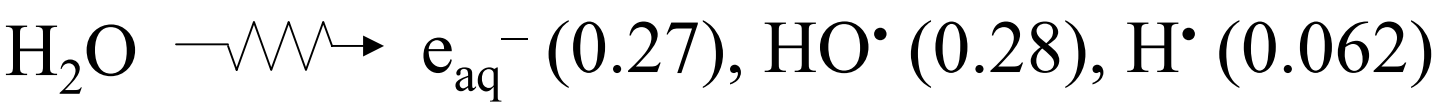
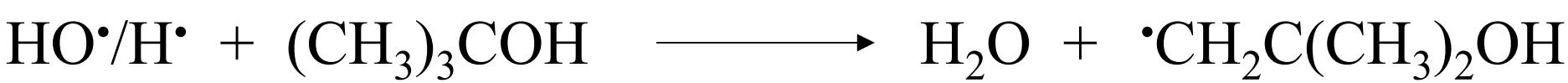
Chrys Chatgilialoglu, Bologna (Italy)

Interaction of hydrated electrons with 8-bromoadenine and 8-bromoguanine derivatives

Radiolytic Methods



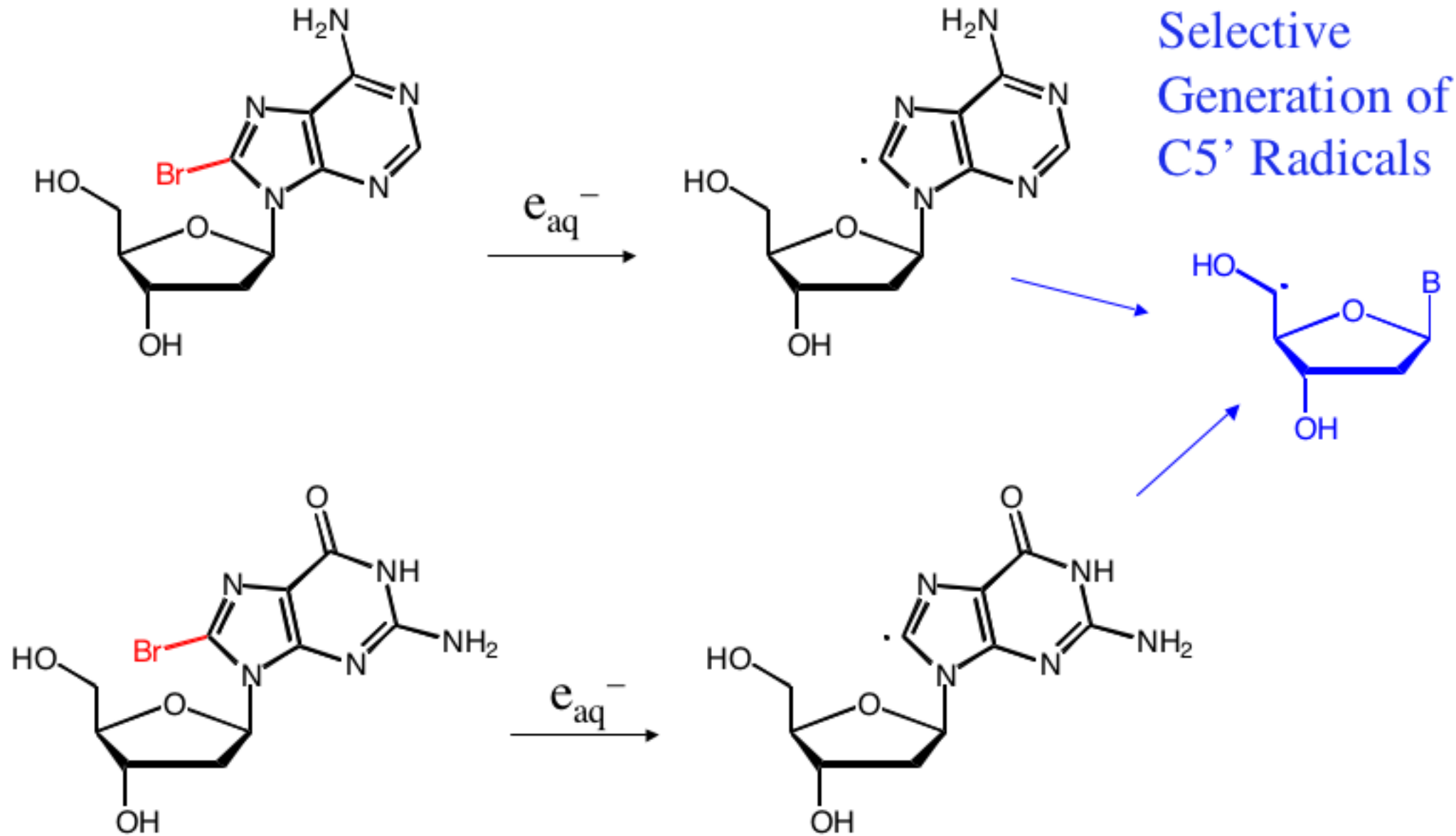
(radiation chemical yields (G) in μmol/J)



$$k(HO^\bullet) = 6.0 \times 10^8\ M^{-1}\ s^{-1}$$
$$k(H^\bullet) = 1.7 \times 10^5\ M^{-1}\ s^{-1}$$

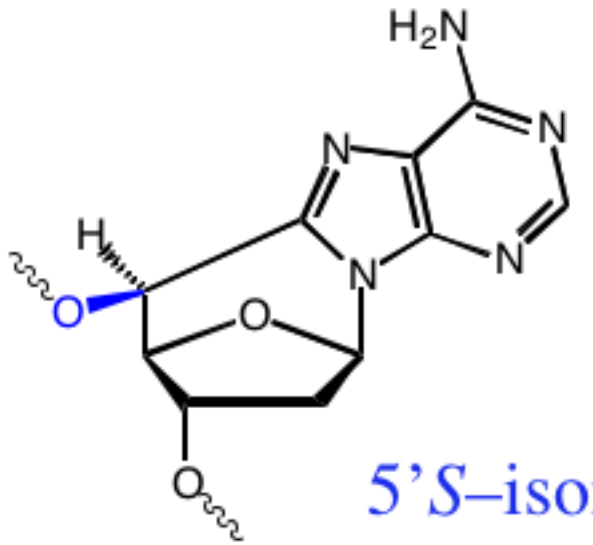
$$G(e_{aq}^-) + G(H^\bullet) = 0.33\ \mu mol/J$$

Proposal



5',8-Cyclopurine Lesions

5'*R*–isomer



5'*S*–isomer

Diastereoisomeric ratio (5'*R*):(5'*S*) ≈ 2 in both ss-DNA and ds-DNA

Dizdaroglou et al. *Int. J. Radiat. Biol.* **1988**, *54*, 195; *Free Radical Biol. Med.* **2001**, *41*, 774.

Synthesis of both diastereoisomers

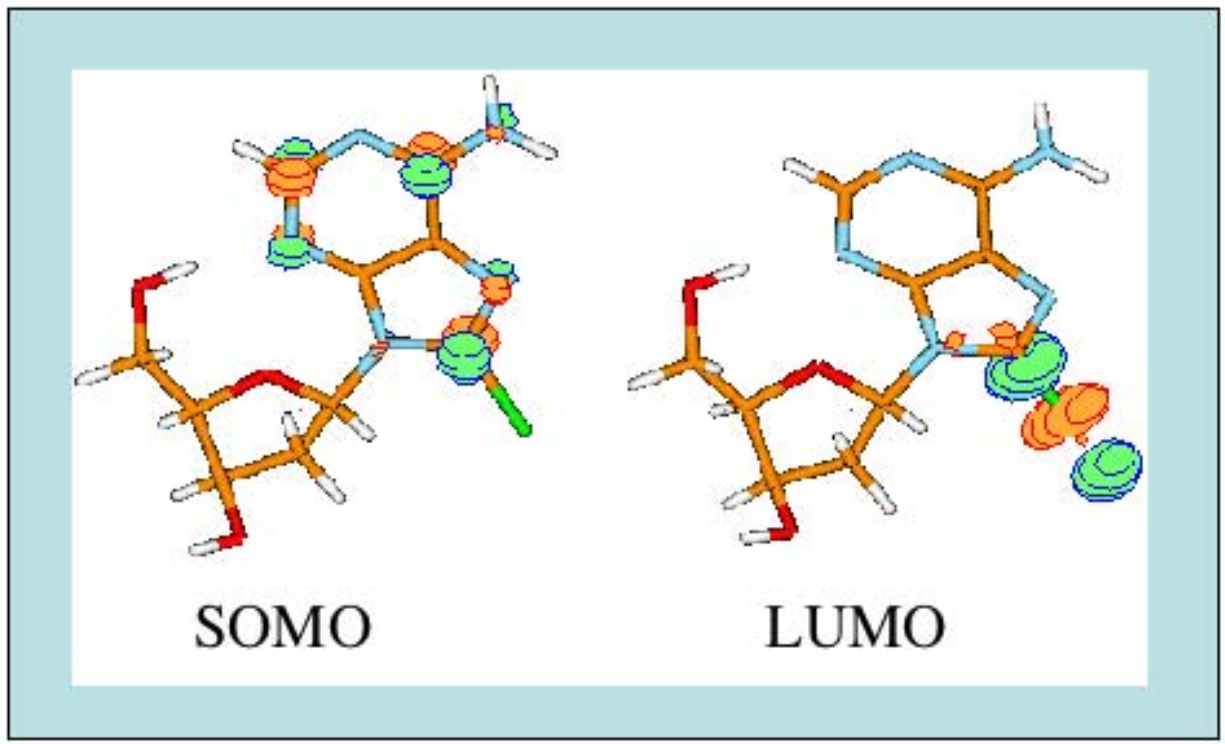
Cadet et al. *J. Org. Chem.* **1998**, *63*, 5245.

Biochemical and biophysical features of such lesions

Cadet, Lindahl and coworkers,
Proc. Natl. Acad. Sci. USA **2000**, *97*, 3832; *J. Biol. Chem.* **2001**, *276*, 49283.
Brooks and coworkers, *J. Biol. Chem.* **2000**, *275*, 22455; *J. Biol. Chem.* **2001**, *276*, 36051.

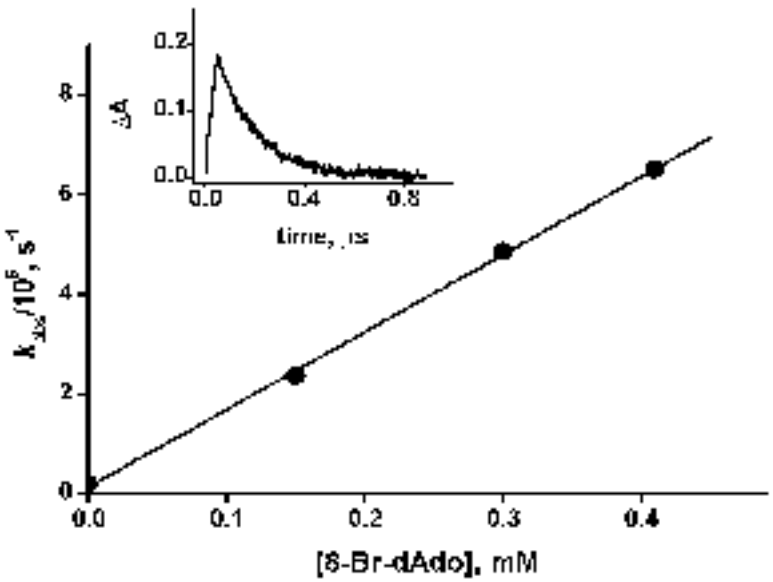
DFT-UB3LYP/6-31G*

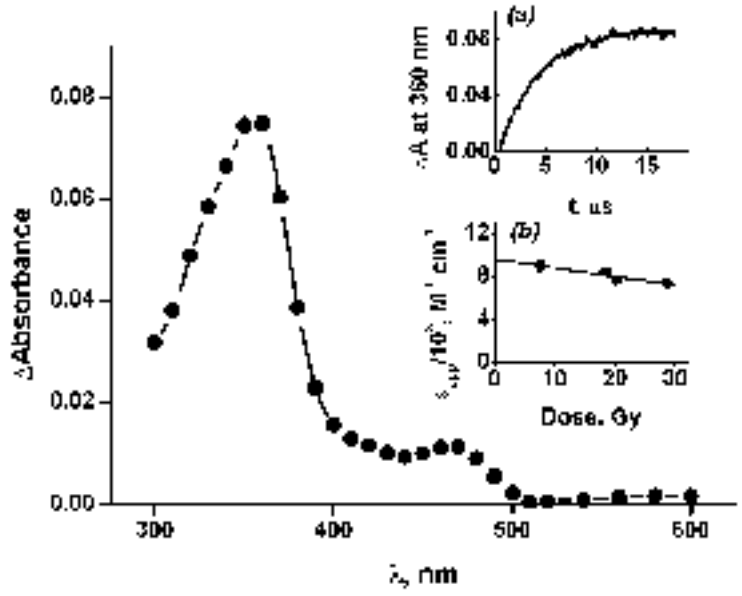
8-Br-dA $^{\bullet-}$

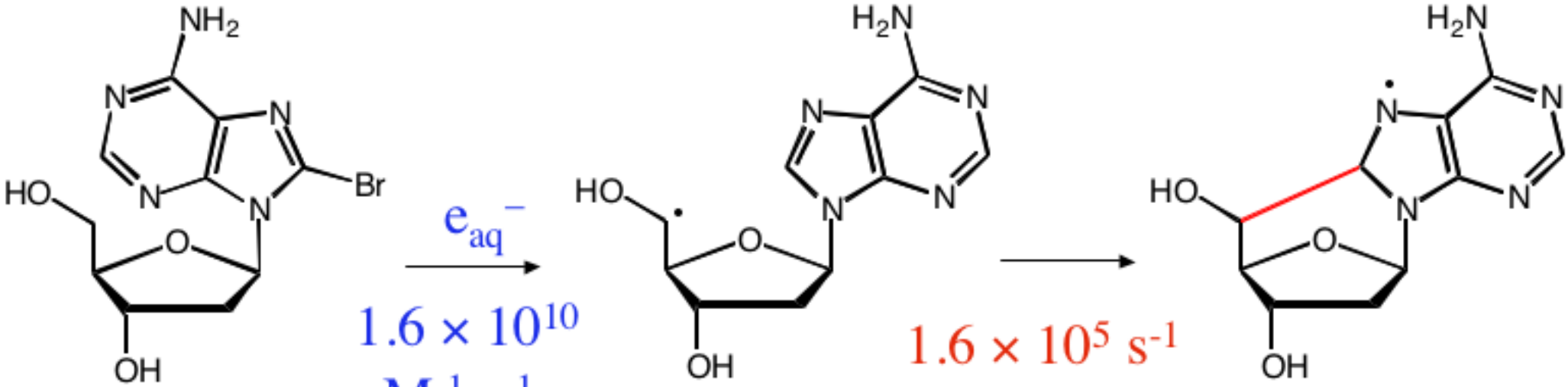


Computed at the geometry of 8-Br-dA

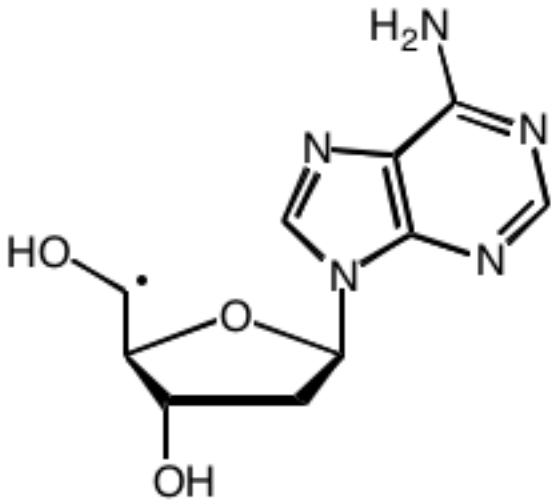
Pulse Radiolysis

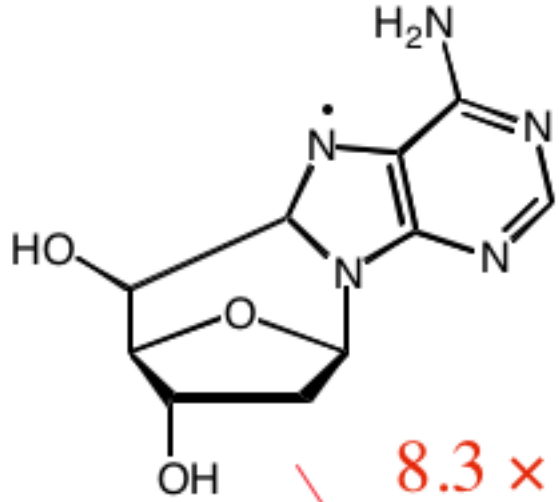






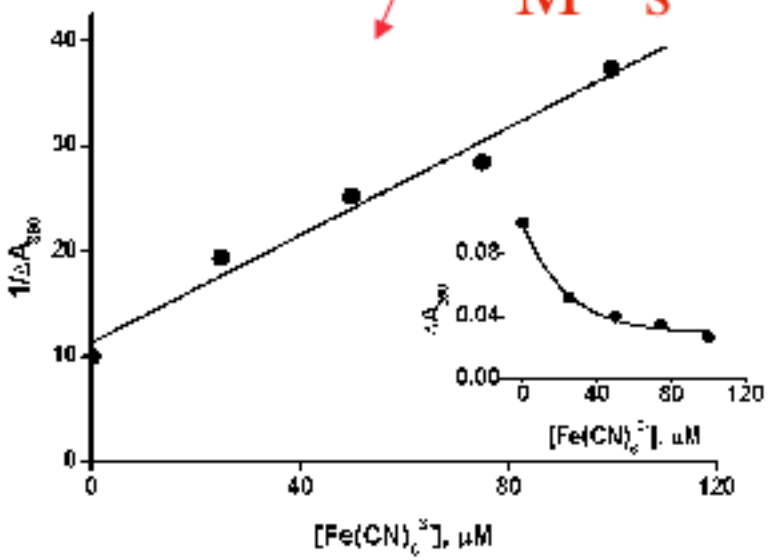
Kinetic Studies by Pulse Radiolysis

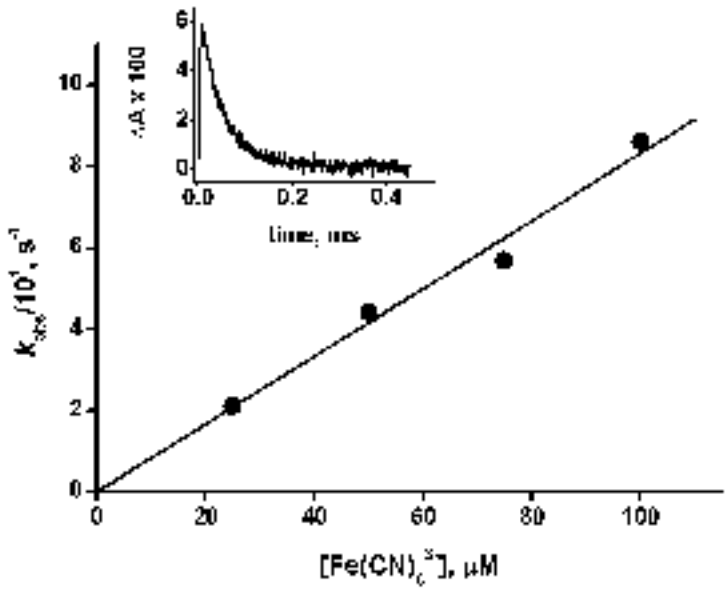




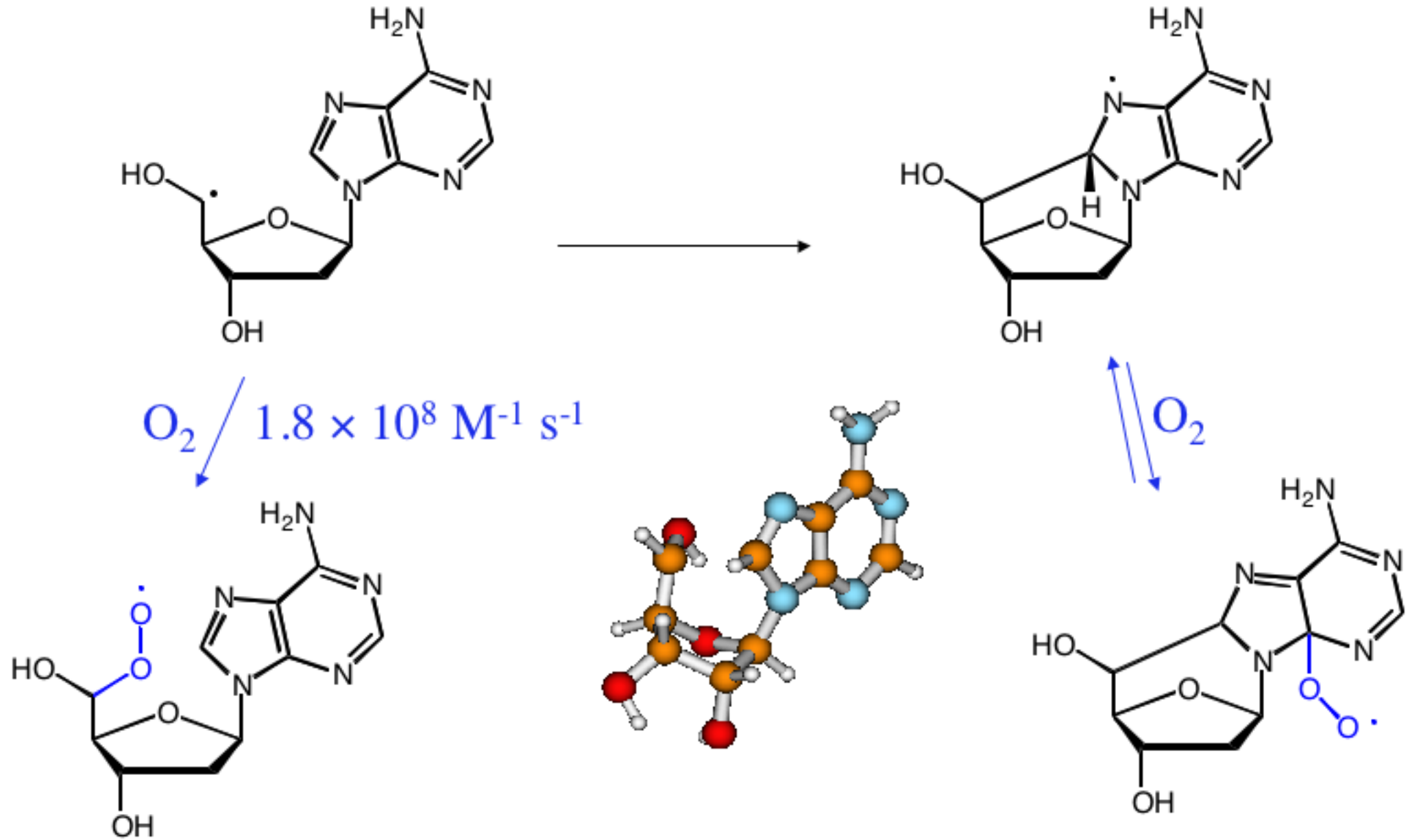
$Fe(CN)_6^{3-}$ 4.2×10^9 $M^{-1}\ s^{-1}$

$Fe(CN)_6^{3-}$ 8.3×10^8 $M^{-1}\ s^{-1}$

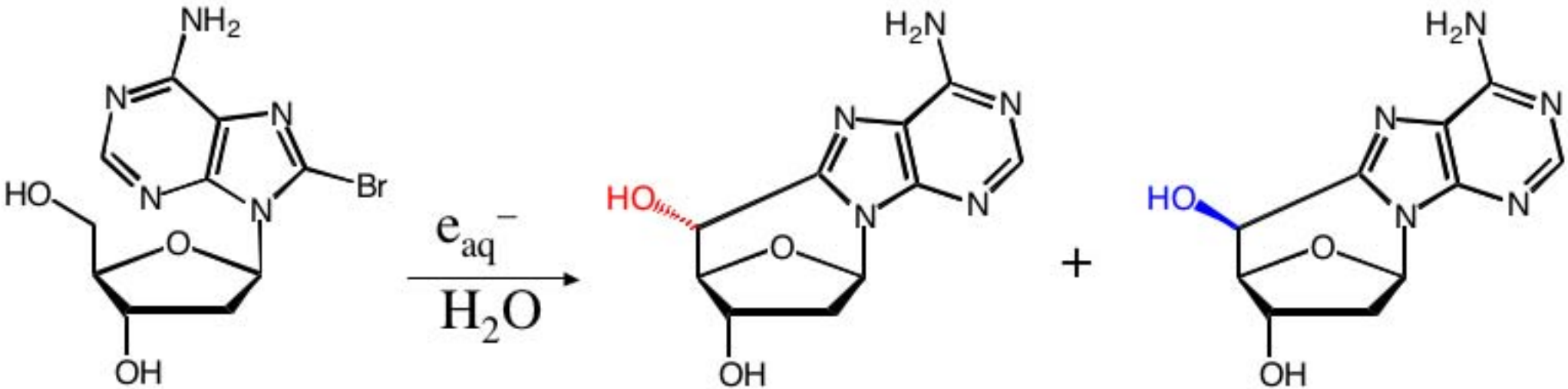




In the presence of Molecular Oxygen



Product studies



5'*R*–isomer 5'*S*–isomer

t-BuOH (0.25 M)

$Fe(CN)_6^{3-}$ steady-state μM level

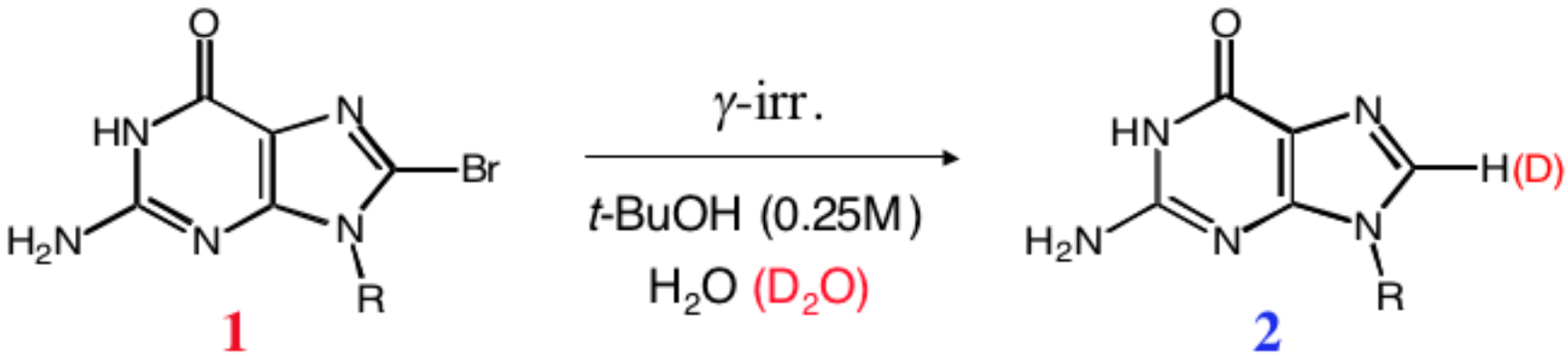
Dose: 3 kGy

Consumption 38%

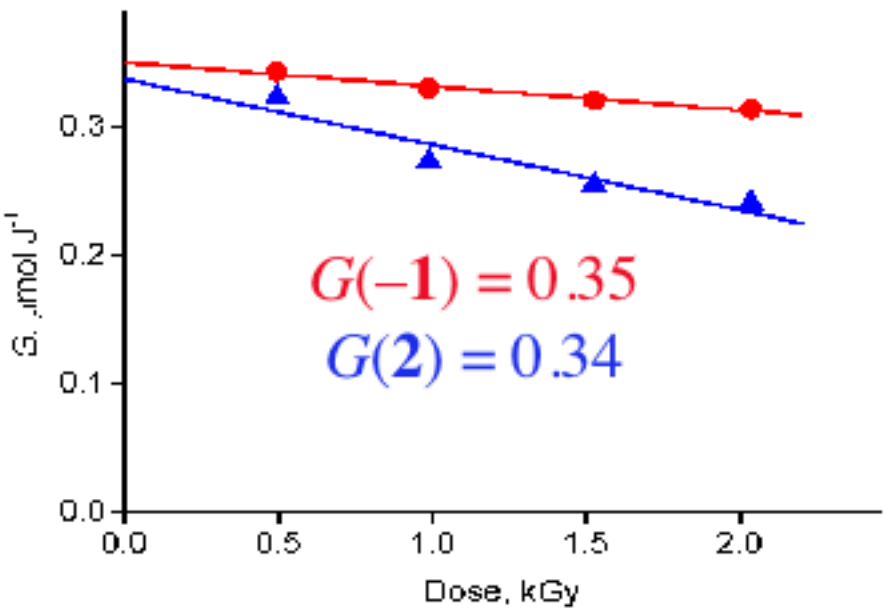
70% yield

(5'*R*):(5'*S*) = 6:1

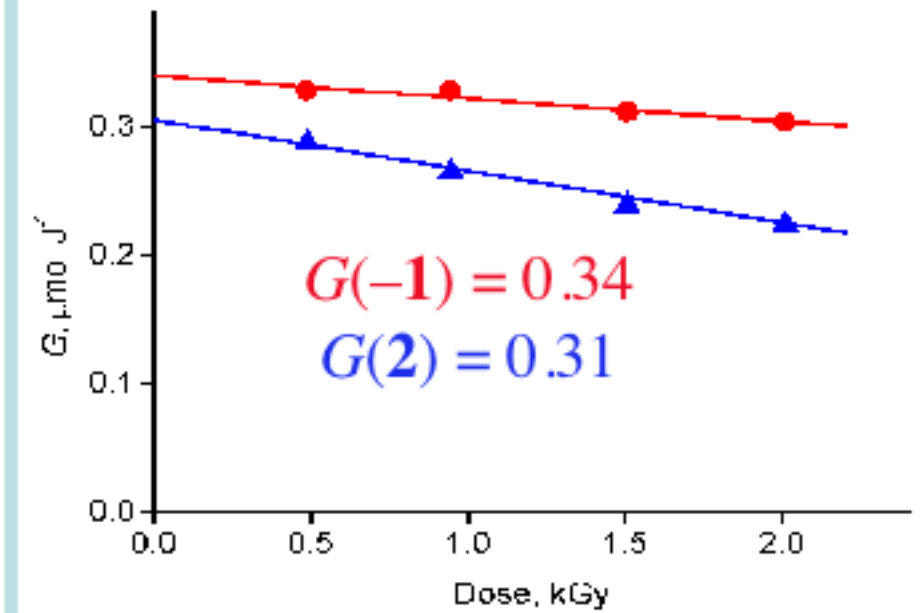
8-Bromoguanine derivatives



R = ribose

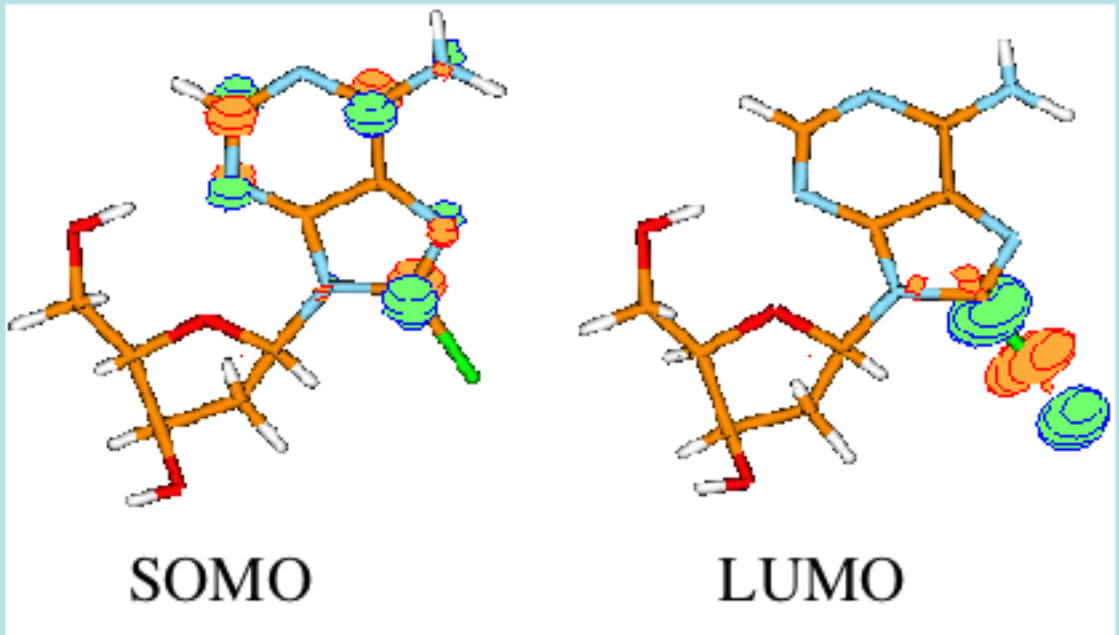


R = 2-deoxyribose

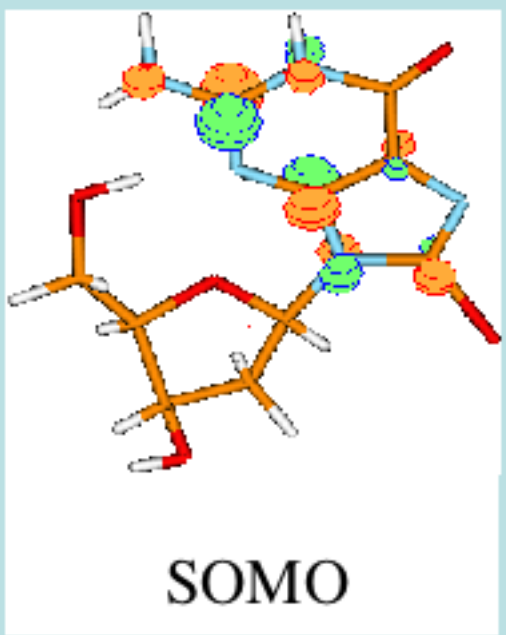


DFT-UB3LYP/6-31G*

8-Br-dA $^{\bullet-}$

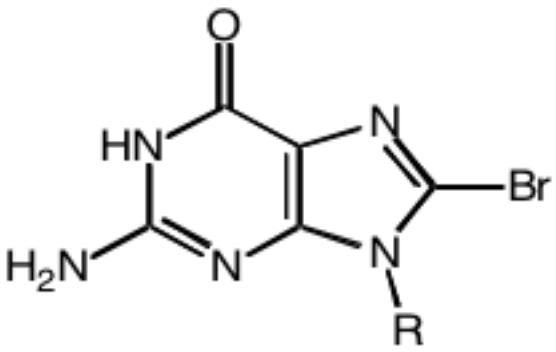


8-Br-dG $^{\bullet-}$

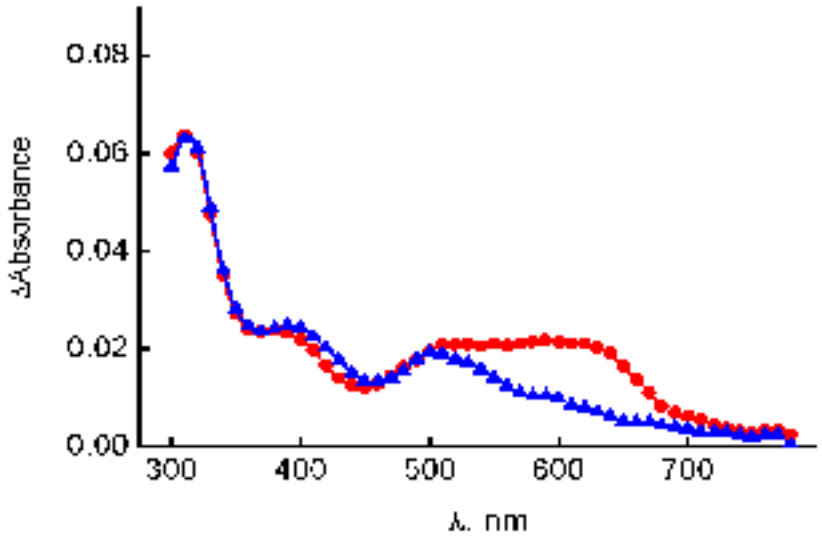


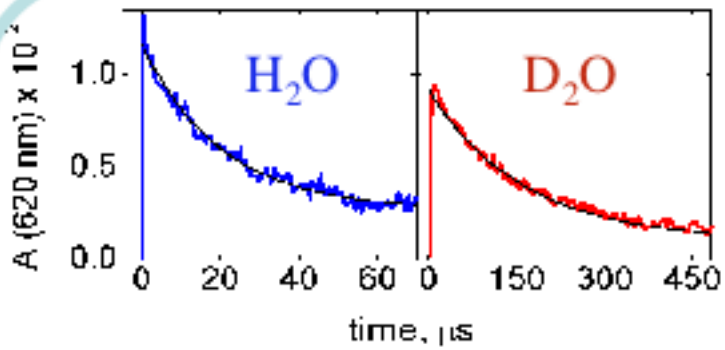
Computed at the geometry of 8-Br-dA and 8-Br-dG

Pulse radiolysis



e_{aq}^-, H^+



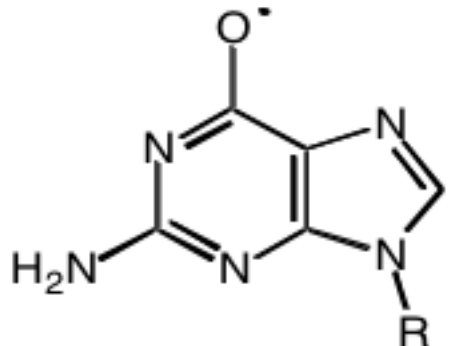


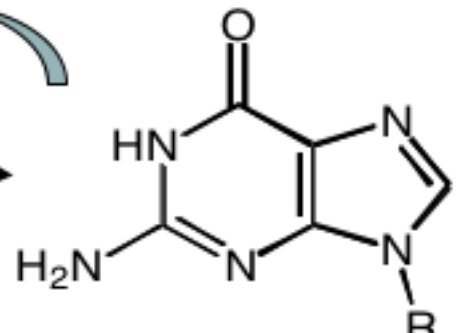
$k_H = 5.0\times10^4\ s^{-1}$

$k_D = 6.2\times10^3\ s^{-1}$

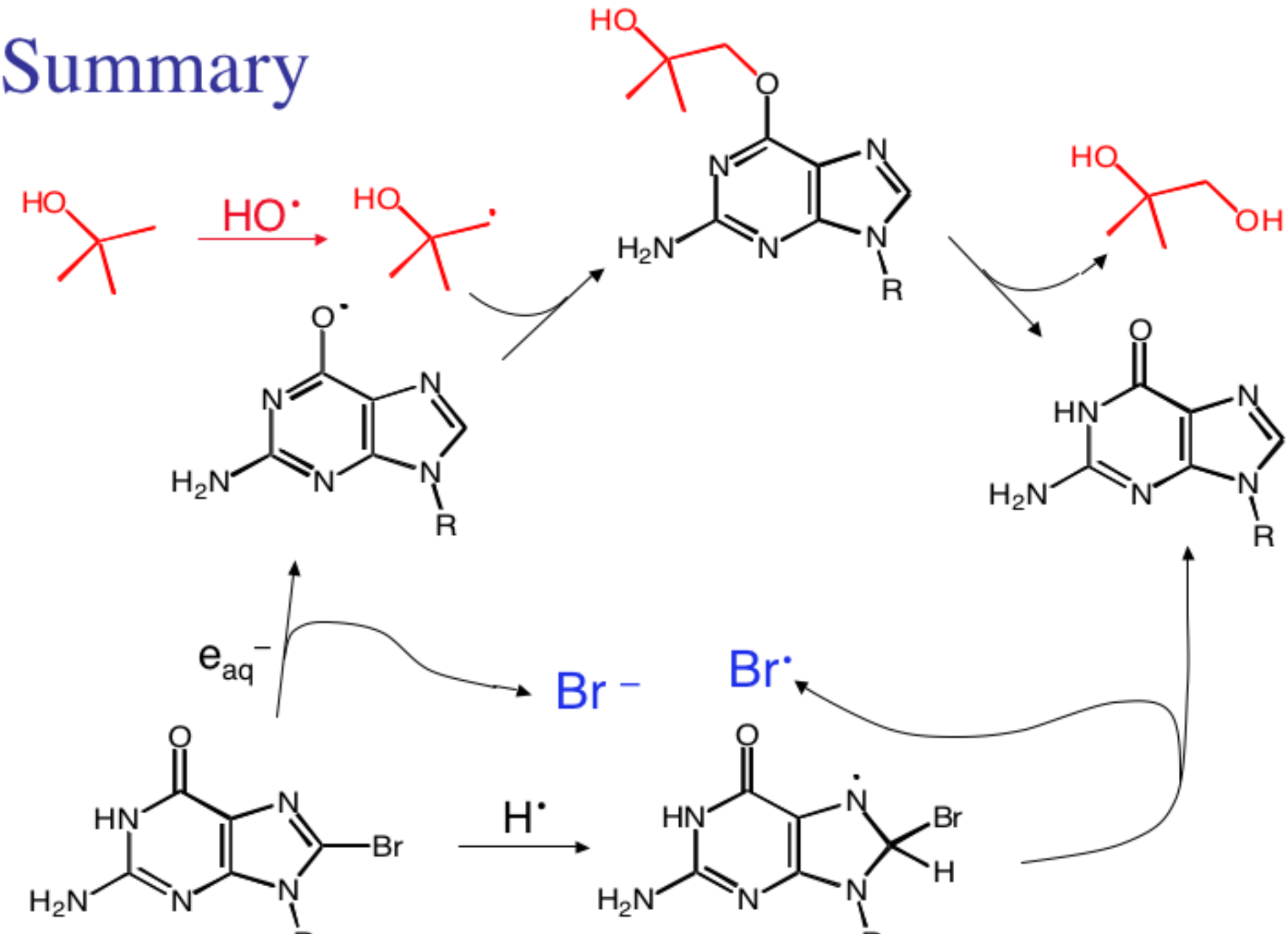
$k_H/k_D = 8$

$SO_4^{\bullet-}$ or $Br_2^{\bullet-}$





Summary

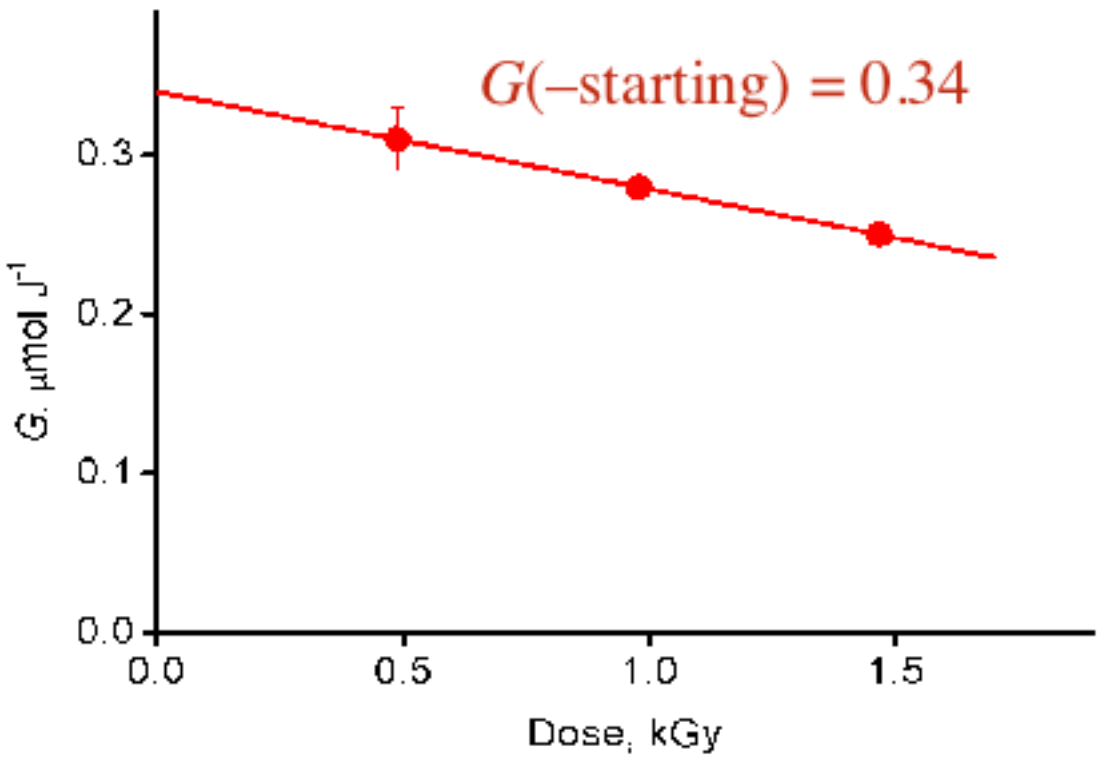

HO
HO•
HO
HO
O
N
N
N
N
H2N
R
HO
OH
O•
N
N
N
N
H2N
R
O
HN
N
N
H2N
N
R
eaq−
Br −
Br•
O
HN
N
Br
H2N
N
N
H•
O
HN
N
Br
H
H2N
N
N

γ-Radiolysis of oligonucleotide trimers

Starting trimer	5'TXT3'	5'TXG3'	5'XTT3'	5'CXA3'
G(–starting)	0.31	0.33	0.33	0.37

$G(e_{aq}^{-}) = 0.27\ \mu mol/J$

$G(H^{\bullet}) = 0.062\ \mu mol/J$

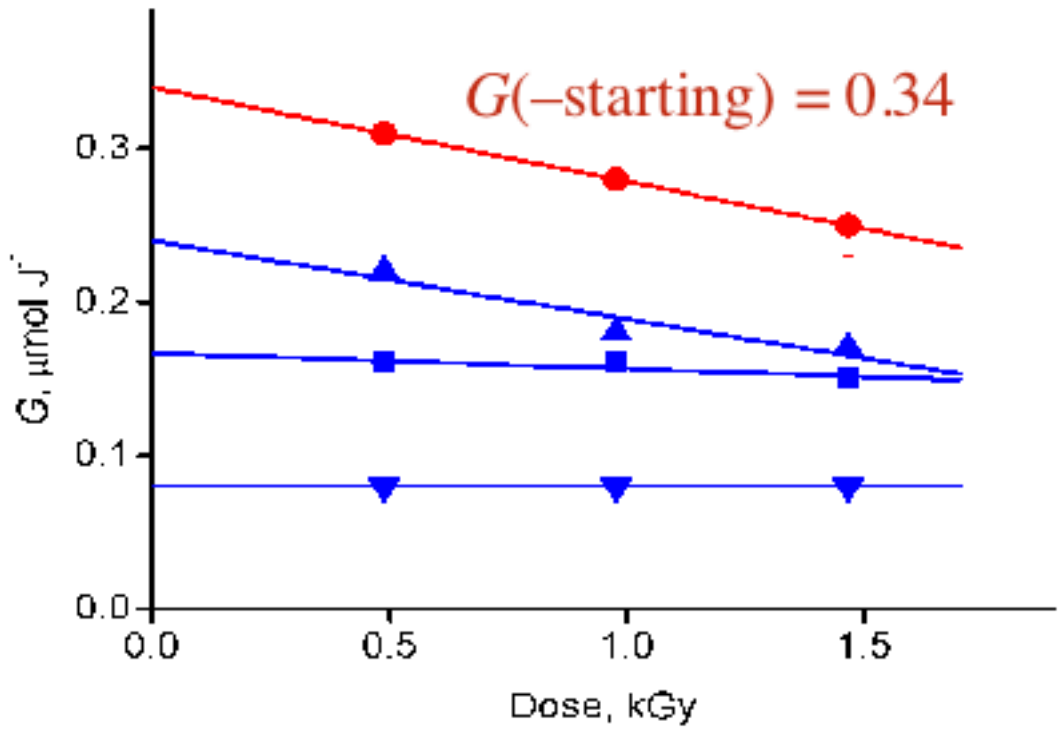


γ-Radiolysis of oligonucleotide trimers

Starting trimer	5'TXT3'	5'TXG3'	5'XTT3'	5'CXA3'
G(–starting)	0.31	0.33	0.33	0.37
G(Product)	0.24	0.24	0.17	0.08

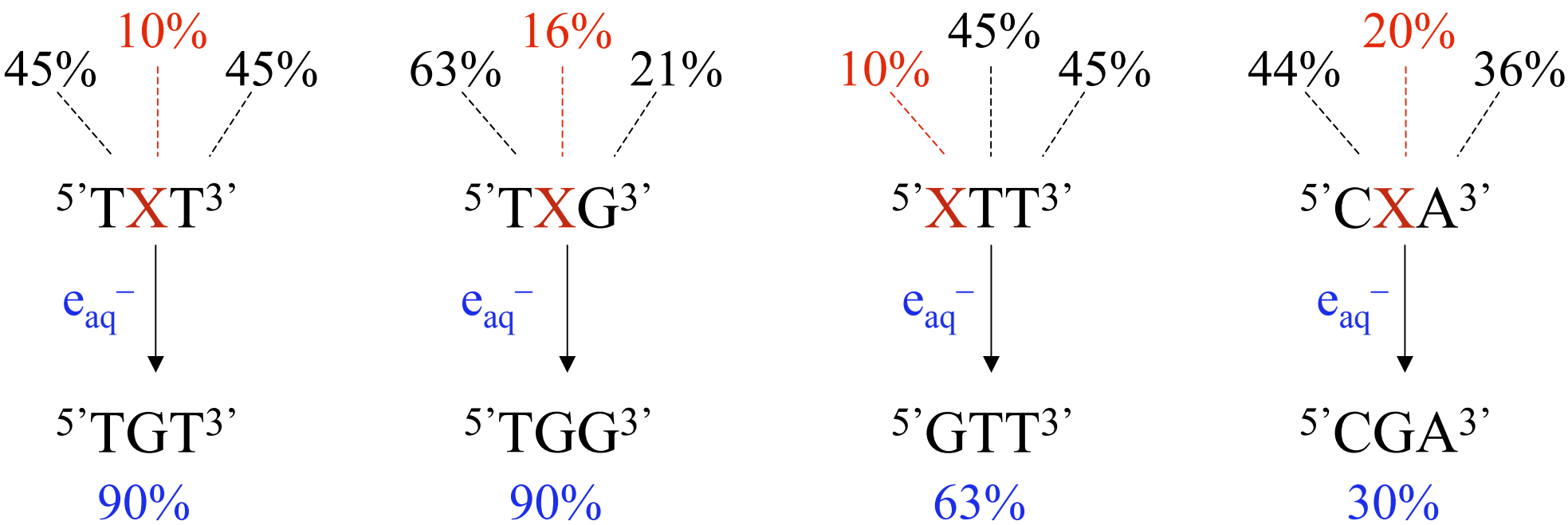
$G(e_{aq}^{-}) = 0.27\ \mu mol/J$

$G(H^{\bullet}) = 0.062\ \mu mol/J$



Electron transfer in oligonucleotide trimers

Nucleoside (N)	X	G	A	C	T
$k(e_{aq}^- + N)$, 10^{10} M^{-1} s^{-1}	0.45	0.60	0.82	1.0	1.8



Electron transfer in oligonucleotide trimers

5’TXT3’	5’TXG3’	5’XTT3’	5’CXA3’
e_{aq}^- 90% ↓	e_{aq}^- 90% ↓	e_{aq}^- 63% ↓	e_{aq}^- 30% ↓
5’TGT3’	5’TGG3’	5’GTT3’	5’CGA3’

Flanking bases

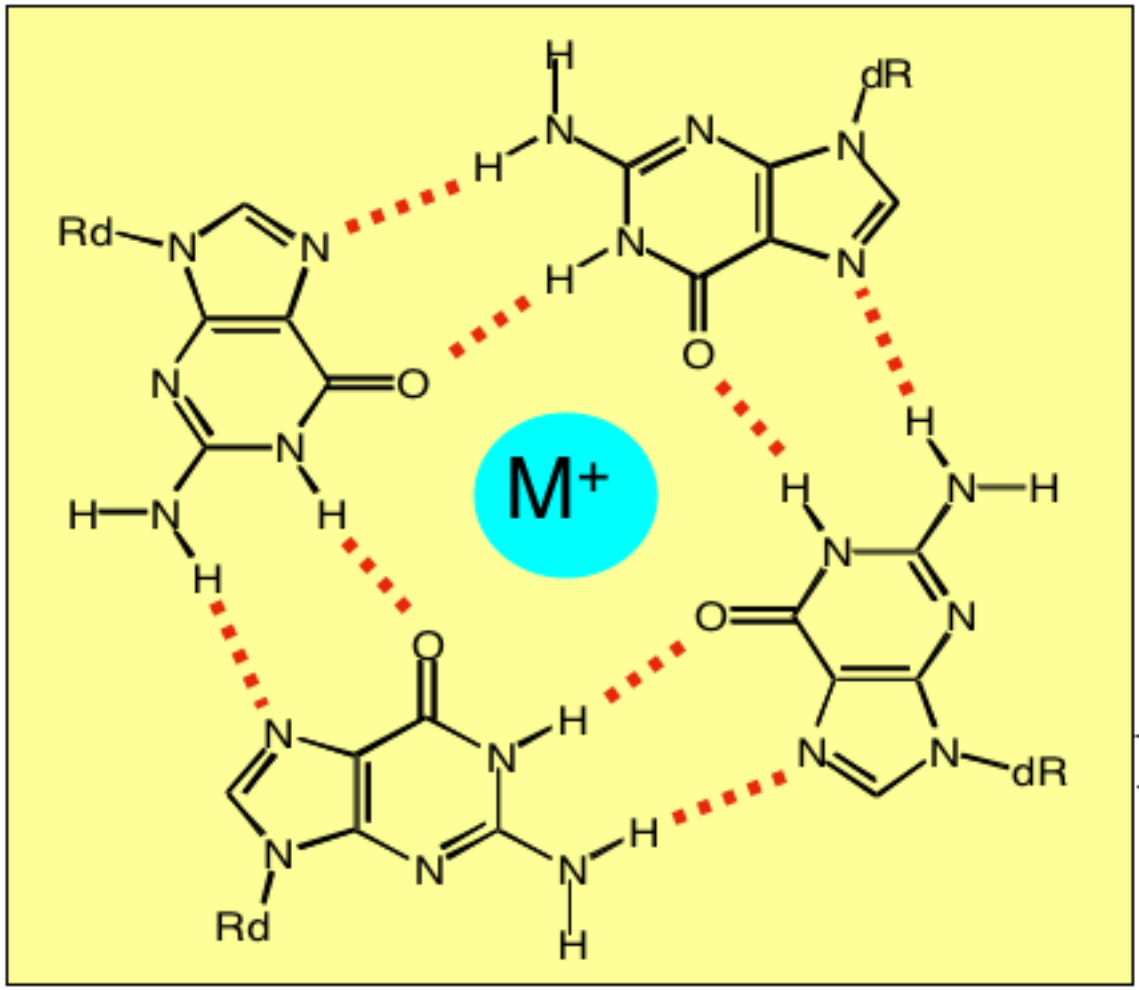
weak bases ← → strong bases

$N^{\bullet -}$ (pK_a) $T^{\bullet -}$ (7.0), $G^{\bullet -}$ (10.8), $A^{\bullet -}$ (12.1), $C^{\bullet -}$ (>13)

electron transfer ← → protonated

G-quadruplex

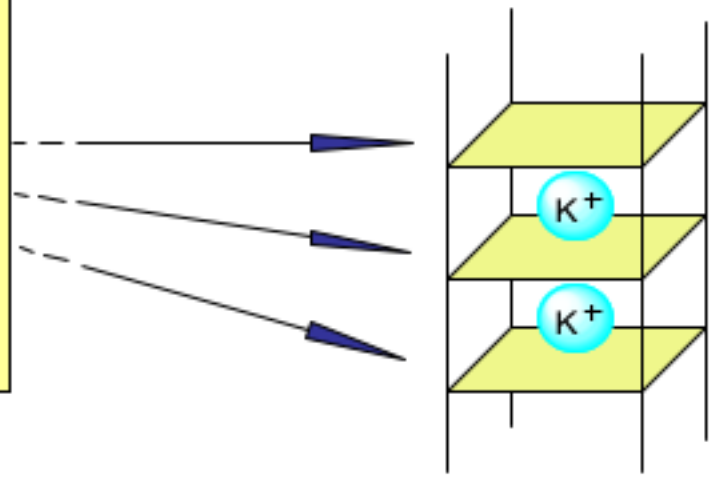
Tetrad



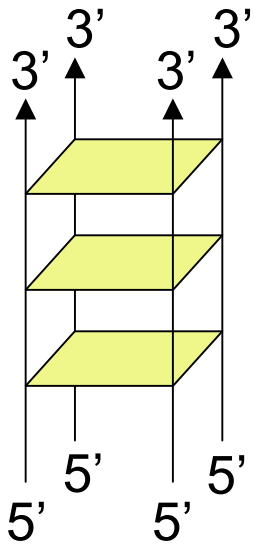
Four Hoogsteen-Bonded guanines

Four-stranded DNA structure formed by G-rich sequence

Stacked tetrads



Different G-quadruplex DNA structures

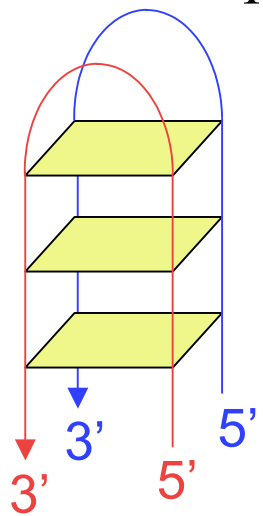


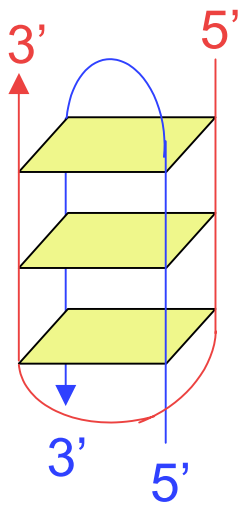
Tetramolecular complex

d(TGGGT) with K^+

"edgewise" TTA loops

"diagonal" TTA loops

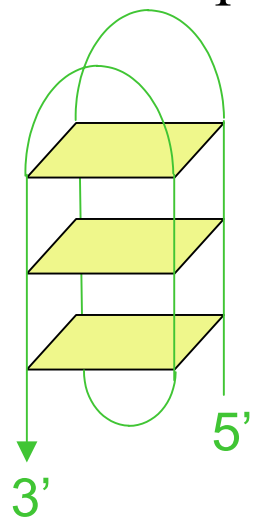




Bimolecular complexes

d($TTAGGG)_2$ with K^+

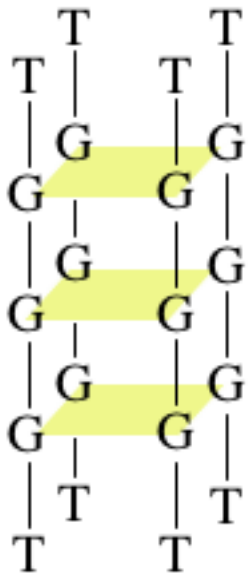
TTA loops

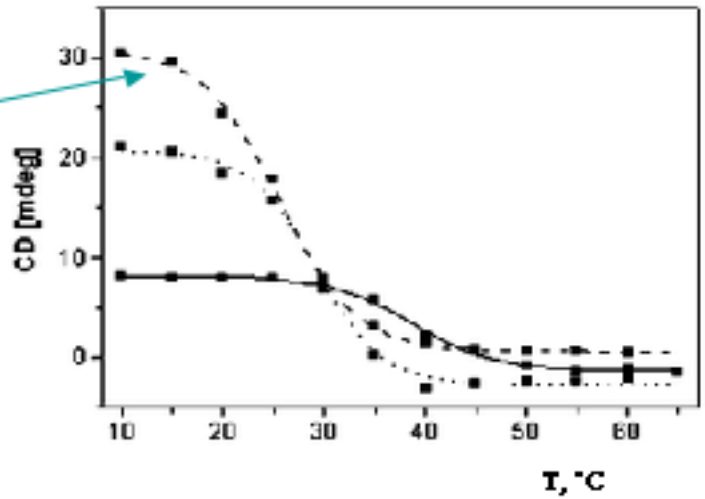


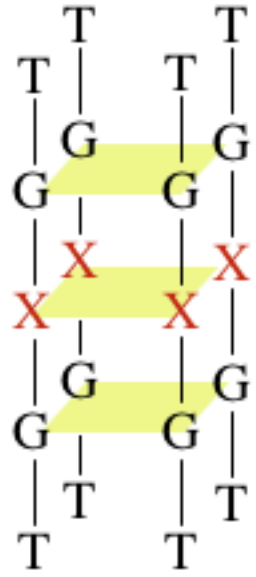
Unimolecular complex

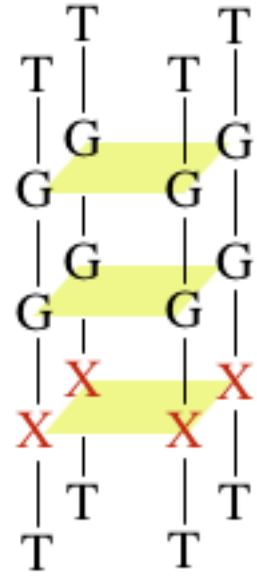
d[$AGGG(TTAGGG)_3$] with K^+

8-Br-dG incorporation

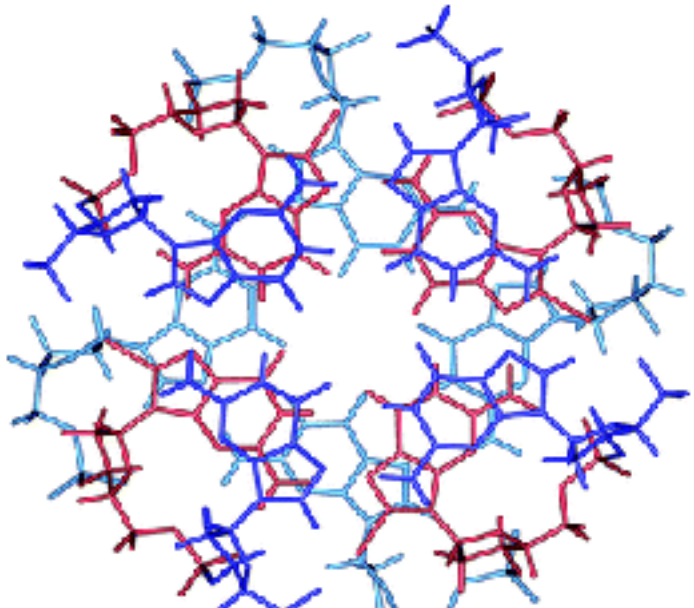


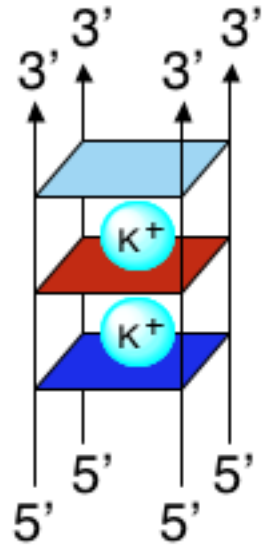






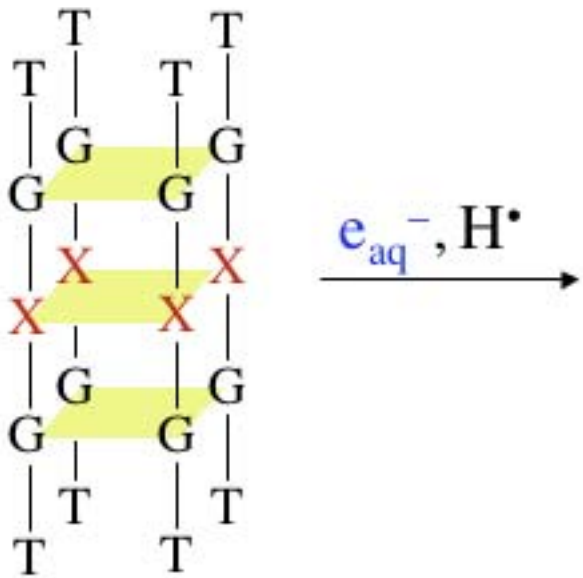
Bottom view

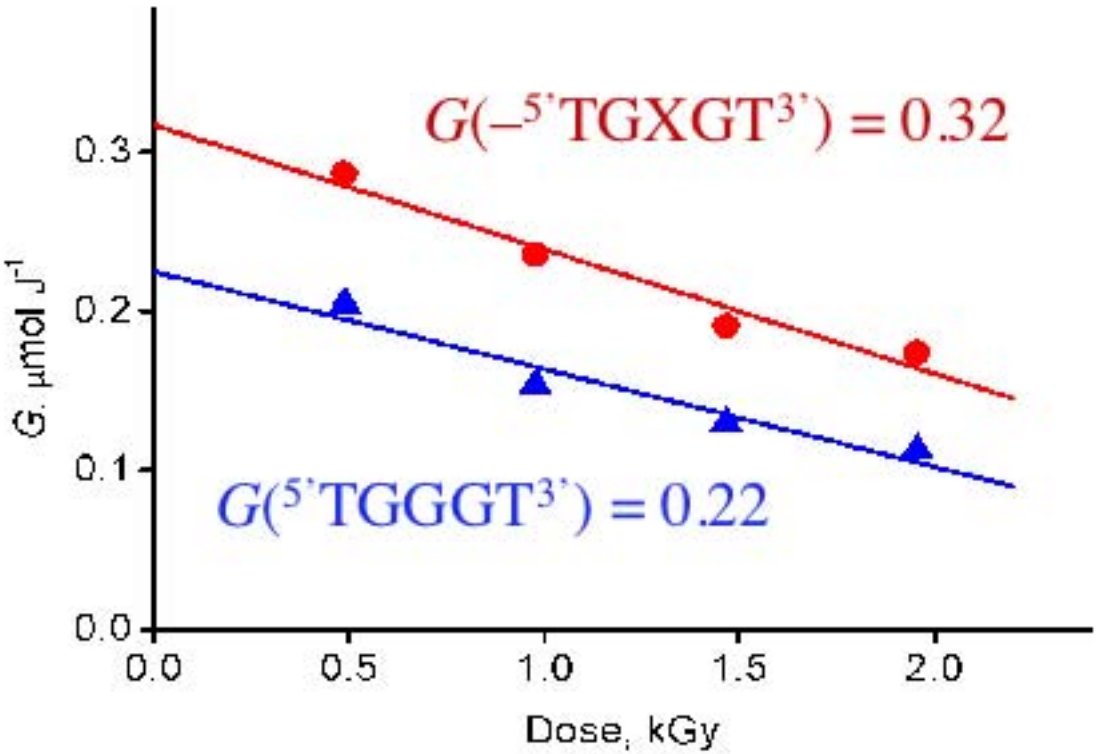




Parallel stranded tetraplex

γ-Radiolysis





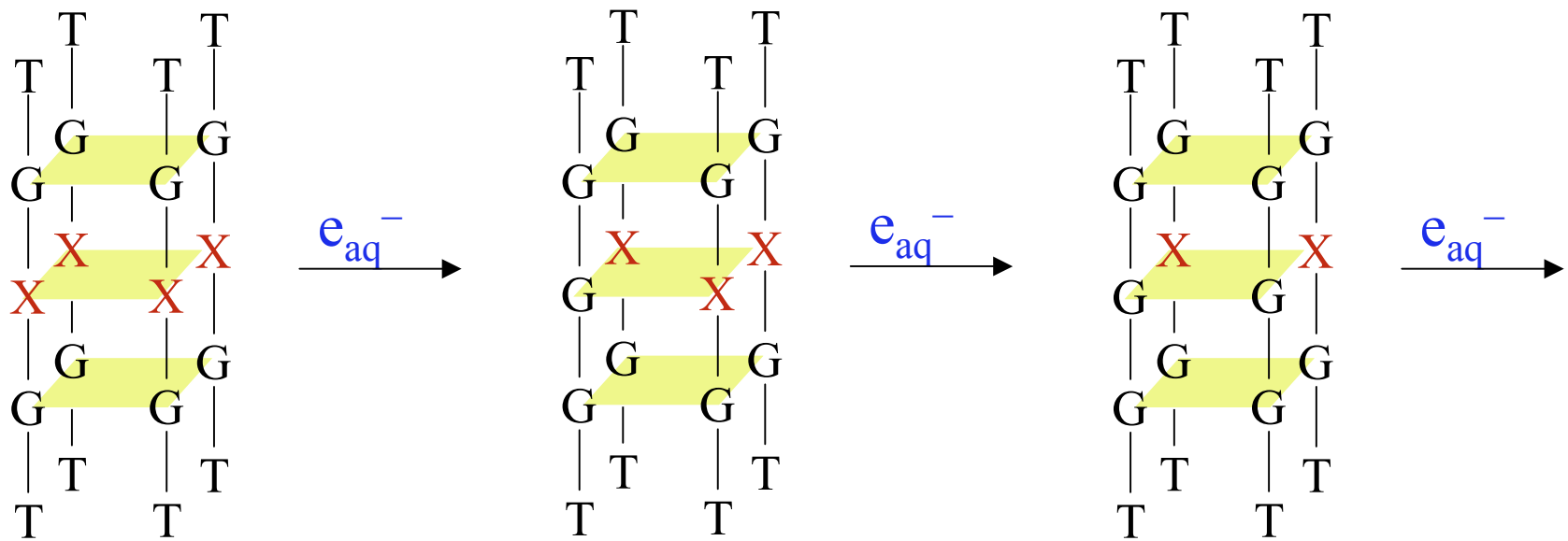
$G(e_{aq}^-) = 0.27\ \mu mol/J$

$G(H^\bullet) = 0.062\ \mu mol/J$

82% yield

Excess electron transfer in G-quadruplex

e_{aq}^- add ~70% to T, ~23% to G and ~7% to X



G–quartets very effective in excess electron transfer

8-Br-dG moieties very good as detection system

Acknowledgements

Quinto G. Mulazzani
Maurizio Guerra
Carla Ferreri
M. Luisa Navacchia

Clara Caminal
Liliana Jiménez
Antonio Manetto
Antonio D'Aurizio

Marcella Ioele
Roman Flyunt
Rita Bazzanini
Maria Duca
M. De Champdoré

Collaborations

Lorenzo De Napoli, University of Napoli
Gennaro Piccialli, University of Napoli
P. Carlo Montevecchi, Bologna University
Thanasis Gimisis, Athens University
Miguel A. Miranda, University of Valencia

RESEARCH
Marie Curie Actions
Human Resources and Mobility Activity