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CHIMIE INORGANIQUE
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Radiation quality and DNA damage: Chemical aspects

Jean-Luc RAVANAT

Laboratoire Lésions des Acides Nucléiques,

INAC-SCIB UMR E-3 CEA-UJF, CEA Grenoble, 17 rue des Martyrs, 38054 Grenoble cédex 9, France

jravanat@cea.fr

UNIVERSITE JOSEPH FOURIER SCIENCES TECHNOLOGIE MEDECINE

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Difference between endogenous oxidative stress and radiation-induced DNA lesions

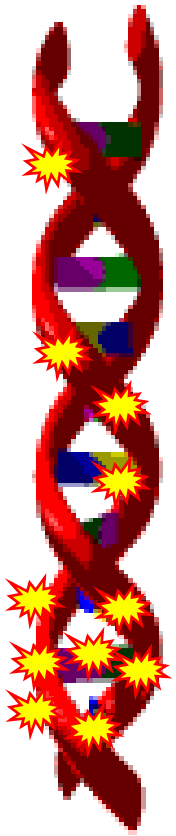
Endogenous generation of ROS

Low particule

Few ionization processes

High particule

Multiple ionization processes



Single lesions

Locally multiple damage sites (LMDS)

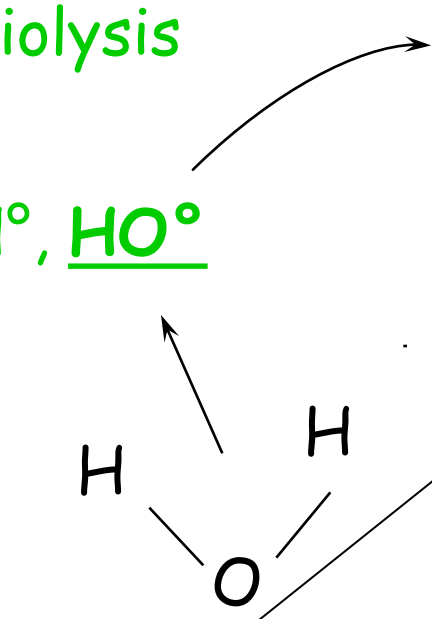
Biological consequences of radiation could be explained by the formation of LMDS (including DSB)

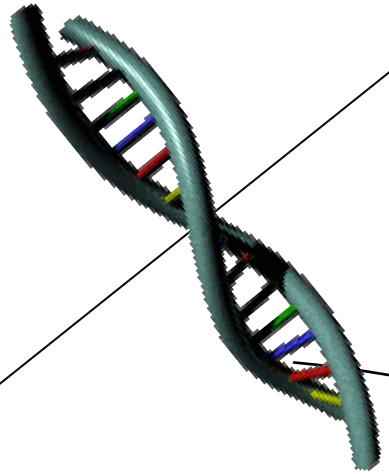
The complexity of damage increases with LET

Ionzing radiation : two effects

Indirect effect
Water radiolysis

e^-_{aq}, H°, **HO°**





Direct effect:
Ionization

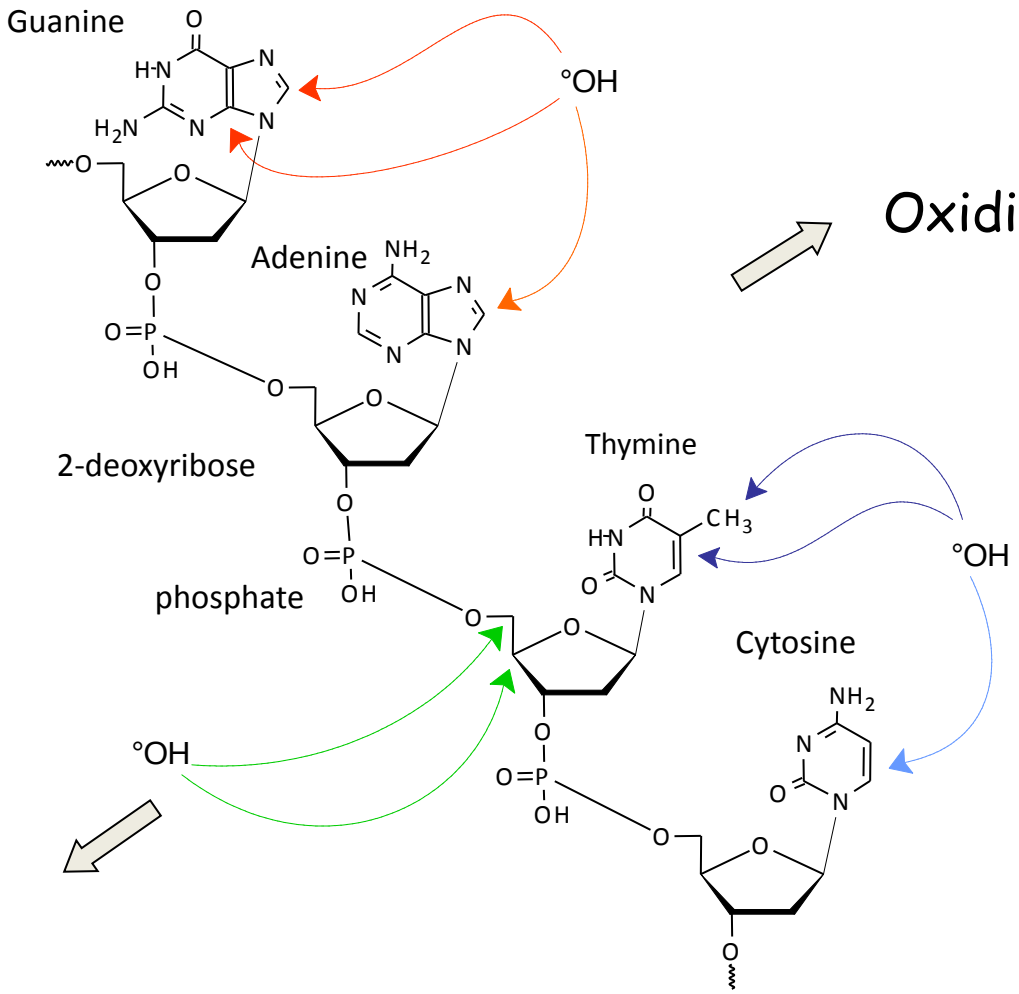
e^-

Particle

Relative importance : Direct/indirect effect ?

Direct effect increases with LET

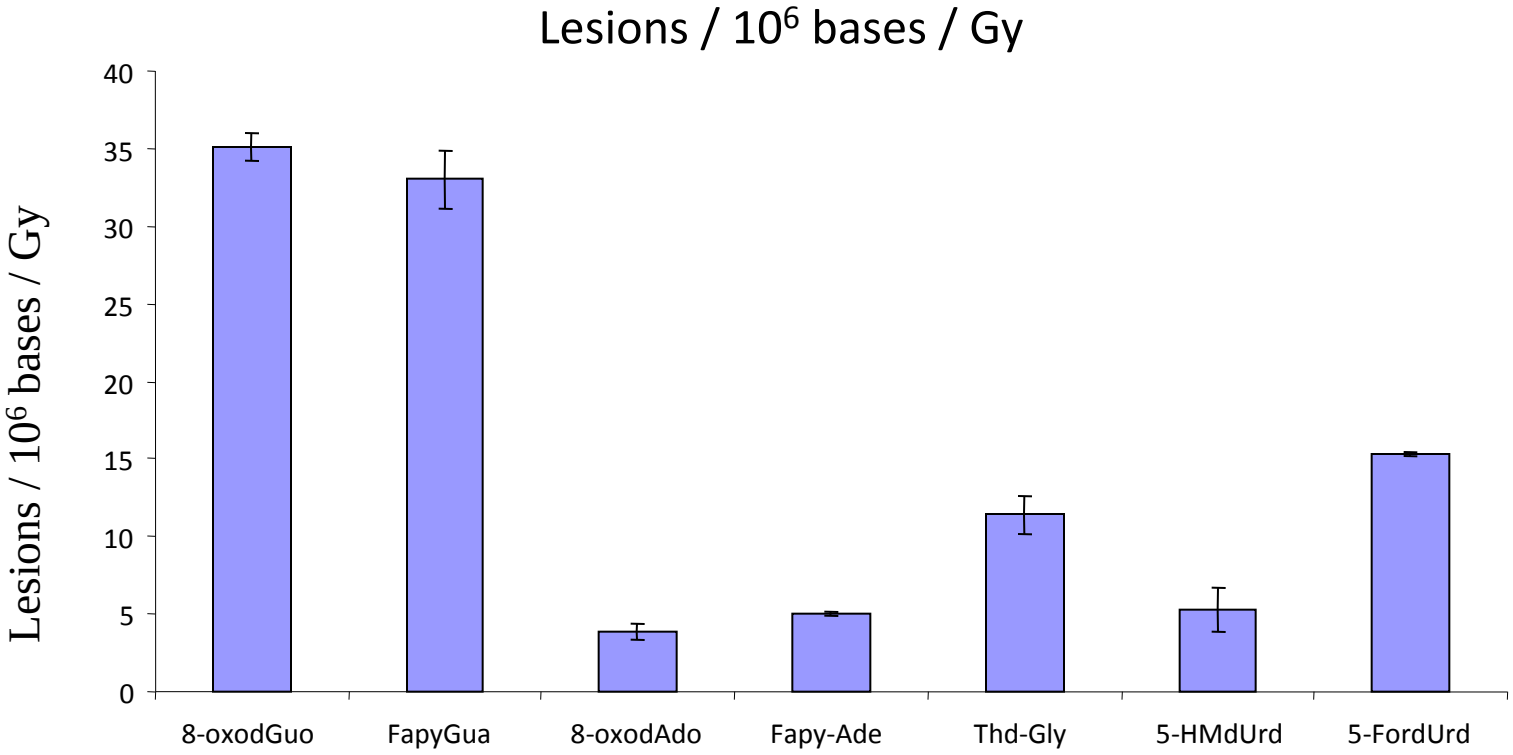
Indirect effect : °OH-mediated DNA damage



Methods were developed to measure these damages in cells (Comet, HPLC-MS/MS)

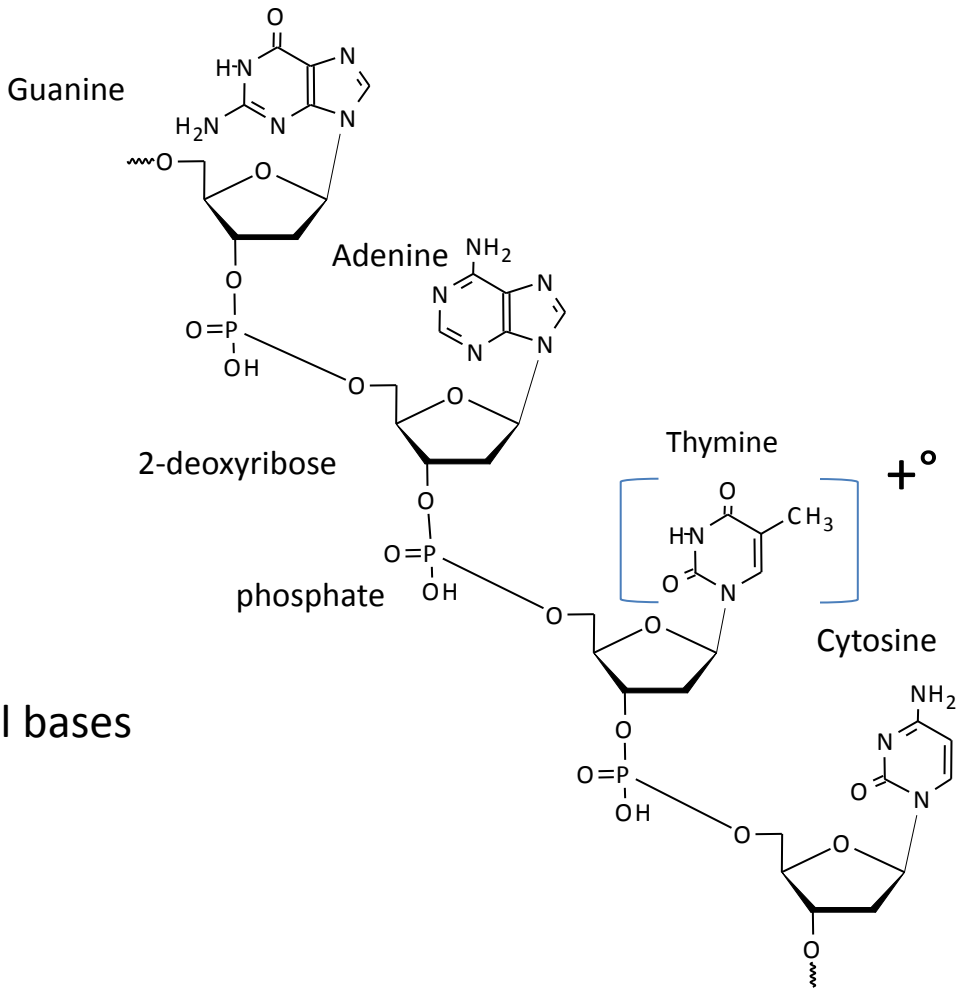
Gamma irradiation : dsDNA aerated aqueous solution

(HPLC-MS/MS subsequently to DNA digestion)



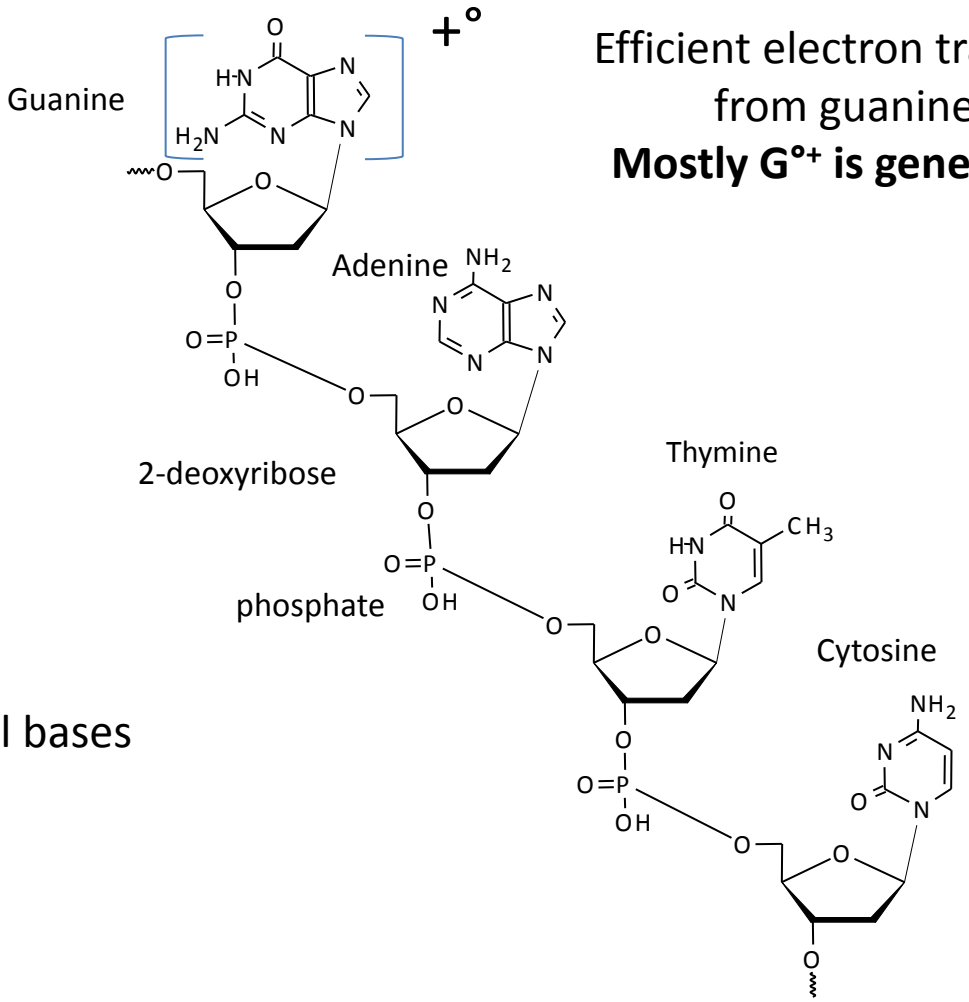
Isolated DNA : About 100 lesions per 10^6 bases per Gy (indirect effect)
All bases are modified (profile similar under Fenton type reaction conditions)

Direct effect : One electron oxidation



Ionisation of all bases
but …

Direct effect : One electron oxidation

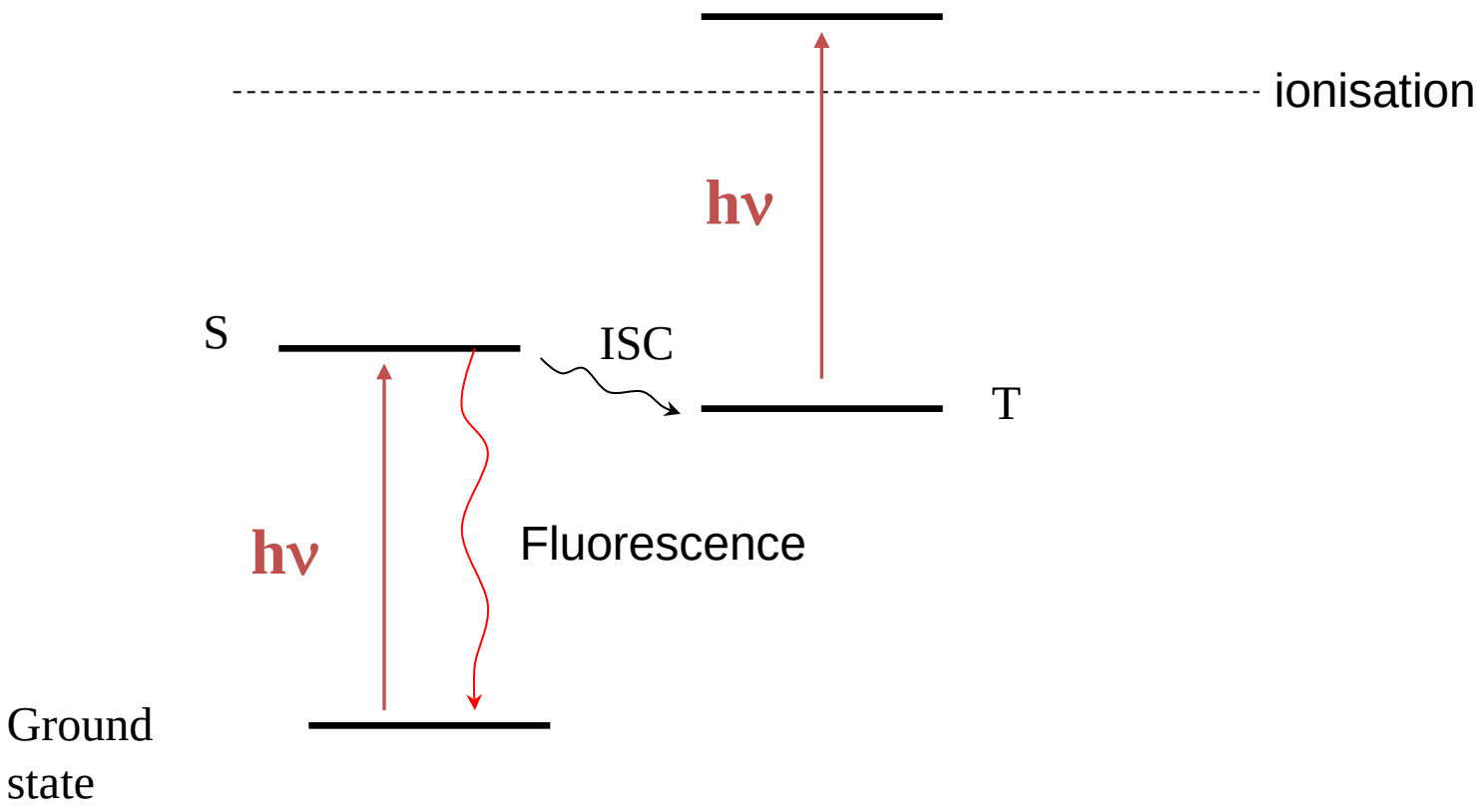


Efficient electron transfert from guanine :
Mostly G°+ is generated !

Ionisation of all bases but …

Direct effect : mostly damage on guanine bases

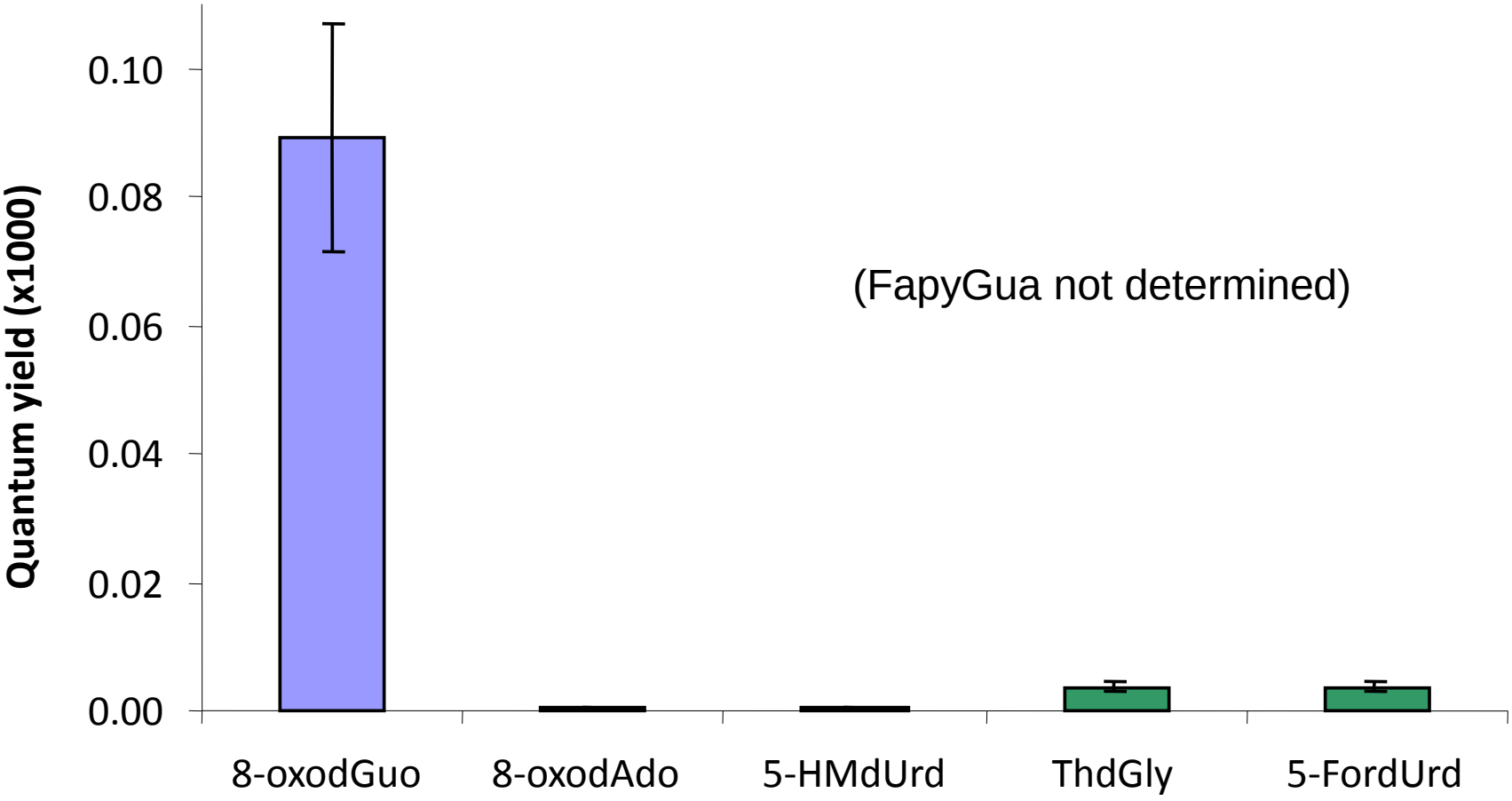
Direct effect of ionization
High intensity UV (266) laser irradiation



Excitation produces pyrimidine dimers (UVB)

Pulsed (5 ns) YAG laser 266 nm produces ionization

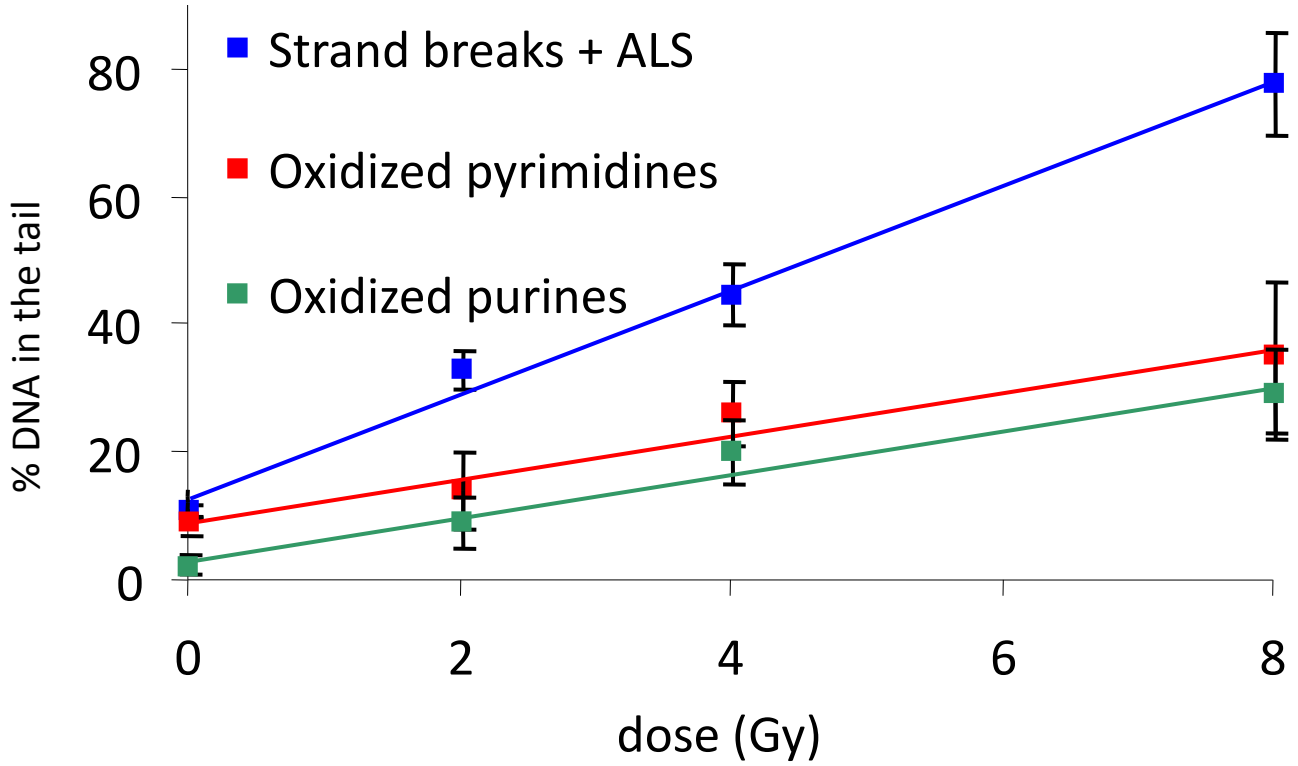
Direct effect of ionization on isolated DNA



Majority of guanine lesions: electron transfer mechanism

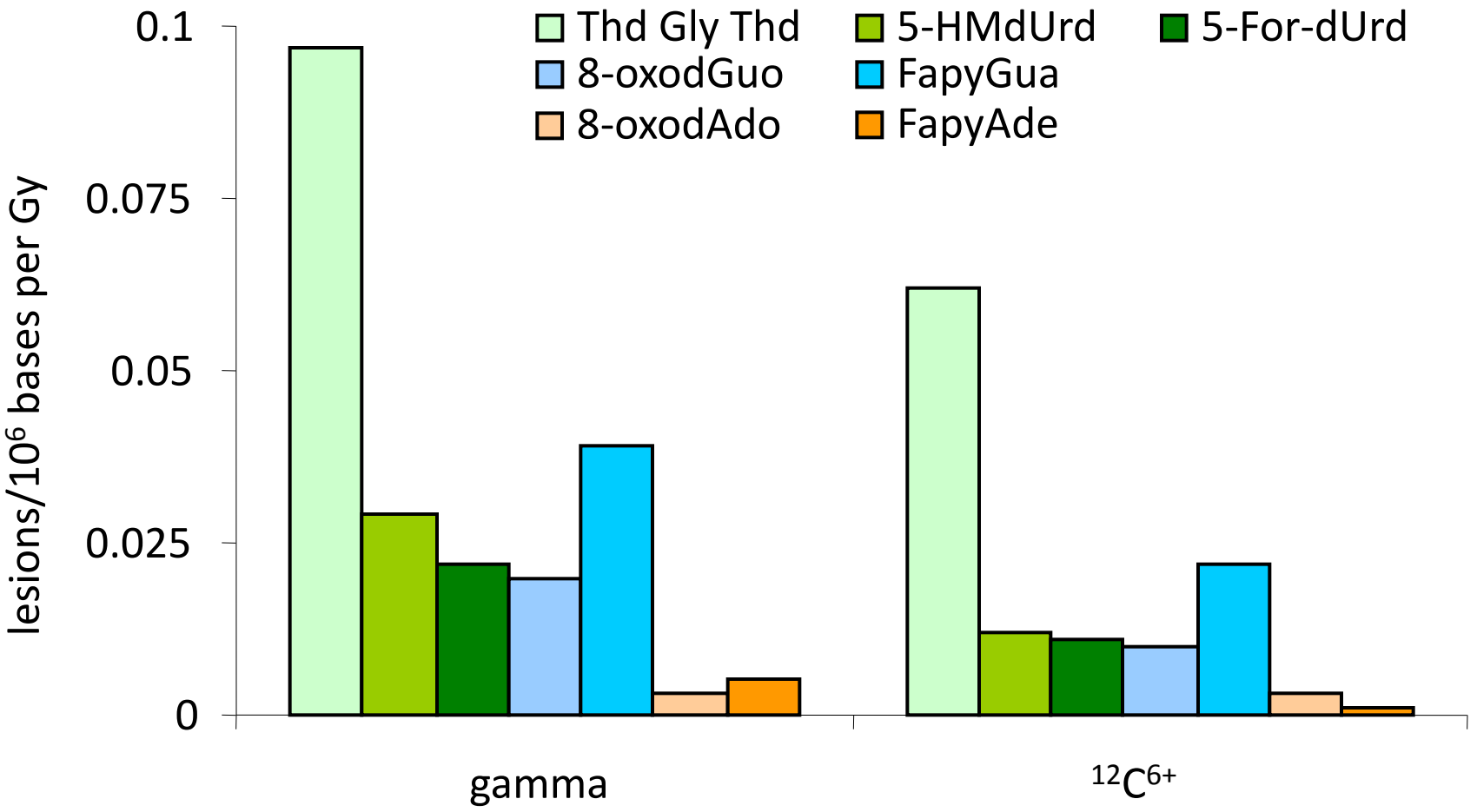
DNA lesions produced in cells

Comet assay : single cell gel electrophoresis + specific DNA repair enzymes



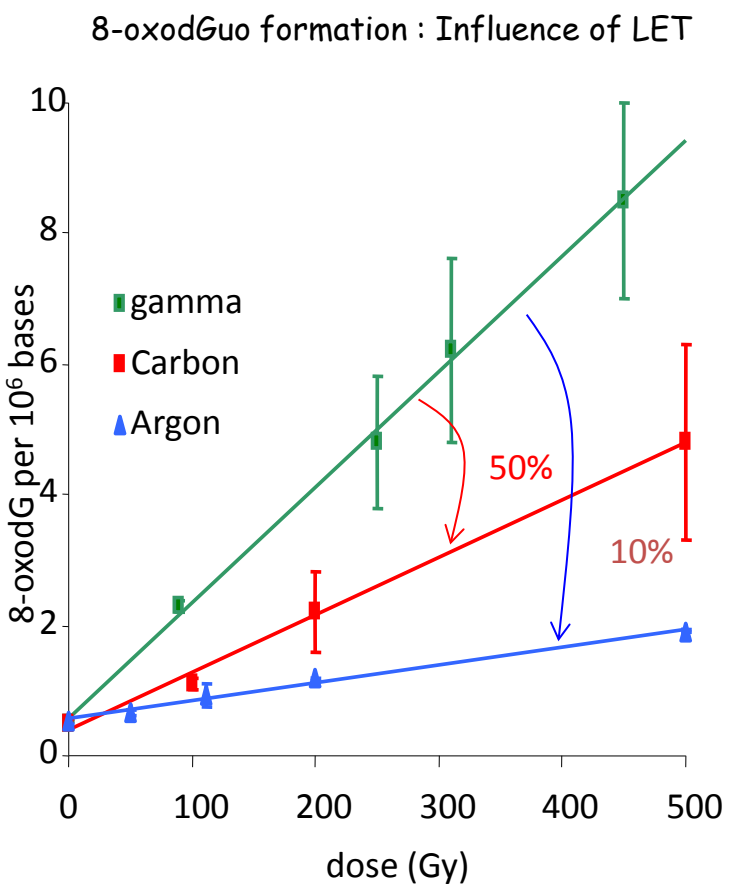
Linearity of formation of the lesions (from 0.1 to 10 Gy)
Very sensitive but not specific and semi-quantitative

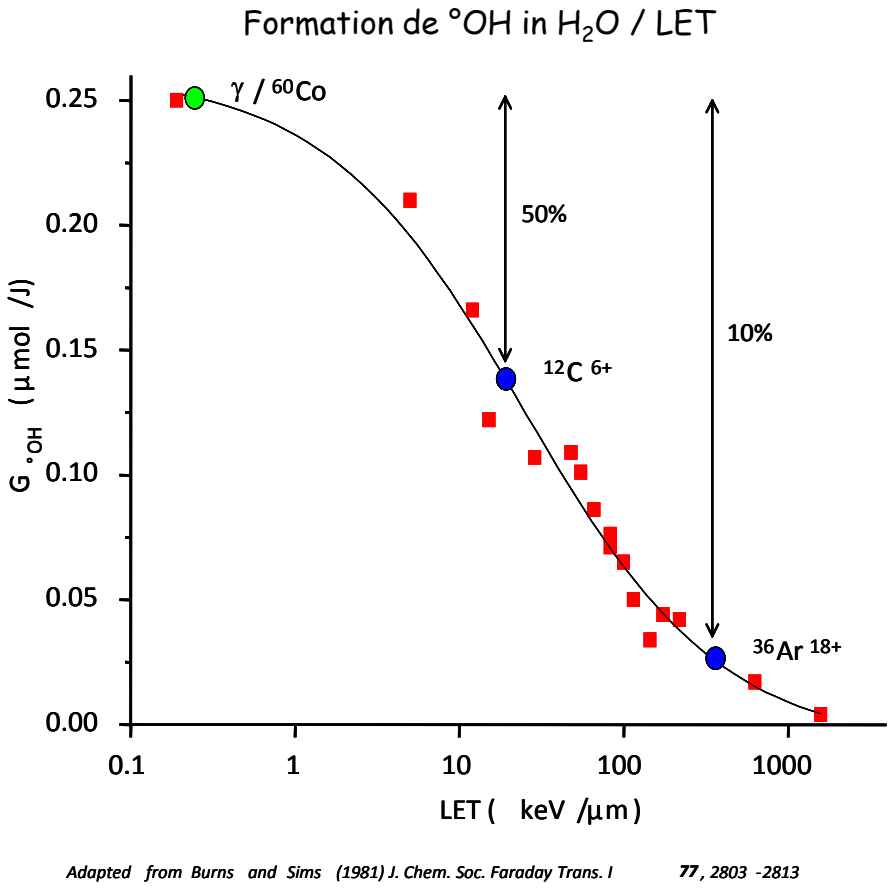
DNA lesions produced in cells



0.3 lesions per 10^6 bases / Gy (3000 times less than in isolated DNA)
8-oxodGuo is not the main lesion (minor importance of direct effect ?)
Yield gamma / $^{12}C^{6+}$ # 2 (identical profile)

Formation of 8-oxodGuo in cellular DNA : Influence of LET





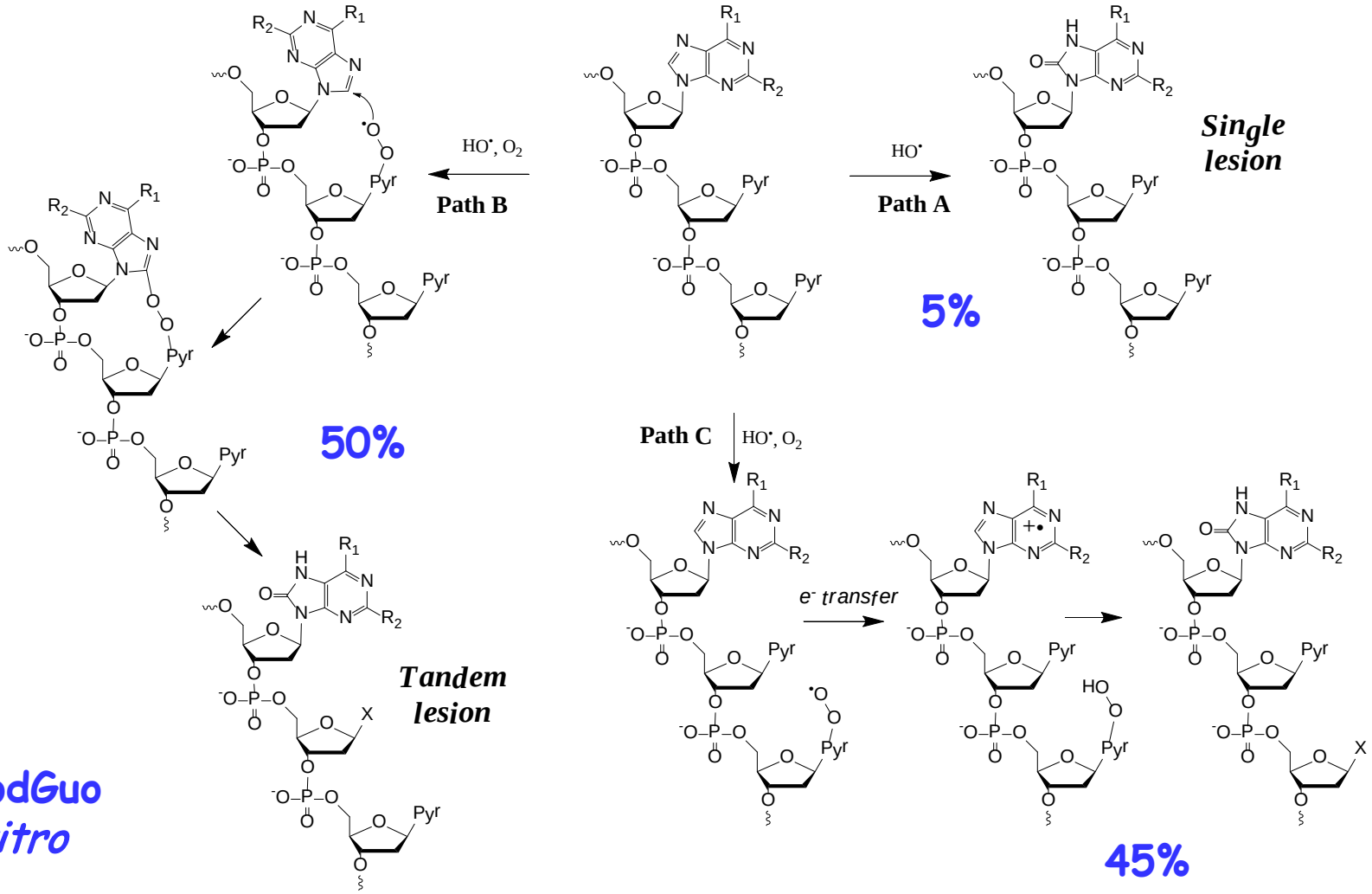
Adapted from Burns and Sims (1981) J. Chem. Soc. Faraday Trans. I ***77****, 2803 -2813*

The yield of 8-oxodGuo formation correlates with that of HO°

Direct effect a minor process ?

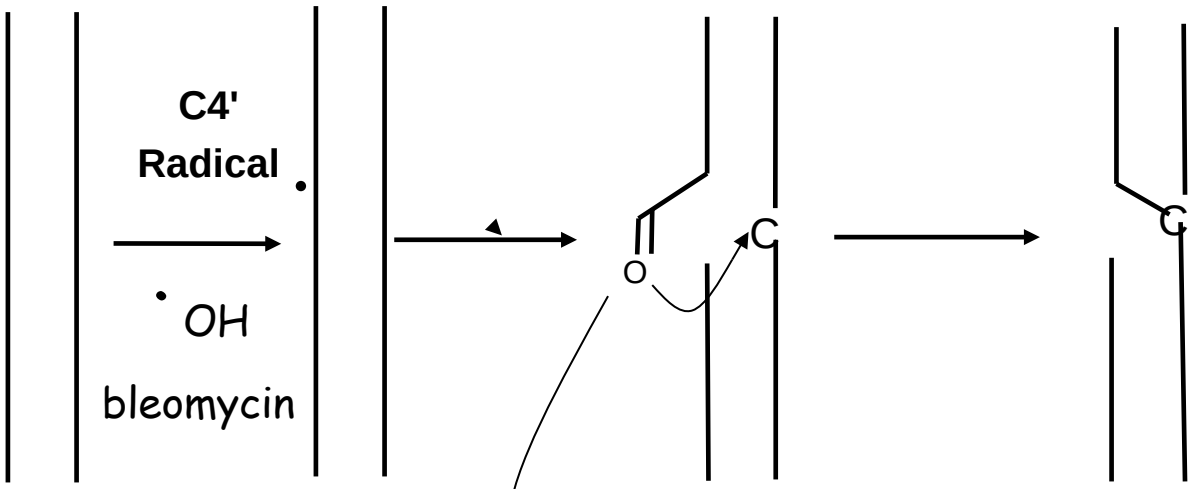
Douki, T.; et al. Int. J. Radiat. Biol. ***2006****, 82, 119-127*

It is not so simple : HO°-mediated 8-oxodGuo formation

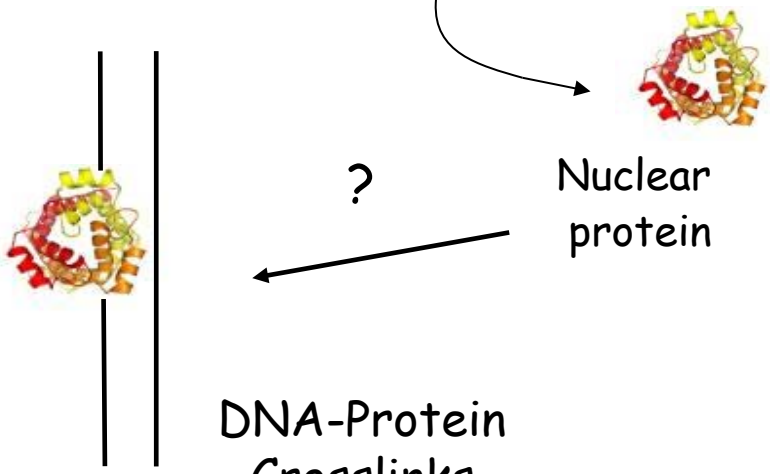


Limited variation of 8-oxodGuo with LET…

It is not so simple : a single oxidation event could induce the formation of complex DNA lesions



Regulus, P. *et al.*, (2007) *Proc. Natl. Acad. Sci., U.S.A.* **104**, 14032-14037
Sczepanski et al. (2008) *J. Am. Chem. Soc.*, **130** , 9646-7



Sczepanski, J. T. *et al. (2010), Proc. Natl. Acad. Sci., U.S.A.* **107**, 22475-22480

Importance of sugar oxidation in the genotoxicity of ionizing radiation

What do we need to know : chemical aspect

Are there specific DNA lesions generated by ionizing radiation? (non DSB)

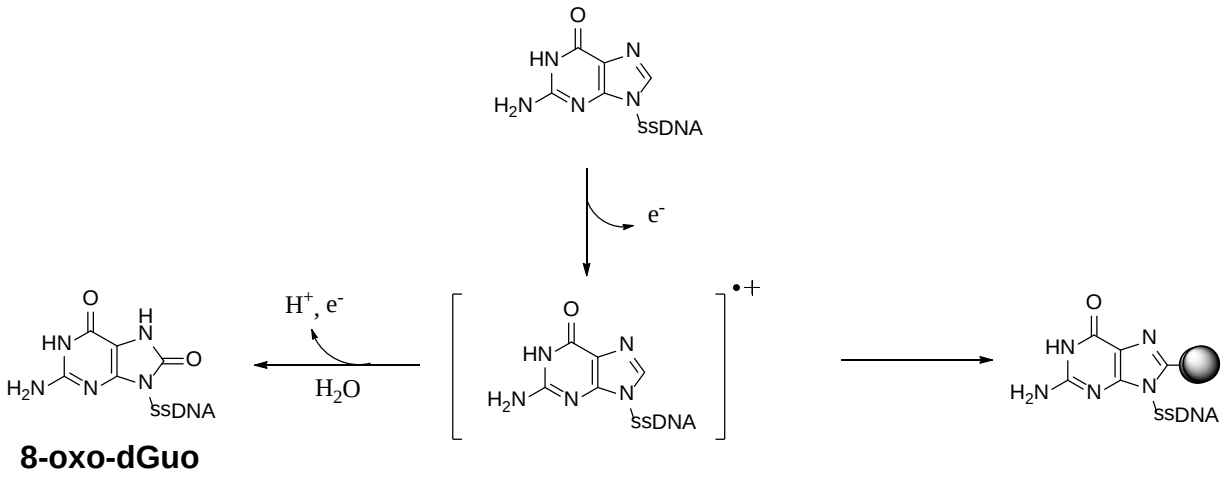
- **Direct effect** (one electron oxidation)
- Indirect effect ?

Qualitative data

Quantitative data

Biological consequences

Potential candidates of specific DNA lesions : Chemistry of guanine radical cation!

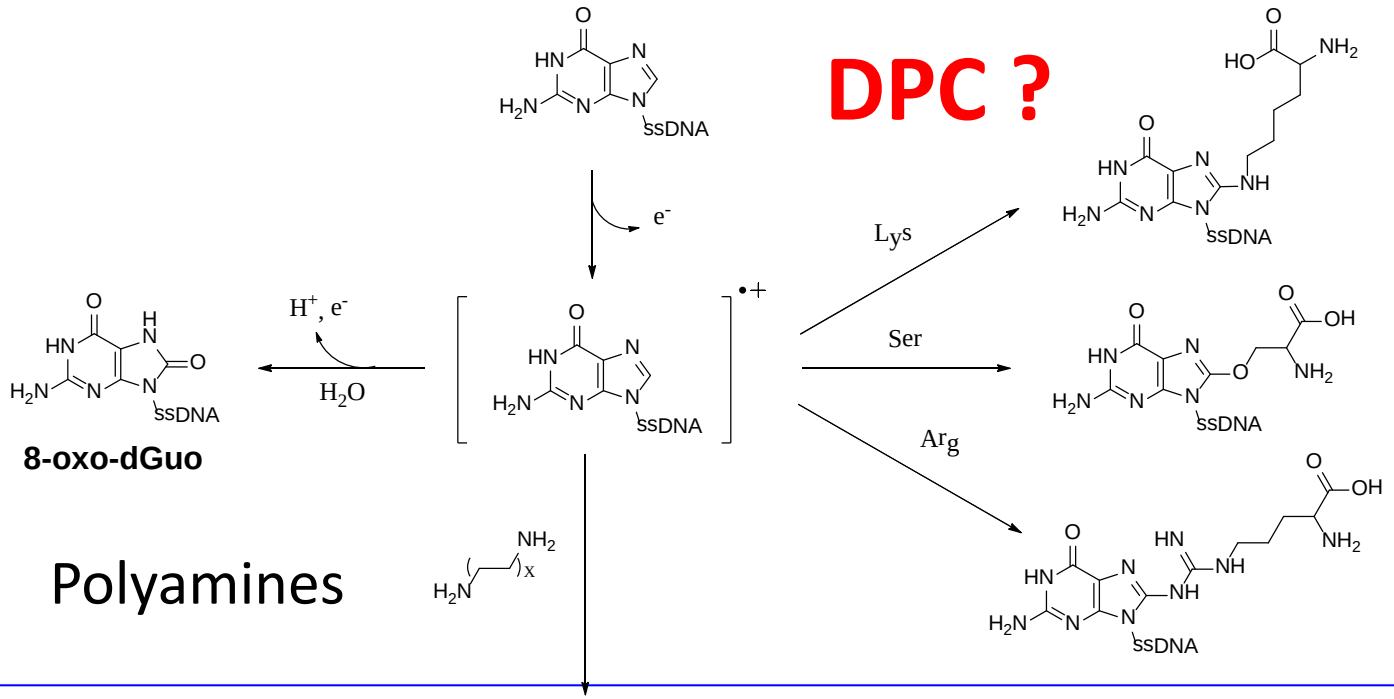


Nucleophilic addition at C8 of guanine

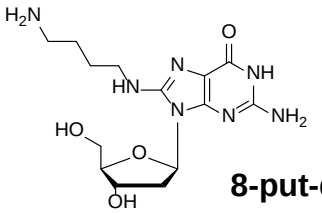
- H_2O : 8-oxodGuo
- in vitro : Lysine (Perrier et al. JACS 2006)
- in vivo ? : DPC ?

Other endogenous nucleophiles ?

Chemistry of guanine radical cation

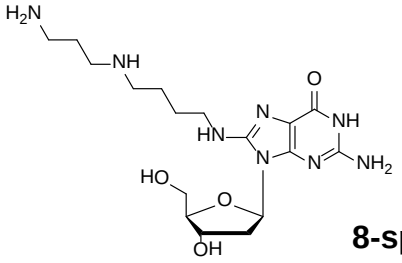


X = $(CH_2)_4$



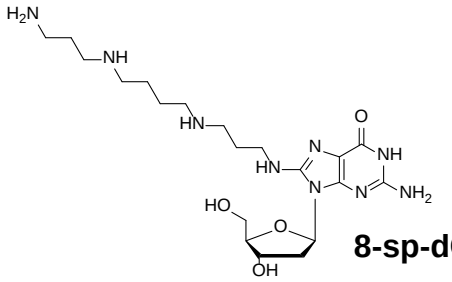
8-put-dGuo

X = $(CH_2)_3NH(CH_2)_3$



8-spd-dGuo

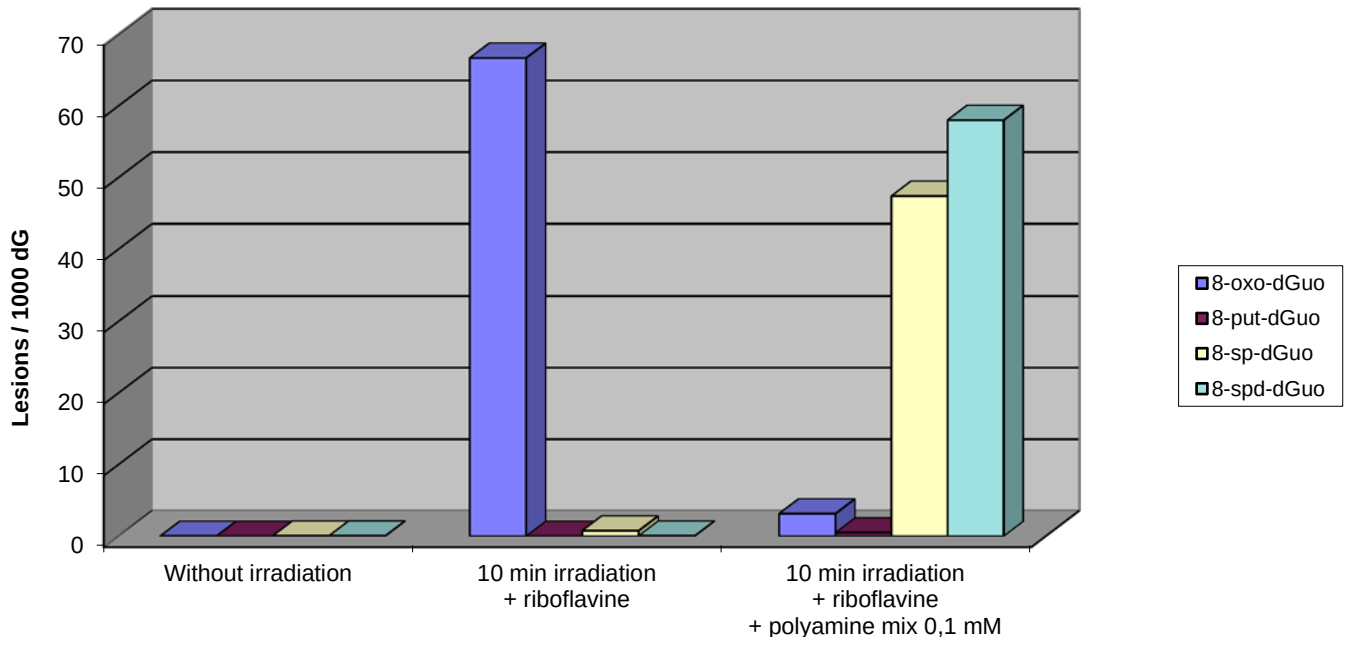
X = $(CH_2)_3NH(CH_2)_4NH(CH_2)_3$



8-sp-dGuo

Markers of direct effect ?

Formation of polyamine adducts in dsDNA UVA + riboflavine



Importance of such reactions in cells ?

Markers of direct effect of radiation ? (UVA)

Specific DNA lesions induced by hight LET particles ?

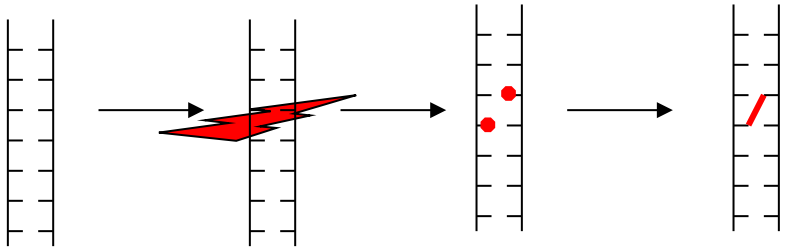
High LET particles : High density of ionisation!

Chemistry of radical :

- Initiation (radical formation)
- Propagation (reaction of radicals)
- Termination (recombination)

High density of ionisation :

- Radical recombination is favored ?



Yield of formation should increase with increasing LET !

Ex: H_2O_2 in water radiolysis

Search for new DNA lesions in dsDNA exposed to high LET particles (HPLC-MS/MS)

Preliminary data : Interstrand crosslinks in dsOligo exposed to high intensity UV laser

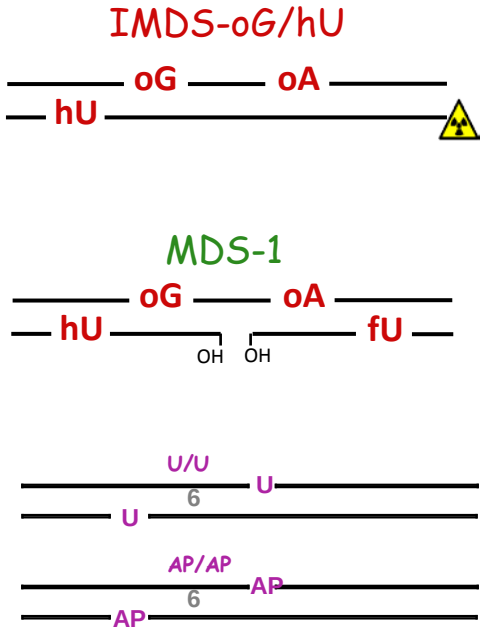
- Radical recombinaison ? (*D. Angelov, ENS, Lyon*)

Study of biological consequences of LMDS Contribution of chemistry !

Chemical Synthesis of oligonucleotides containing LMDS

- In vitro study with purified enzymes
- Insertion into plasmid and transfection into cells !

LAN (D Gasparutto) Collab E. Sage (Fr)
Group of P. O'Neill, M. Lomax (UK)



➡ Hierarchy of repair : reducing probability to generate DSB but increase mutagenicity

Interactome : Bounaix Morand du Puch**, Breton, J**. et al. (2011) Tools and strategies for DNA damage interactome analysis. *Mutat Res, **752, 72-83.***

DNA Repair Biochips : Millau, J.F **Sauvaigo, S**. et al. (2008) A microarray to measure repair of damaged plasmids by cell lysates. *Lab. Chip., **8, 1713-1722.***

Adaptable to LMDS !

Conclusion

Chemistry of radical decomposition in dsDNA is different to that observed for single nucleosides !

What about cellular DNA …

There is still a lot to do in « Radiation chemistry »

Or « Molecular Radiobiology »

Not limited to DNA (proteins and lipids)

Experimental difficulties (Theoritical studies)

« Low dose » is a nonsense for « Chemical studies »!