-3.9}$$

$$C \underset{-H^+}{\overset{+H^+}{\rightleftharpoons}} C(+H^+) \qquad K_a = 10^{4.4}$$

$$G^{\bullet +} : C \rightleftharpoons G(-H)^{\bullet} : C(+H^+)$$

$$K_a = 10^{0.5}$$

Steenken, S. *Free Rad. Res. Commun.*,*16*, **1992**, 349 – 379.

Intra-base pair proton transfer is NOT expected from C•$^+$ to G based on the *p*K$_a$ values

$$C^{\bullet+} \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} C(-H)^{\bullet} \qquad K_a = 10^{-4.4}$$

$$G \underset{-H^+}{\overset{+H^+}{\rightleftharpoons}} G(+H^+) \qquad K_a = 10^{2}$$

$$C^{\bullet+} : G \rightleftharpoons C(-H)^{\bullet} : G(+H^+)$$

$$K_a = 10^{-2.4}$$

Steenken, S. *Free Rad. Res. Commun.*,*16*, **1992**, 349 – 379.

Intra-base pair proton transfer is NOT expected in one-electron oxidized A:T based on the *p*K$_a$ values of the respective species

$$A^{\bullet +} \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} A(-H)^{\bullet} \qquad K_a = 10^{-1}$$

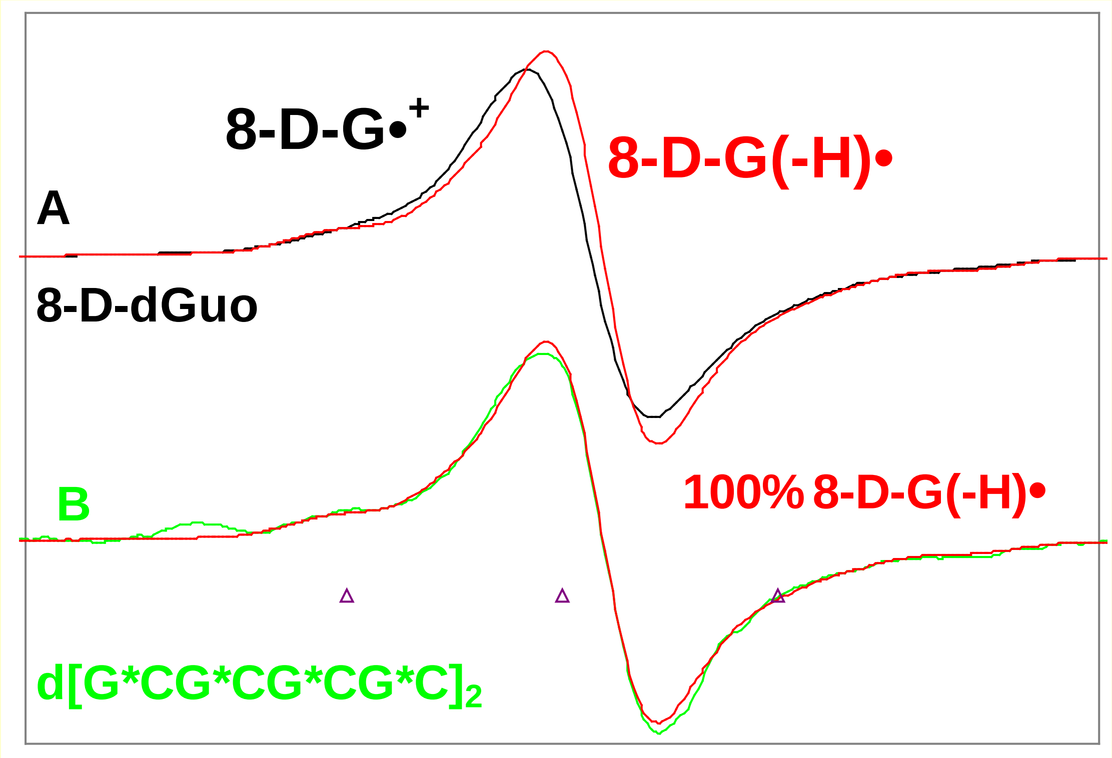
$$T \underset{-H^+}{\overset{+H^+}{\rightleftharpoons}} T(+H^+) \qquad K_a = 10^{-5}$$

$$A^{\bullet +} : T \rightleftharpoons A(-H)^{\bullet} : T(+H^+)$$

$$K_a = 10^{-6}$$

Steenken, S. *Free Rad. Res. Commun.*,*16*, **1992**, 349 – 379.

G•$^+$ protonation state in ds DNA-oligomers: Intra-base pair proton transfer

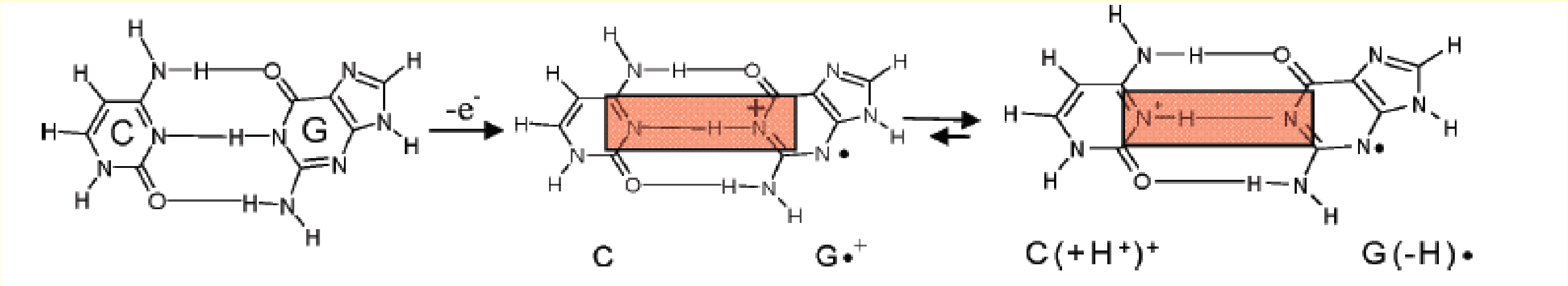


Adhikary et al., JACS 2009, 131 (24), pp 8614–8619.

The ESR spectra of 8-D-G•$^+$ is distinguishable from 8-D-G(-H)•

In a ds DNA model we find 100% deprotonation of 8-D-G•$^+$

Charge is separated from spin by this process and as a result the spin (hole) is localized on a single G



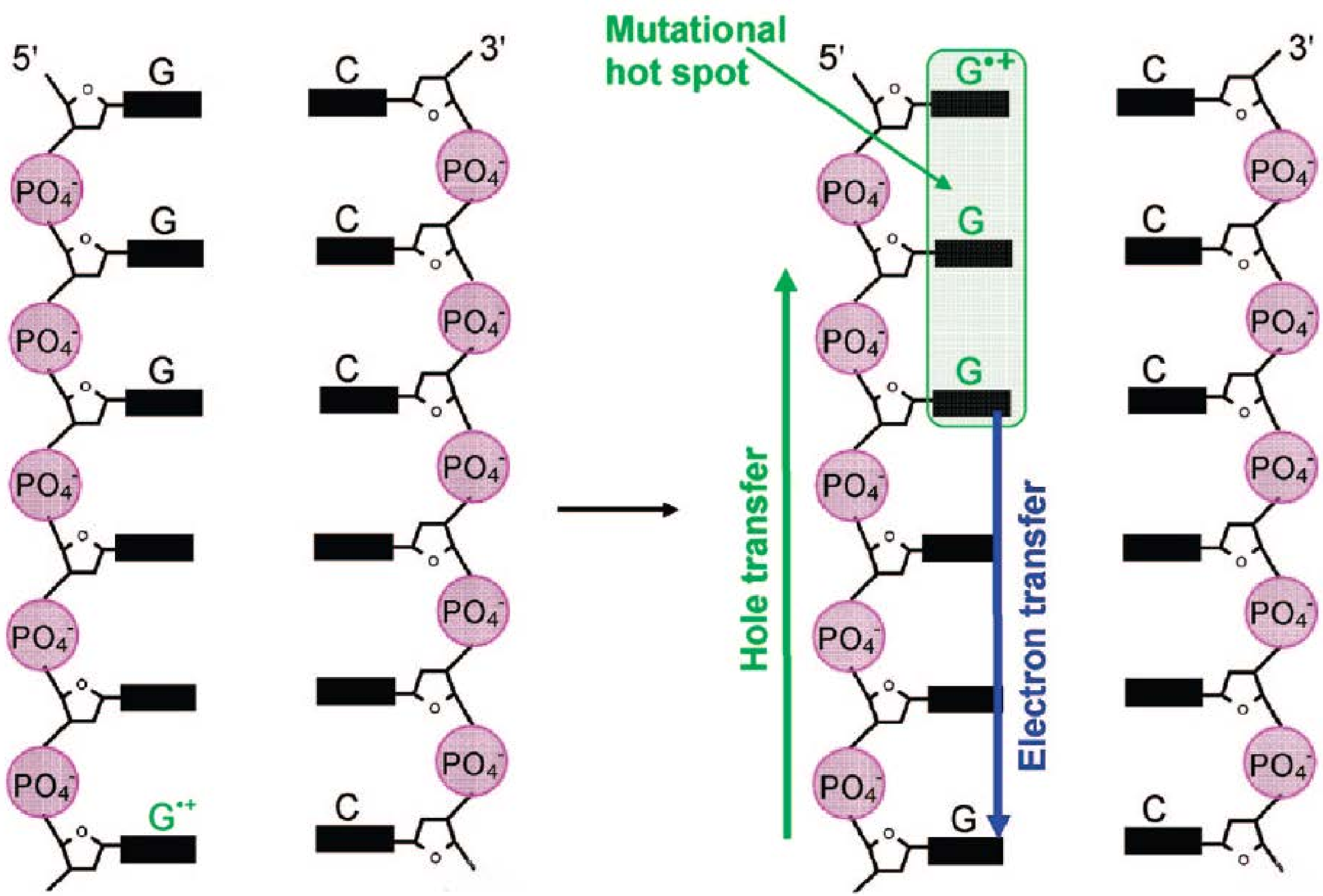
Hole localizes on G

Guanine has the lowest one-electron oxidation potential and the lowest ionization energy amongst the DNA bases; as a consequence, the hole localizes on G.

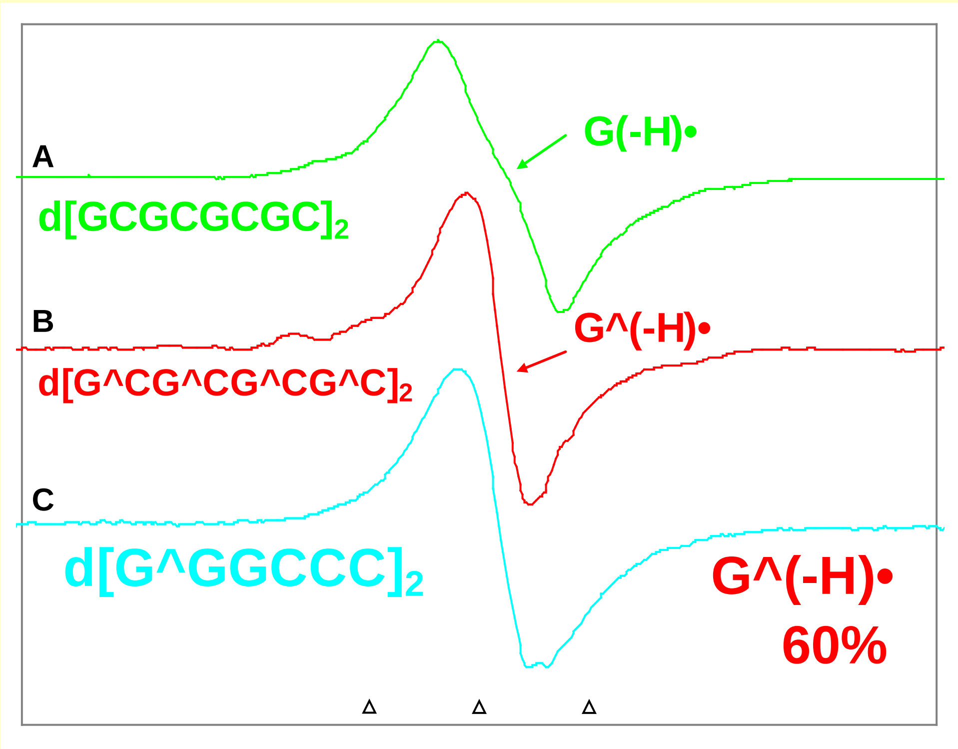
DNA —Radiation / Or one-electron oxidant e.g., $Cl_2^{\bullet -}$→ $G^{\bullet +}$

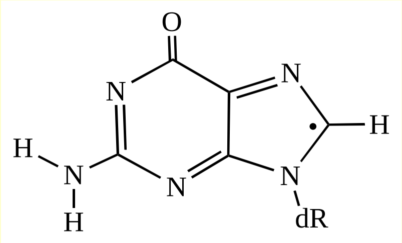
Extent of hole localization in GGG-oligos at low temperature?

Hole Migration to GGG

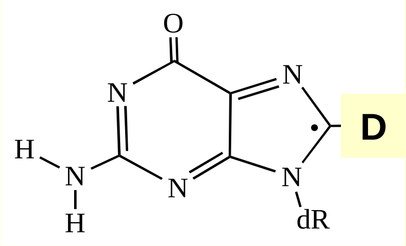


Hole Localization in DNA GGG oligos





G(-H)•



G^(-H)•

G(-H)• = G(N1-H)• :C(+H$^+$)

↑ Hole ↑ Charge

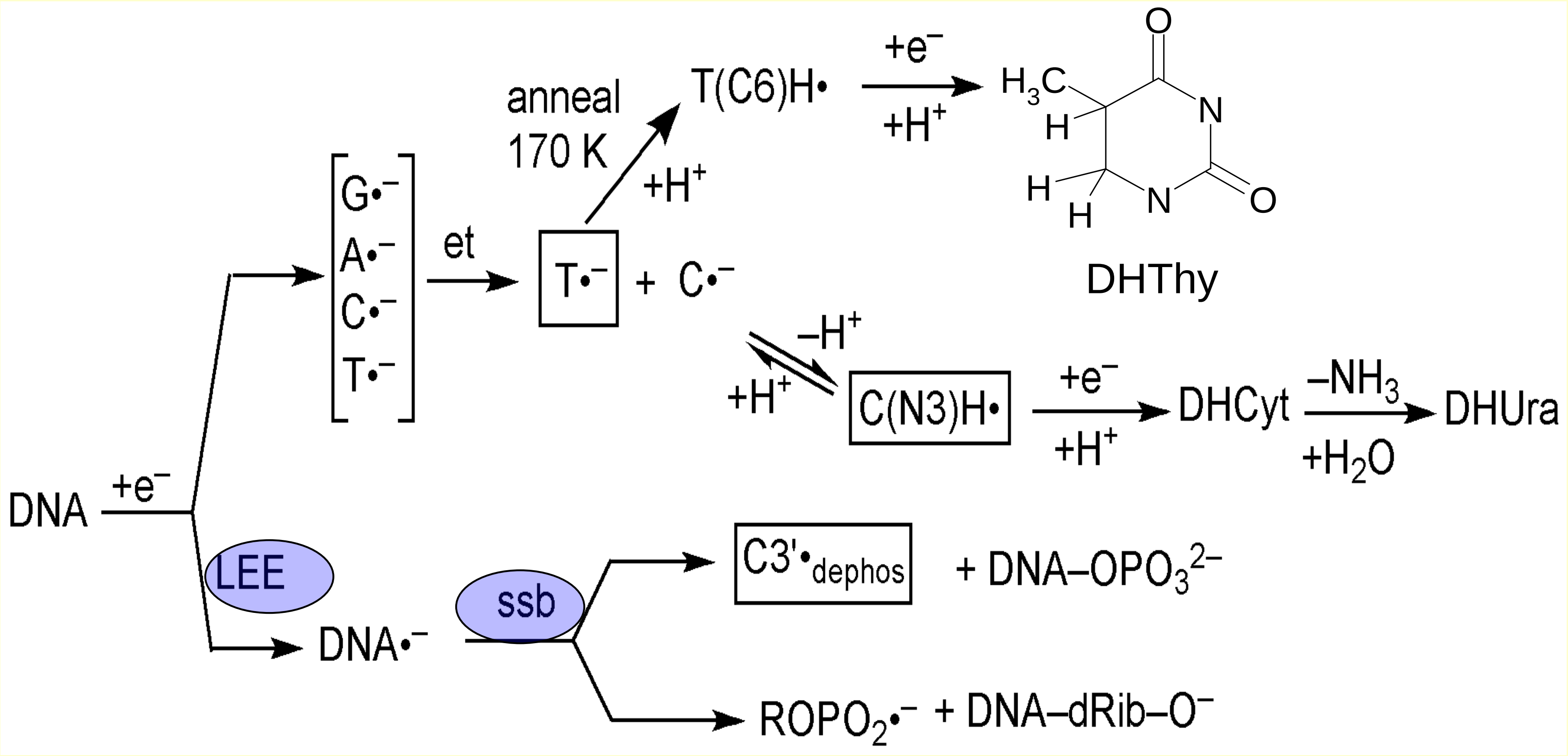
60%	15%	20%
↓	↓	↓
d[G^GGCCC]$_2^{•+}$	d[GG^GCCC]$_2^{•+}$	d[GGG^CCC]$_2^{•+}$

The hole is fully localized at one base

1. Adhikary et al., JACS 2009, 131 (24), 8614 – 8619.

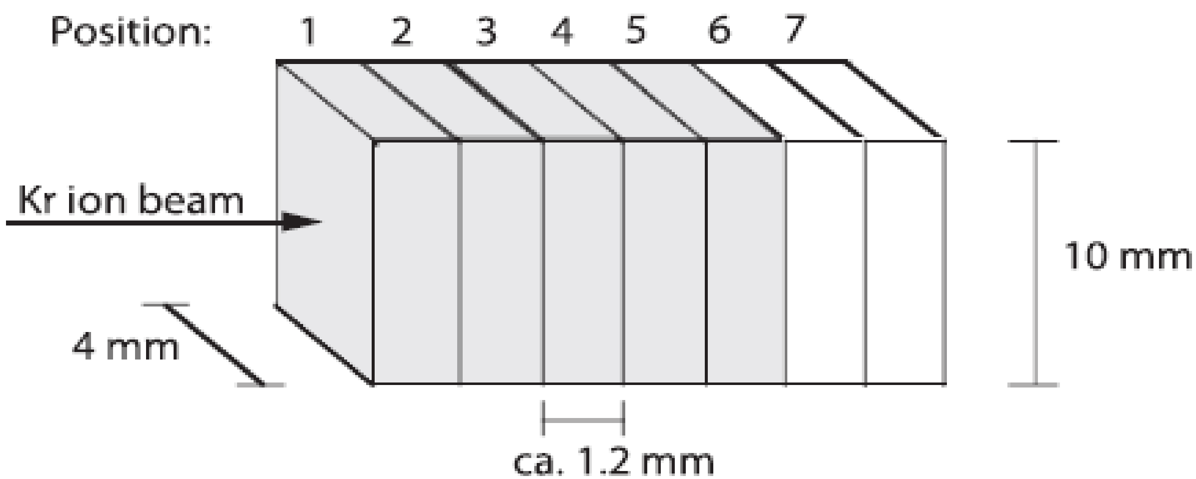
2. Adhikary and Sevilla, J Phys Chem B, 2011, 115, 8947.

DNA-radicals formed via the reductive (i.e., electron-gain) pathway



Adhikary, A., Kumar, A., Becker, D., and Sevilla, M. D. (2012) Theory and ESR spectral studies of DNA-radicals. In **Encyclopedia of Radicals in Chemistry, Biology and Materials**. (C. Chatgilialoglu, A. Struder (Eds.)), John Wiley & Sons Ltd, Chichester, UK, pp 1371-1396.

DNA-sample configuration for ion-beam radiation



SCHEME 1. This scheme shows the DNA sample configuration for one sample packet. There are five such packets in this work. The Kr-86 beam penetrated partially into sample 5, and samples 6 and 7 were unirradiated. Sample 5 contains the Bragg peak.

Becker, D., Adhikary, A., Tetteh, S. T., Bull, A. W., and Sevilla, M. D. (2012) Kr-86 ion-beam irradiation of hydrated DNA: Free radical and unaltered base yields. *Radiation Research*, **178**, 524 – 537.

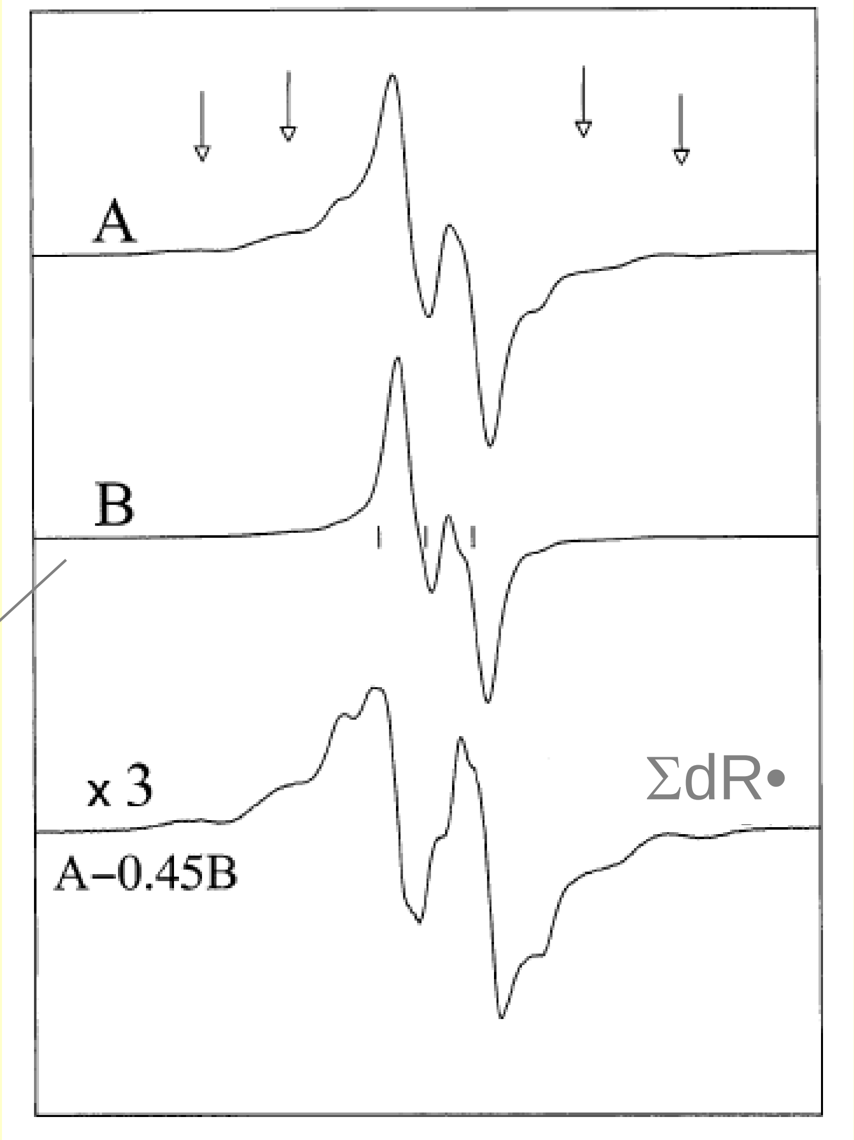
ESR spectra of Ion-beam and γ-irradiated DNA, 77 K

- 19% (C•⁻)
- 18% T•⁻
- 25% G (-H)•
- 38% ΣdR•

All to ±10%

- 25% (C•⁻) C(N3)H•
- 25% T•⁻
- 40% G(-H)•
- 10% ΣdR•

All to ±5%



^{40}Ar beam (energy = 60 MeV/nucleon, LET = 800 keV/μm) irradiated DNA (Γ = 12), 77 K

γ-irradiated (Dose = 35 kGy) DNA (Γ = 12), 77 K

Becker et al., (2003) *Radiat. Res.*, **160**, 174 - 185

Radical Percentages: Kr-86 Irradiated DNA vs. γ-irradiated

Kr-86	γ-irradiation
38% G(–H)•	40% G(–H)•
11% $T^{•-}$	25% $T^{•-}$
16% C(N3)H•	25% C(N3)H•
34% ΣdR•	10% ΣdR•

Far lower percentage of base radicals [G(–H)•, $T^{•-}$, C(N3)H•] in Kr-86 irradiated samples than in γ-irradiated.

Becker, D., Adhikary, A., Tetteh, S. T., Bull, A. W., and Sevilla, M. D. (2012) Kr-86 ion-beam irradiation of hydrated DNA: Free radical and unaltered base yields. *Radiation Research*, **178**, 524 – 537.

Table 8.6 Radical Yields and Energy Partition in ^{86}Kr ion-beam irradiated hydrated ($G = 12\ D_2O$/nucleotide) DNA[a]

LET (keV/μm)	G_{Base} (μmol/J)	G_{sugar} (μmol/J)	Fraction energy in penumbra[b]	Fraction energy in core
1080	0.056	0.027	0.29	0.71
1175	0.070	0.027	0.37	0.63
1460	0.051	0.027	0.27	0.73
2000	0.038	0.025	0.20	0.80
4000	0.013	0.0075	0.068	0.93

[a] Adapted from Ref. [35]

[b] Fraction energy in penumbra $= G_{base}(Kr)/G_{base}(\gamma)$, with $G_{base}(\gamma) = 0.19$ μmol/J

Adhikary, A., Becker, D., and Sevilla, M. D. (2014) Electron spin resonance of radicals in irradiated DNA. In **Applications of EPR in radiation research** (A. Lund, M. Shiotani (Eds.)), Springer-Verlag, Berlin, Heidelberg, 299 - 352.

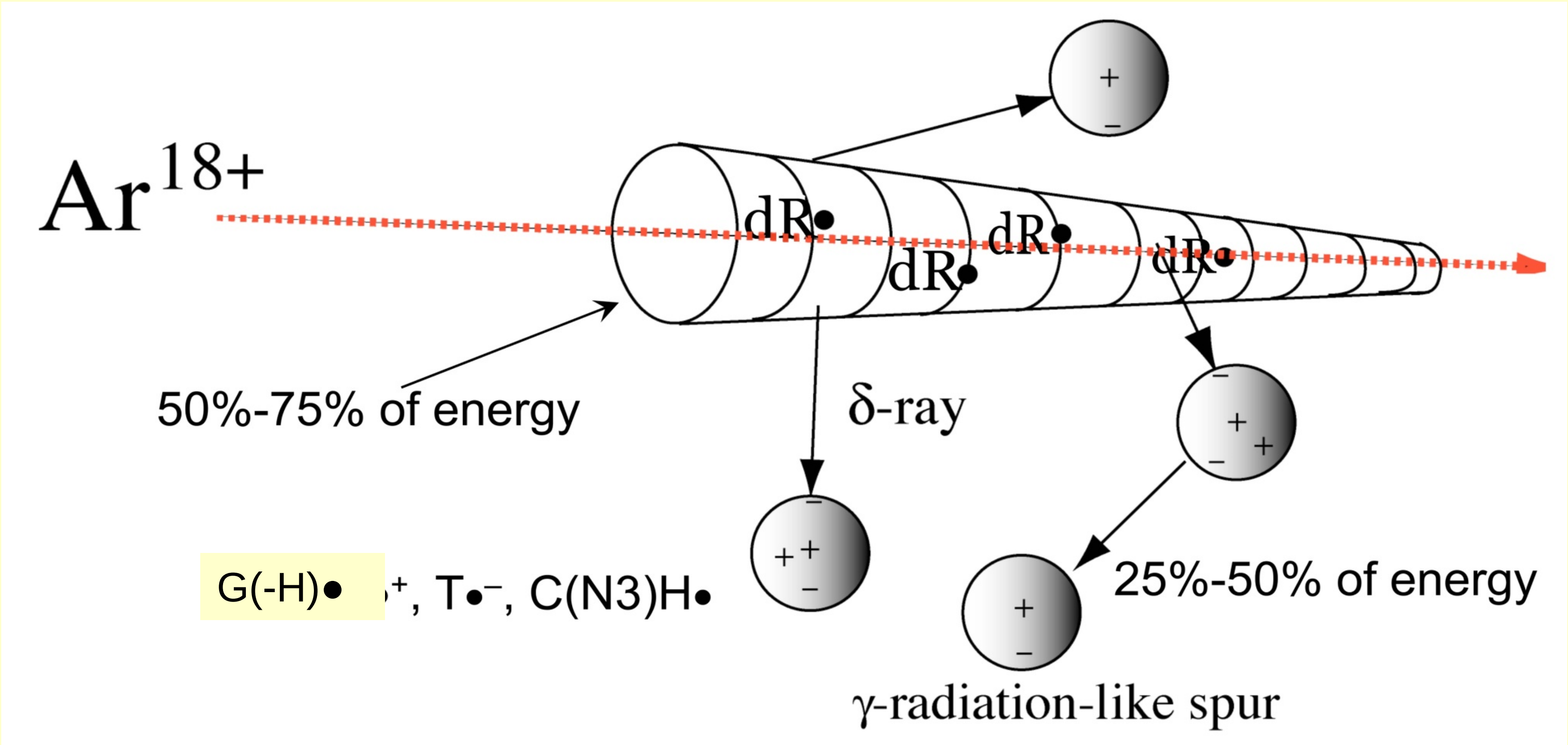
G–values for Sugar Radical Formation and Base Release

LET (keV/μmol)	G (dR•)[a]	G (base release)[a]
1080	0.027	0.028
1175	0.027	0.026
1460	0.027	0.024
2000	0.025	0.025
4000	0.0075	0.011

[a] (μmol/J). Reprinted with permission from Ref. [35]. *Radiation Research*, Copyright (2012), Radiation Research Society

Adhikary, A., Becker, D., and Sevilla, M. D. (2014) Electron spin resonance of radicals in irradiated DNA. In **Applications of EPR in radiation research** (A. Lund, M. Shiotani (Eds.)), Springer-Verlag, Berlin, Heidelberg, 299 - 352.

Sugar radical formation in ion-beam core



Becker, D., Adhikary, A. and Sevilla, M. D. (2010) Mechanism of Radiation Induced DNA Damage: Direct Effects. In Recent Trends in Radiation Chemistry (B.S.M. Rao and J. Wishart (Eds.)), World Scientific Publishing Co., Singapore, New Jersey, London, 509 - 542.

Ion-recombination in the core cannot account fully for the higher relative yields of the neutral sugar-phosphate backbone radicals formed in ion-beam irradiated DNA.

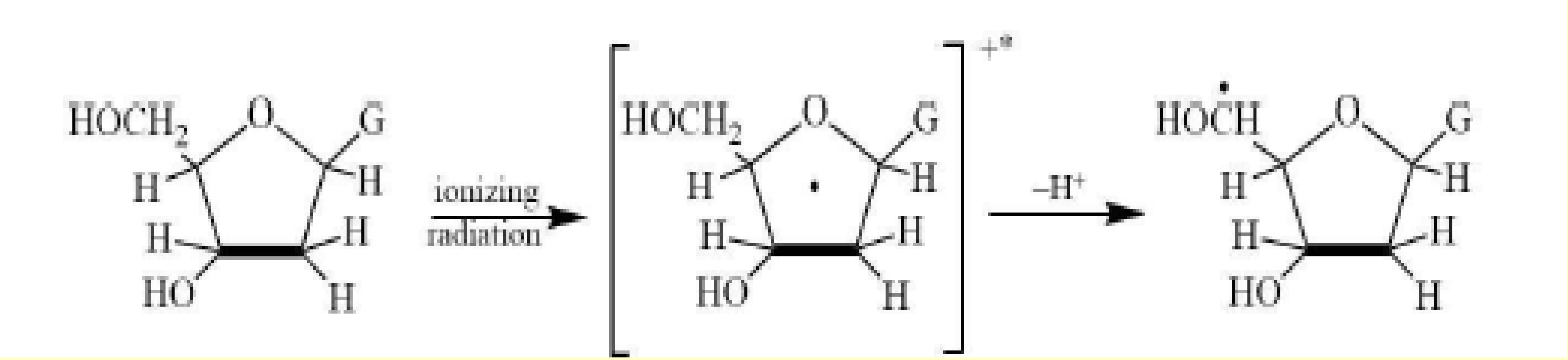
Two additional mechanisms have been proposed:

- Excited state processes

- Low Energy Electrons (LEE) processes via dissociative electron (DEA) attachment

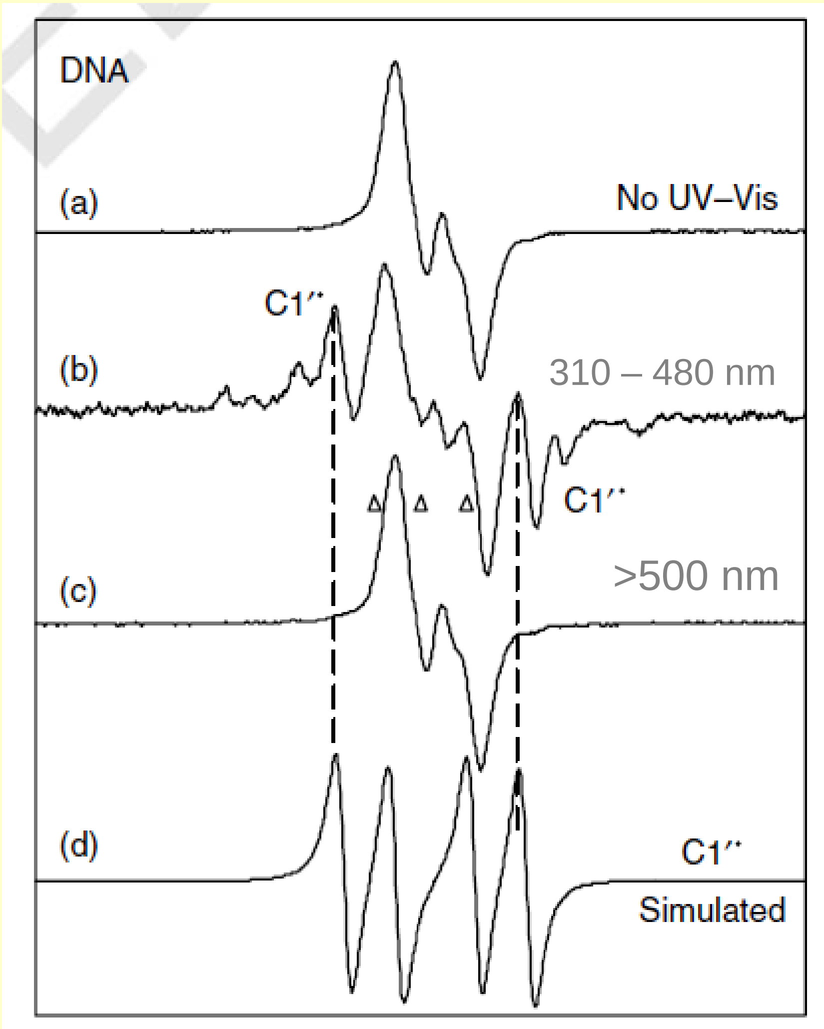
Excited State Processes

Hypothesis – excited states of base ion radicals produce sugar radicals



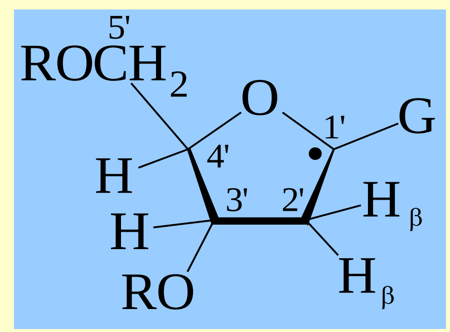
Becker, D., Adhikary, A. and Sevilla, M. D. (2007) The Role of Charge and Spin Migration in DNA Radiation Damage. In *Charge Migration in DNA* (T. Chakraborty (Ed.)), Springer-Verlag, Berlin, Heidelberg, 139 - 175

Test of hypothesis: Photo-excitation of one-electron-oxidized guanine in DNA



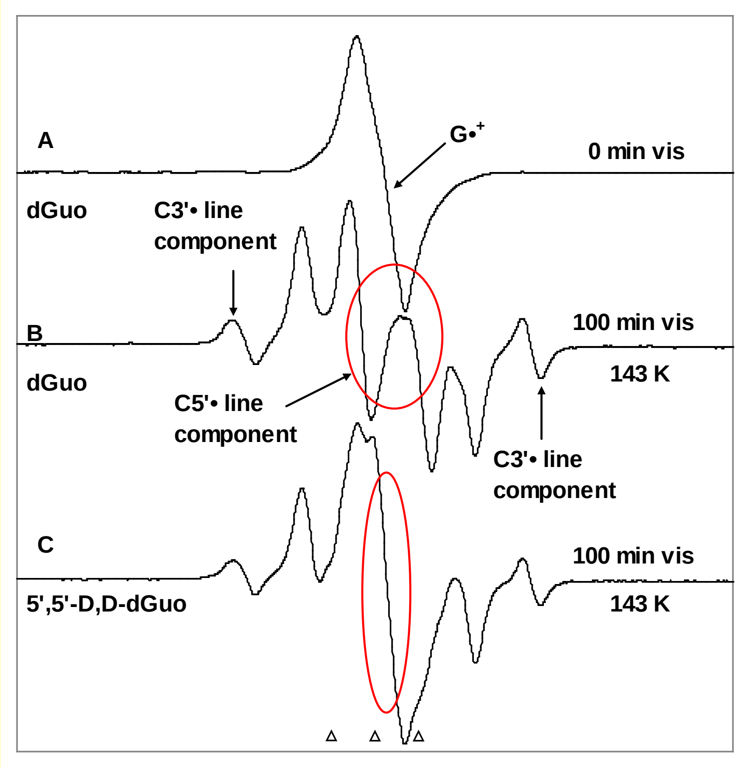
After 15 kGy γ-irradiation at 77 K

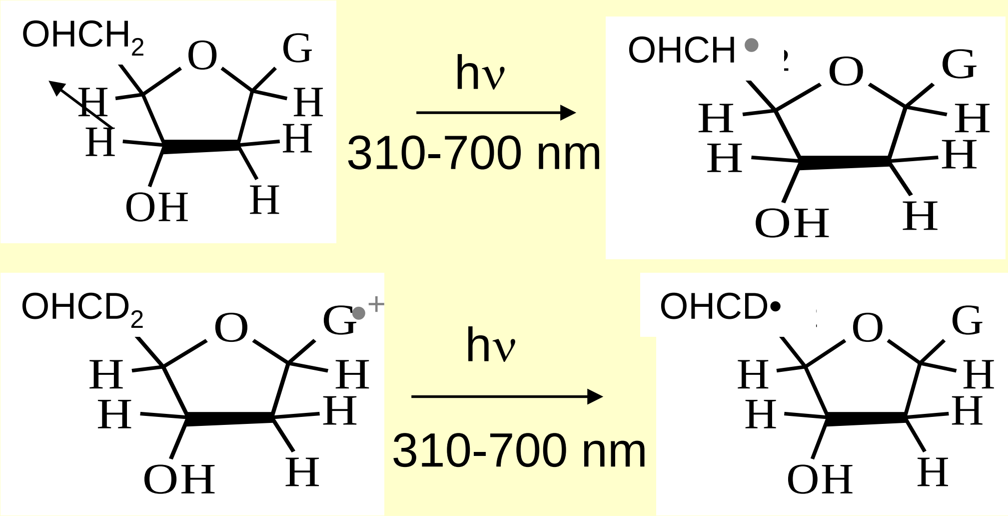
UV-vis produces prominent line components from C1’•.



Adhikary et al., (2005) *Nucleic Acids Res.*, **33**, 5553 - 5564

Identification of C5'• by selective deuteration at C5'-in dGuo

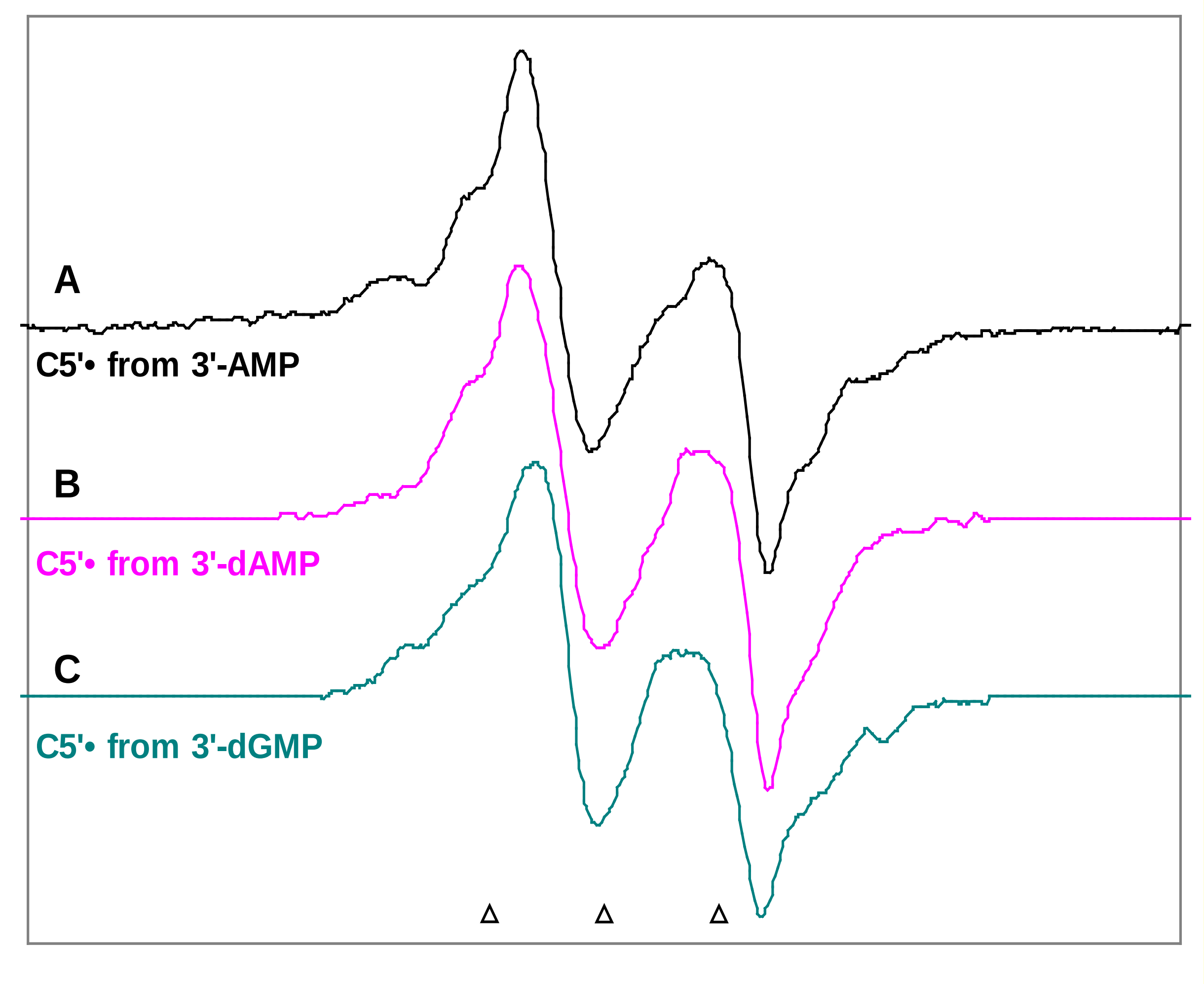




For C5'•: Deuterium substitution collapses the central doublet

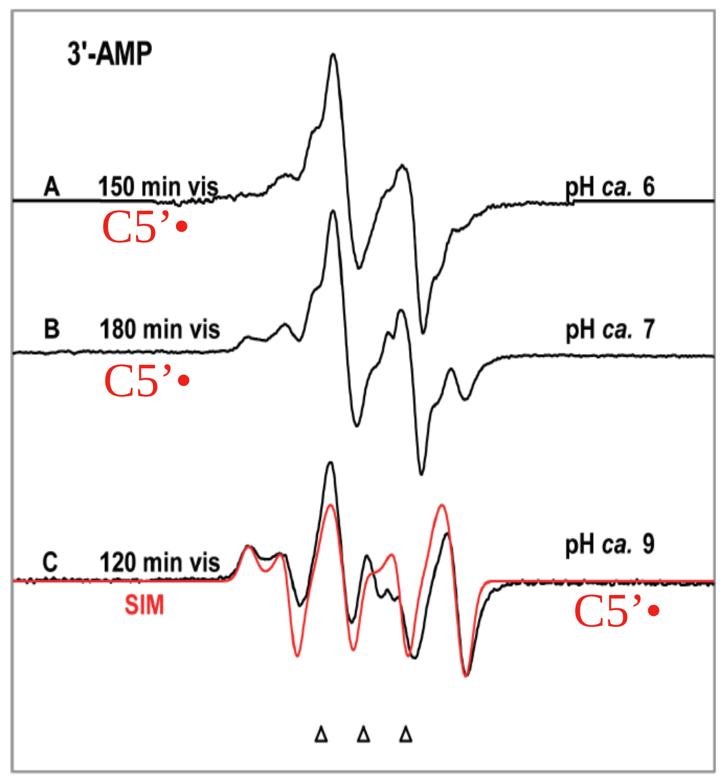
Adhikary et al., (2005) *Nucleic Acids Res.*, **33**, 5553 - 5564

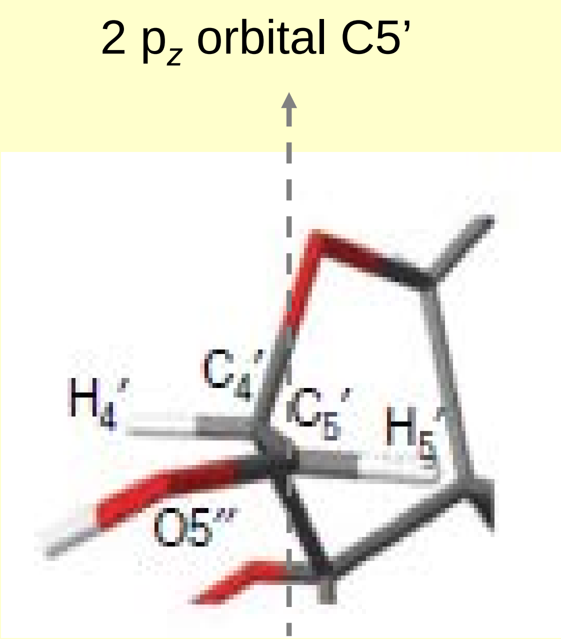
C5'• from DNA and RNA monomers



A. Adhikary, D. Khanduri, A. Kumar, and M. D. Sevilla, *J. Phys. Chem. B*, 2008, **112**, 15844–15855.

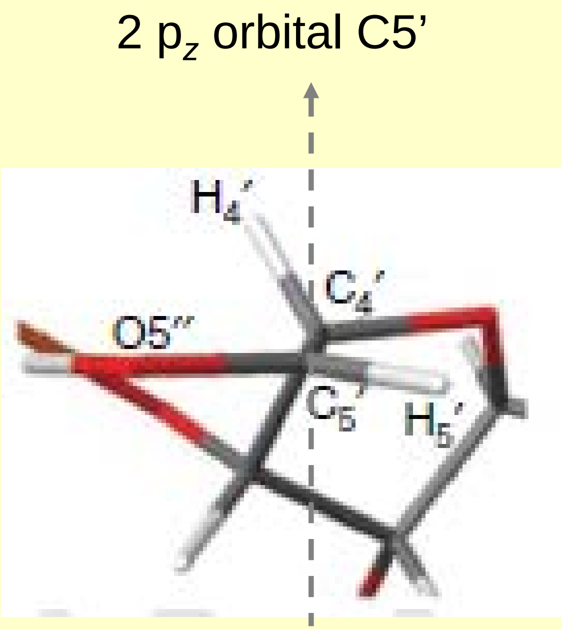
Identification of conformations of C5'• via photoexcitation of A•$^+$ in 3'-AMP





H4'-C4'-C5'-O5" = 0°

C4'-βH HFCC small

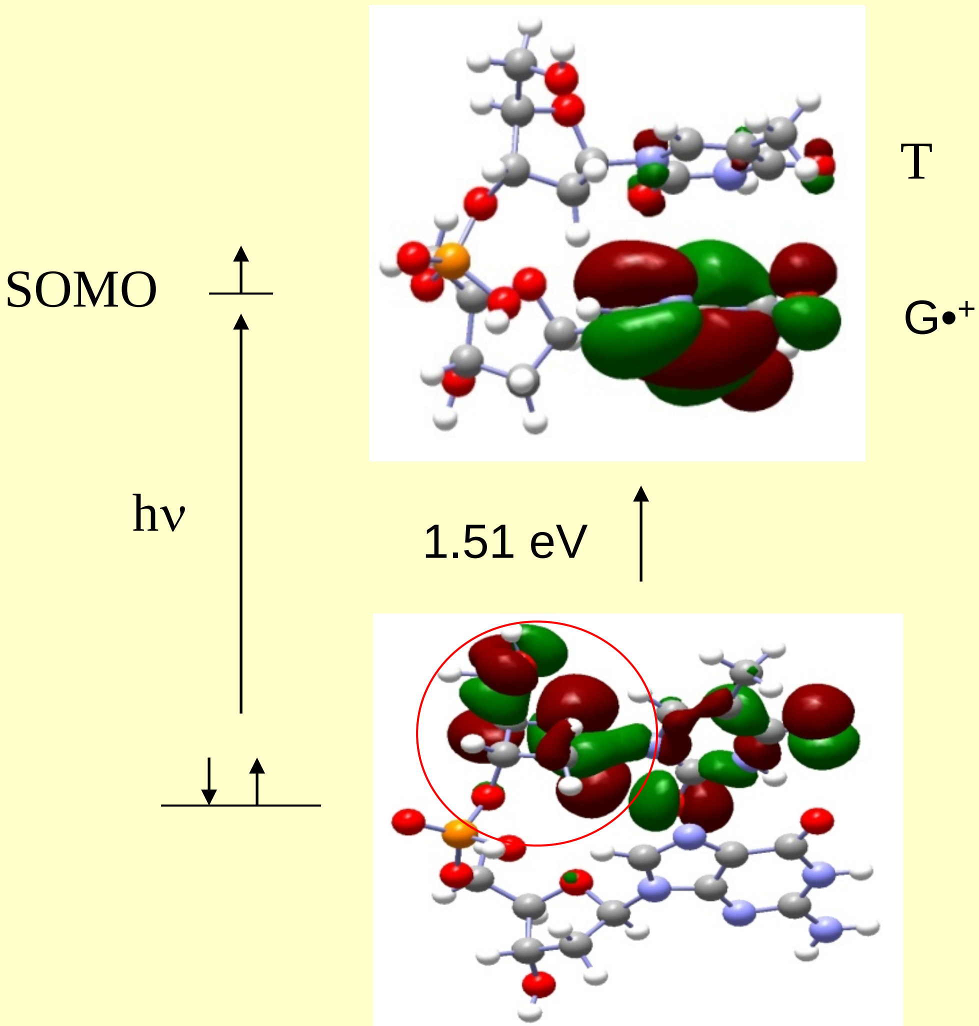


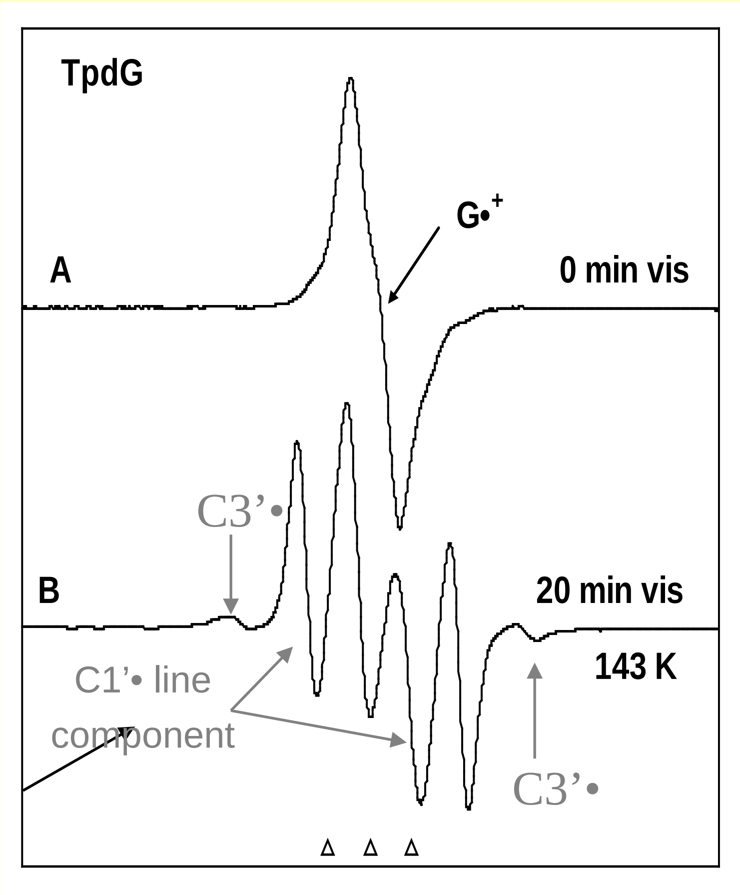
H4'-C4'-C5'-O5" = 54°

C4'-βH HFCC = 34.5 G

A. Adhikary, D. Khanduri, A. Kumar, and M. D. Sevilla, *J. Phys. Chem. B*, 2008, **112**, 15844–15855.

TD-DFT calculations and photo-excitation studies for one-electron oxidized **TpdG**

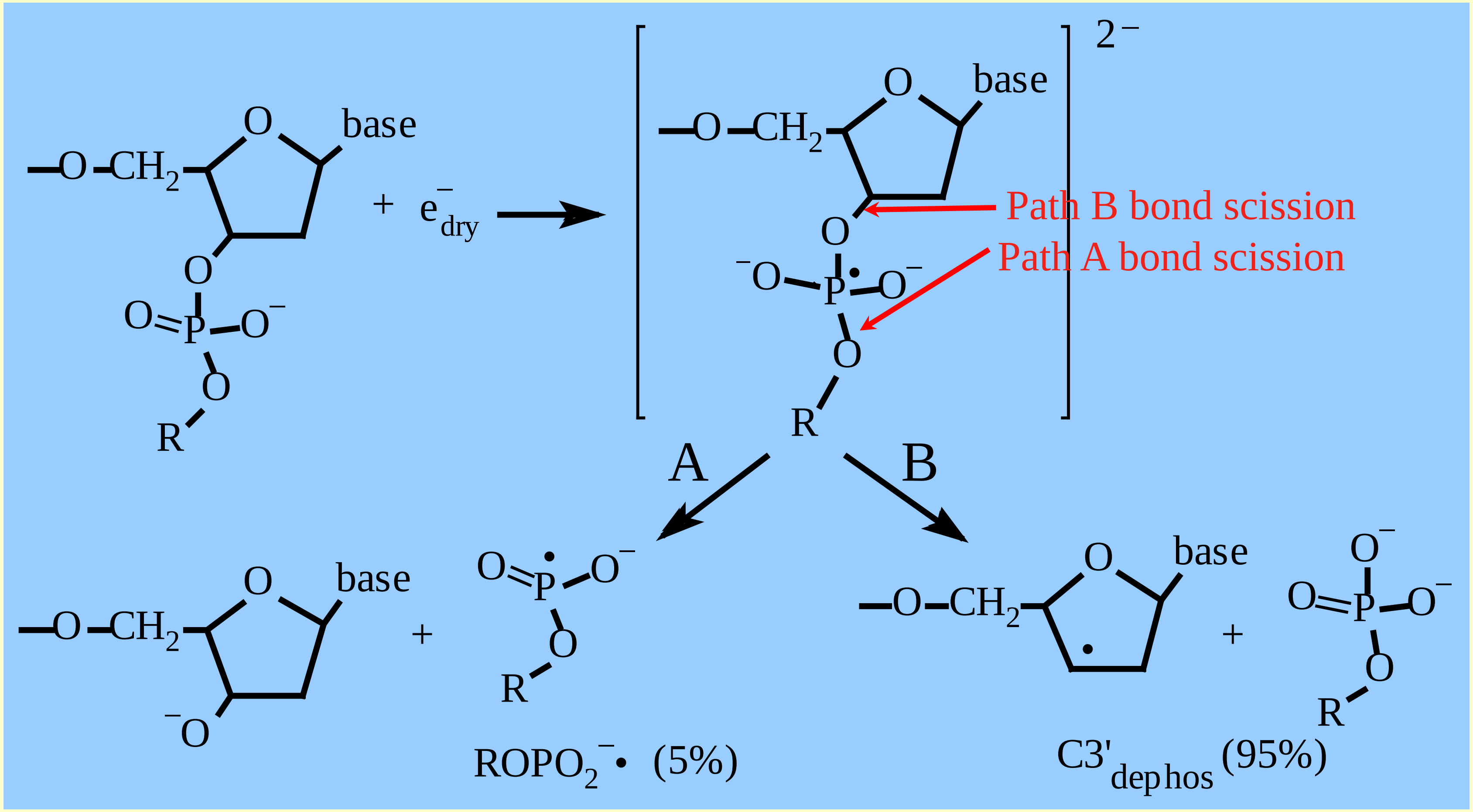




Adhikary, A.; Kumar, A; Sevilla, M. D. *Radiat. Res.*, **2006**, *165*, 479

Low Energy Electron Processes

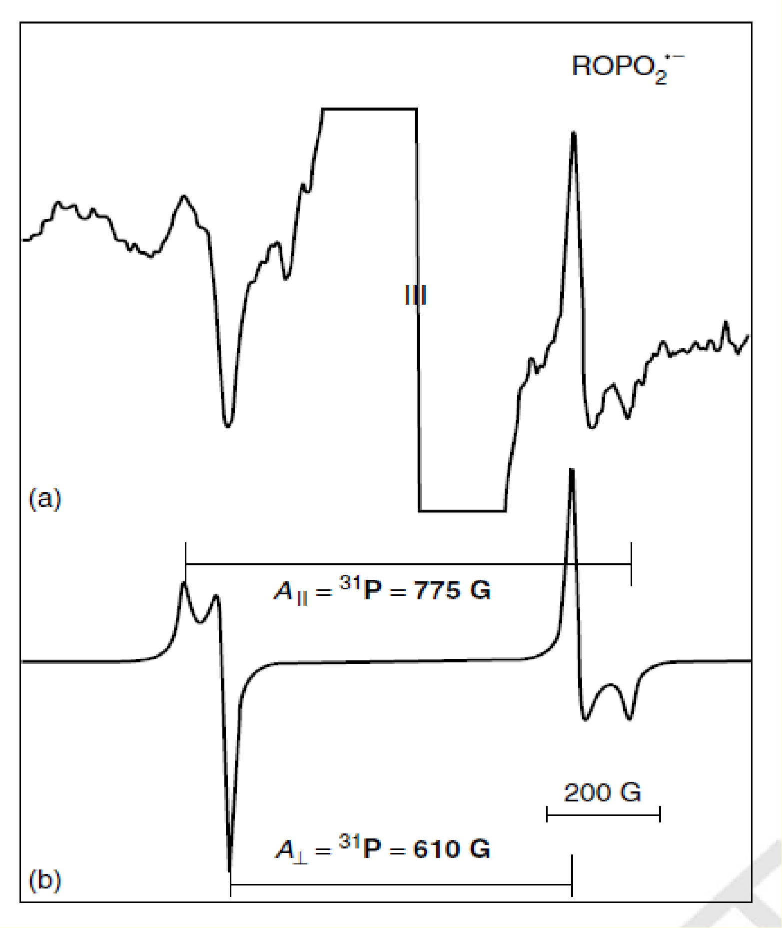
Meachanism of formation of phosphoryl radical at C3’ position

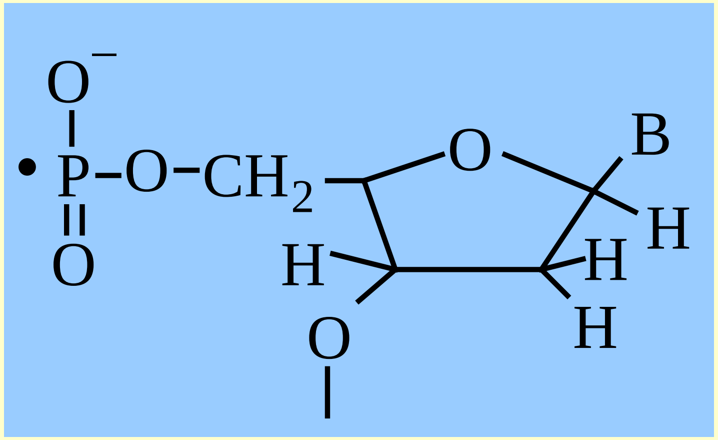


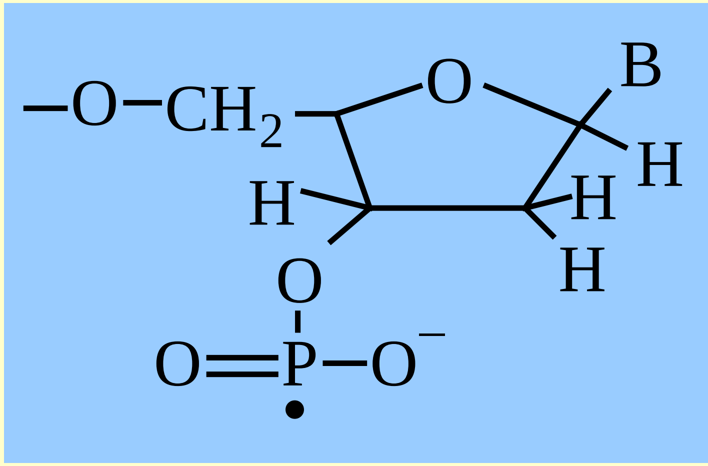
Becker et al., (2003) *Radiat. Res.*, **160**, 174 - 185

LEE effects.

Phosphoryl radicals - strand break radical(s)

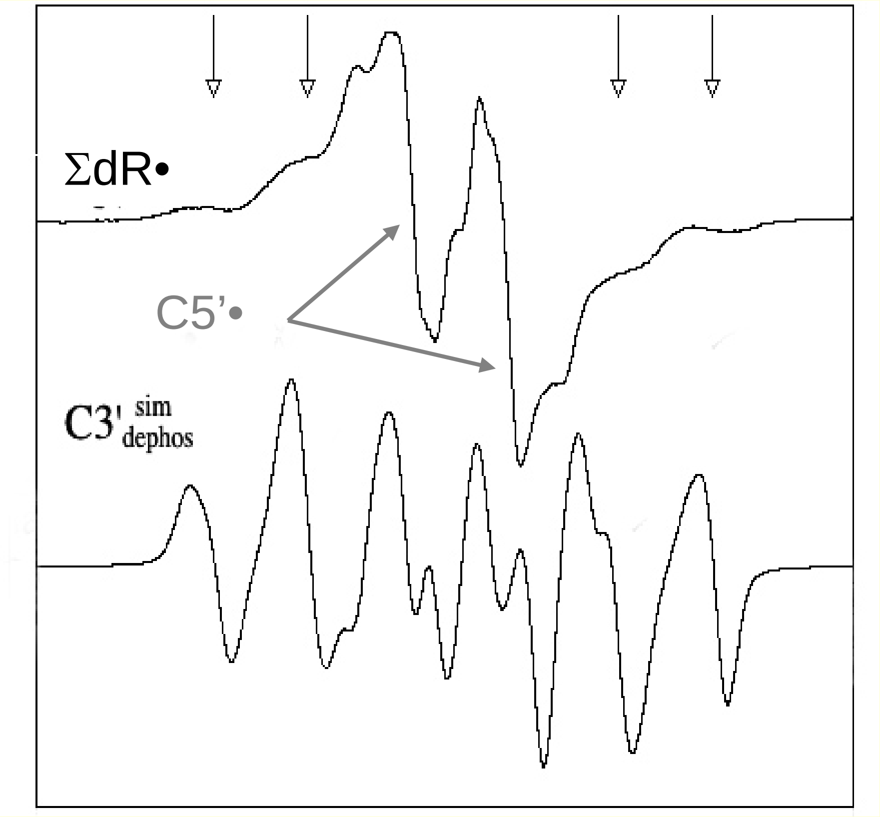






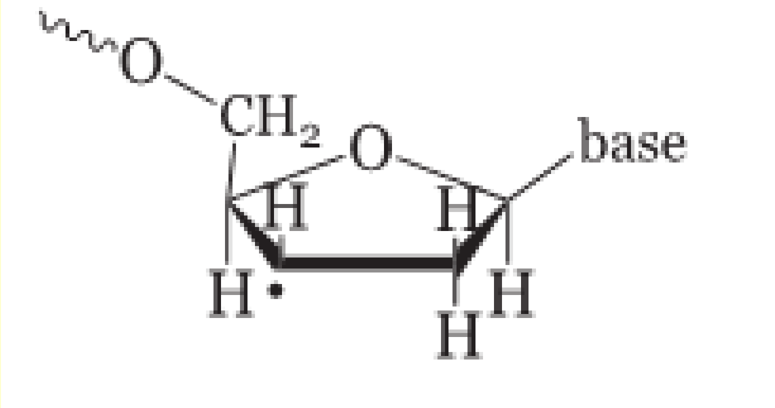
Becker et al., (2003) *Radiat. Res.*, **160**, 174 - 185

2nd Strand Break radical found in ion beam irradiated DNA



ΣdR• from DNA

C3'$_{dephos}$•

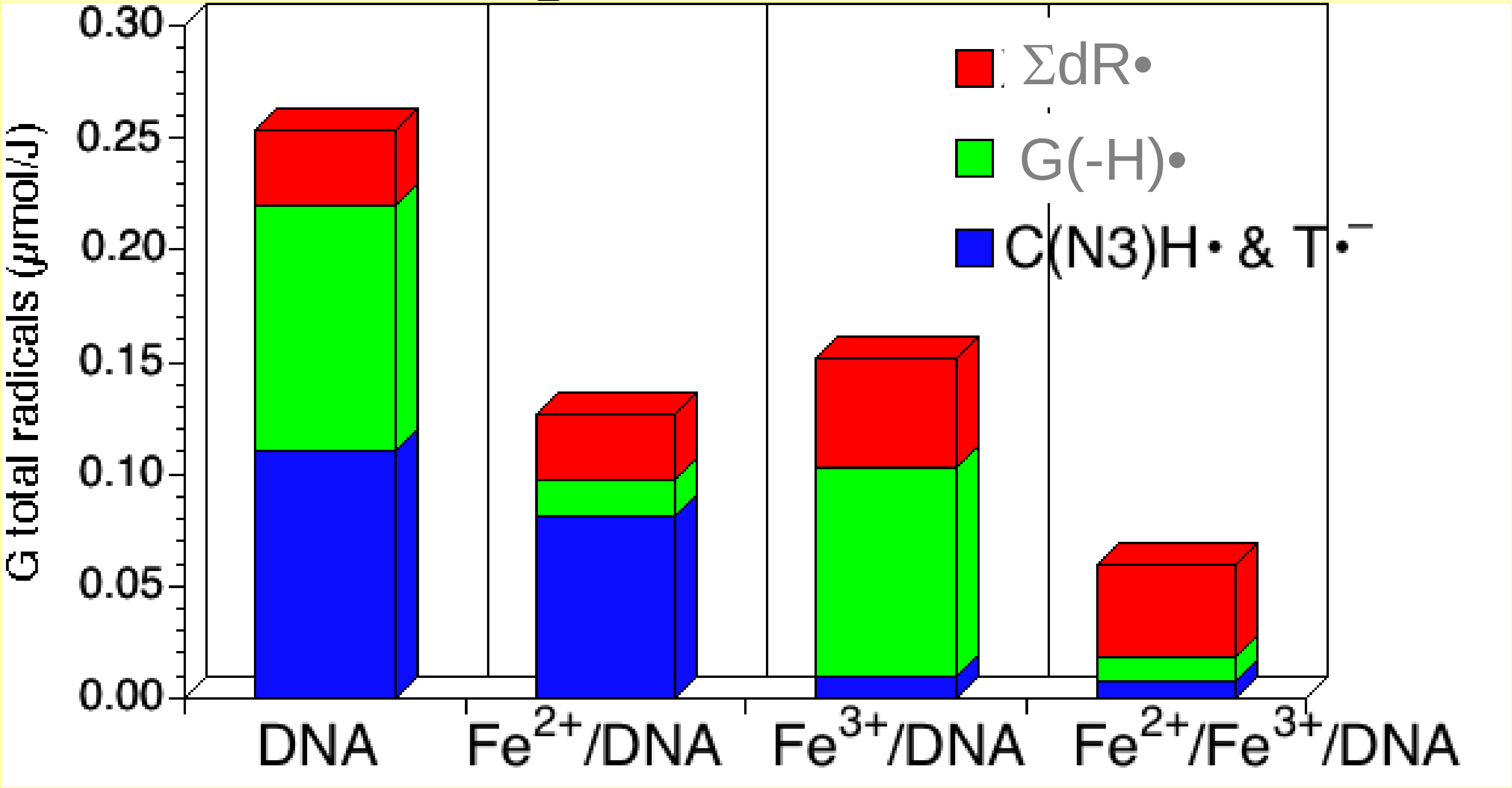


Simulation using theoretical spectral parameters

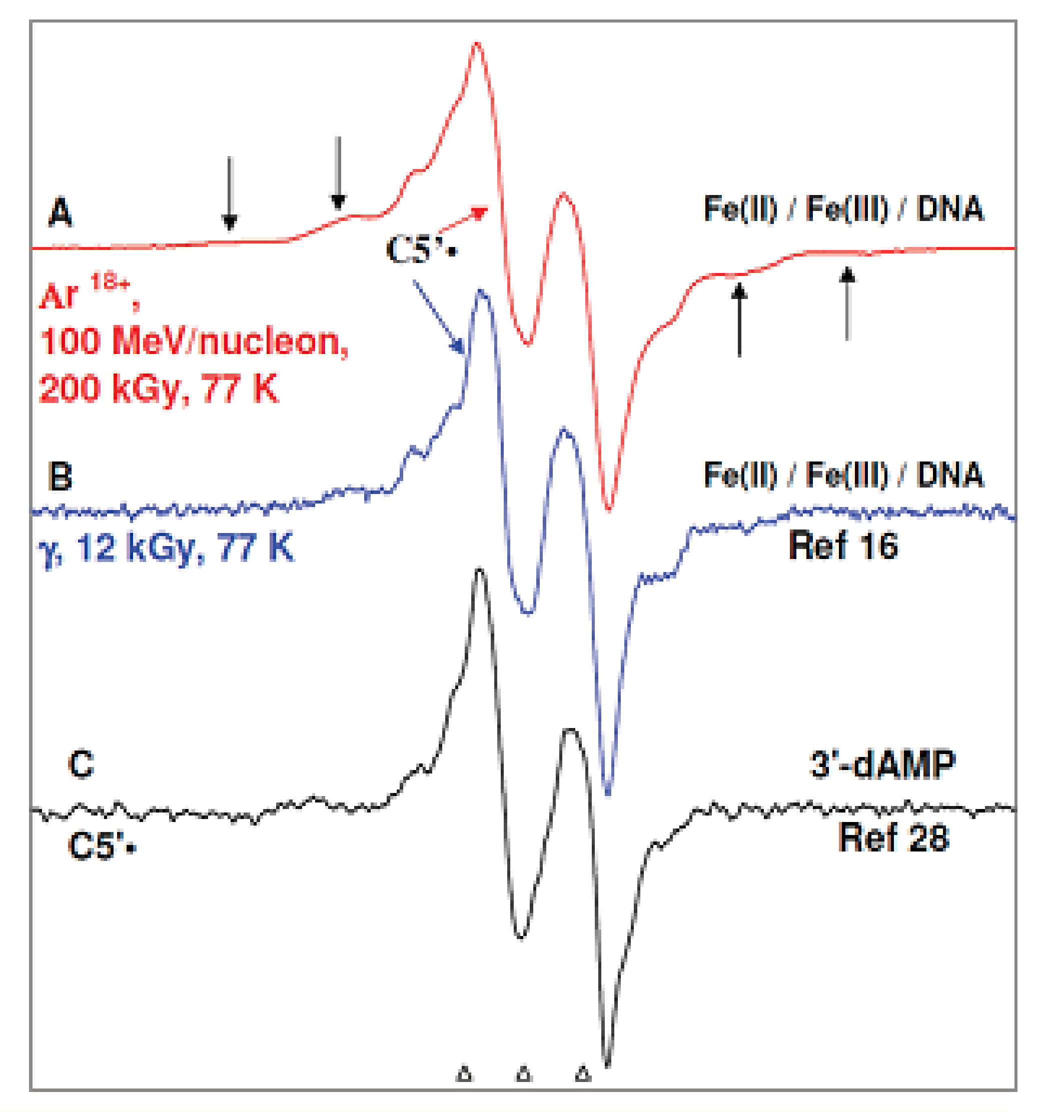
Becker et al., (2003) *Radiat. Res.*, **160**, 174 - 185

Ionization of The Sugar-Phosphate Backbone

Effect of hole and electron scavengers on G-values



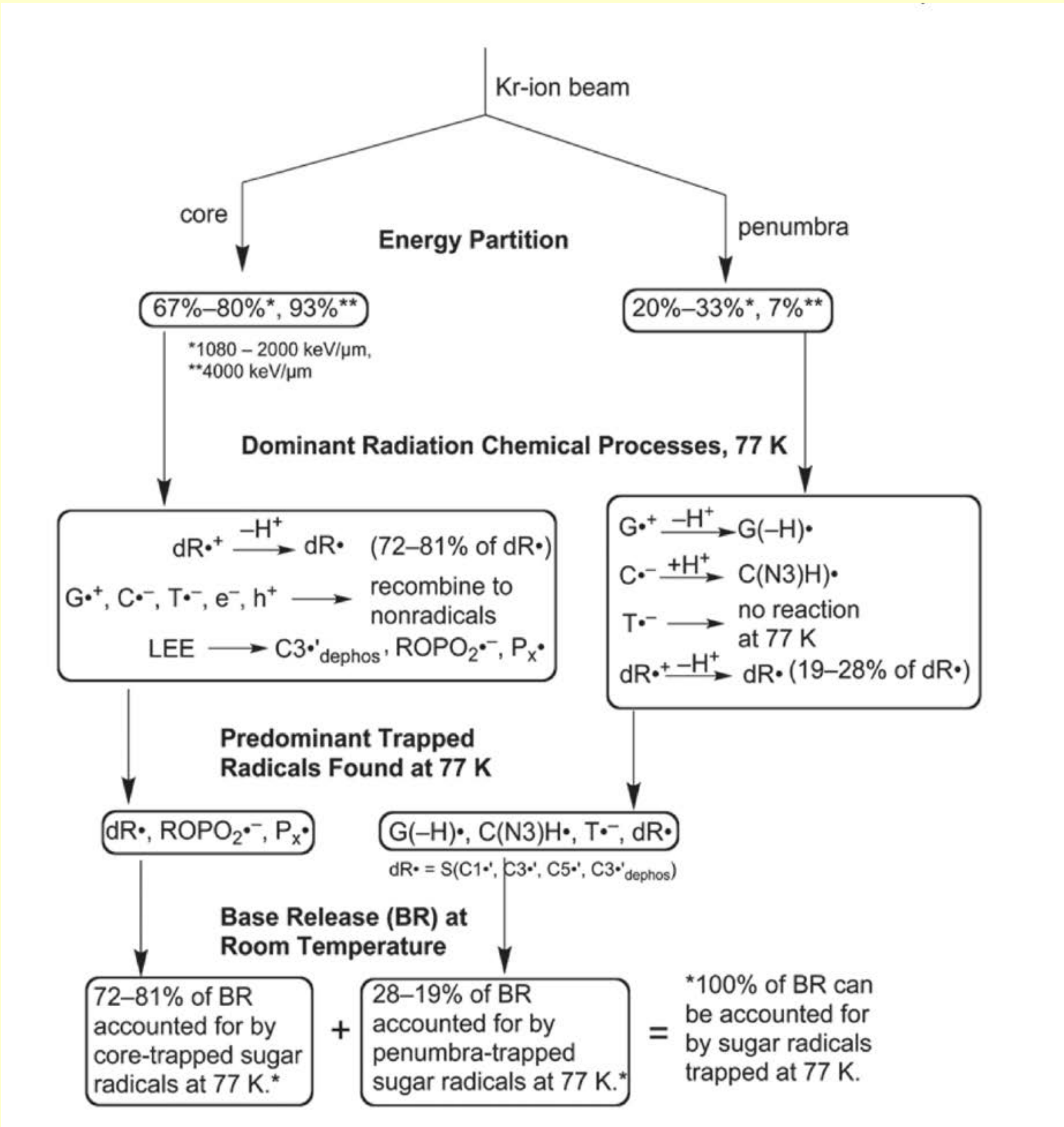
C5'• is significant in the γ-irradiated and ion-beam irradiated DNA



Adhikary et al.

dx.doi.org/10.1021/jp3023919 | J. Phys. Chem. B 2012, 116, 5900−5906

Radiation Chemistry Track Structure Model



Adhikary, A., Becker, D., and Sevilla, M. D. (2014) Electron spin resonance of radicals in irradiated DNA. In **Applications of EPR in radiation research** (A. Lund, M. Shiotani (Eds.)), Springer-Verlag, Berlin, Heidelberg, 299 - 352.

Conclusions

- Both oxidative and reductive (LEE) paths produce radicals in the sugar-phosphate backbone which are strand break precursors.

- C5'• (35% of ΣdR•), C3'•$_{dephos}$ (8% of ΣdR•), $RO_2PO^{•-}$ (0.1 % of ΣdR•) in γ-irradiated DNA.

- Exited state processes lead to sugar radicals. UV-vis excitation of guanine cation radical in DNA leads to C1'•. This explains the higher yield of G base release in ion-beam irradiated DNA.

- CNPs protect significantly against radiation-induced unaltered base release (a surrogate of radiation-induced strand breaks). CNPs do not reduce the extent of intial damage but cause repair of this damage.

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Thank you for your attention!