OXIDATIVELY GENERATED DAMAGE TO DNA: FROM MODEL STUDIES TO THE CELL

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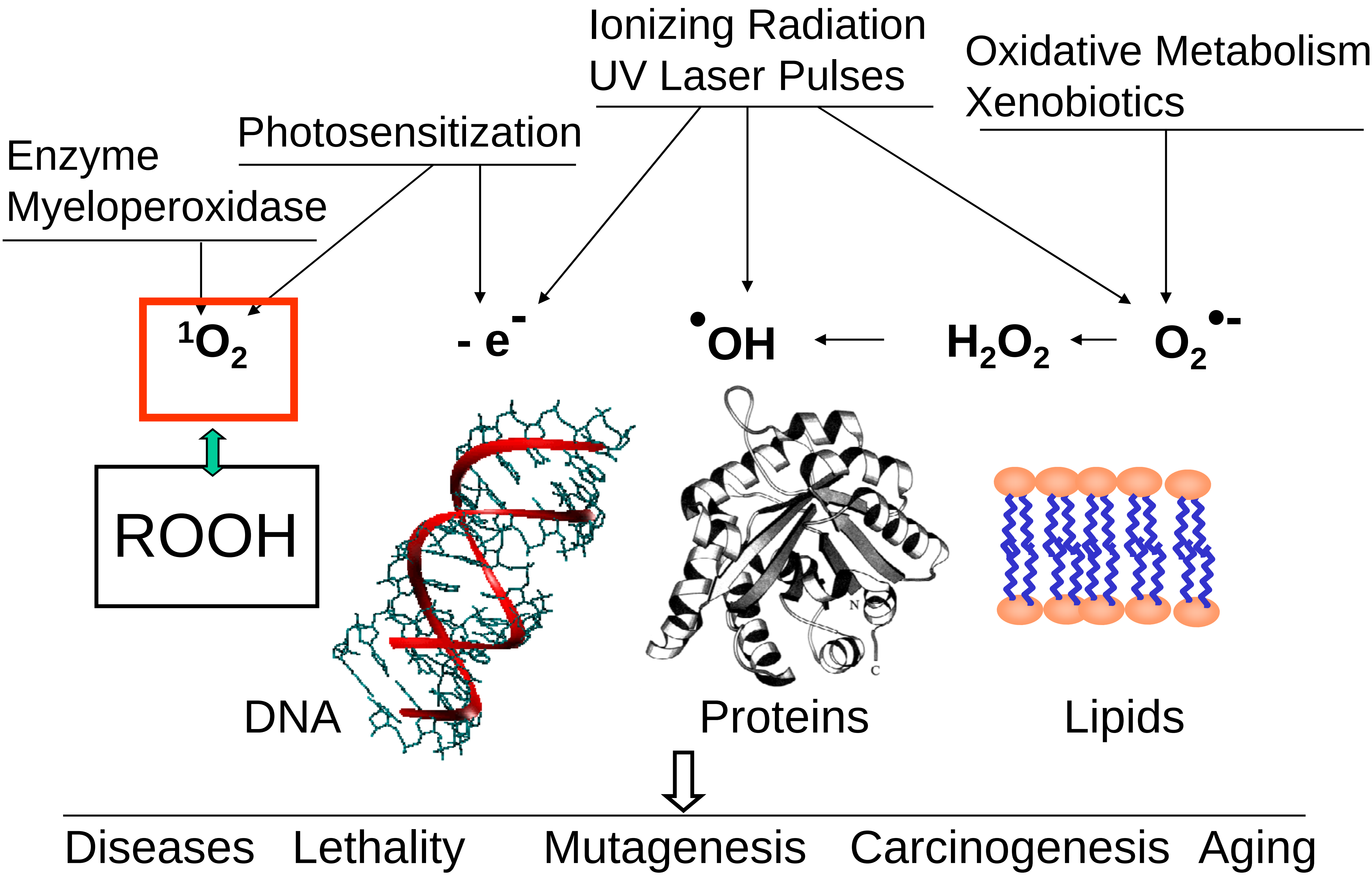


(Cadet & Di Mascio, Peroxides in Biological Systems, 2006)

OXIDATIVELY GENERATED DAMAGE TO DNA

- OLIGONUCLEOTIDE STRAND BREAKS
  (hydrogen abstraction at 2', 4' and 5' carbons)

- ABASIC SITES
  * *hydrolysis of the N-glycosidic bond (modified bases)*
  * *oxidation at C1‘ (2-deoxyribonolactone)*

- BASE LESIONS *(about 80 modifications identified in model studies)*

- COMPLEX LESIONS *(tandem and clustered damage)*

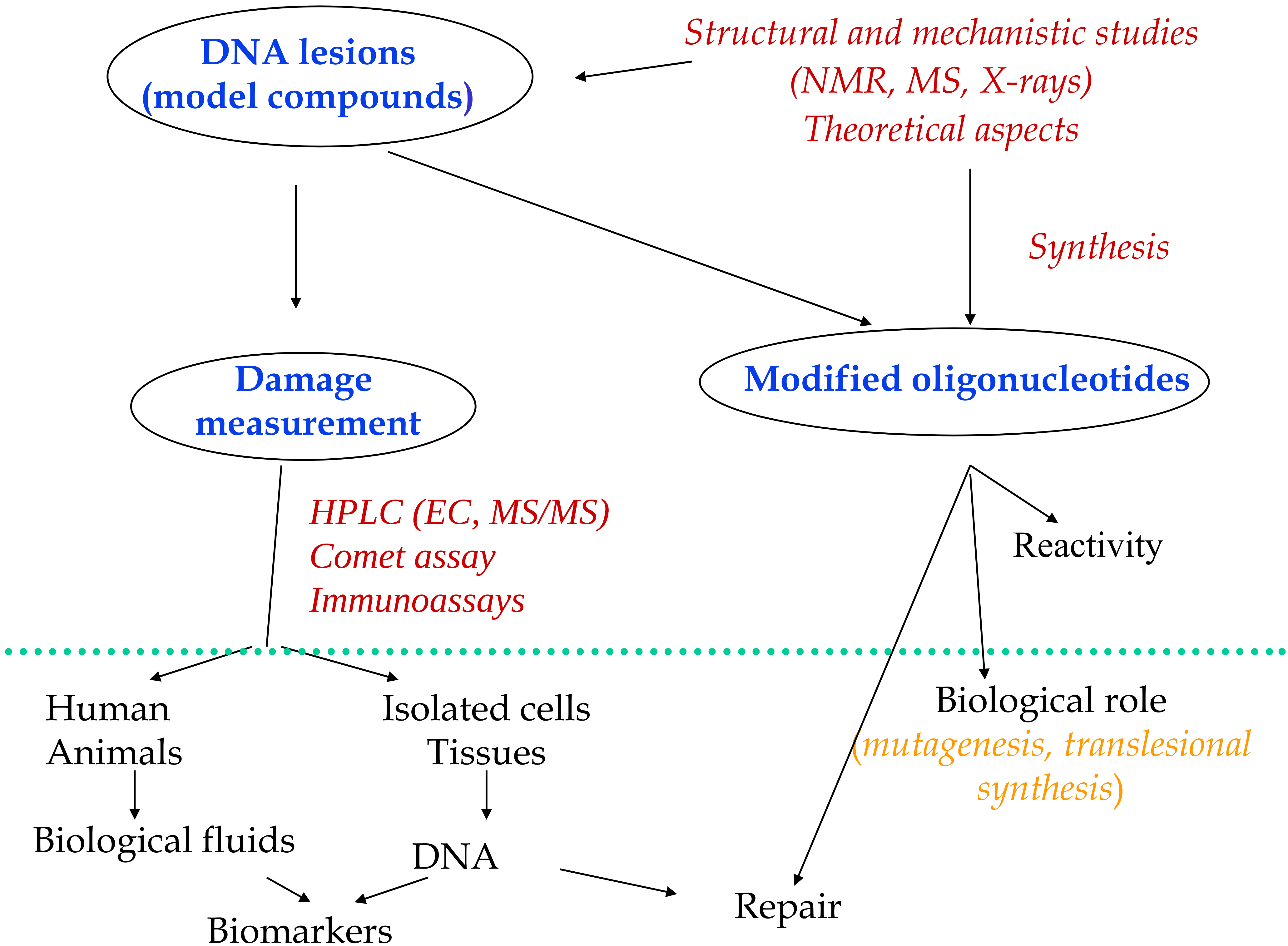
- DNA-PROTEIN CROSSLINKS

- ALDEHYDE ADDUCTS TO AMINOBASES
  (breakdown products of LOOH and oxidation products of 2-deoxyribose)

- ALKALI-LABILE SITES
  (abasic sites and a few oxidized bases including thymine glycols, 5-formymuracil, hydantoins …)

REACTIVE OXYGEN AND NITROGEN SPECIES
(reactivity)

- Superoxide (hydroperoxide) radical: *no detectable reactivity toward DNA*
- Hydrogen peroxide: *low reactivity with adenine and implication in Fenton reaction*
- ·OH radical: *reacts with all bases and the sugar moiety*
- Singlet oxygen ($^1O_2$): *[4+2]-cycloaddition to guanine*
- Ozone (O_3): *only reacts with pyrimidine bases*
- HOCl: *halogenation of purine and pyrimidine bases*
- Peroxynitrite ($ONOO^-$): *reacts with guanine (addition, oxidation)*
- One-electron oxidizing agents: *hydration & deprotonation of base radical cations*


DNA lesions
(model compounds)
Structural and mechanistic studies
(NMR, MS, X-rays)
Theoretical aspects
Synthesis
Damage
measurement
Modified oligonucleotides
HPLC (EC, MS/MS)
Comet assay
Immunoassays
Reactivity
Human
Animals
Isolated cells
Tissues
Biological role
(mutagenesis, translesional
synthesis)
Biological fluids
DNA
Biomarkers
Repair

Oxidatively generated damage to cellular DNA
(*outlines*)

- Single base modifications

  * singlet oxygen oxidation of guanine
  * damage induced by OH radical to the bases
  * one-electron oxidation of the bases

- Complex modifications (one radical oxidation hit)

  * DNA protein cross-links
  * Tandem base lesions

OXIDATIVELY GENERATED BASE DAMAGE TO CELLULAR DNA
(current situation)

* Isolated DNA and model compounds:

More than 80 lesions have been identified as oxidative degradation products of thymine, cytosine, adenine, guanine and 5-methylcytosine

* Cellular DNA: only 14 base lesions have been accurately measured:

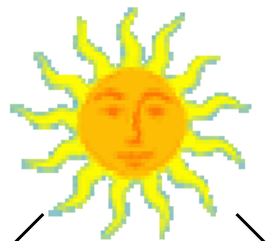
Adenine (2)

Guanine (2)

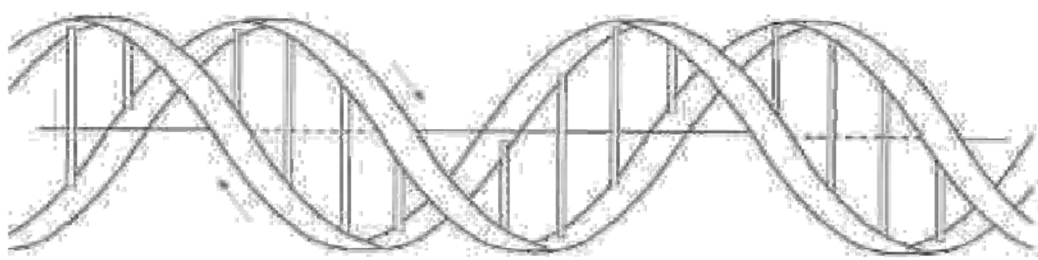
Thymine (6)

Cytosine (4)

Effects of solar radiation on cellular DNA



UVA (320-400 nm) **UVB** (290-320 nm)

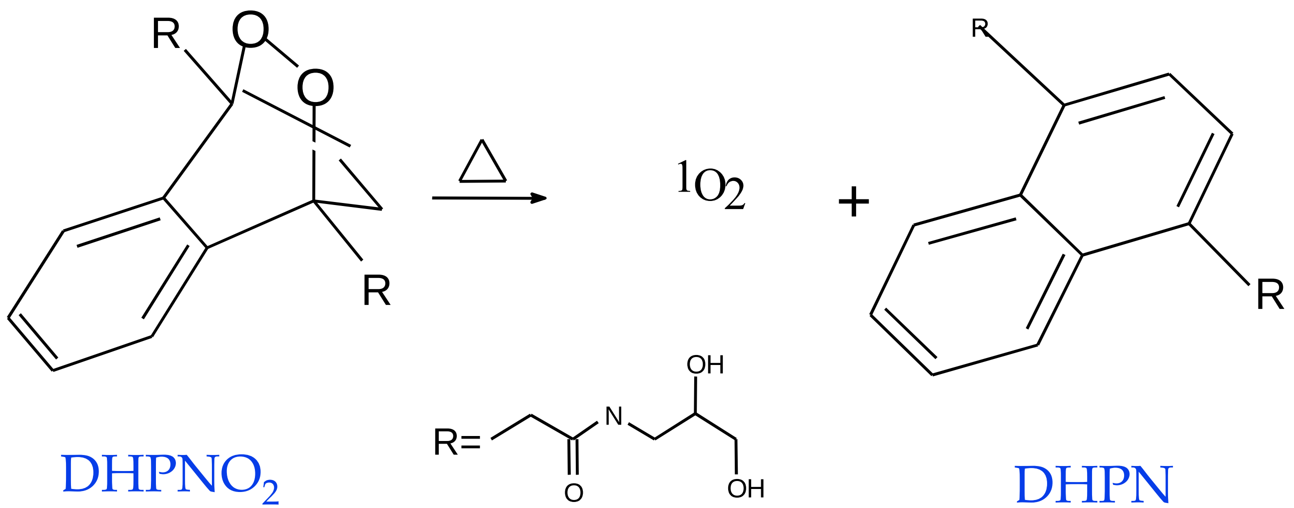


Indirect effect:
photosensitization
→base oxidation
→pyrimidine dimers

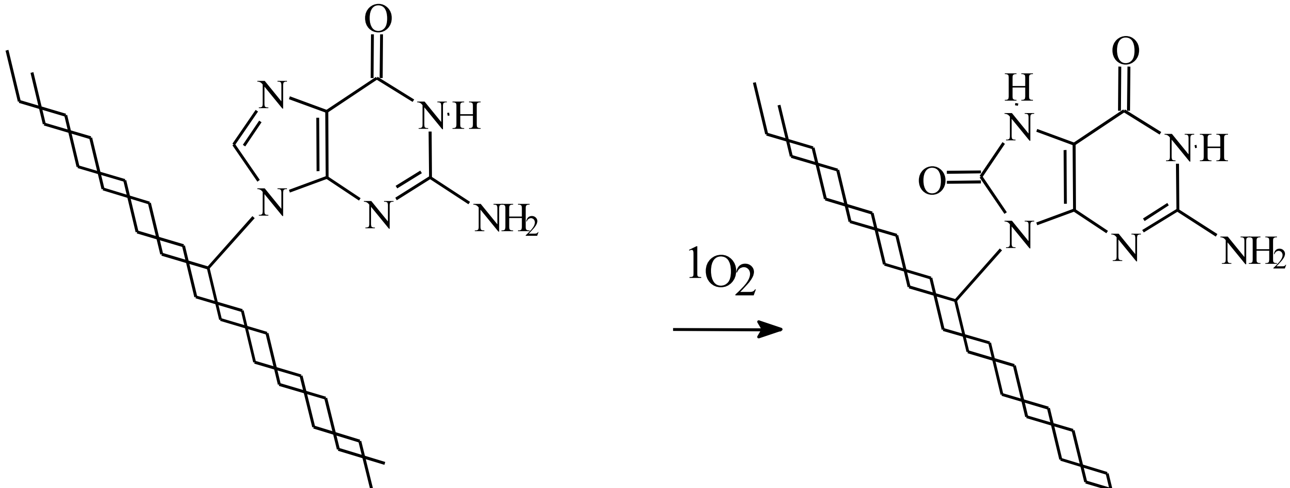
Direct effect:
dimeric pyrimidine
base photoproducts

(Cadet et al, Photochem. Photobiol. Sci 2009)

Singlet oxygen oxidation of cellular DNA

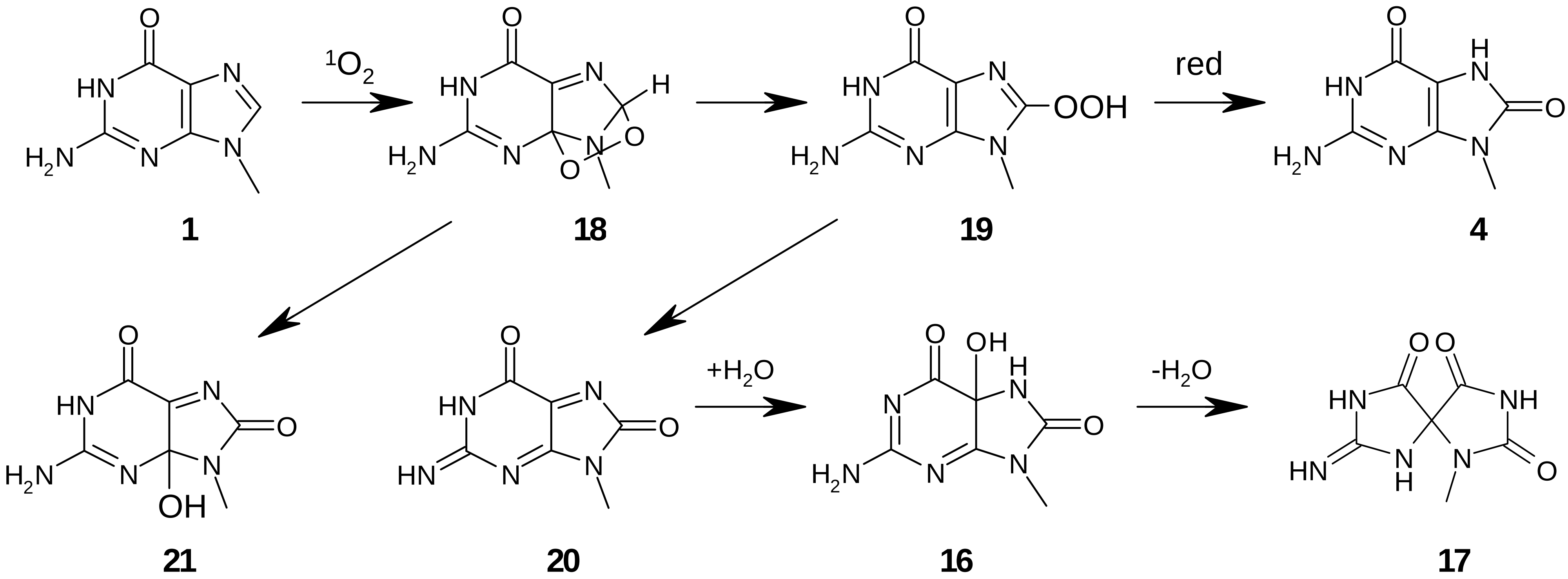


DHPNO$_2$ DHPN



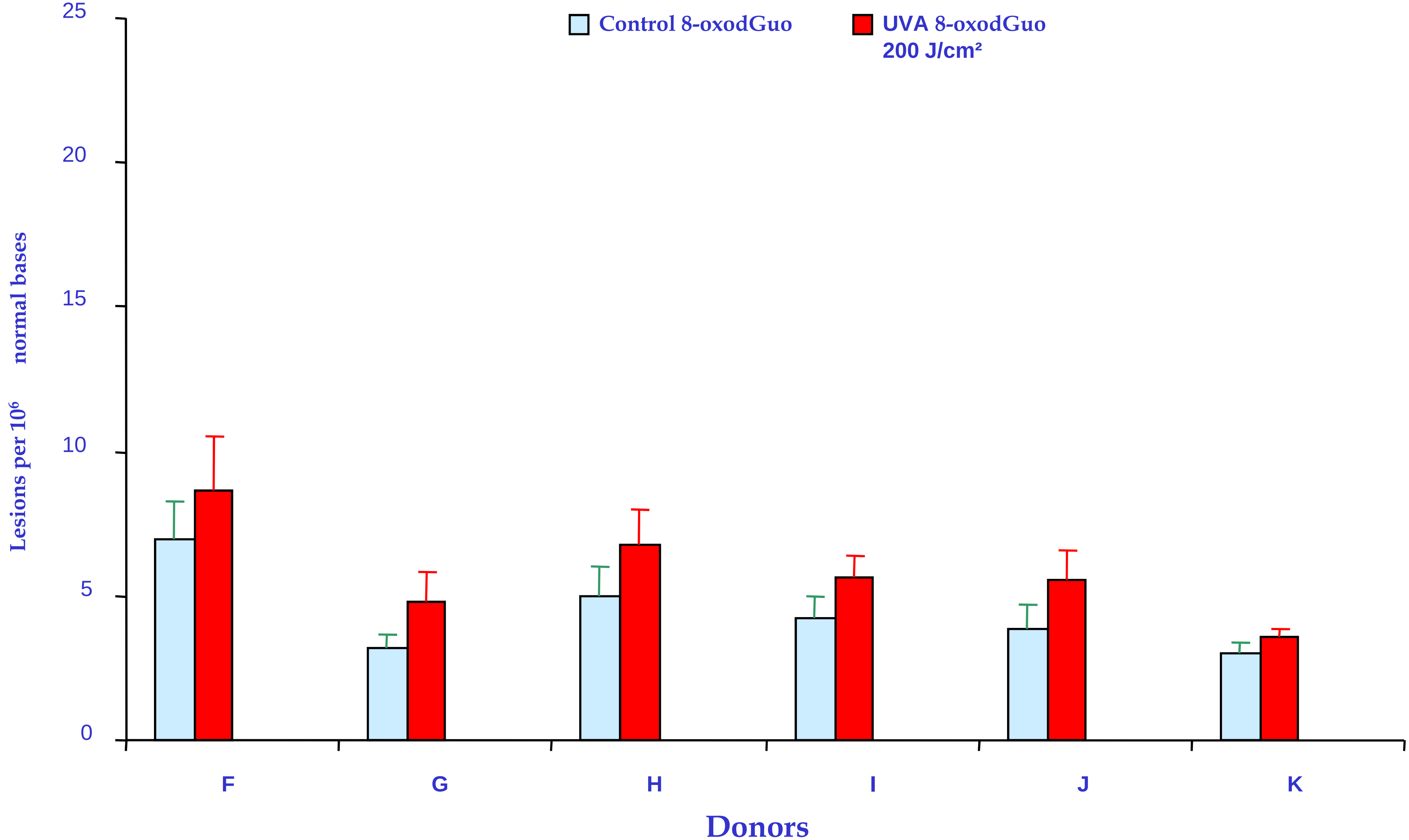
(Martinez et al, J. Am. Chem. Soc., 2000; Ravanat et al, J. Biol. Chem., 2000; Cadet et al, Photochem.Photobiol., 2006)

Singlet oxygen oxidation of guanine



(Ravanat *et al*, 2001; Niles *et al*, 2001; Martinez *et al*, 2002; Ye *et al*, 2003; McCallum *et al*, 2004: Cadet *et al*, 2008)

Formation of 8-oxodGuo in the DNA of human skin upon exposure to UVA radiation

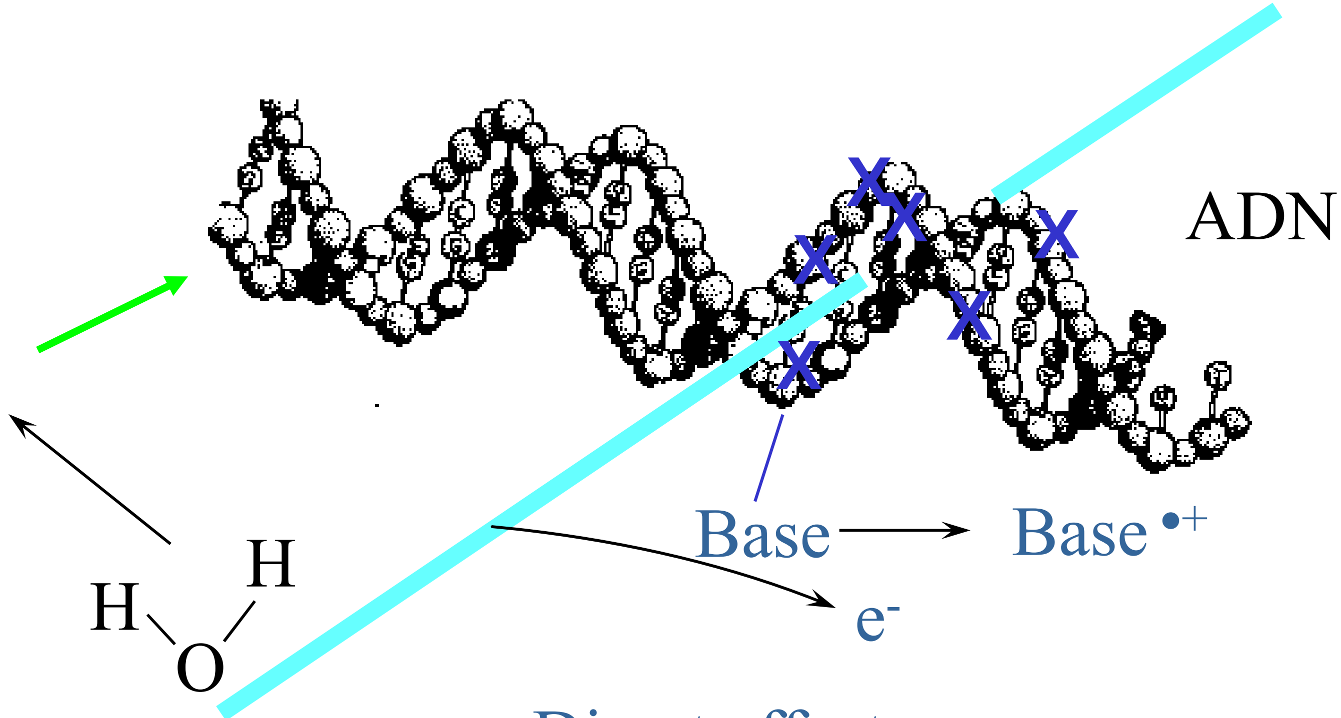


Mouret *et al*, *Proc. Natl. Acad. Sci. USA*, 103 (2006) 13765-70

Radiation-induced damage to DNA

Indirect effect:
(radiolysis of water)

e^-_{aq}, $H^\bullet$, **$HO^\bullet$**



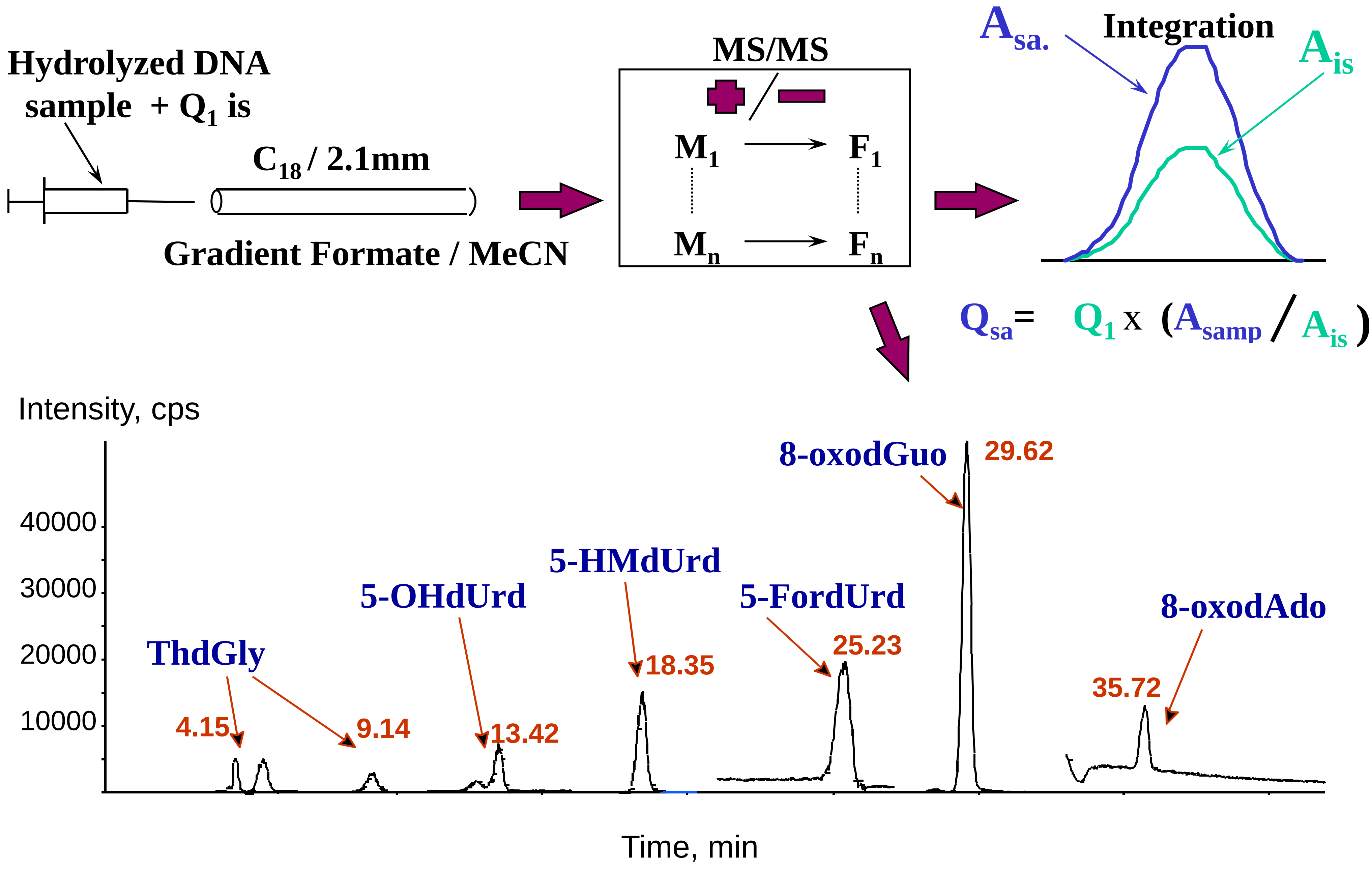
Direct effect:
(ionization of DNA bases)

Aims of the work:

- identification of the lesions (biomarkers)
- measurement of DNA damage in cells

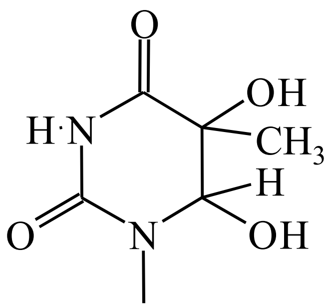
HPLCMS/MS of oxidized nucleosides

(separation - detection – quantitation)



Gamma ray-mediated formation of DNA damage in the DNA of of human monocytes

(lesions /10^9 DNA bases/ Gy)



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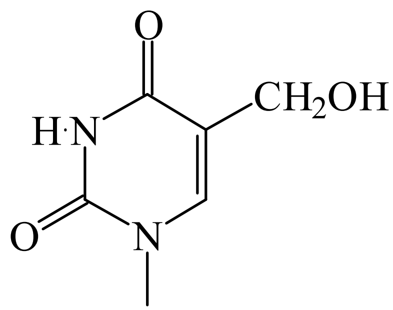

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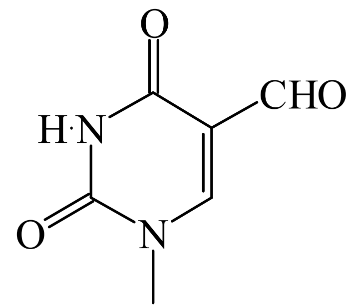

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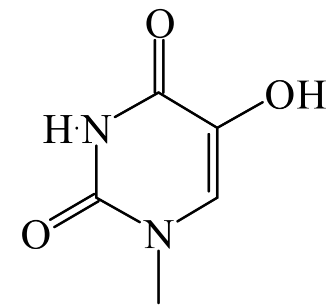
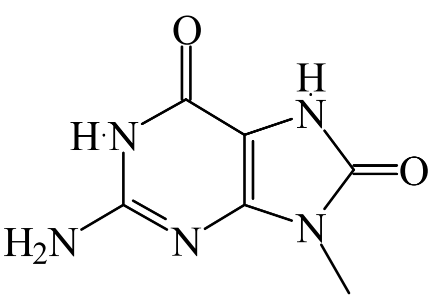


< 0.2



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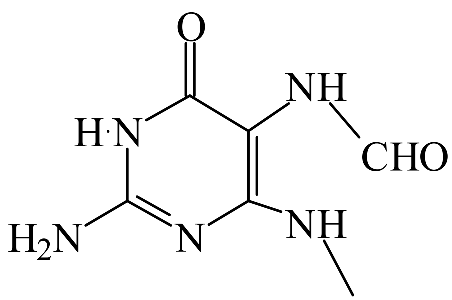

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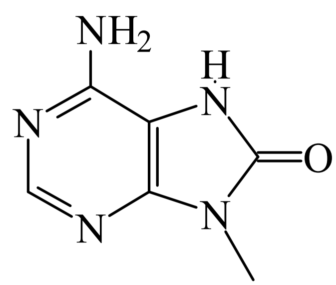
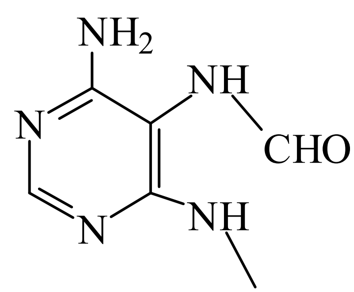


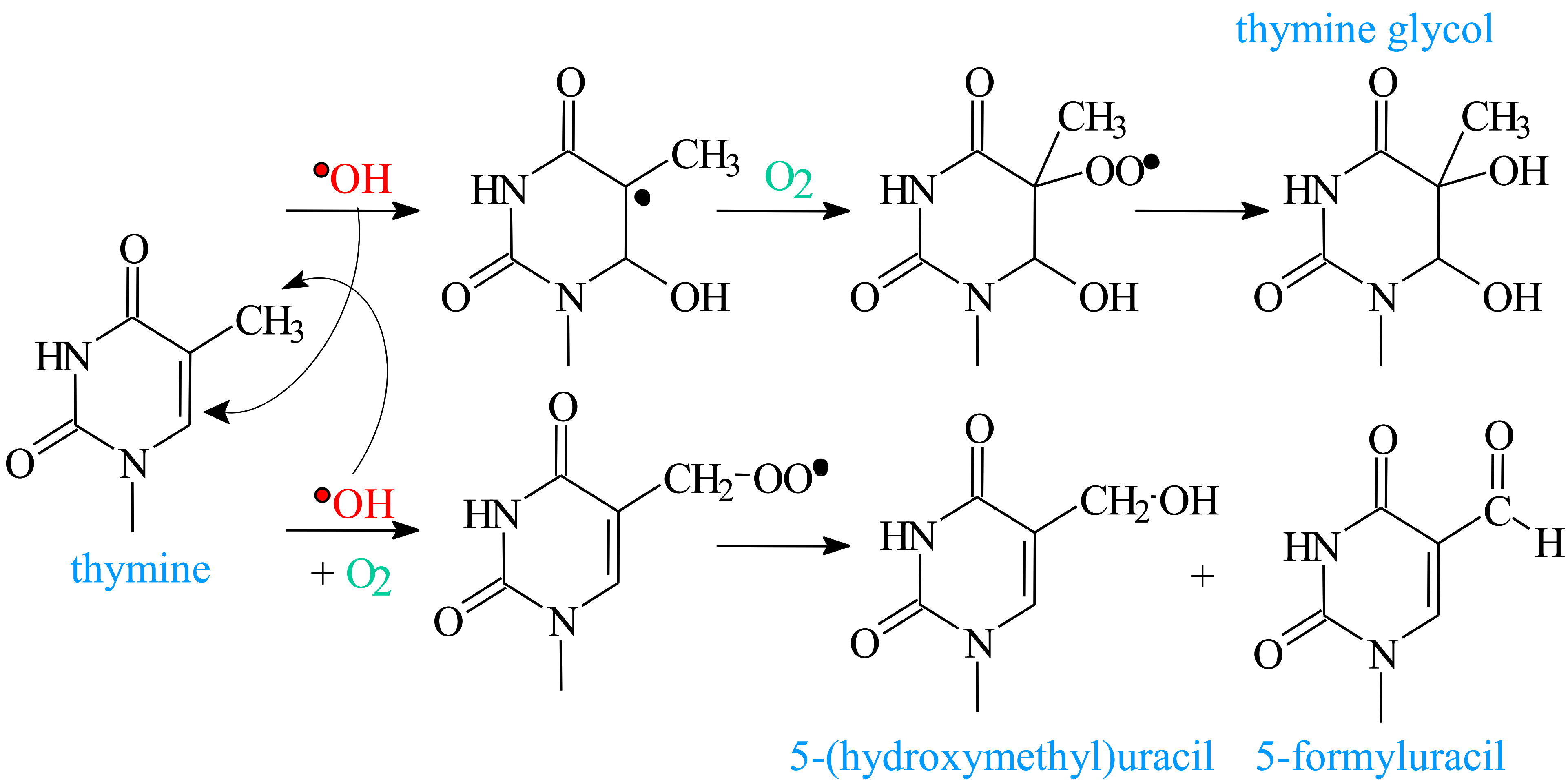
3.0



5.0

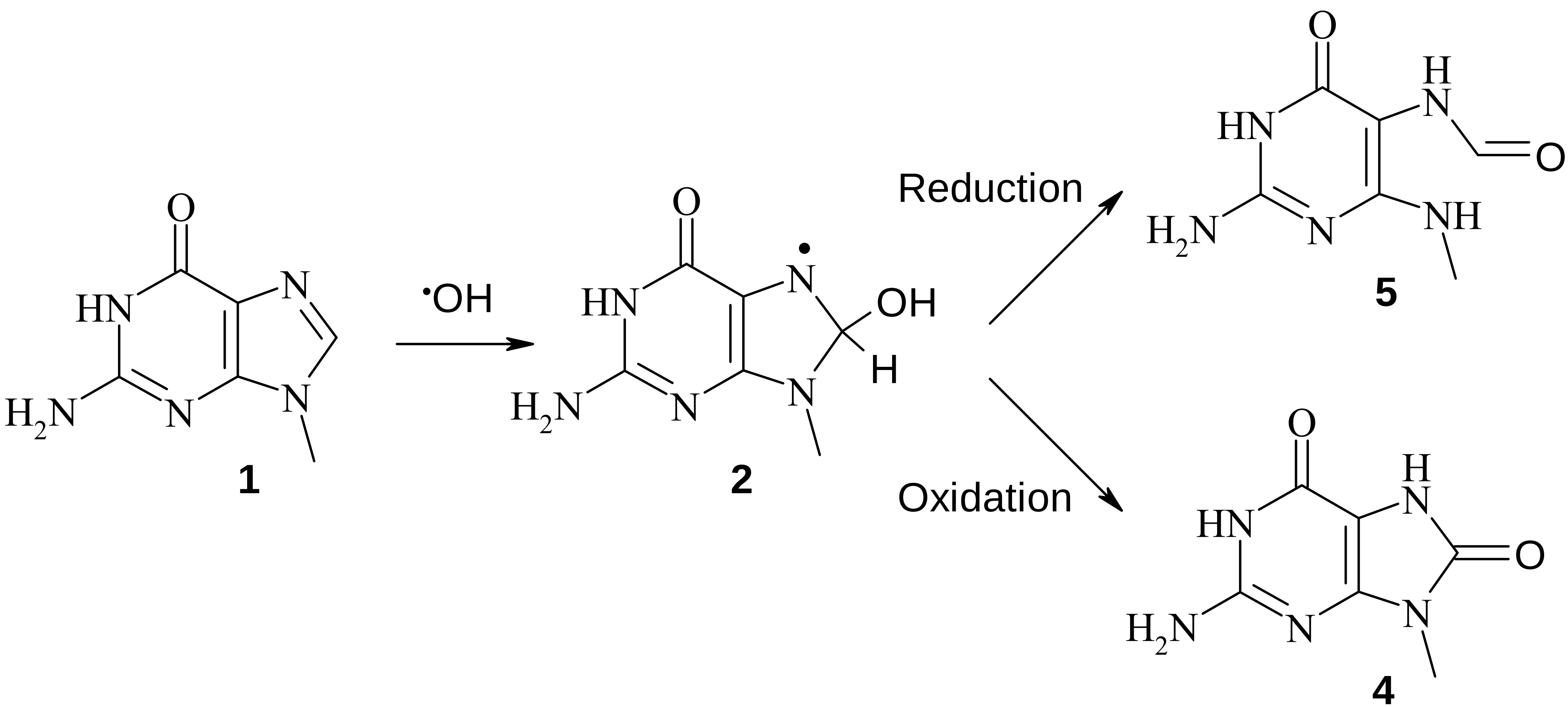
Measurement within the dose range: 0 - 100 Gy

MAIN OXIDATIVELY GENERATED THYMINE DAMAGE IN CELLULAR DNA



(Cadet et al, 2005, 2007; Douki et al, 2006, Douki & Cadet, 2007)

OH radical reactions of the guanine moiety at C8



(Cadet et al, Acc. Chem. Res. 41, 1075-1083, 2008)

One-electron oxidation of cellular DNA

- High intensity UV laser photolysis (266 nm)

  suitable way to generate purine and pyrimidine radical cations

- Specific oxidation of guanine:

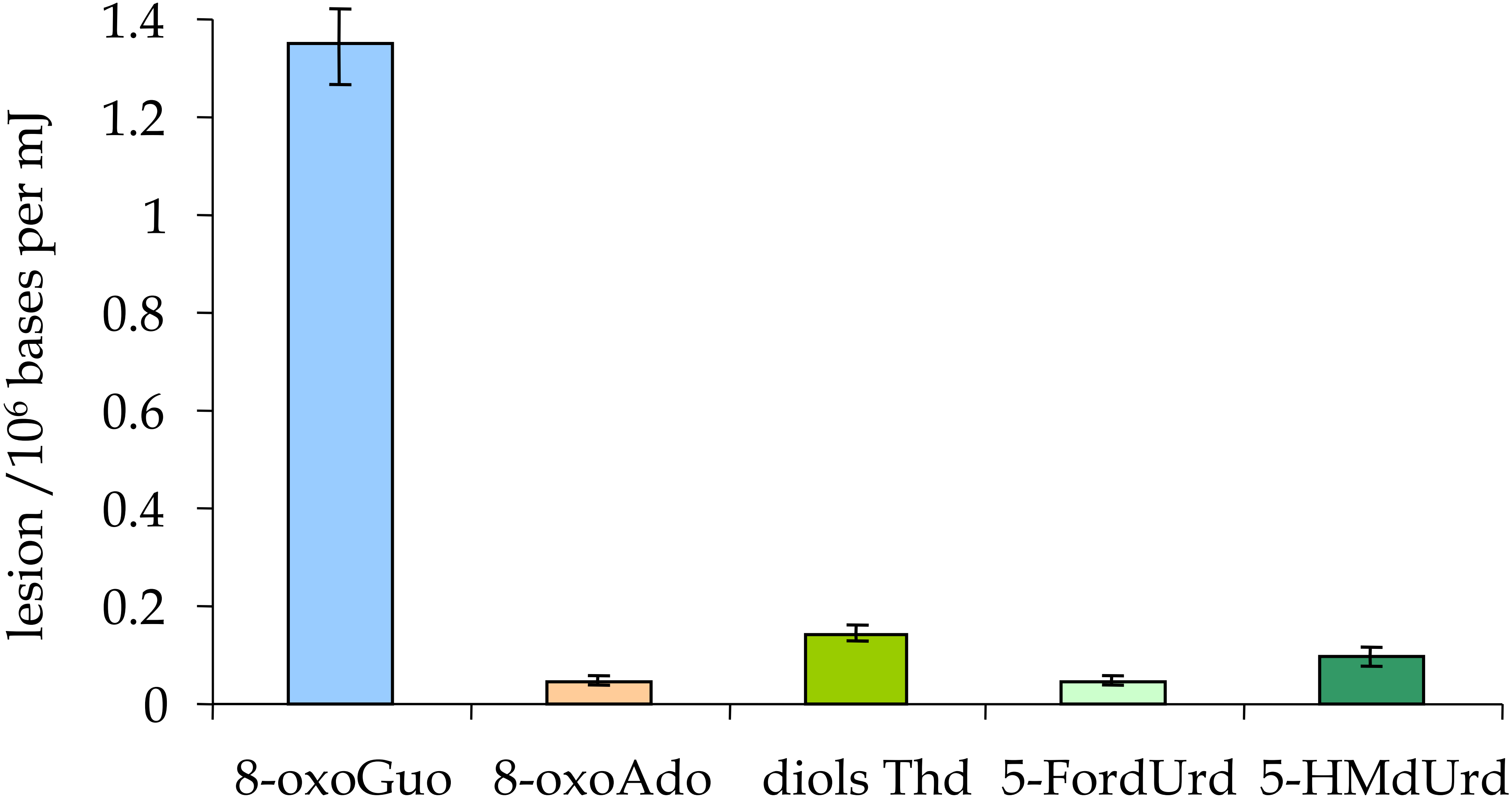
  predominant formation of 8-oxo-7,8-dihydroguanine

- Charge transfer reaction

  with guanine bases the likely sinks of hole transfer

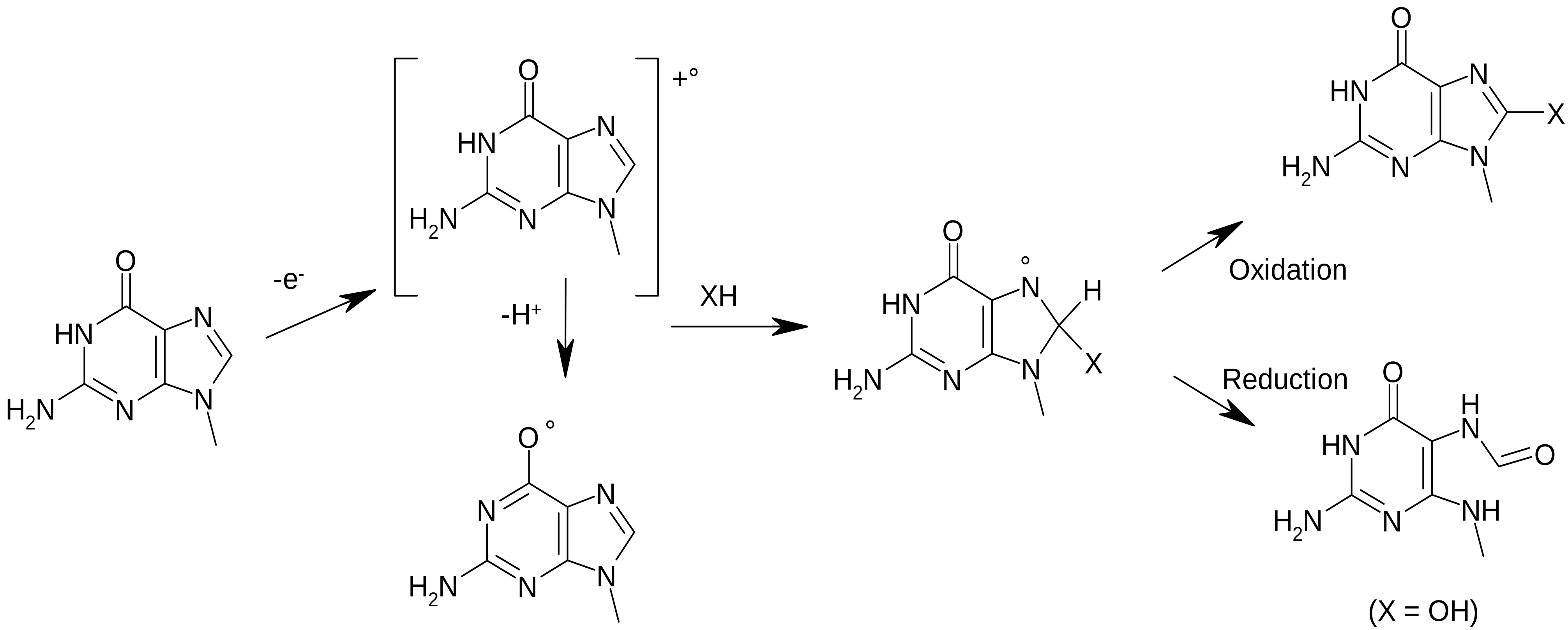
(Angelov *et al*, *JACS* 1997, Douki *et al* , *Topics Curr Chem*, 2004), Cadet et al, in press)

Oxidized nucleosides induced in cellular DNA upon exposure to high intensity 266 nm laser pulses (biphotonic ionization)



(Douki *et al*, *Int J Radiat Biol*, 2006)

Nucleophilic reactions of the guanine radical cation



XH = H_2O Kasai et al, JACS 1992

XH = $_2$HN-R (lysine) Perrier et al, JACS 2006

Cytosine (NH_2): DNA interstrand cross-link *(cooperation with D.A. Angelov)*

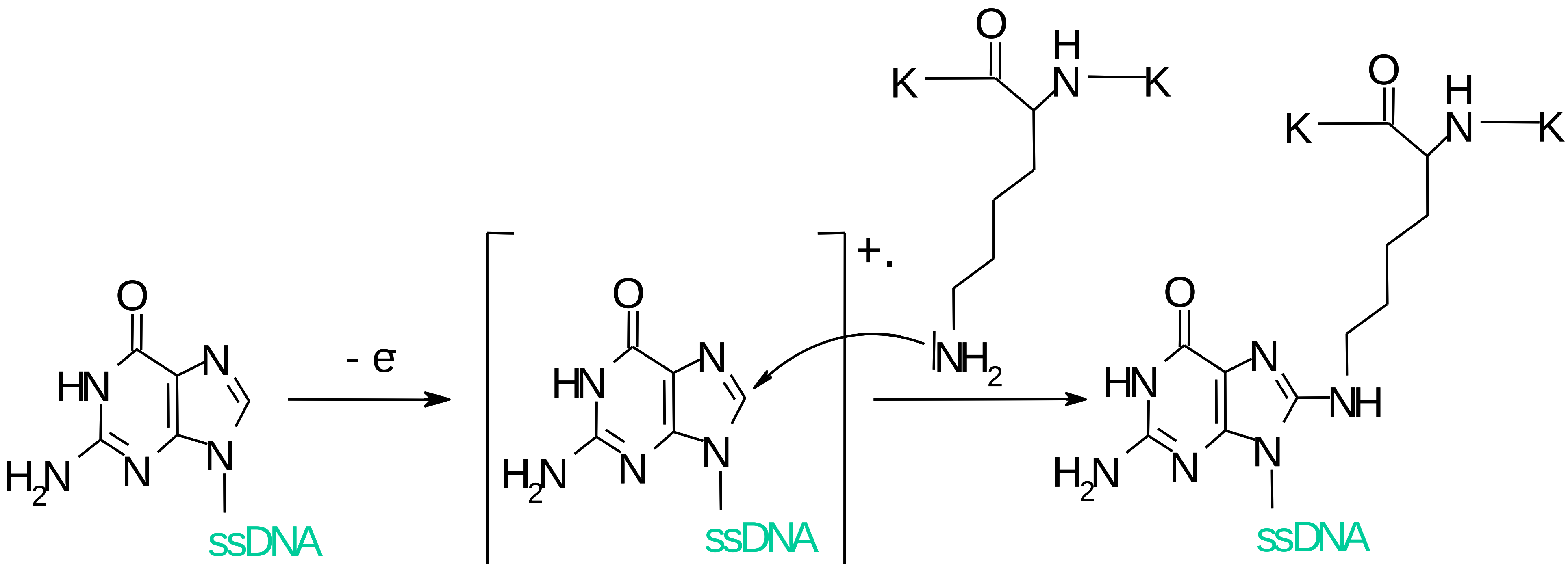
OXIDATIVELY GENERATED DAMAGE TO DNA
(clustered modifications)

- DNA-protein crosslinks

- Tandem lesions
    - Intranucleoside (sugar-base)
    - Vicinal bases

Formation of a guanine-lysine cross-link

(one-electron oxidation reaction)

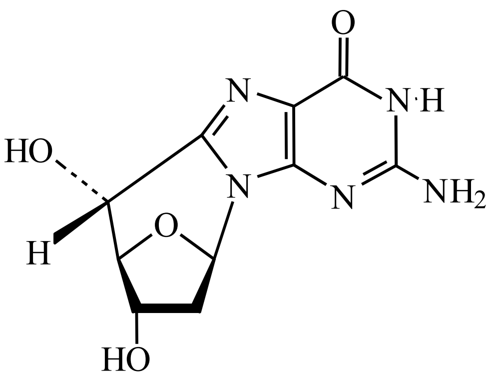


TGT **A** **B**

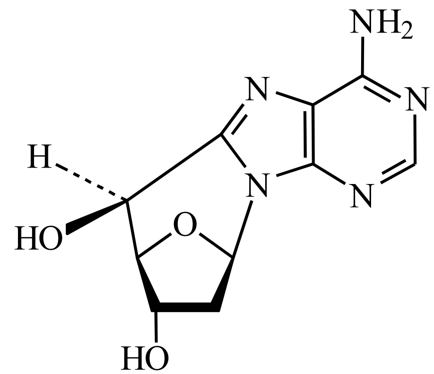
Nucleophilic addition of a free amino group at C8 of the guanine radical cation

(Perrier et al, JACS, 2006)

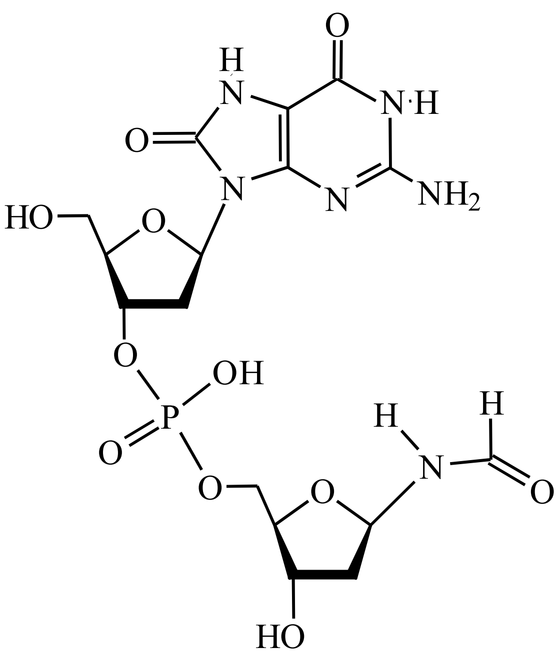
Oxidatively generated tandem DNA damage
(one initial radical hit)



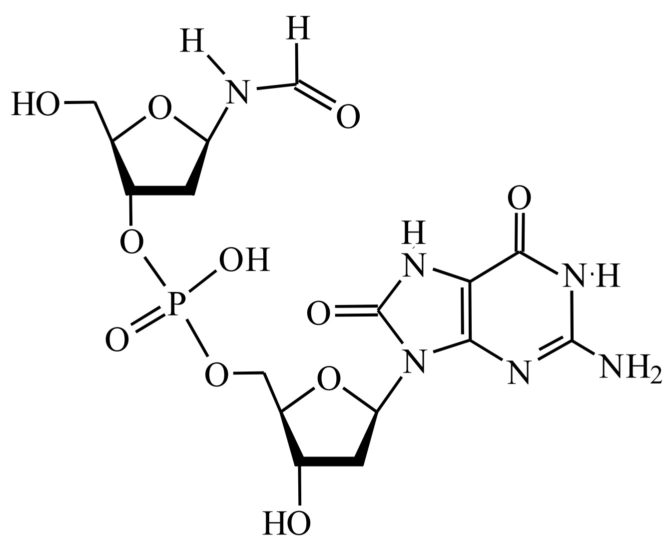
(5′*R*)-5′,8-cyclodGuo



(5′*S*)-5',8-cyclodAdo

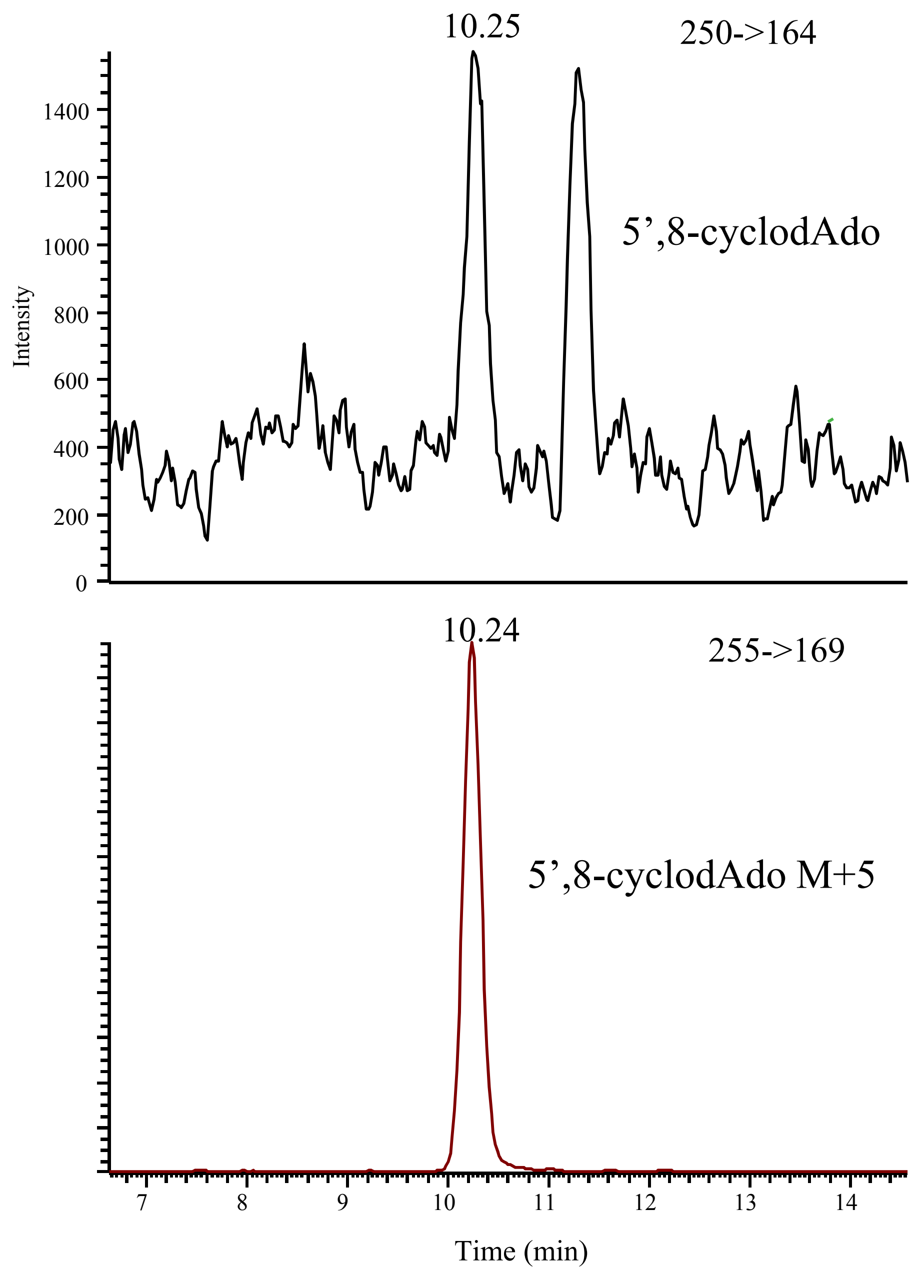


8-oxodGuo/Fo



Fo/8-oxodGuo

HPLC-MS/MS detection of (5’*R*)-5’,8-cyclodAdo in the DNA of γ-irradiated cells



- The cells were exposed to 2 kGy
- The yield of formation is 0.2 (5’*R*) 5,8-cyclodAdo/10^9nucleosides/Gy
- The yield of formation of 8-oxodGuo is 20 8-oxodG/10^9nucleosides/Gy
- The background level of (5’*R*)-5’,8-cyclo dAdo lower than the limit of detection, i. e. 0.05 lesions per 10^9 nucleosides.

Radiation-induced formation of (5'*S*)-5',8-cyclo-2'-deoxyadenosine in cellular DNA

- The role of CSA in the response to oxidative DNA damage in human cells. M D'Errico et al. *Oncogene* (2007) 26, 4336–4343

Human keratinocytes KN1RO exposed to X-rays (5 Gy)

Measurement of (5'*S*)-cyclodAdo by HPLC-MS

Rate of formation of (5'*S*)-cyclodAdo : 20 lesions per 10^9 normal nucleosides and per Gy

- Radiation-induced formation of purine cyclonucleosides in DNA: product distribution and inhibiting effects of oxygen. N Belmadoui, F BoussicaultT, JL Ravanat, C Chatgilialoglu and J Cadet (in preparation)

Human monocytes exposed to gamma-rays (2 kGy)

Measurement of (5'*R*)-cyclodAdo by HPLC-MS /MS

Rate of formation of (5'*R*)-cyclodAdo : 0.2 lesions per 10^9 normal nucleosides and per Gy

Shielding effect against damage in nuclear DNA with respect to naked DNA: at least 3 orders of magnitude

The radiation-induced formation of purine 5',8-cyclonucleosides is, at least, 2 orders of magnitude lower than than reported previously!

OXIDATIVELY GENERATED DAMAGE TO CELLULAR DNA
(conclusions)

- *The steady-state level of the main oxidized bases is within the range of 1 lesion per 10^6 - 10^7 normal bases whereas tandem lesions are generated at best with a much lower efficiency than single lesions.*

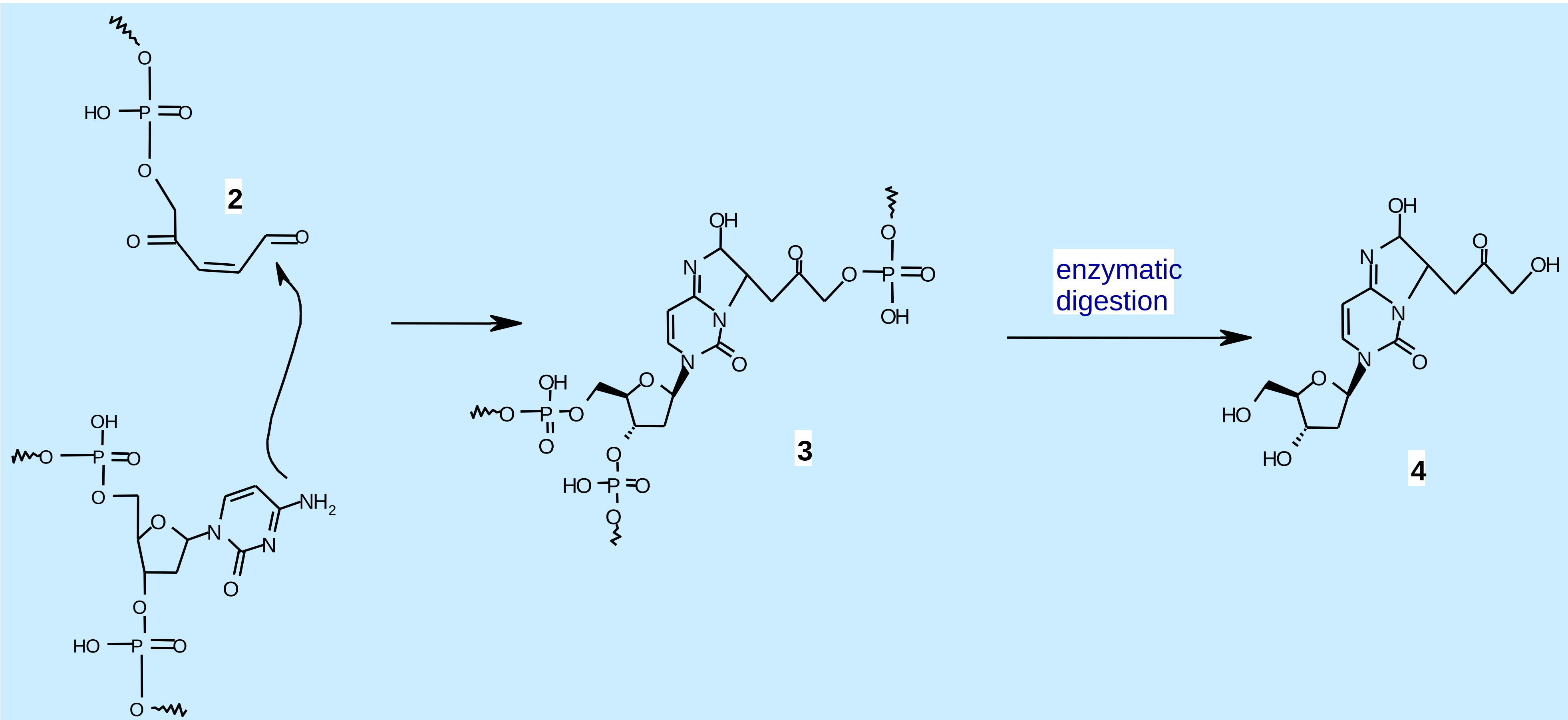
- *HPLC-MS/MS and HPLC-ECD are operative for measuring acute effects of strong oxidizing agents and for level of DNA > 30 µg .*

- *Enzymic assays are appropriate for low amounts of DNA and to deal with slights variations in the level of oxidized bases (typically chronic exposure, antioxidants studies).*

- *Still a paucity of information on several lesions (secondary oxidation products, tandem modifications, DNA-protein cross-links)*

- *Aldehyde-aminobase adducts (oxidation of the 2-deoxyribose moiety)*

Reactions mediated by H atom abstraction at C4 of 2-deoxyribose within cellular DNA



(Regulus *et al*, *Proc. Natl. Acad. Sci. USA*, 2007)