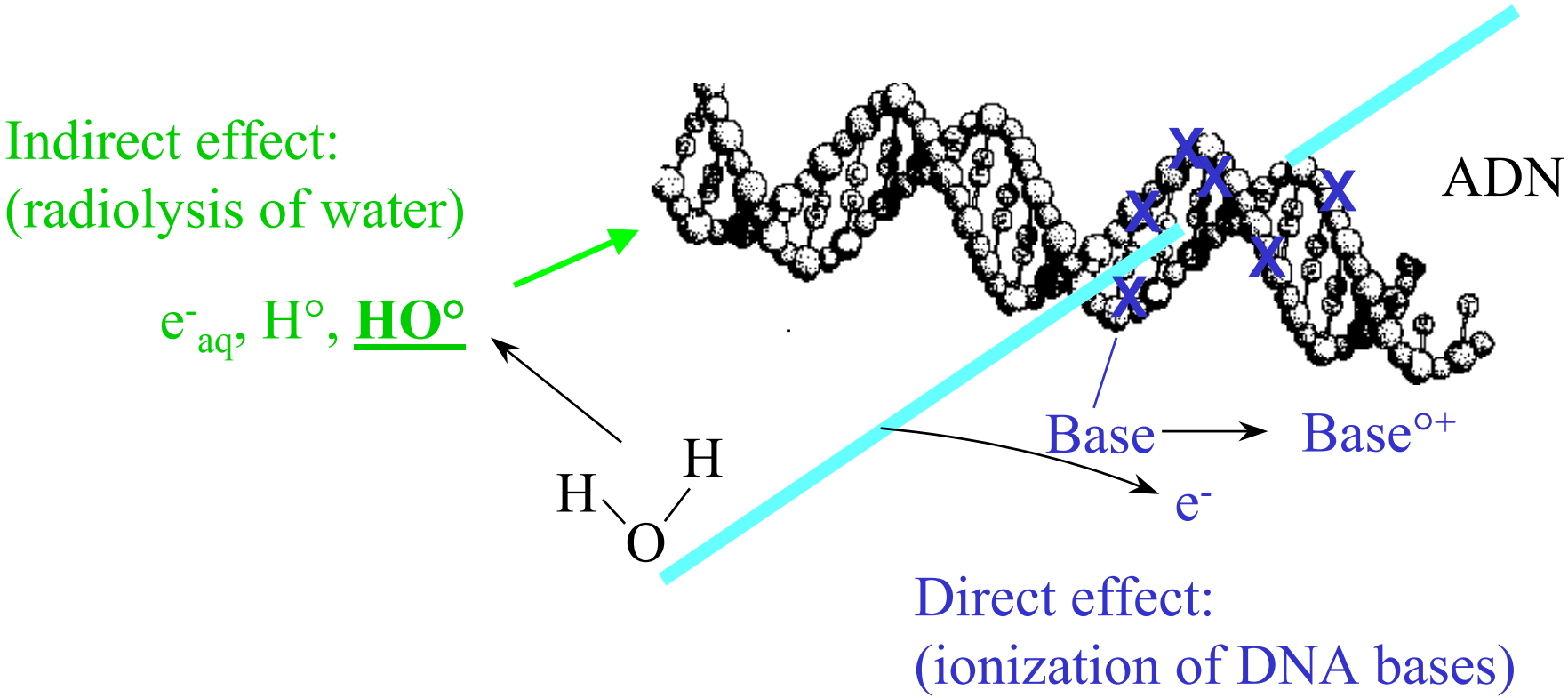
Laboratoire des Lésions des Acides Nucléiques

Direction des Sciences de la Matière
DRFMC/SCIB, CEA-Grenoble



Aims of the work:

- identification of the lesions
- quantification of their formation in cells
- determination of their biological properties

OXIDATIVE DAMAGE TO DNA

PHOTOSENSITIZATION

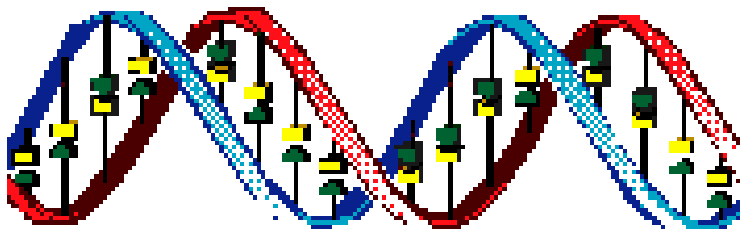
IONIZING RADIATION

UV LASER PULSES

OXIDATIVE METABOLISM

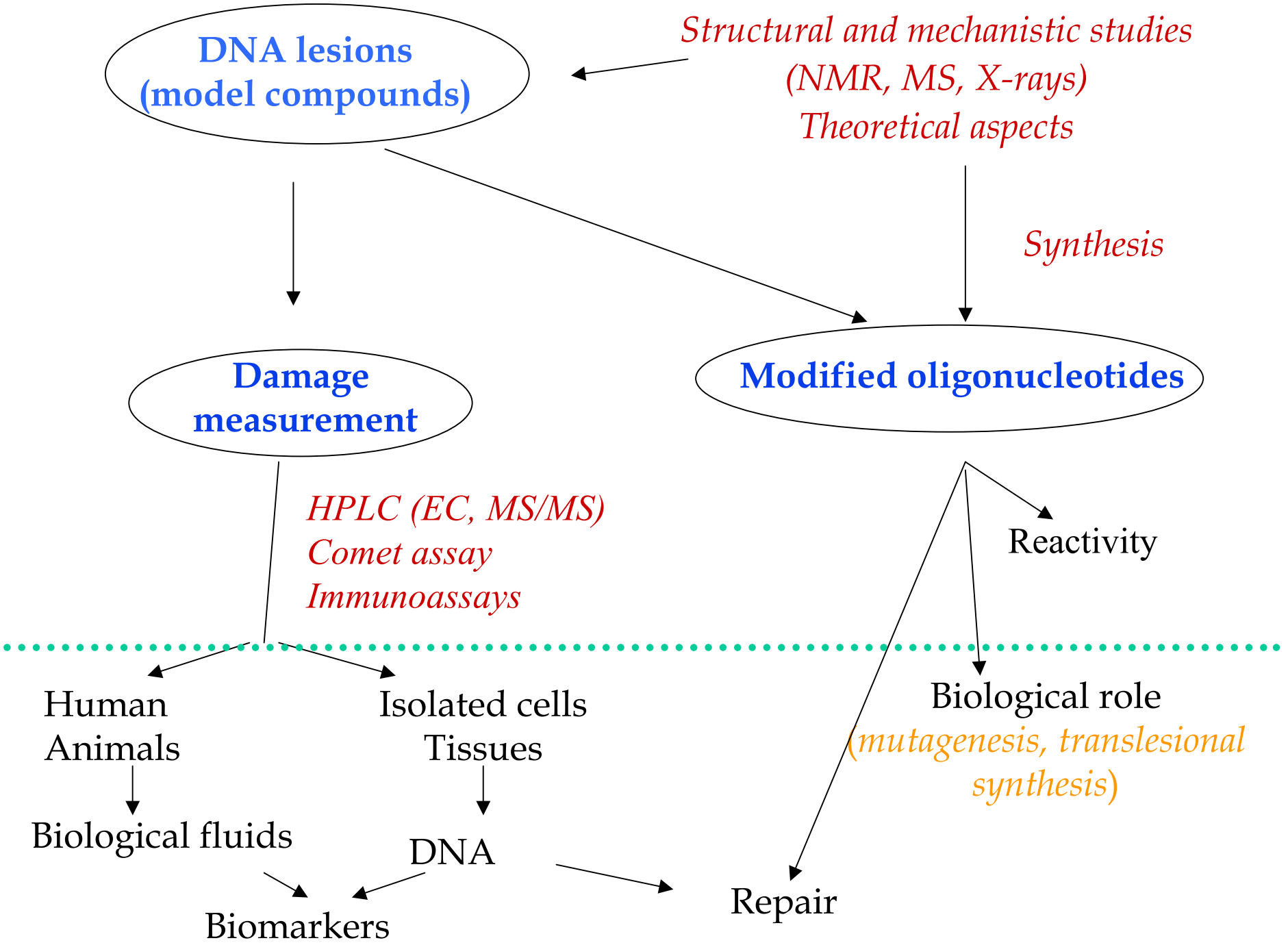
XENOBIOTICS

$^1O_2$ $- e^-$ $\cdot OH$ $\leftarrow$ H_2O_2 $\leftarrow$ $O_2^{-\cdot}$



- Modified bases
- Abasic sites
- Single & double strand breaks
- DNA-protein cross-links
- Aldehyde adducts to aminobases

LETHALITY MUTAGENESIS CARCINOGENESIS AGING


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

MODEL SYSTEMS

•identification of over **<u>70 radiation-induced base damage</u>**
•several major mechanistic pathways established
•correlations with theoretical approaches

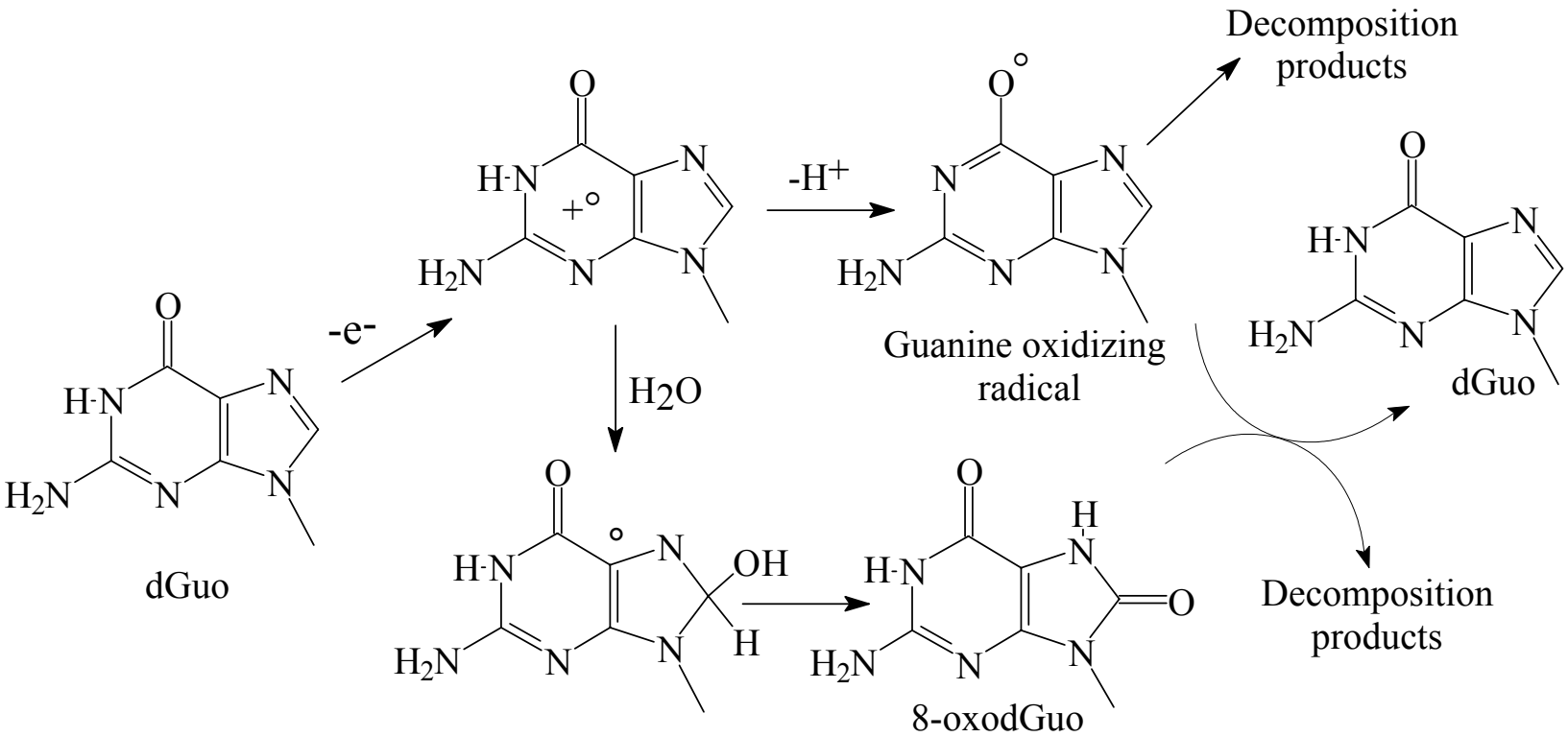
MEASUREMENT OF DAMAGE IN CELLULAR DNA (chemistry in the cells)

•twelve modified bases singled out by chromatographic assays
•damage quantified in cells exposed to low doses (0.2 Gy) using biochemical assays

DETERMINATION OF BIOLOGICAL FEATURES

•synthesis of 20 site-specifically modified oligonucleotides (*mostly base damage*)
•substrate specificity of repair enzymes
• mutagenic properties of lesions (*shuttle vector methods, polymerases*)

Radical oxidation reactions of isolated 2'-deoxyguanosine



Yields of formation of 11 base modifications in the DNA of monocytes upon exposure to ^{60}Co γ-rays (LET: 0.2 keV/μm) and $^{12}C^{6+}$ ions (LET: 25 keV/μm)

DNA base modifications *(per 10^9 bases and per Gy)*	γ-rays	$^{12}C^{6+}$
5,6-Dihydroxy-5,6-dihydrothymidine *(4 diastereomers)*	97	62
5-(Hydroxymethyl)-2'-deoxyuridine	29	12
5-Formyl-2'-deoxyuridine	22	11
5-Hydroxy-2'-deoxyuridine	< 0.2	< 0.2
8-Oxo-7,8-dihydro-2'-deoxyadenosine	3	3
Adenine formamidopyrimidine	5	1
8-Oxo-7,8-dihydro-2'-deoxyguanosine	20	10
Guanine formamidopyrimidine	39	22

Estimated damage in a mammalian cell nucleus for 1 Gy of low-LET radiation

- *Initial physical damage*

Ionizations in cell nucleus	~ 100 000
Ionizations directly in DNA	~ 2 000
Excitations directly in DNA	~ 2 000

Selected biochemical damage (Ward 1988)

DNA strand breaks DNA	1 000
8-Hydroxyadenine	700
Diol de thymine	200
DNA double-strand breaks	40
DNA-proteins crosslinks	150

Selected cellular effect

Lethal events	~ 0.2 - 0.8

D.T. Goodhead, IJRB, 65, 7-17 1994

RADIATON DAMAGE TO DNA

(future work)

- Complex damage

- Effects of electrons of low energy

- DNA-protein crosslinks

- DNA-aldehyde adducts (LOOH)

- Bystander effects