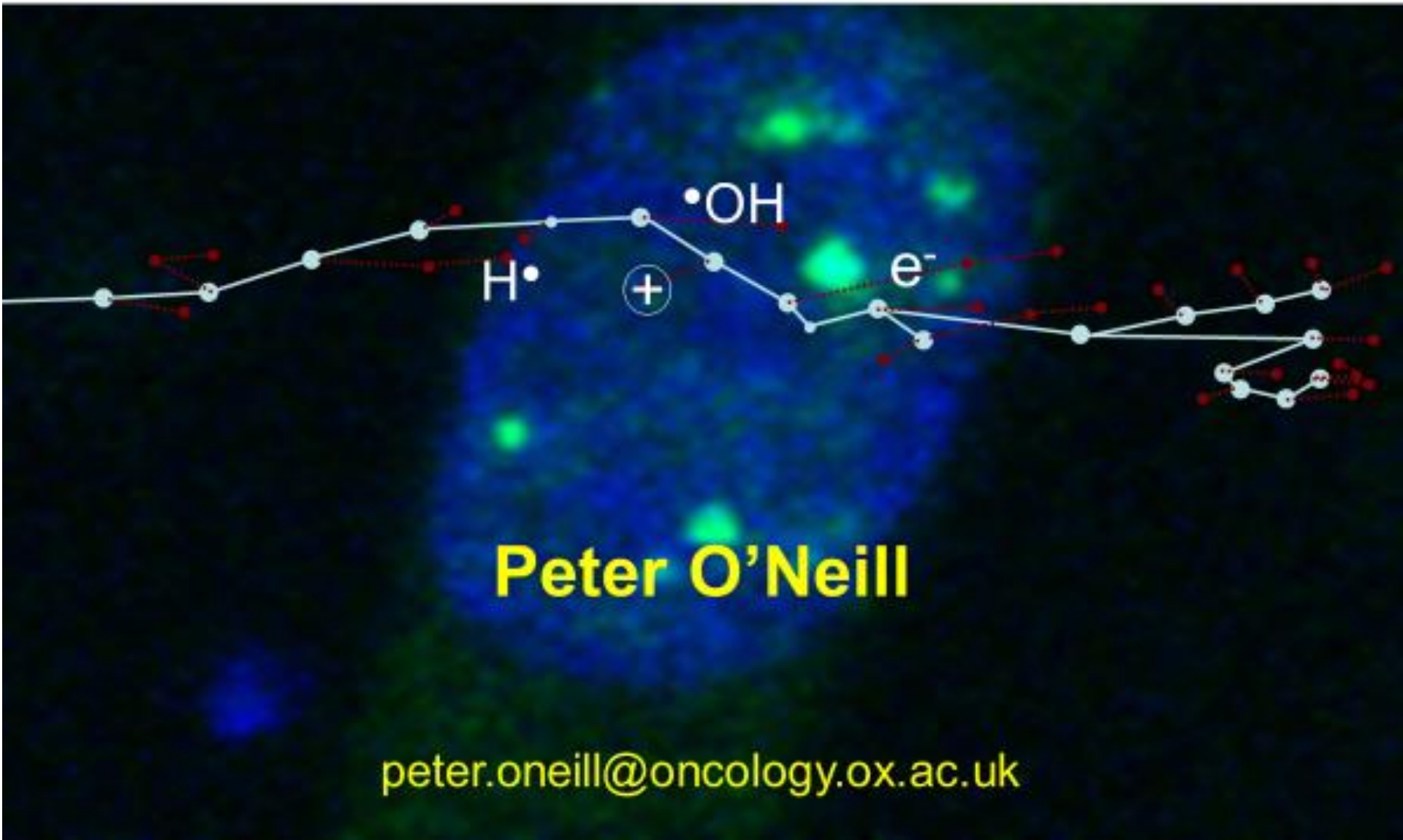
,Radiation Chemistry and DNA Damage - Peter O'Neill
https://three.jsc.nasa.gov/articles/NASA%20chemistry%202021%20THREE%20revised%20v2.pdf. Date posted: 10/05/2021.

Radiation Chemistry and DNA Damage/repair



Learning Outcomes:

- The timescales of initial interactions of water radicals, induced in the radiation track, and during expansion of the radiation track, until the remaining radical species become homogeneously distributed (at times >0.1μs after their initial formation).

- The consequences of early chemical events during track expansion involving non-homogeneous interactions and the subsequent homogeneous interactions at later times (>0.1 μs).

- DNA damage induced by direct effects or indirect effects involving with water radicals

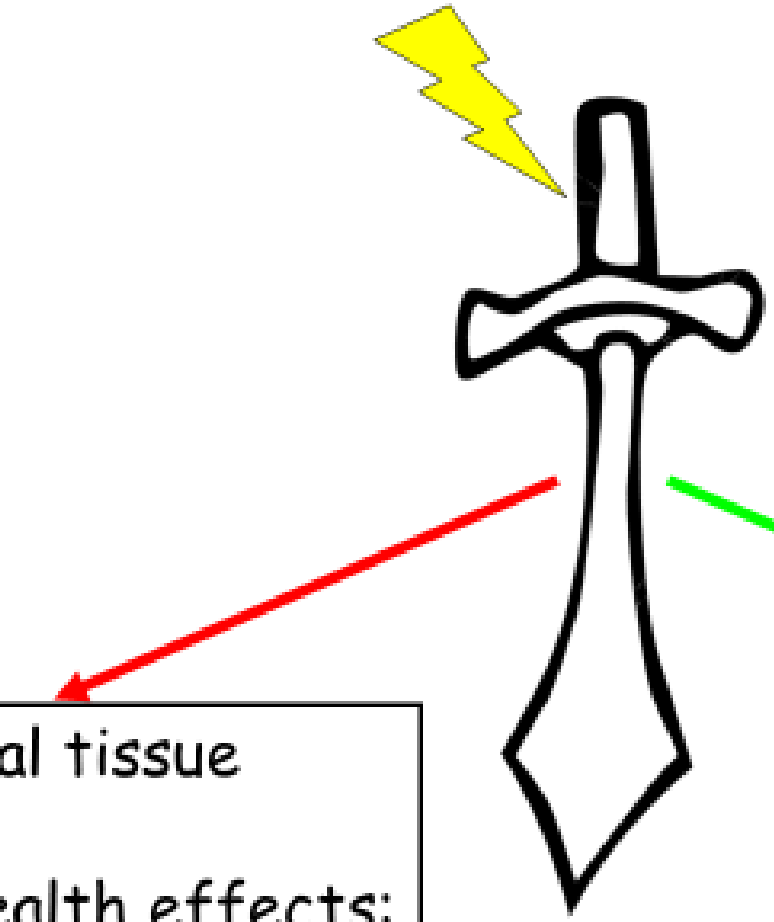
- Understand how the different types of damage are formed in cellular DNA by ionising radiation of different LET.

- Spatial distribution of reactants, particularly when comparing the **SPATIAL DISTRIBUTION** of radiation-induced DNA damage, particularly clustered DNA damage, with DNA damage induced endogenously. Subsequent consequences for damage repair.

- Understand the significance of clustered DNA damage induced by ionising radiation relative to endogenously induced DNA damage.

- Describe the effect of oxygen on enhancing radiosensitivity of ionising radiation for low LET radiation.

Ionising radiation is like a two-edged sword

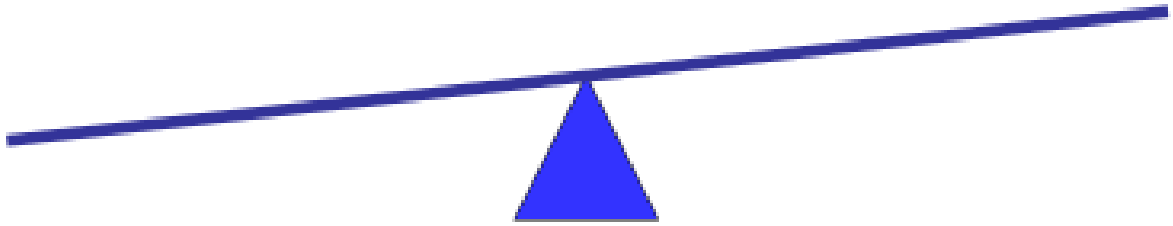


Normal tissue

Adverse health effects:
e.g. 2nd cancer

Tumour tissue

Cellular inactivation:
e.g. cancer cure

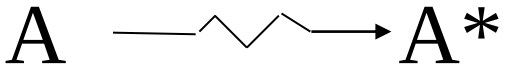


Initial primary events initiated by ionising radiation

Primary events initiated through direct energy deposition in target molecules

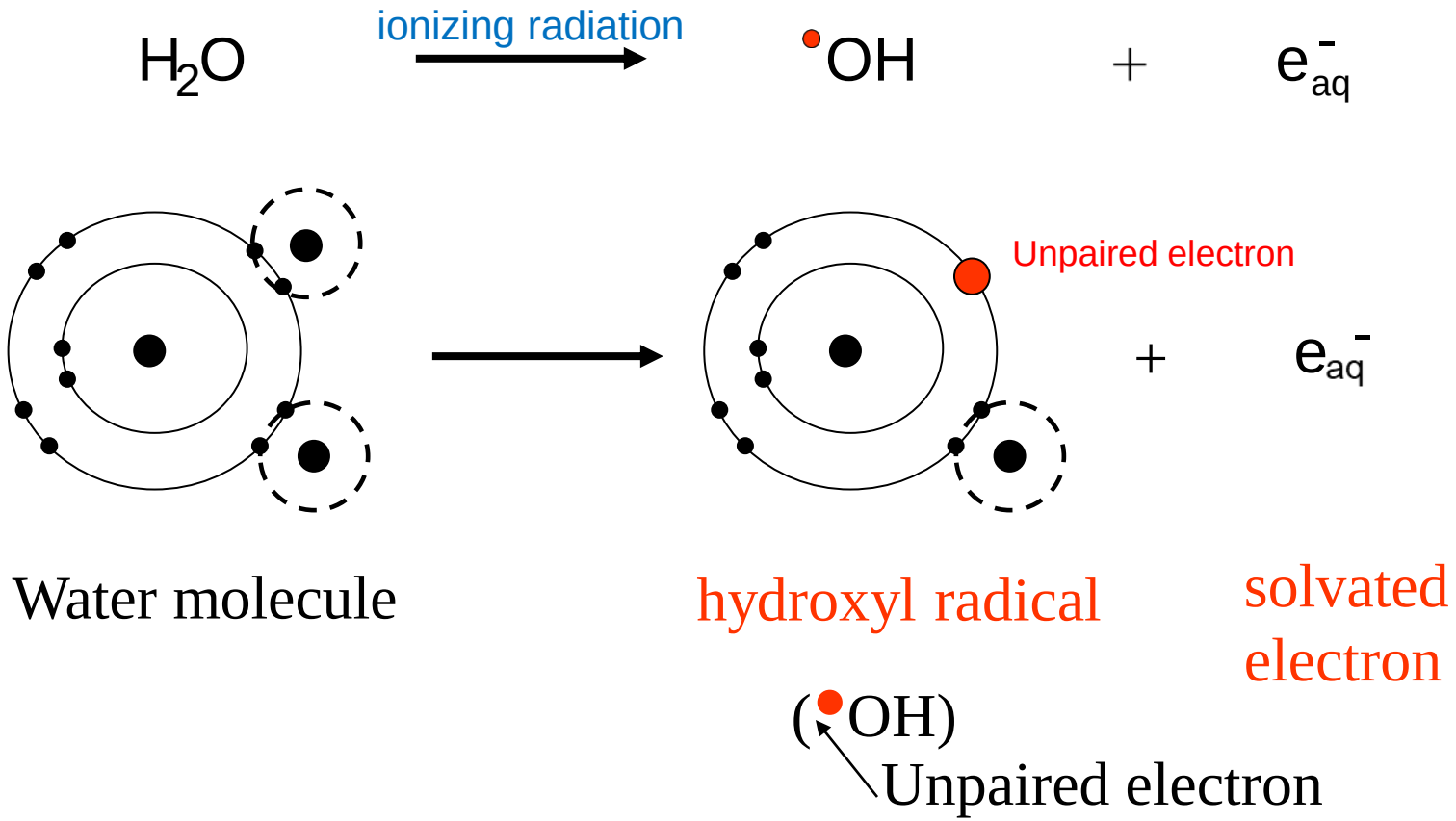
ionization (electron ejected from an atom or molecule)

Excitation



Definition of a free radical, using water as an example

An atom or molecule which contains
one or more unpaired electron(s)



Radical's name often ends in 'yl'

Major considerations for interaction of ionizing radiation with matter

- **Homogeneous vs. non-homogeneous distribution of reactants**

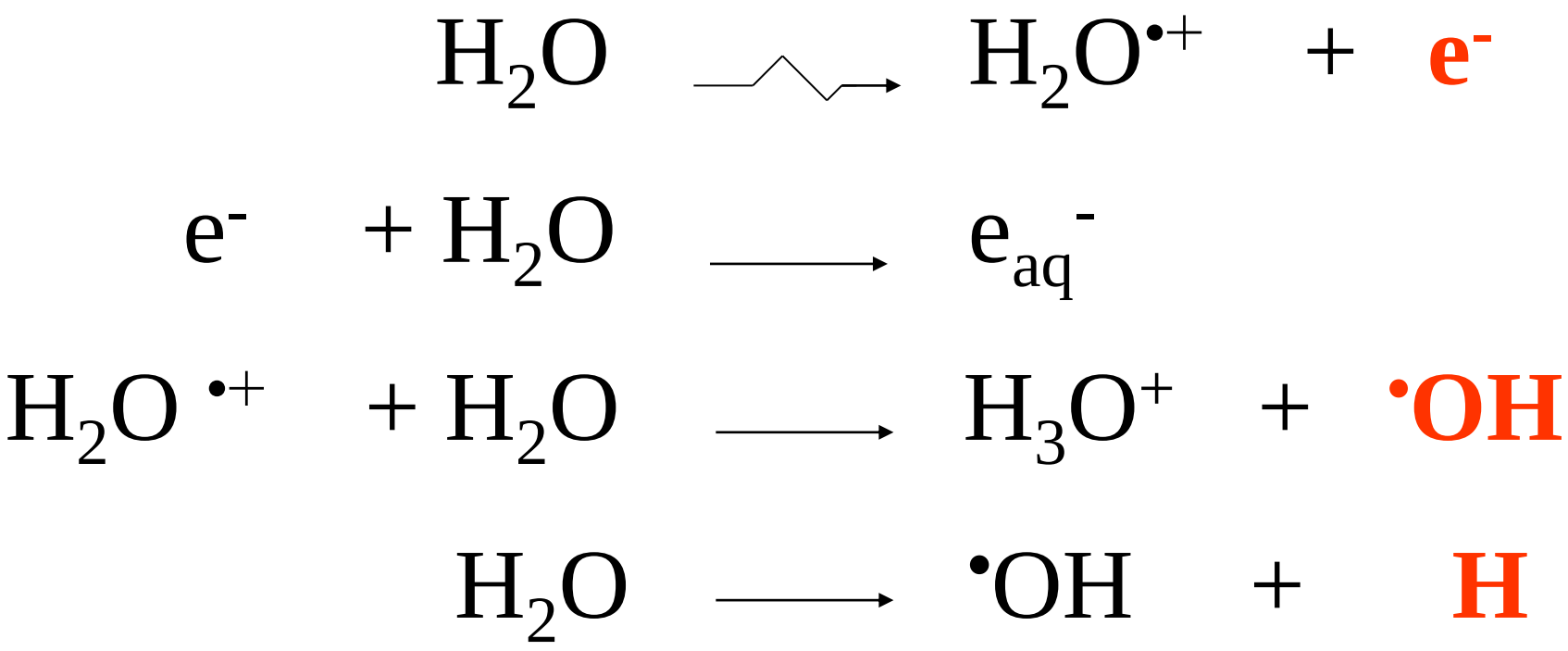
Ionizing radiation results in a non-homogeneous distribution of chemical events at early times, whereas most studies of chemical kinetics involves homogeneous distribution of events.

- **Implications of non-homogeneous distribution of reactants**

Non-homogeneous, spatial distribution of reactants results in radiation-induced clustered DNA damage at early times.

- **Time is an important variable in radiobiology**

Radiolysis of water



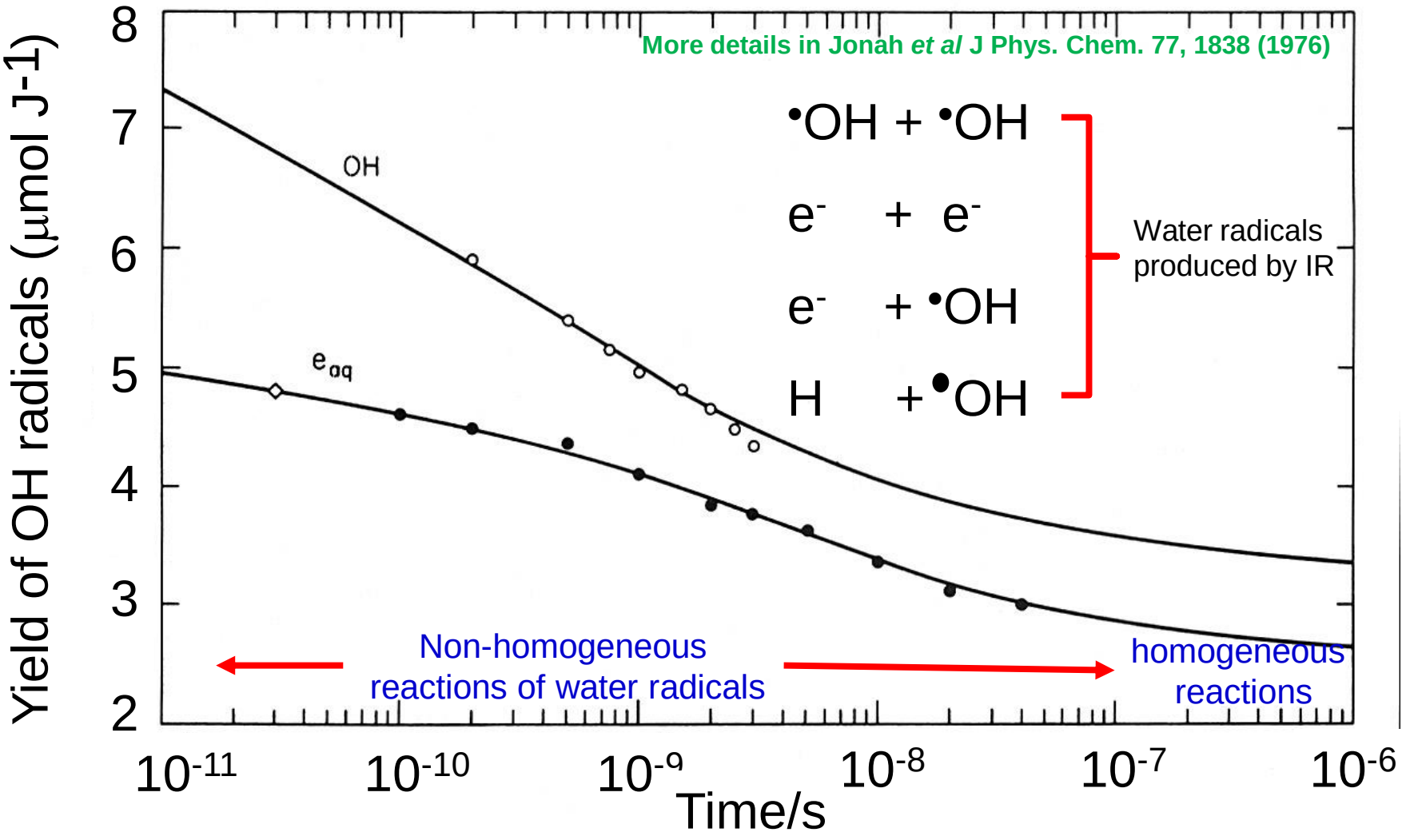
Radiation chemical yields (G-values) for low LET radiation

G(•**OH**) 0.28, G(e_{aq}^-) 0.27, G(H) 0.055 μmol J^{-1}

determined **~1 μs** after a short radiation pulse, when the water radicals have become homogeneously distributed.

About 45% of OH radicals (see next slide) react through non-homologous reactions during track expansion in water at times <1 μs .

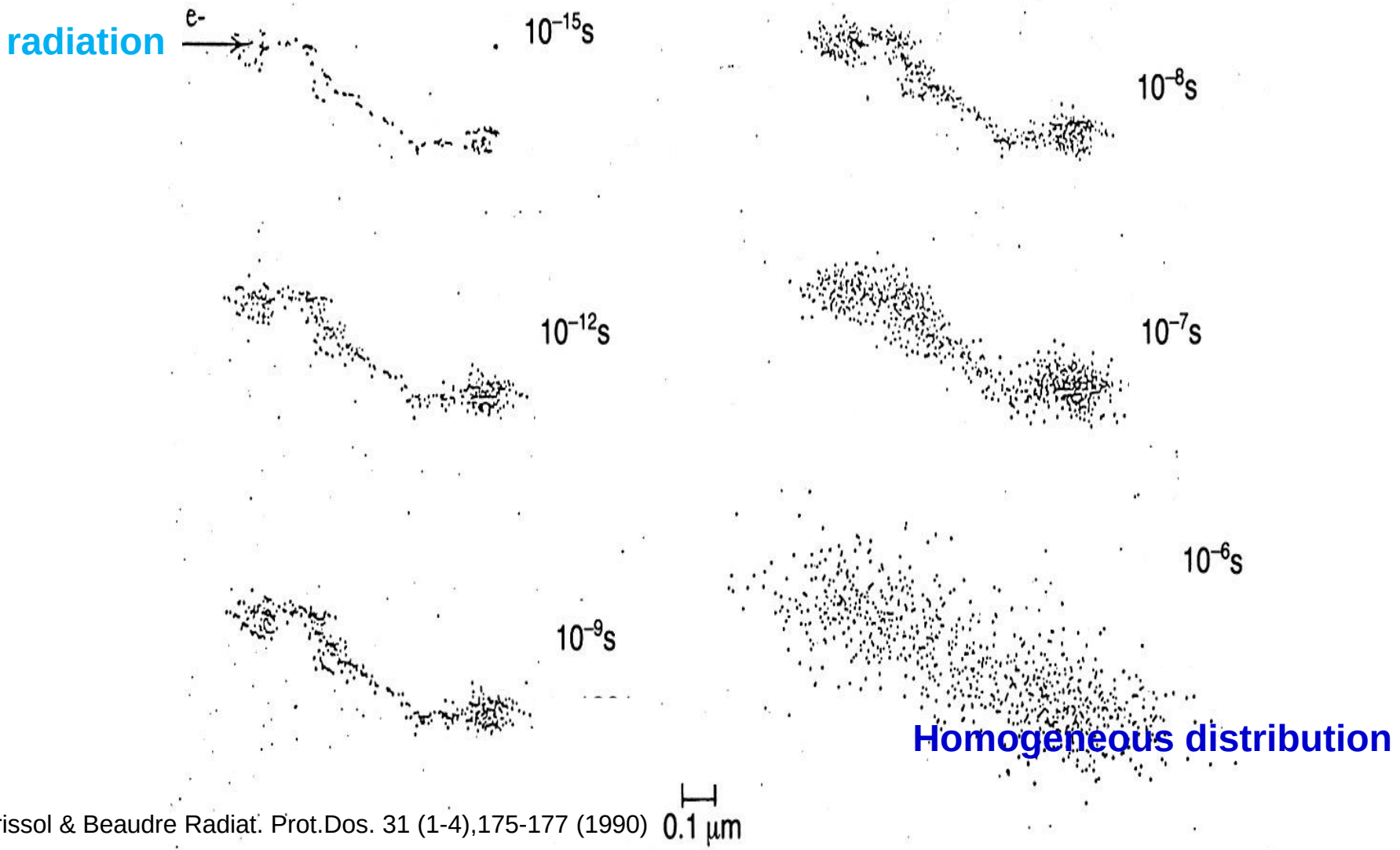
Measured time-dependent yields of OH radicals and electrons (G-values) for low LET radiation in water



At times >0.1 μs, the remaining, radiation-induced water radicals become homogeneously distributed when conventional reaction kinetics apply, with the lifetime of the radicals depending on dose rate and dose.

Expansion of a 10 keV electron track in liquid water in space and time (Terrissol and Beaudré 1990)

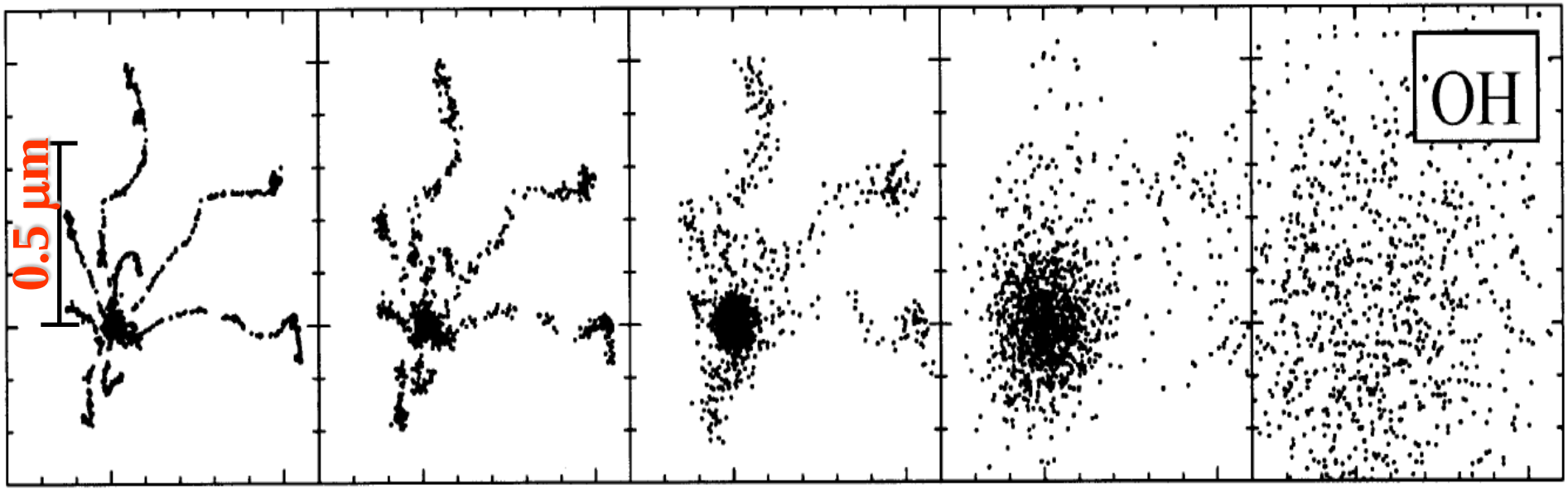
Dots represent radical species at the various times shown.
Non-homogeneous distribution of radical species at time <0.1 μs



Homogeneous distribution

Terrissol & Beaudre Radiat. Prot.Dos. 31 (1-4),175-177 (1990)

Radical diffusion of hydroxyl radical in water – time and spatial distribution scales during radiation track expansion

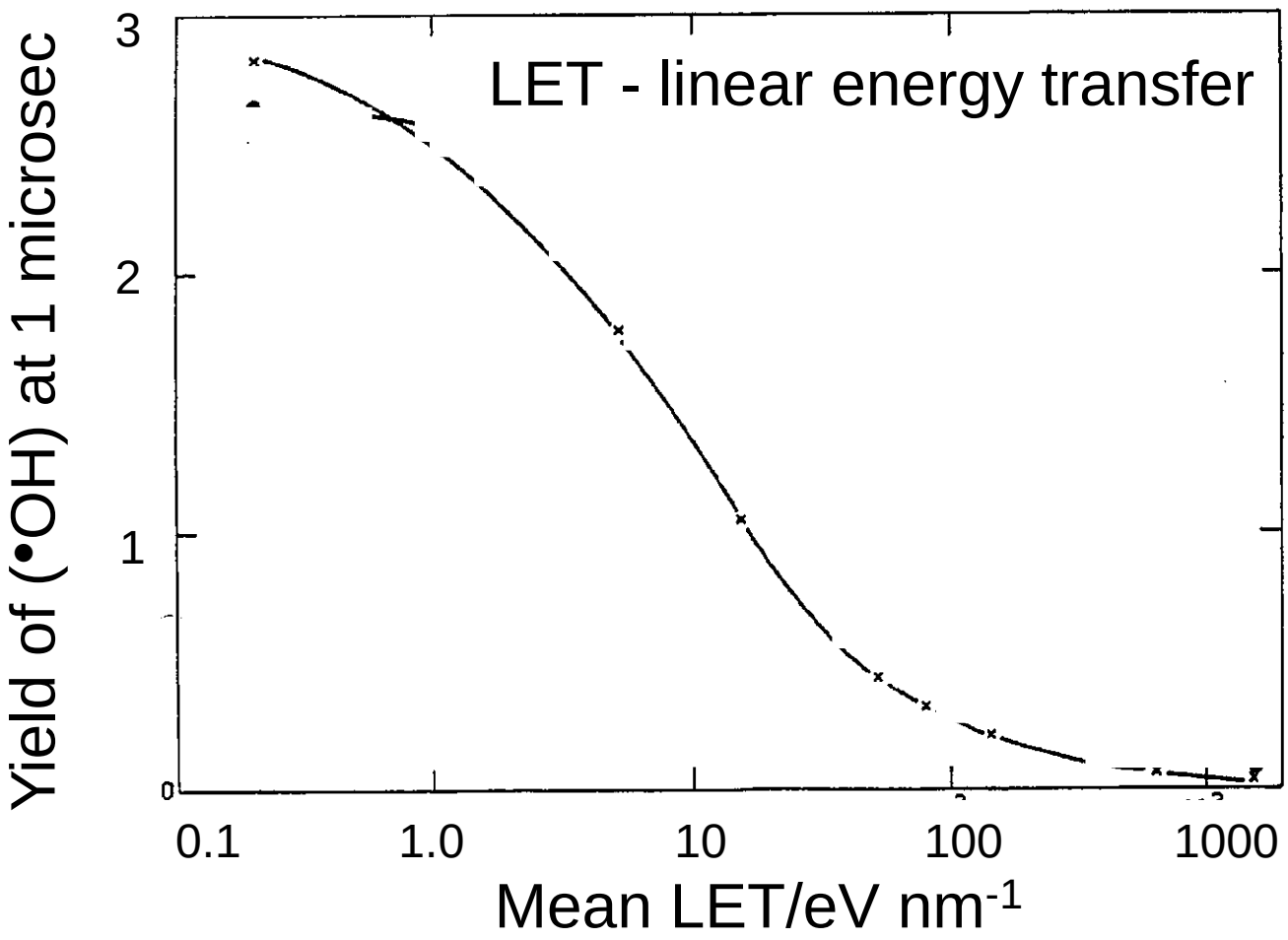


Spatial distribution of ·OH radical in liquid water up to 10 µs irradiated at an LET of ~70 keV/µm

Image adapted from Muroya Y, Plante I, Azzam EI, Meesungnoen J, Katsumura Y, Jay-Gerin JP. High-LET ion radiolysis of water: visualization of the formation and evolution of ion tracks and relevance to the radiation-induced bystander effect. *Radiat Res.* **165**(4), 485-491 (2006).

Yield of hydroxyl radicals in water, determined 1 microsecond after irradiation, decreases on increasing LET of the radiation.

Burns & Sims, JCS Faraday Trans 1 2803 (1981)



Majority of OH radicals interact by non-homologous interactions in water at LET >100 eV/nm

Comparison of the lifetime of hydroxyl radicals in water with that in a mammalian cell

- Irradiation of **water** results in the water radicals interacting by non-homologous reactions during track expansion. At ~0.1-1 μs the water radicals remaining are homogeneously distributed and interact by homogeneous reactions over several tens of μs to ms, depending on dose and dose rate of the radiation and the reaction rate constants.

- Using a model for radiation damage of DNA in cells based on a radiation chemistry approach, the lifetime for an •OH radical in a cellular milieu of ~1-2 ns was estimated, based on estimates of the scavenging capacity.

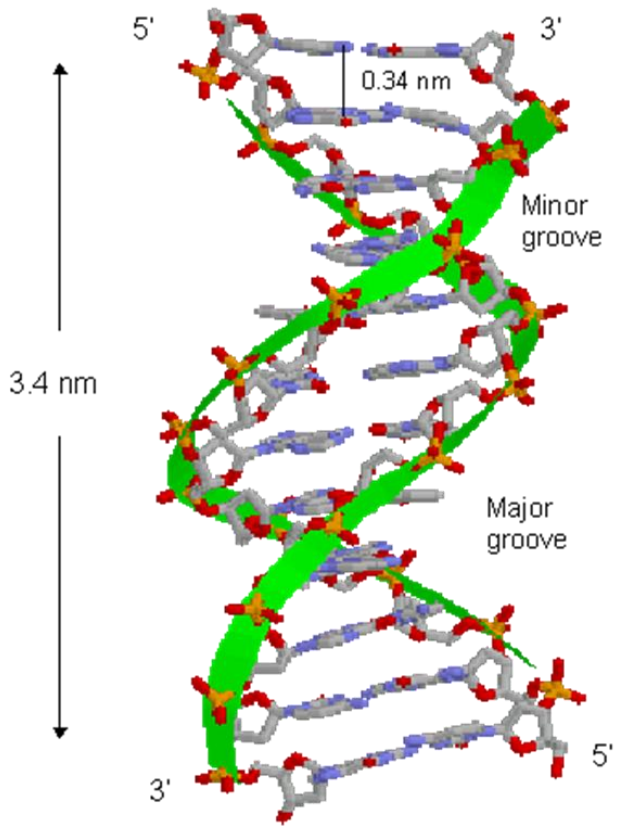
  Michael & Hunt, Rad Res 74, 23-34 (1978)

- It was shown experimentally that the average diffusion distance of an •OH in a cellular milieu is about 4-6 nm with an estimated lifetime of ~4 ns

  Roots and Okada Radiat. Res 64, 306-320 (1975)

Water radicals formed in cells by ionizing radiation interact mainly by non-homologous interactions with biomolecules within ~4 ns during track expansion, reflecting the scavenging capacity in a cell.

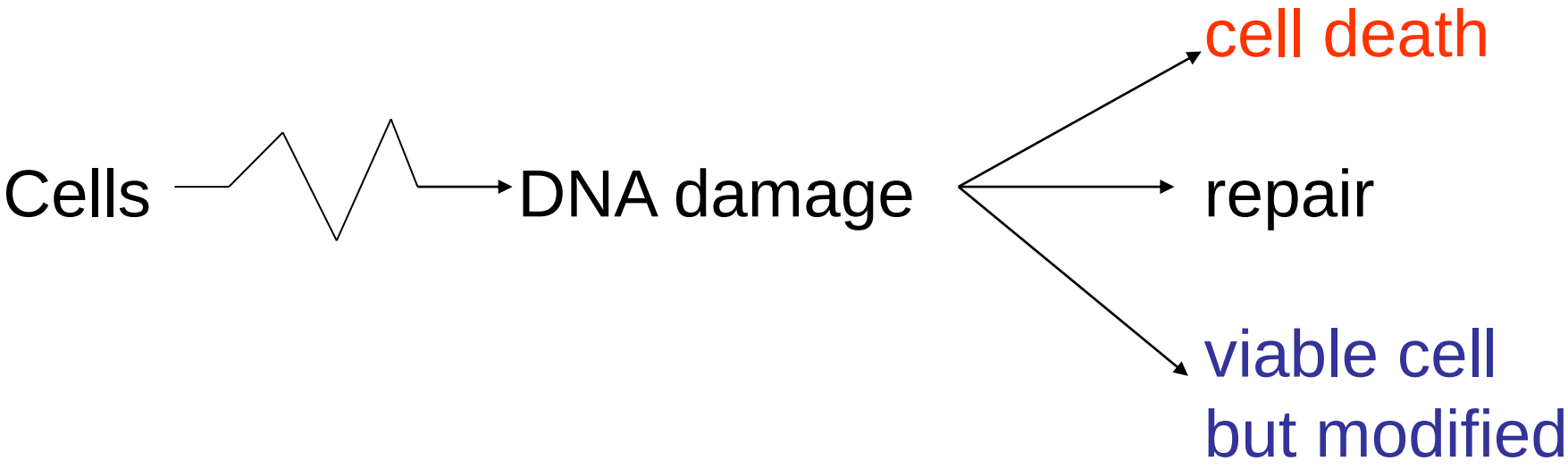
Discussion on the induction of DNA damage in cells by ionizing radiation



Sangeetha Gowda K.R. et al. Mechanism of DNA Binding and Cleavage. Biomedicine and Biotechnology, 2014, Vol. 2, No. 1, 1-9. doi:10.12691/bb-2-1-1

DNA thought to be one of the major targets where changes lead to biologically significant outcomes

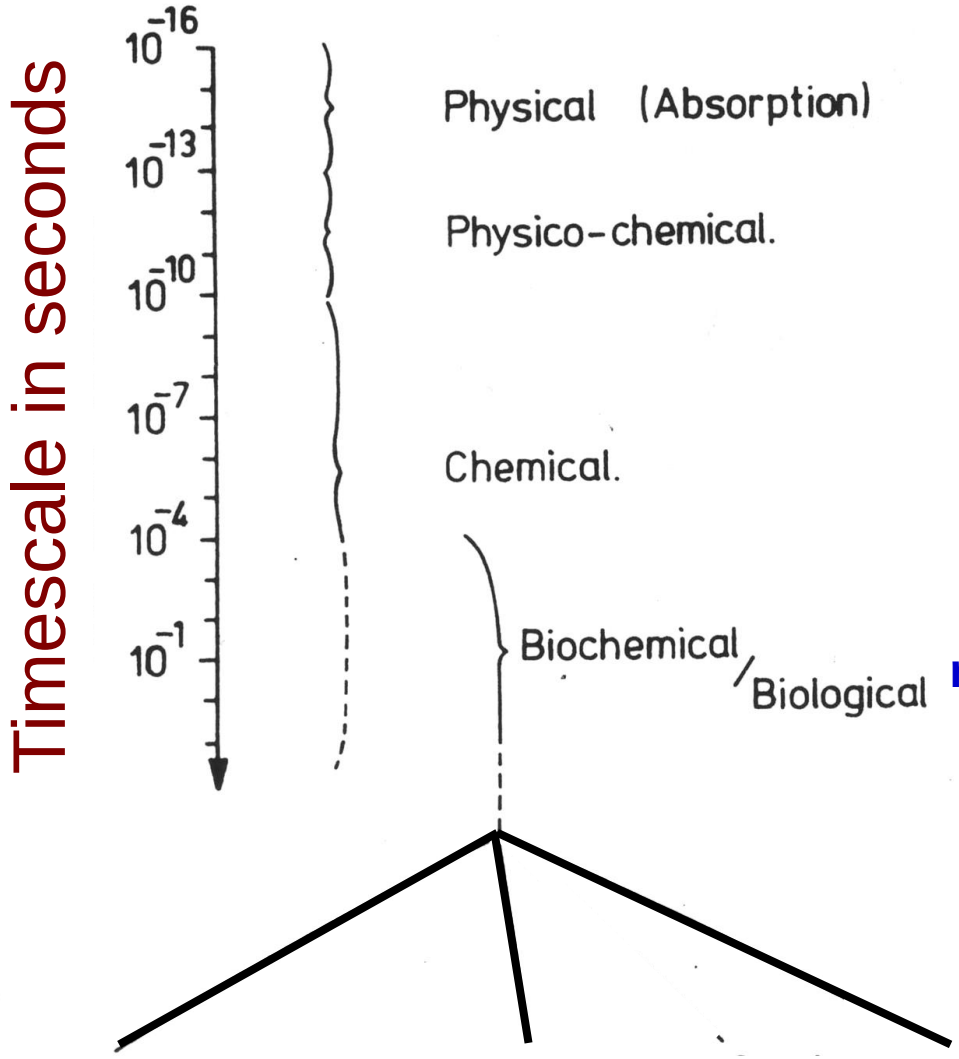
Effect of radiation- targeted effects



Cell death ..... killing of tumour cells important in radiotherapy

Cells modified (transformation) ..... cancer
hereditary defects

Timescale of radiation action in cells



Direct energy deposition in DNA, biomolecules and water

Non-homogeneous reactions of water radicals with DNA during radiation track expansion in a few ns

Interaction of DNA radicals and biomolecule radicals with oxygen, thiols, ascorbate etc

DNA radicals and other biomolecule radicals are converted into non-radical species

In biological cells, DNA repair or mis-repair occurs

Cell death mutation carcinogenesis

Chemical processes leading to bio-damage

Energy deposition

↓

Ionisation, excitation

↓

Free radicals on target molecules

↓

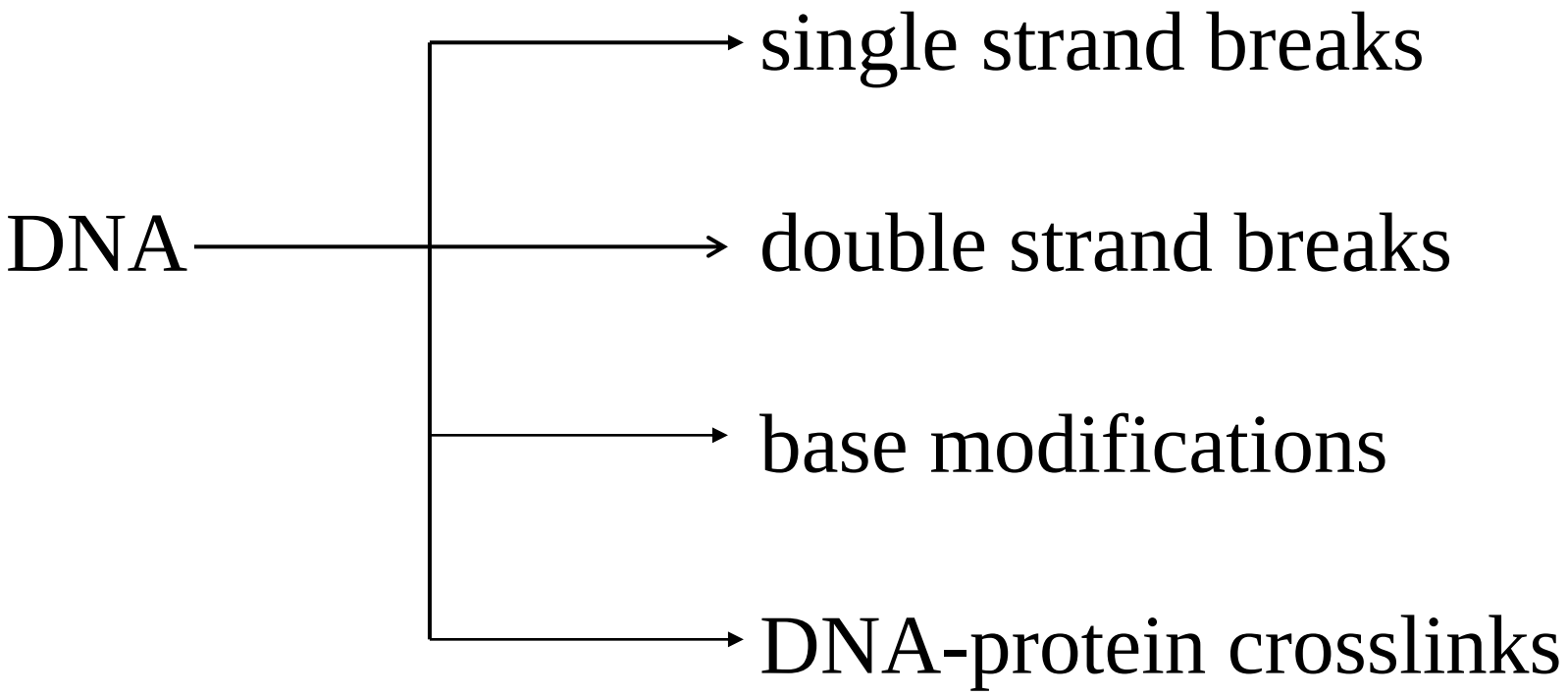
Chemical changes to biomolecules
(DNA, proteins, membranes etc)

↓

Biological effects

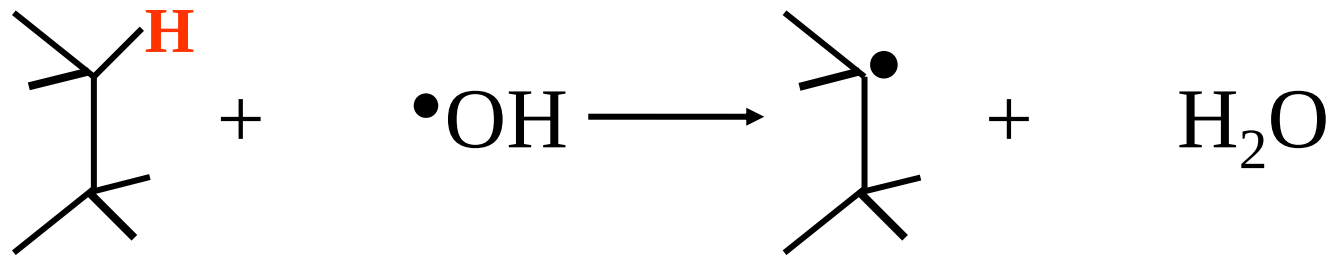
Radiation-induced damage to DNA

DNA thought to be one of the major targets where induced changes may be biologically significant



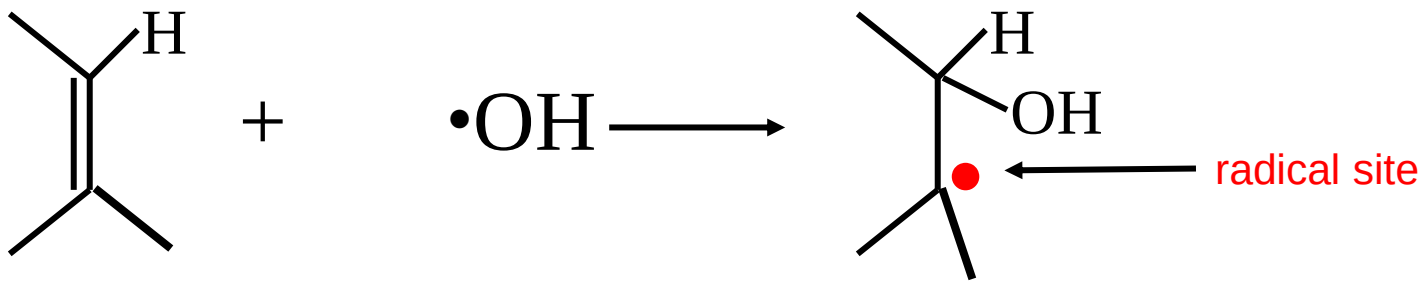
Reactions of hydroxyl radicals

1) **hydrogen atom abstraction** from saturated sites



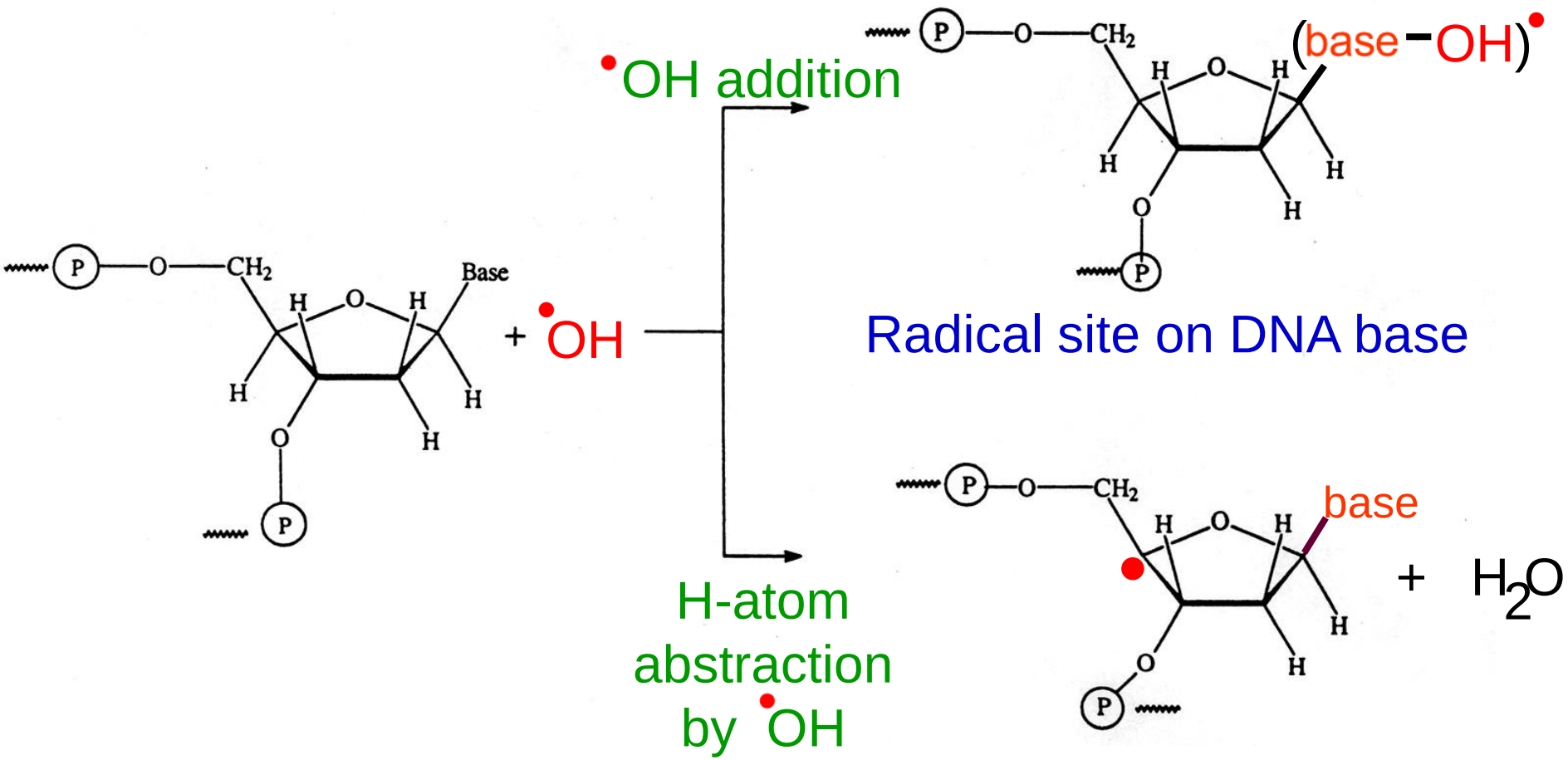
e.g. Abstract H-atom from DNA sugar backbone or proteins.

2) **hydroxyl radical addition** to unsaturated bonds

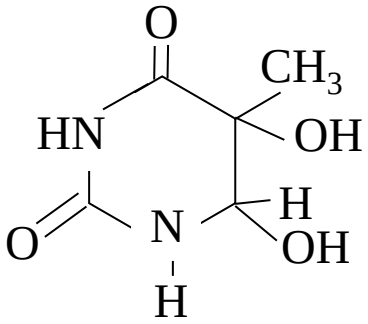


e.g. Adds to DNA bases and biomolecules containing double-bonds

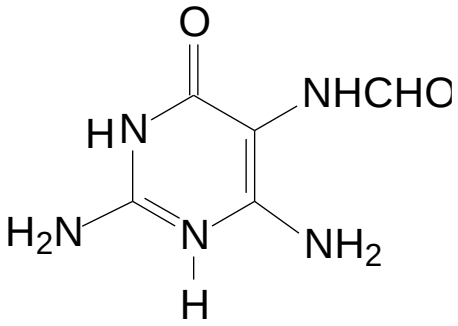
Examples of OH-radical interactions with DNA



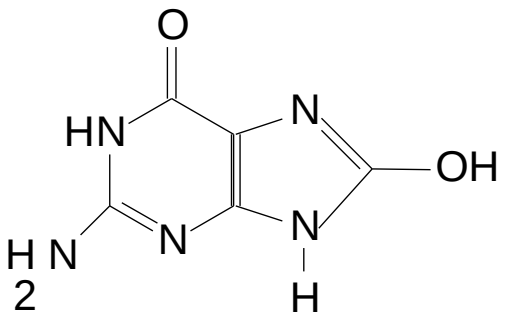
Types and yields of main DNA modifications induced in cells by low LET radiation



5,6-thymine glycol (Tg)



FapyG



8-oxoguanine (8-Go)

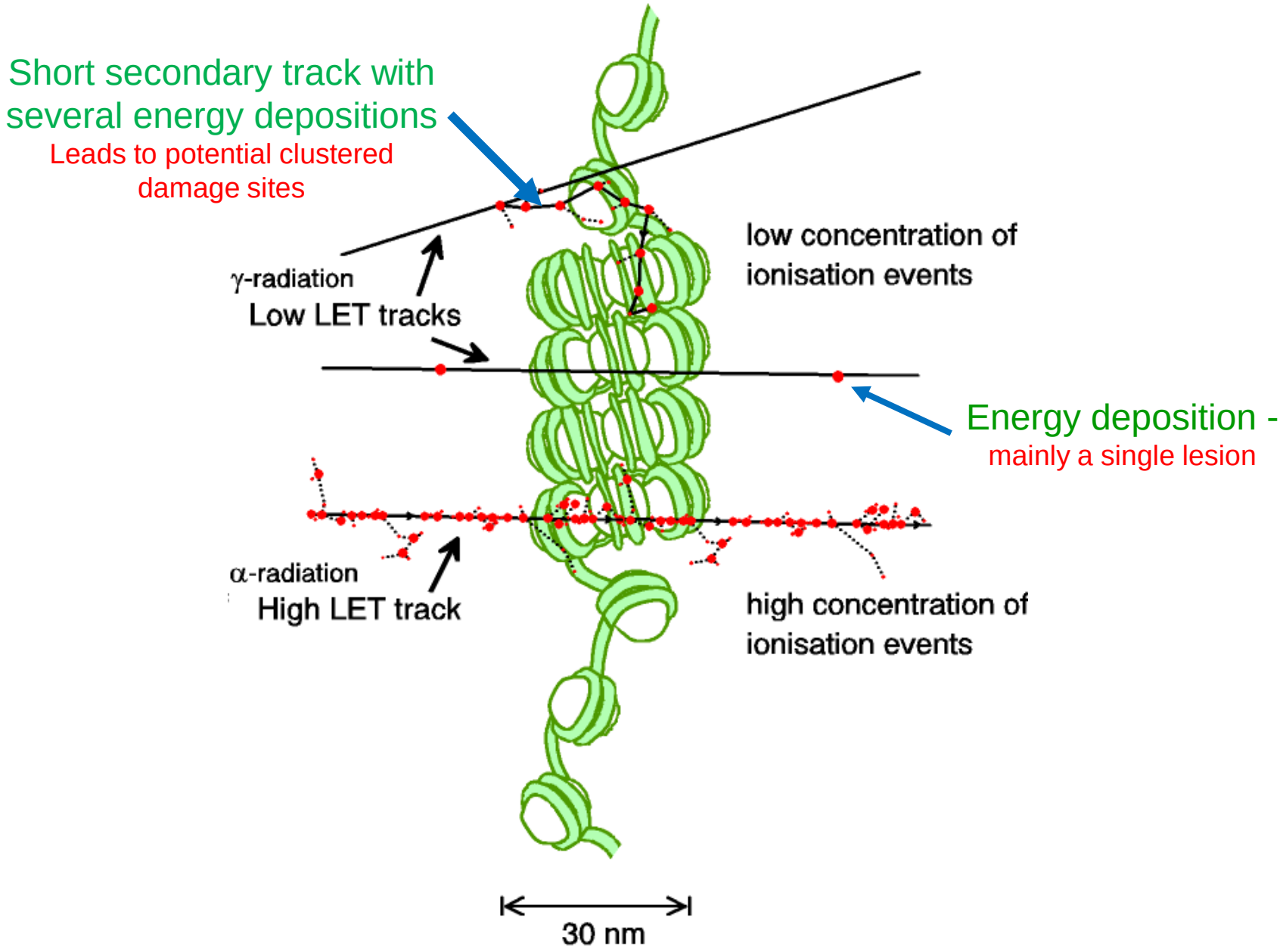
~590 (370) ~240(132) ~120 (60) lesions/cell/Gy

SSB	1000/cell/Gy
DSB	20-40/cell/Gy
5-(hydroxymethyl)-2'-deoxyuridine	~180 (132)/cell/Gy
5-formyl-2'-deoxyuridine	~130 (66)/cell/Gy

Yields in red represent those for $^{12}C^{6+}$ ion (LET 31.5 keV/μm)

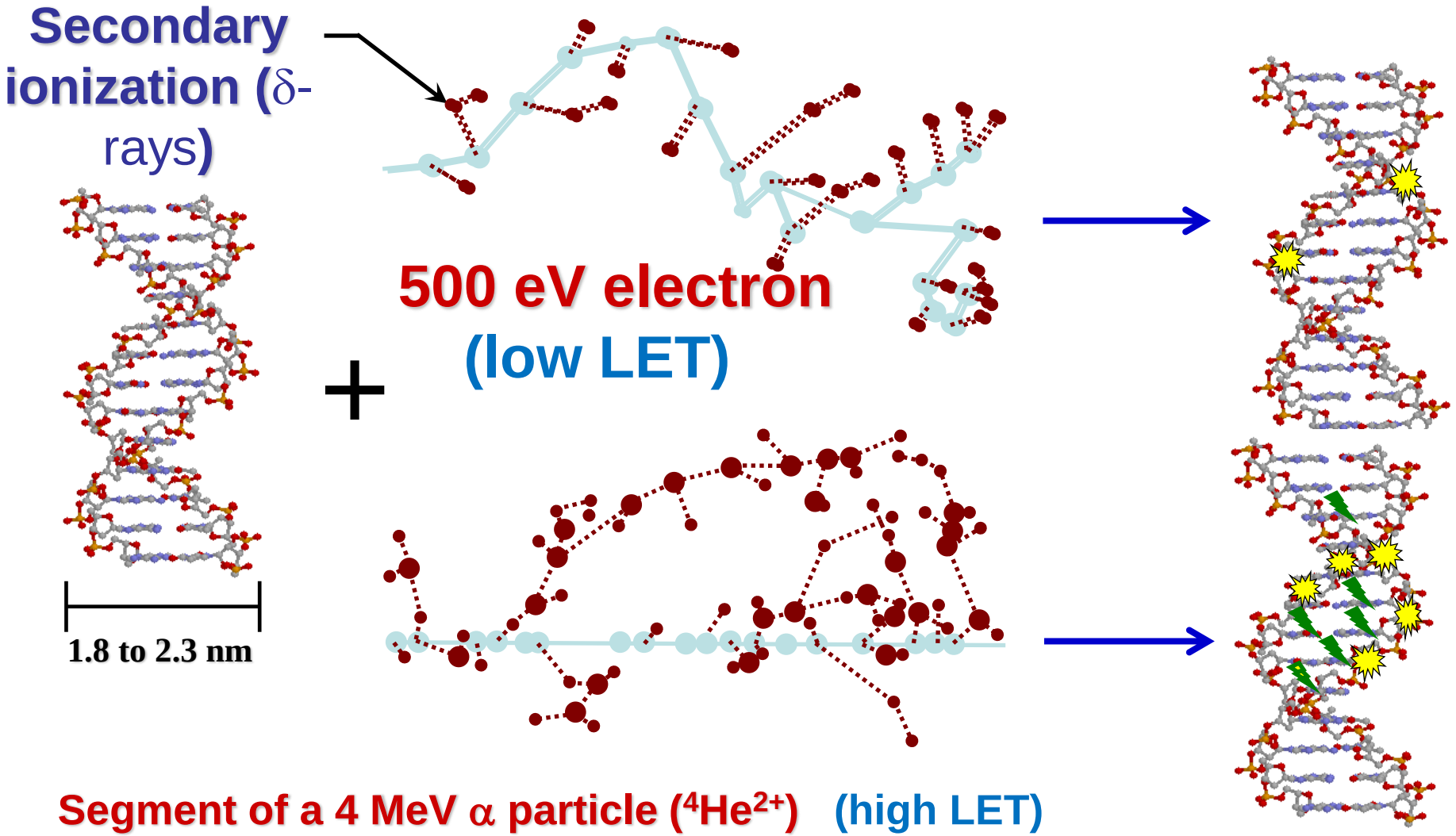
Cadet *et al*., Acc. Chem. Res., **41** 1075 (2008)

Radiation Tracks + DNA wrapped around chromatin



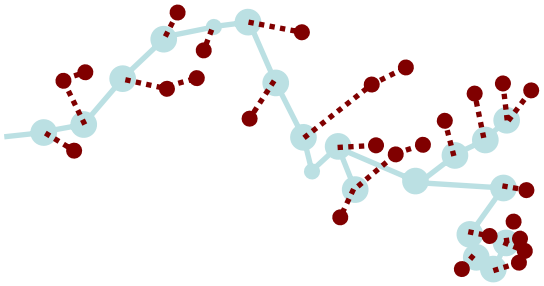
Mechanisms – Direct Effects of inducing DNA damage

Direct effects: ionisation of DNA DNA → $DNA^{\bullet+}$ + e-



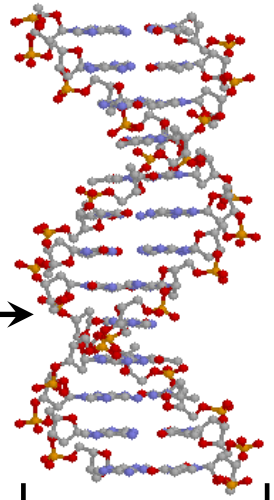
Mechanism - Indirect Effects of inducing DNA damage

Ionization of water produces chemically reactive species (see slide 7) that diffuse short distances to interact with DNA and other biomolecules.



$$H_2O \rightarrow H_2O^{\bullet +} + e^-$$

$$H_2O^{\bullet +} + H_2O \rightarrow {}^{\bullet}OH + H_3O^+$$

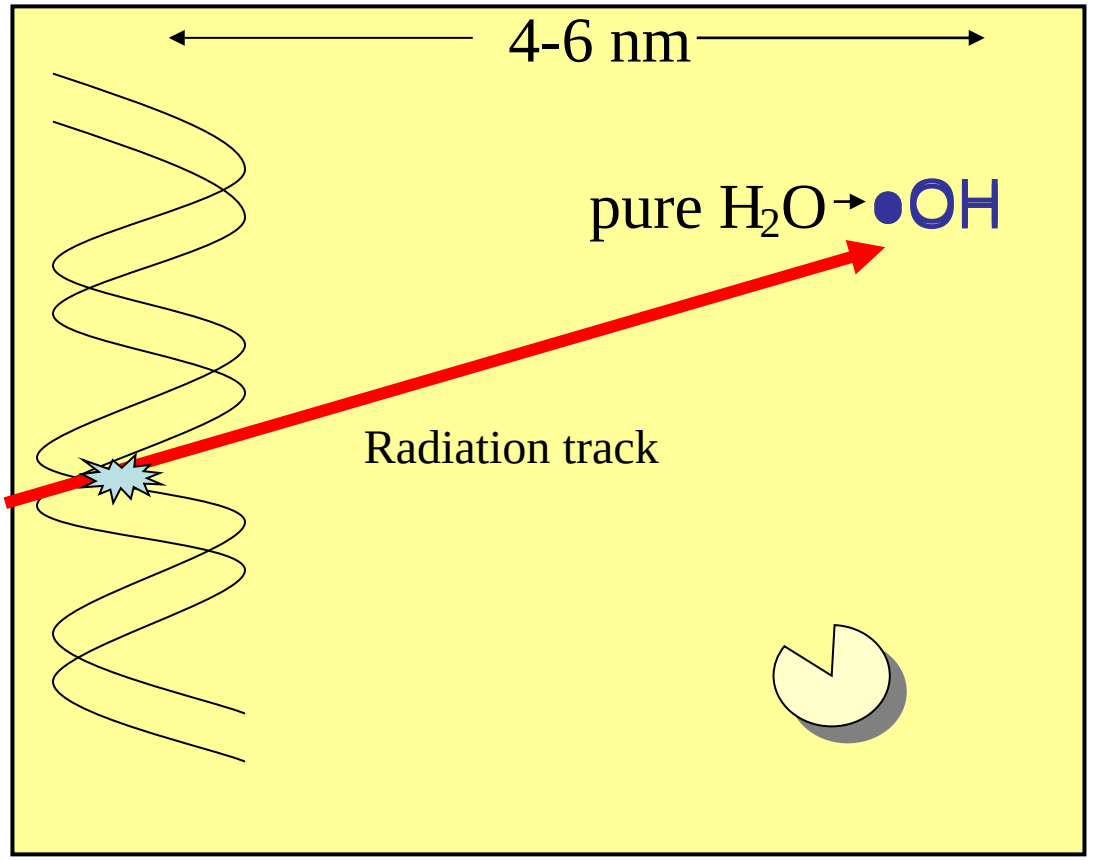


Average diffusion distance of an •OH in a cellular milieu is about 4-6 nm

Roots R, Okada S. Estimation of life times and diffusion distances of radicals involved in X-ray-induced DNA strand breaks of killing of mammalian cells. *Radiat. Res.* **64**, 306–320 (1975).

Scavenging capacity in cells- role of scavengers

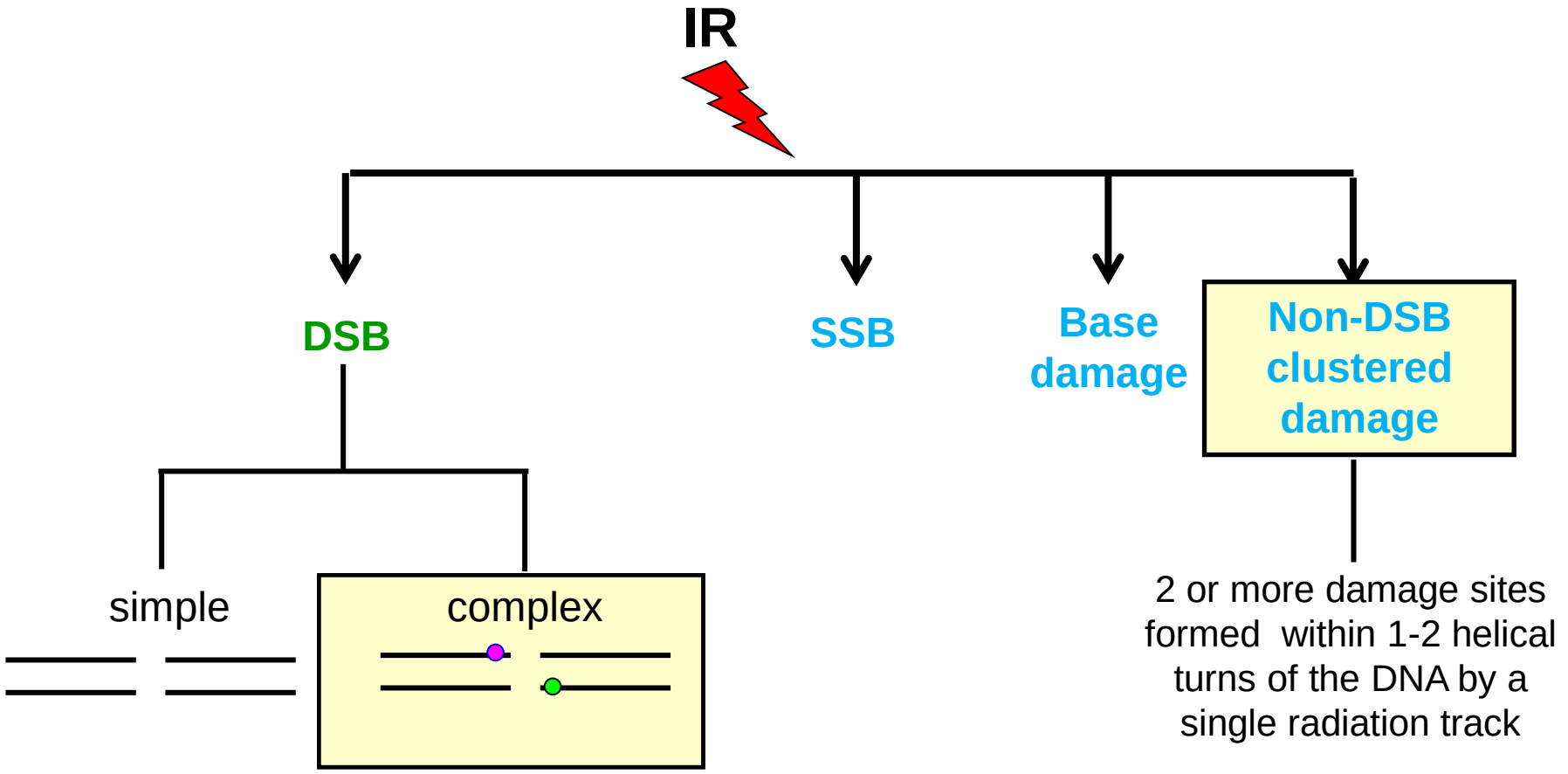
Radiation	% indirect damage
low LET	~60%
high LET	~30%



scavenger

The short diffusion distance of water radicals emphasizes the high scavenging capacity (~8 x $10^8 s^{-1}$) in cells and the non-homogeneous interactions during track expansion (see slide 12)

Types of IR-induced DNA damage



Non-homologous end joining
Homologous recombination
Single strand annealing

Base excision repair
SSB repair

Pathways involved in repair of DNA damage

Clustered DNA damage

Definition:-TWO or more lesions formed within 1 or 2 helical turns of the DNA by **a single radiation track**

Clustered damage

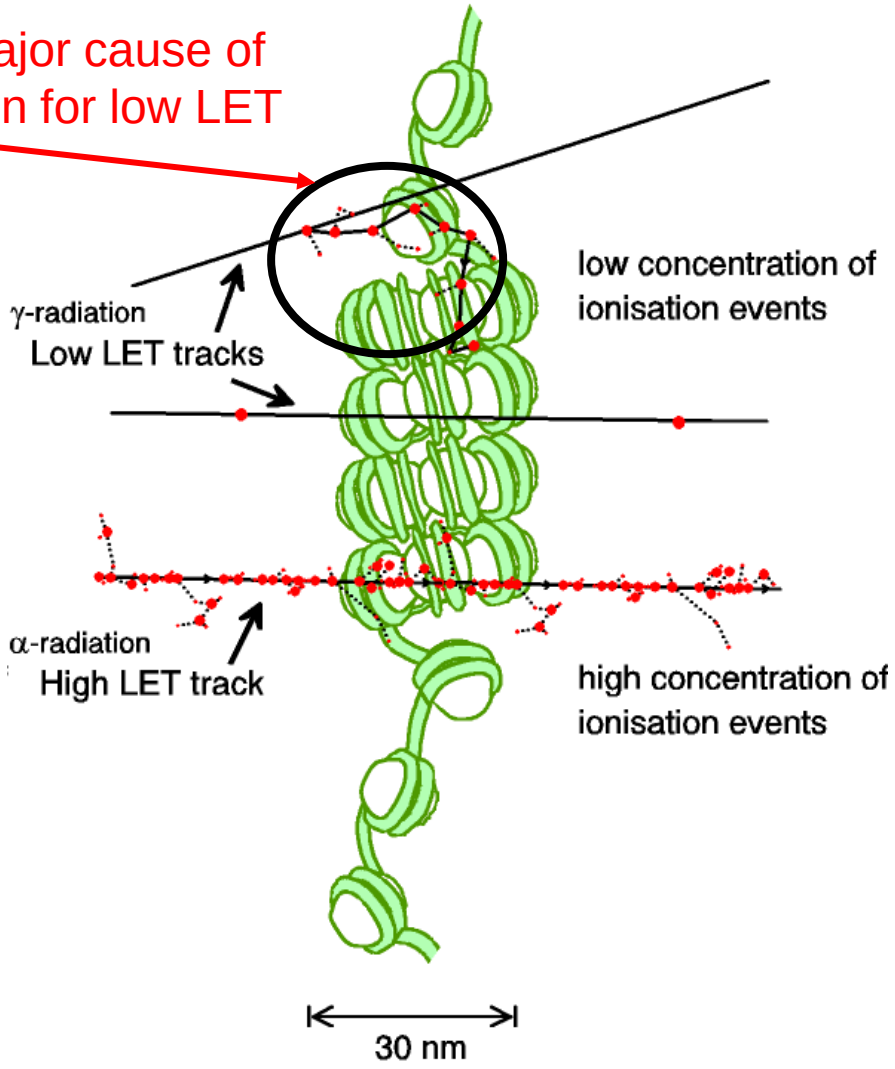
represent single base lesion, abasic site or SSB

Complex or ‘dirty’ DSB

represent single base lesion or abasic site

Complexity of clustered DNA Damage is largely dependent on ionisation density of the radiation

Short track- major cause of cluster formation for low LET



single strand break

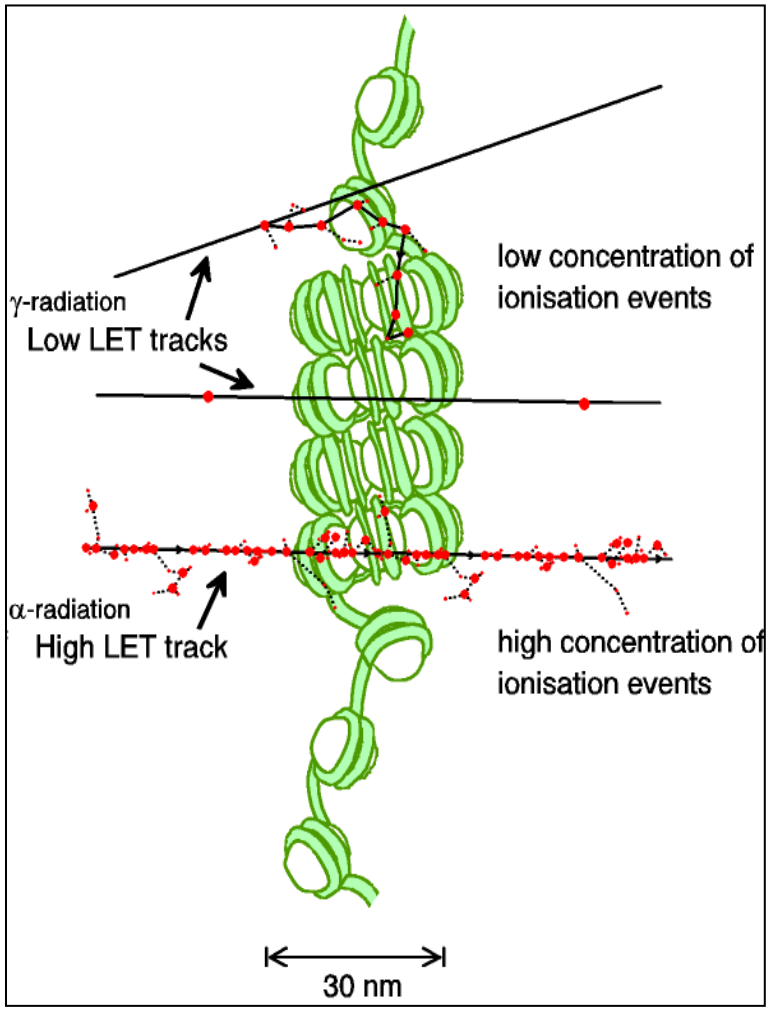
base damage/ sugar damage

clustered damage

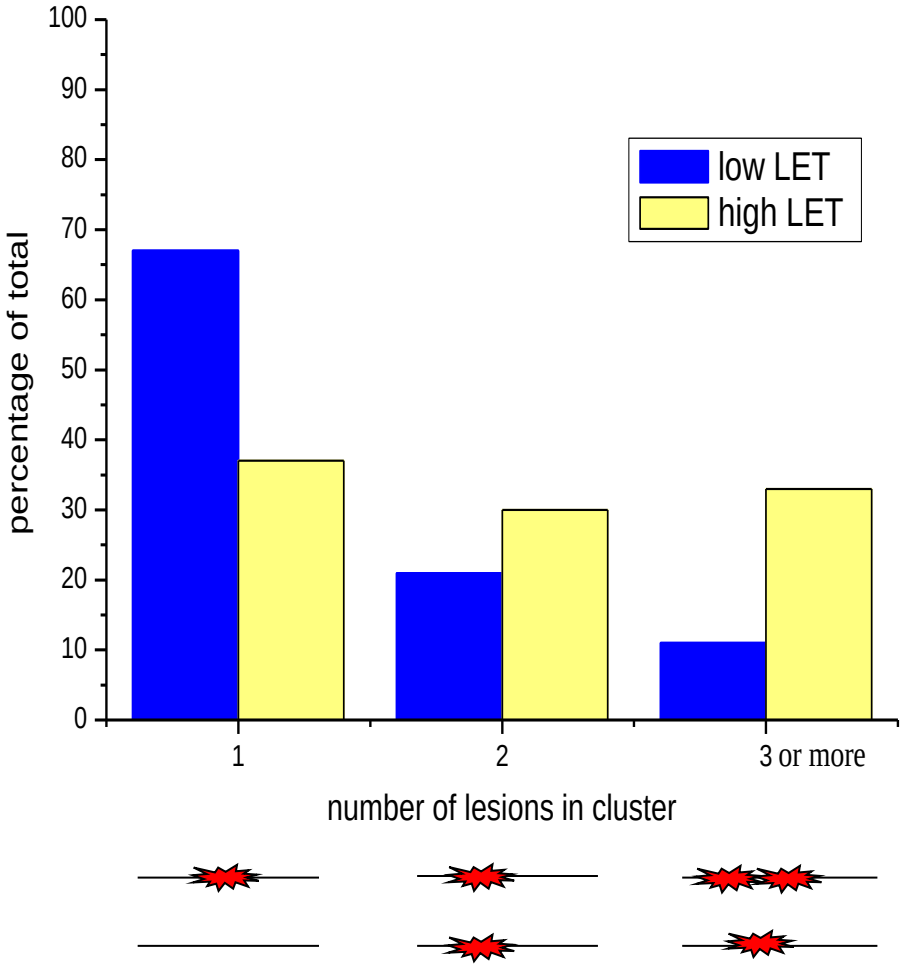
double strand break

Botchway, S.W., Stevens, D.L., Hill, M.A., Jenner, T.J. and O'Neill, P. *Radiat. Res*.148, 317-324 (1997).

Complexity of clustered DNA damage depends on LET



Complexity of DNA damage



Nikjoo, N., O'Neill, P., Goodhead, D.T. et al. Int. J. Radiat. Biol., 71, 467-483 (1997)
Nikjoo, O'Neill et al Rad. Environ. Biophys. 38, 31 (1999)
Nikjoo et al Radiat Res 156, 577 (2001)

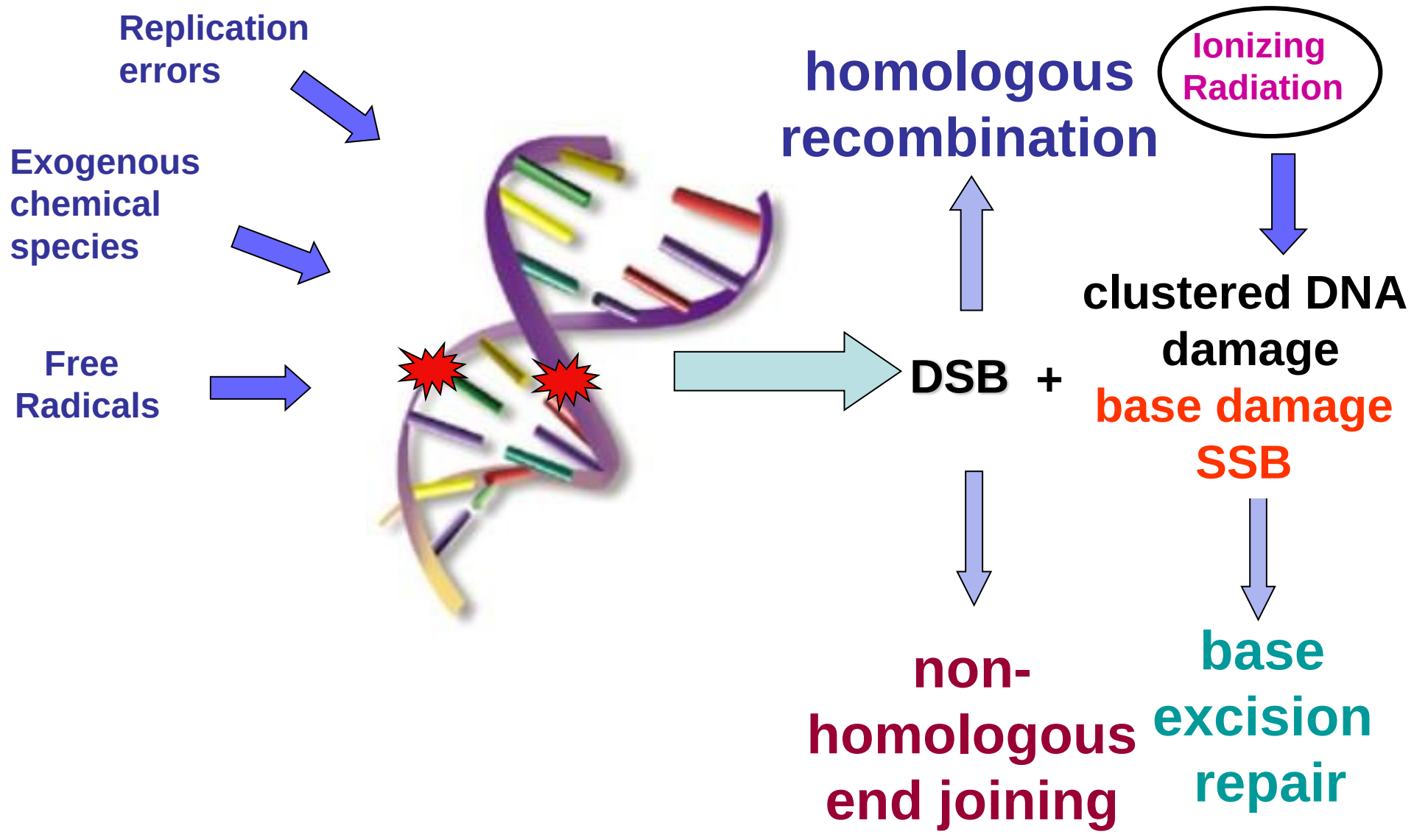
Number of lesions in a cluster increases as LET increases

What do we know about radiation-induced clustered DNA damage

- Prediction from biophysical modelling - radiation induces non-DSB clustered DNA damage
- These clusters are formed in mammalian cells
- Majority of clustered damage induced by radiation are non-DSB clusters (>>4x prompt DSB)
- Yield of complex DSB relative to simple DSB dependents on Linear Energy Transfer of the radiation

Chaudry & Weinfeld, J. Mol. Bio., 249, 914 (1995); Sutherland *et al.* PNAS, 97, 103(2000), Radiat Res. 157, 611 (2002); Jenner *et al.*, Rad. Res, 156, 590 (2001); Gulston *et al*, Nucl. Acids Res. 30, 3464 (2002)

Maintaining Genomic Stability - DNA Repair Pathways



DNA damage also induced endogenous

Types of individual DNA damage induced by radiation and endogenously are chemically similar

- >10000-100000 DNA lesions formed/day/cell endogenously (Ames *et al* PNAS 90, 7915 (1993)
- For a dose of 1 Gy, ionizing radiation induces ~3000 DNA lesions/cell
- Radiation DNA damage induced at an environmental dose rate of 2 mGy/year produces annually about 6 DNA lesions/cell.

What is the difference between low dose radiation and endogenous effects at the DNA level

Spatial distribution of DNA lesions formed in a radiation track within a few ns results in non-homogeneous distribution of DNA lesions

Formation of Clustered DNA Damage and DSB

Goodhead, D Int. J. Radiat. Biol. 65; 7-17 (1994);

Evidence indicates endogenous damage mainly present as isolated DNA lesions

- Endogenous DNA damage is formed over much longer times as the species are homogenously distributed through diffusion, so that most of the DNA damage is seen as isolated lesions. (Ward, W F Blakely, E Joner; Radiation Research, 103 (1985))

- Hydrogen peroxide induced a large number of SSB (> 36 000/cell), equivalent to 36 Gy without impairing cell survival. DSB only seen at very high concentrations of H2O2 so are not formed endogenously at low levels. (J. Dahm-Daphi, C. Sass, W. Alberti,, Int.J. Radiat. Biol. 176; 67–75 (2000)).

Repairability of non-DSB clustered DNA damage pose problems for DNA repair machinery

Processing of non-DSB clustered DNA damage sites containing 2 lesions is retarded

- Repair of both lesions within clustered damage is impaired
- Repair occurs sequentially, first the SSB/AP site then the base lesion. This limits the production of DSB
- Presence of nearby lesions results in the extension of the lifetime of the SSB.
- Depends on
  - the types of lesions
  - inter-lesion distance
  - relative orientation of the lesions

David-Cordonnier, M.-H et al. *Nucl. Acids Res.* 29, 1107 (2001); *J. Biol. Chem*., 275, 11865 (2000); *Biochemistry* 40, 5738 (2001); *Biochemistry* 40, 11811 (2001); *Biochem.* 41, 634 (2002), Lomax, M. E., Cunniffe, S.,O'Neill, *DNA Repair*, 3, 289 (2004), *Biochem.* 43, 11017 (2004); Bellon, S., Shikazonon, N., Cunniffe, S., Lomax, M. O'Neill, P. *Nucleic Acids Res*., 37, 4430 (2009)

Clustered DNA damage sites may persist to replication

non-DSB clustered DNA damage

↓

retarded repair by BER pathway

↓

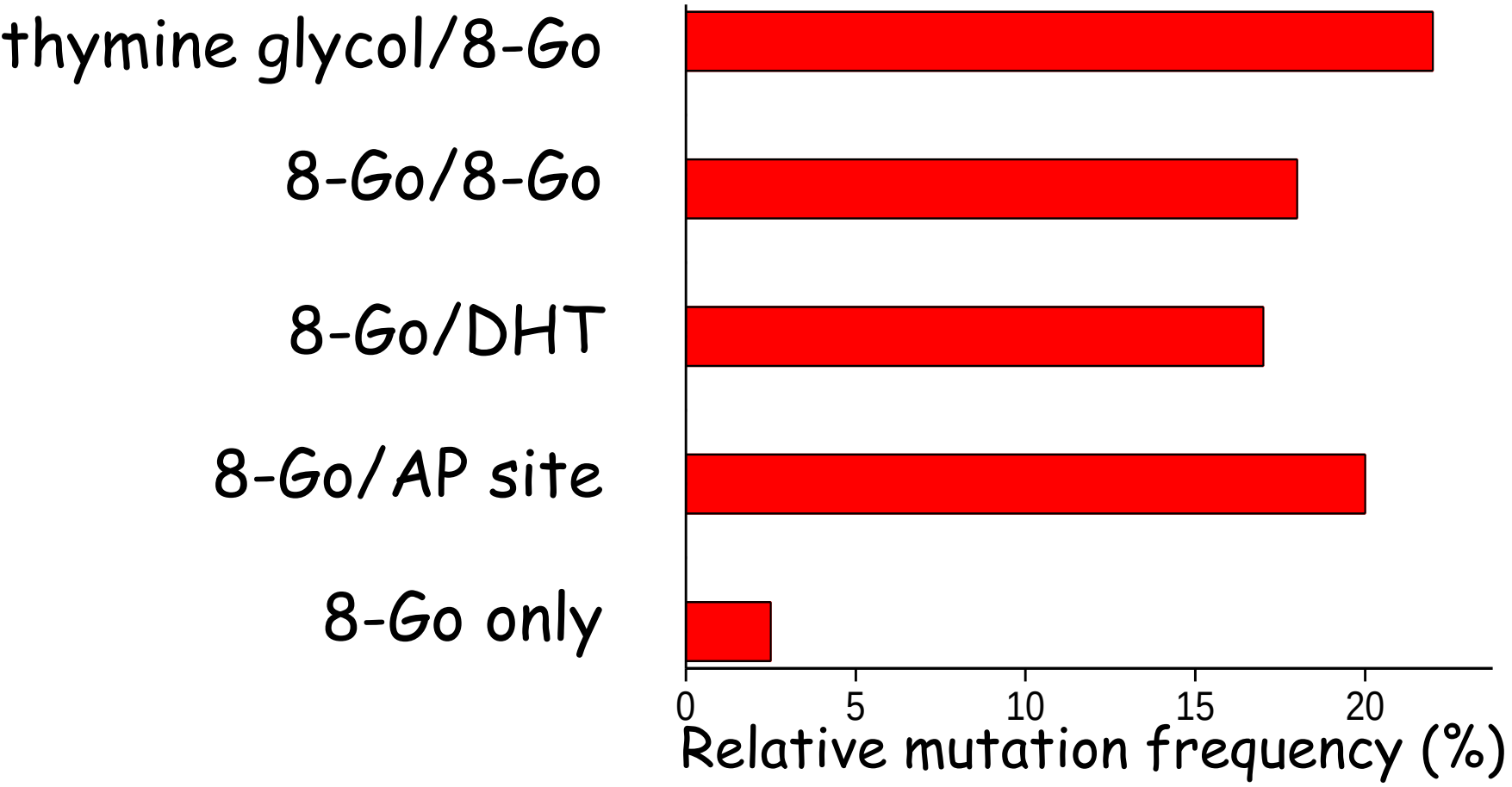
increased probability that clusters are present at replication

⇣

clustered sites - highly mutagenic

David-Cordonnier et al. Nucl. Acids Res. 29, 1107 (2001); J. Biol. Chem., 275, 11865 (2000); Biochemistry 40, 5738 (2001); Biochemistry 40, 11811 (2001); Biochem. 41, 634 (2002), Lomax, Cunniffe, O'Neill, DNA Repair, 3, 289 (2004), Biochem. 43, 11017 (2004); Bellon, Shikazono, Cunniffe, Lomax, O'Neill. Nucleic Acids Res., 37, 4430 (2009); Mourgues et al, Nucl. Acids Res., 35, 7676 (2007); Shikazono, Akamatsu, Takahashi, Noguchi, Urushibara, O'Neill, Yokoya. Mutation Research 749, 9 (2013).

Example of similar mutability of different bistranded (-1) clustered damage sites in *mutY* null *E. coli*



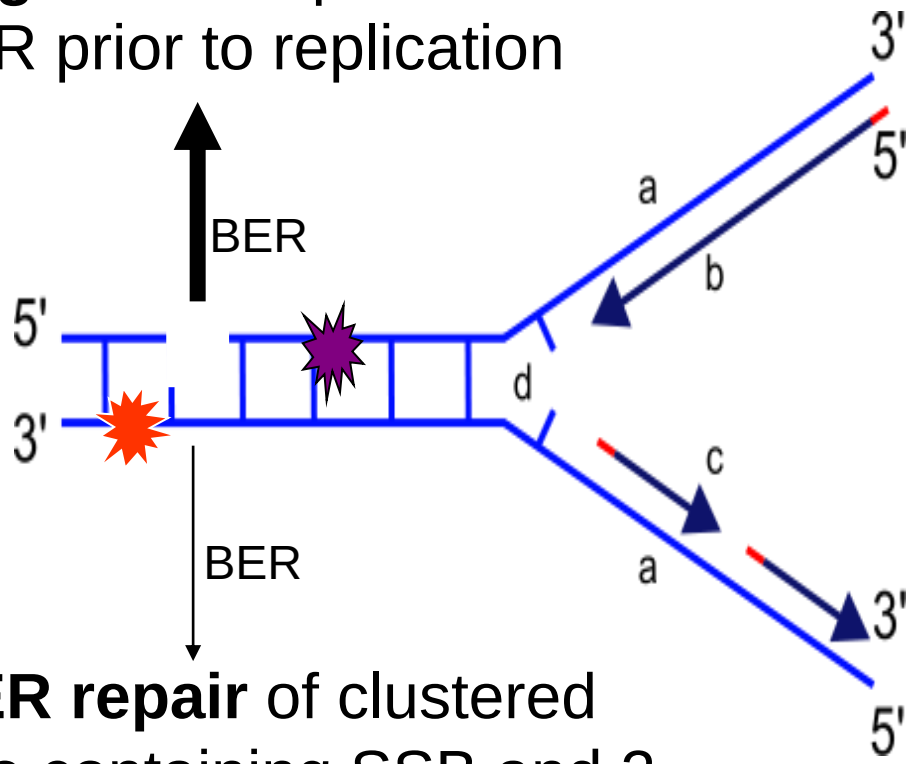
Single lesion far less mutagenic than clustered damage

Dominant role of MutY as an anti-mutagen in repair

Pearson, C. G., Shikazono, N., Thacker, J. and O'Neill, P. *Nucl. Acids. Res*., 32, 263-270 (2004); 34, 3730-3738 (2006); Bellon, S., Shikazonon, N., Cunniffe, S., Lomax, M. O'Neill, P. *Nucleic Acids Res*., 37, 4430 (2009).

Stalled replication due to extended lifetime of cluster

Single SSB repaired
by BER prior to replication



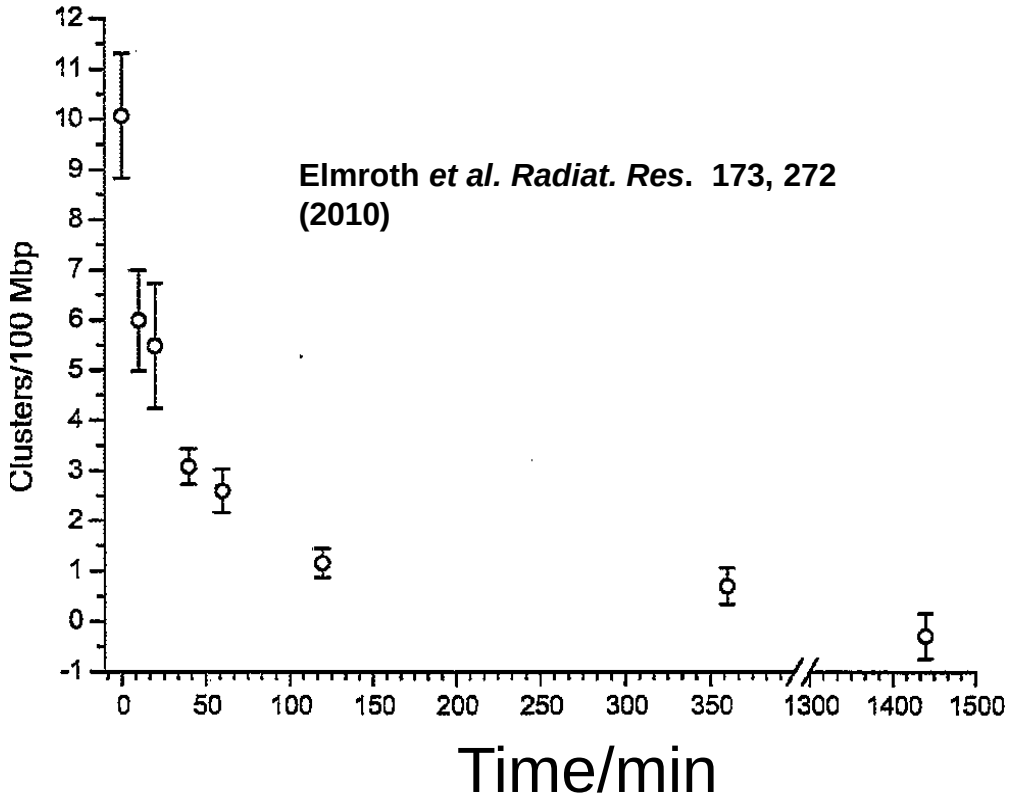
Slow BER repair of clustered damage site containing SSB and 2 base lesions

Results in entering and stalling replication with damage

Outcome- potential mutations

Lomax, Cunniffe, O'Neill, DNA Repair, 3, 289 (2004); Eccles, Lomax, O'Neill. Nucl. Acids Res., 38, 1123-1134 (2010); Cunniffe, O'Neill, Greenberg, Lomax Mutation Research 762, 32–39 (2014).

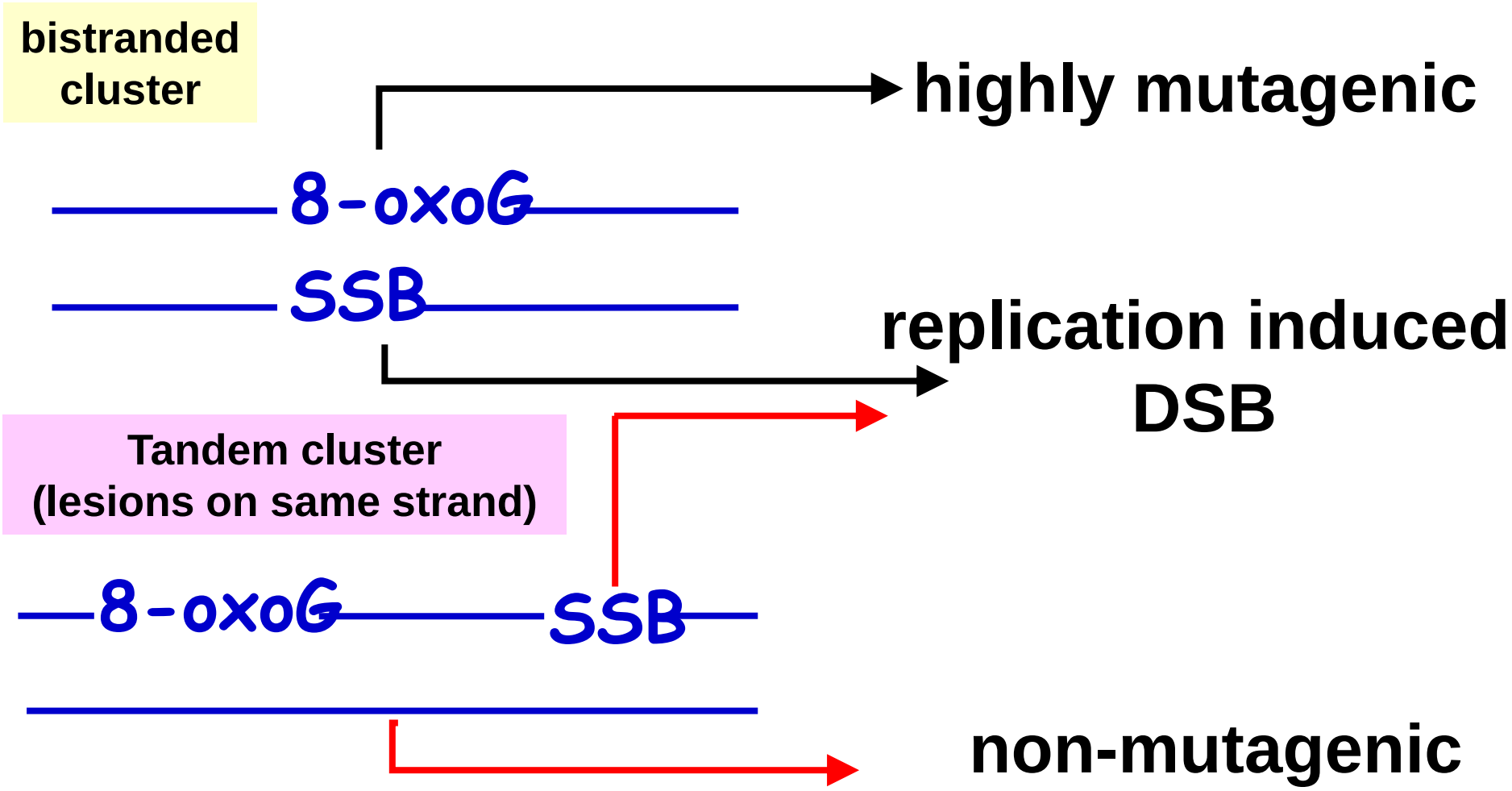
Dynamics of loss of clustered DNA damage in cells following irradiation is significantly slower than for isolated base lesions/SSB



Lifetime of clustered DNA damage sites significantly longer than single base lesions or SSB in cellular DNA

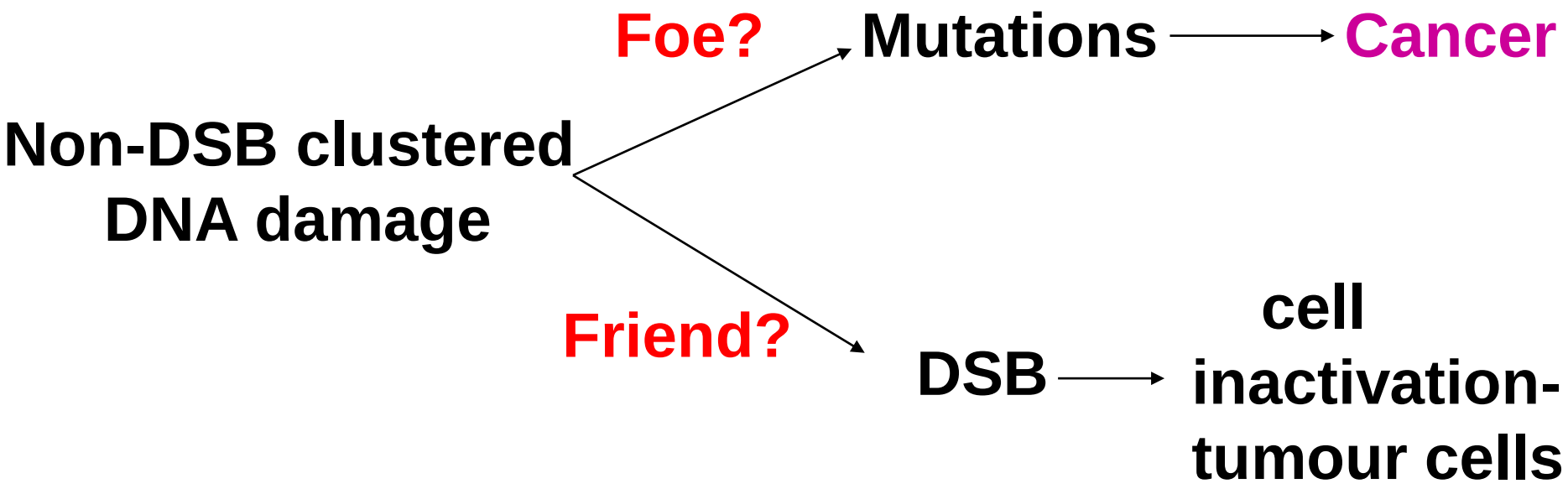
Eccles et al Mutation Res. 711, 134-141 (2011); Georgakilas, O'Neill, Stewart, Radiat. Res. 180, 100-109 (2013); Sage, E., Shikazono, N.; FRBM 107, 125-135 (2017)

Consequences of a persistent SSB at stalled replication



Cunniffe *et al*, DNA Repair, 6, 1839 (2007); Shikazono & O'Neill, Mutation Res., 669, 162 (2009)
Eccles, L.J., Lomax, M. E., O'Neill, P. *Nucleic Acids Res*., 38, 1123-1134 (2010).

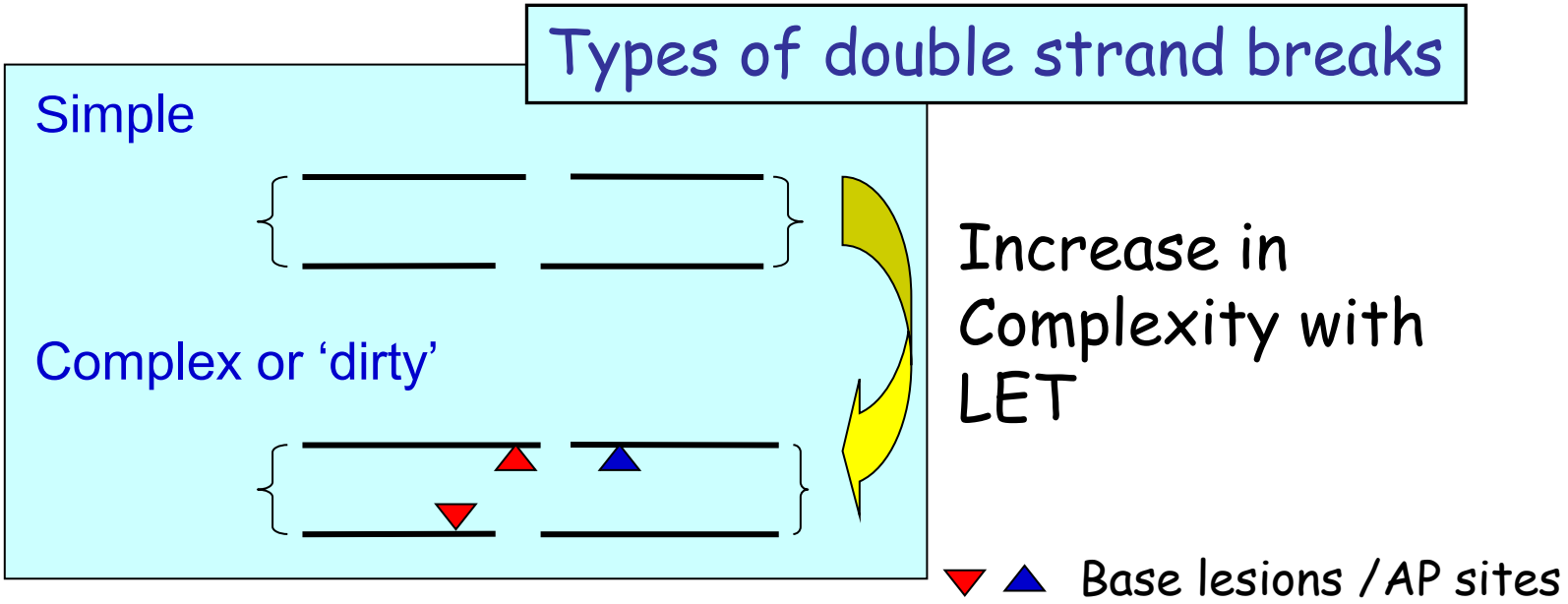
Radiation-induced clustered DNA damage: friend or foe?



Role for clustered damage sites in causing genomic instability

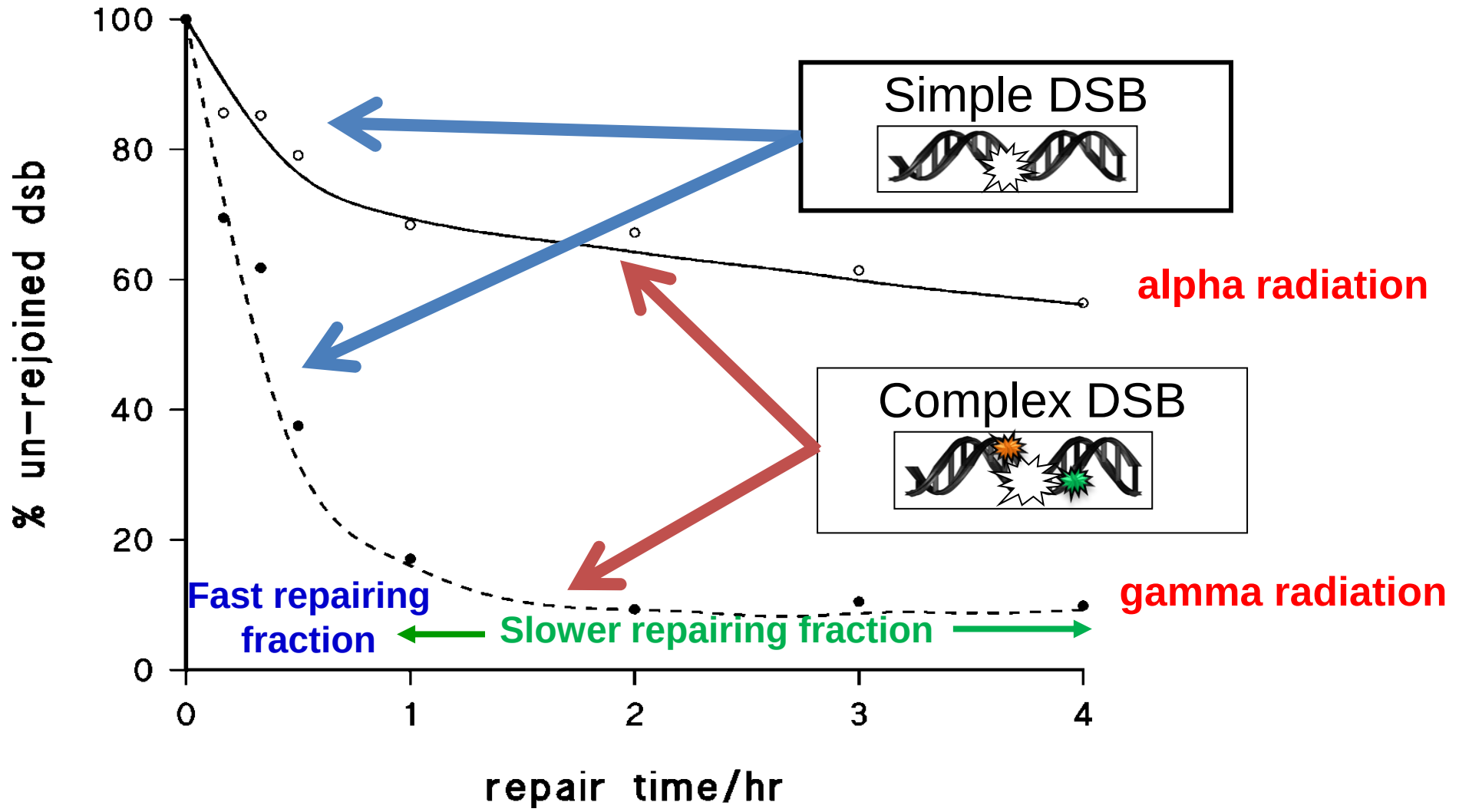
Cunniffe et al, DNA Repair, 6, 1839 (2007); Shikazono & O'Neill, Mutation Res., 669, 162 (2009); Eccles, Lomax, O'Neill, Nucleic Acids Res., 38, 1123 (2010); Eccles, O'Neill, Lomax Mutation Research 711, 134 (2011)

Complex (dirty) DNA double strand breaks



Increase in complexity of 'dirty' DNA double strand breaks leads to slower rejoining – longer lived DSB

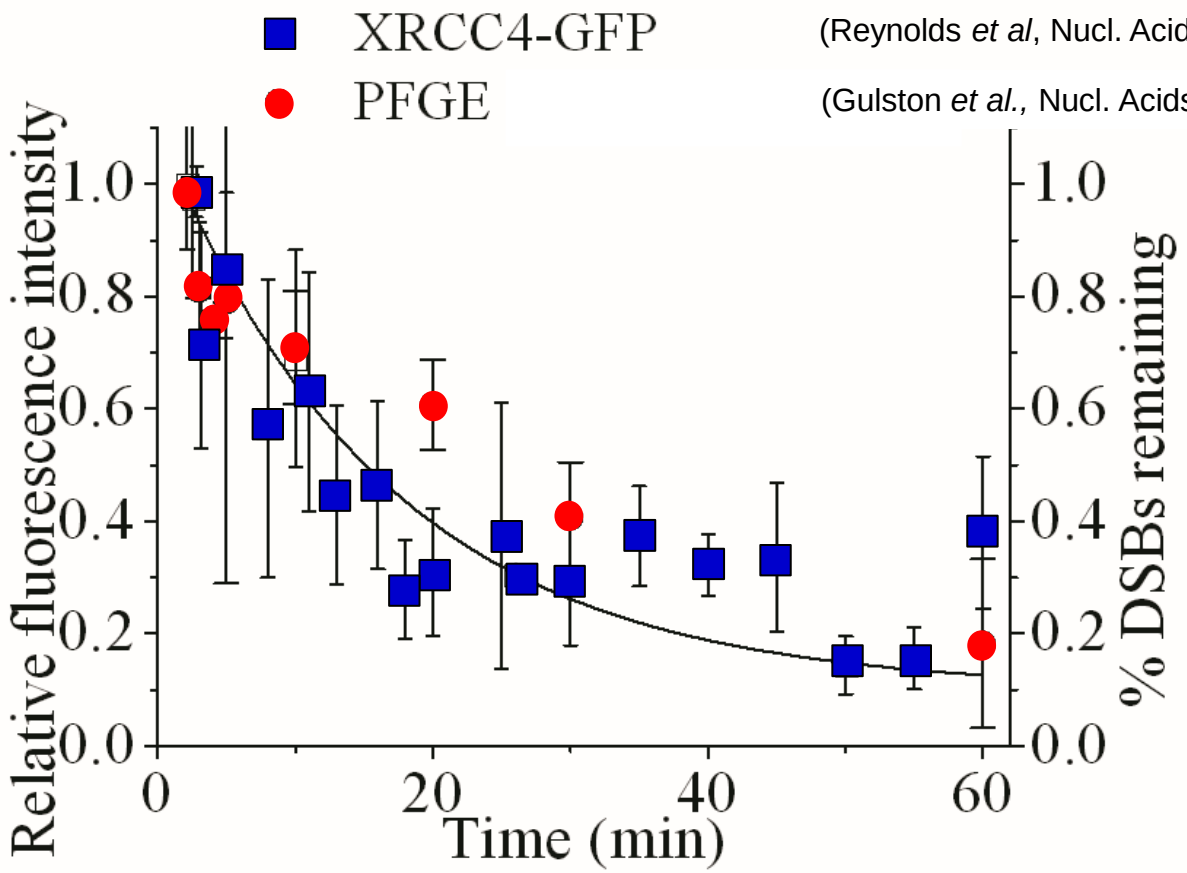
Complex (dirty) double strand breaks are difficult to rejoin



Fraction and complexity of dirty DSBs increase with LET of the radiation

Jenner, T.J., de Lara, C.M., O'Neill, P. and Stevens, D.L. *Int. J. Radiat. Biol*, 64, 265-273 (1993)
de Lara, C.M., Jenner, T.J., Townsend, K.M.S., Marsden, S.J. and O'Neill, P. *Radiat. Res*. 144, 43-49 (1995).

DSB rejoining (PFGE) step corresponds with final ligation step of NHEJ, seen as loss of XRCC4

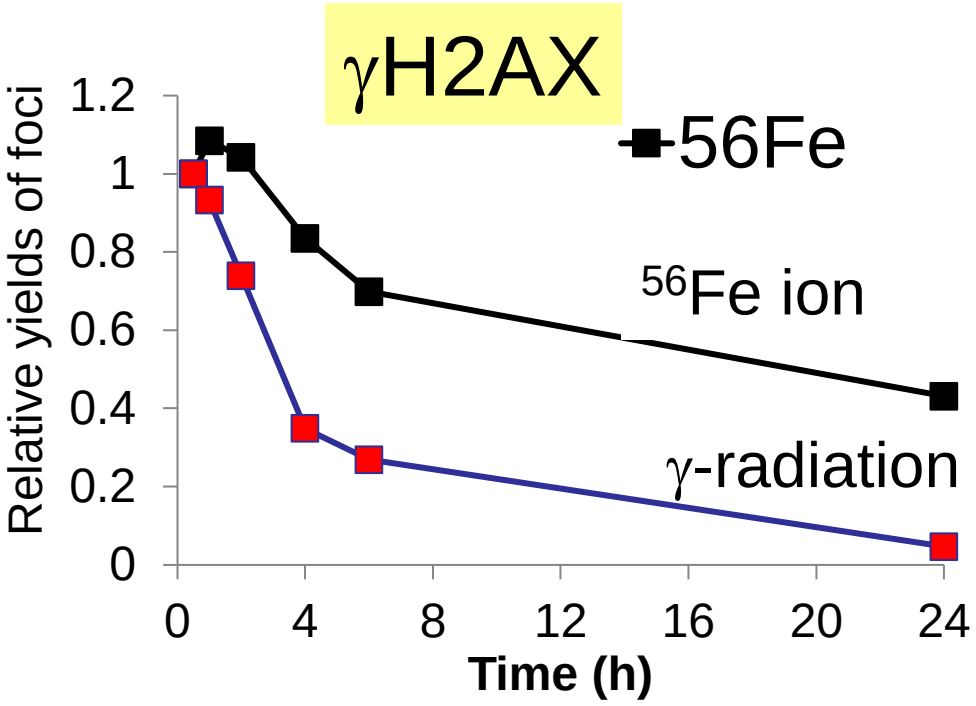


(Reynolds *et al*, Nucl. Acids Res., 40, 10821-31 (2012))

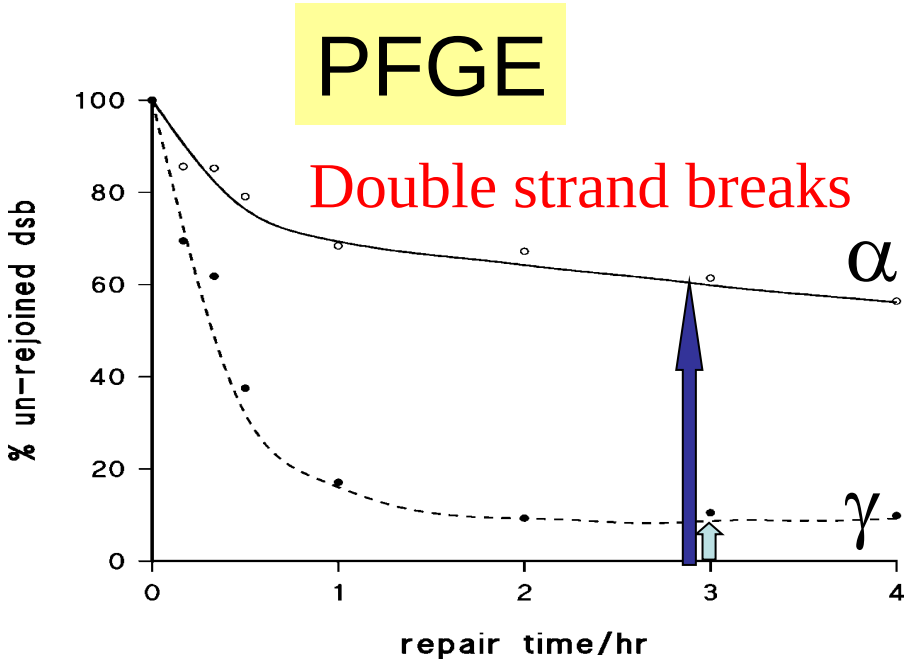
(Gulston *et al.,* Nucl. Acids. Res. **32**, 1602-1609 (2004))

For low LET radiation, majority of DSB (simple) rejoined within 40-60 min

Variation in DSB rejoining timescales following irradiation



Anderson, Harper, Cucinotta, O'Neill.
Radiat. Res. 174, 195-205 (2010)

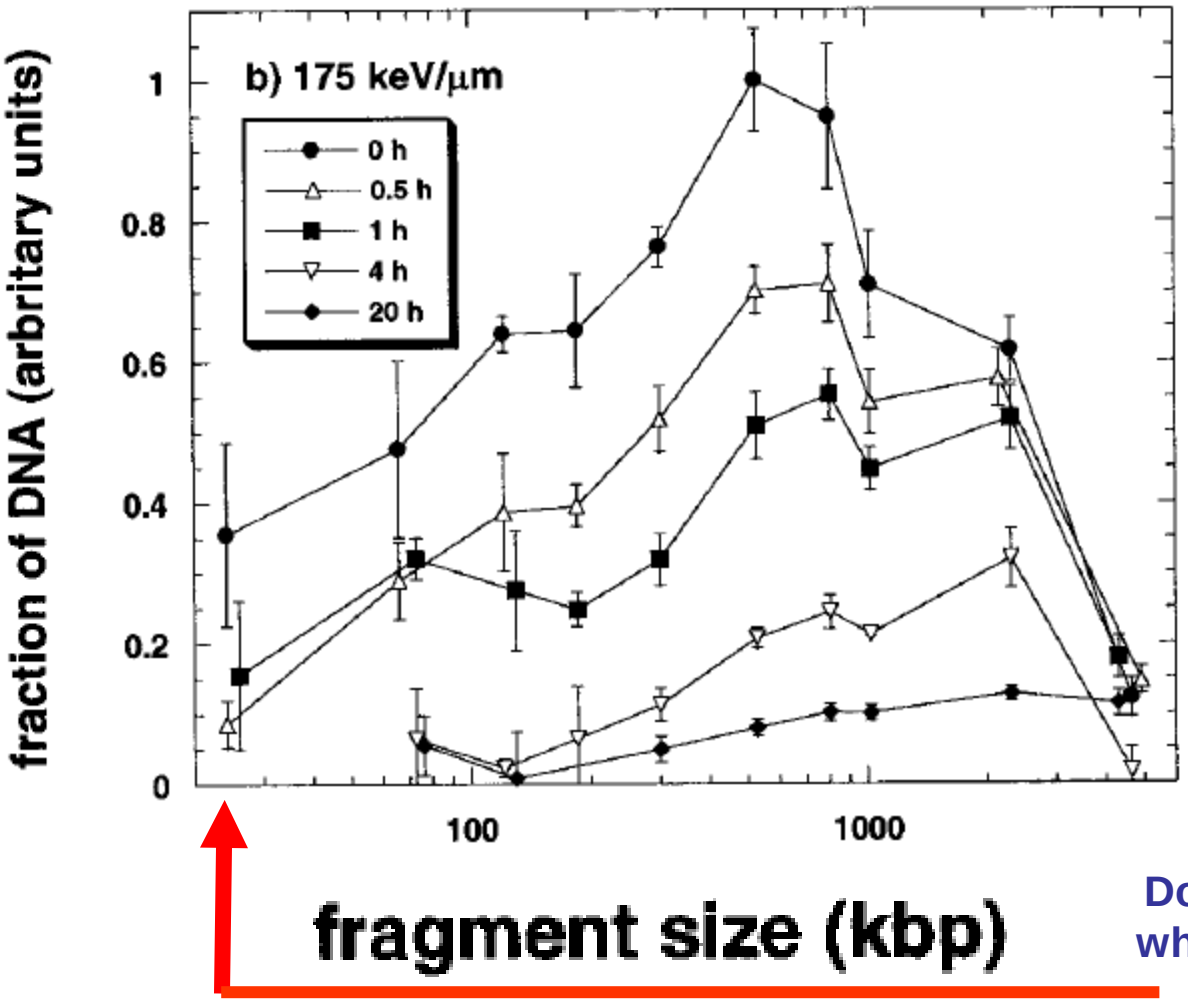


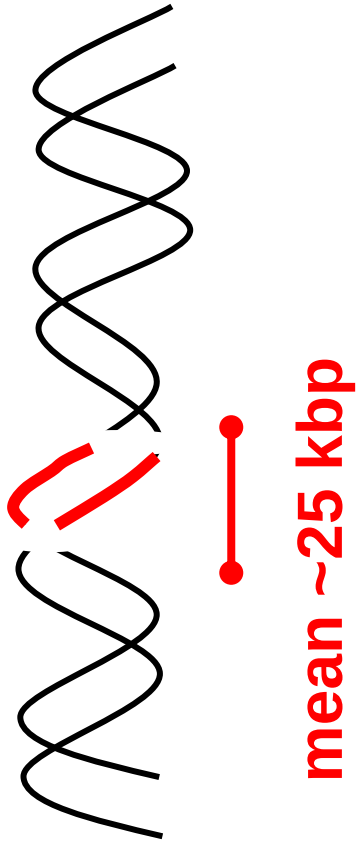
Jenner, de Lara, O'Neill,Stevens,
Int. J. Radiat. Biol, 64, 265-273 (1993)

Reflects γH2AX is only a surrogate marker of DSB and gives qualitative information

Differences in repair kinetics reflect different yields of complex (dirty) DSBs on LET

Small, non-random distribution of DNA fragments induced by nitrogen ions whereas low LET radiation produces random distribution of DSB





Do closely formed DSB as above, when detected by γH2AX, show as one focus?

Does the attempted repair of DSB, when clustered, occur independently for high LET?

Hoglund and Stenerlow, Radiation Research 155, 818-825 (2001)

Consequences of clustered damage

- **Complex damage sites pose problems to the DNA repair machinery**

- **Clustered DNA damage lead to high levels of mutations through retarded repair of base excision repair**

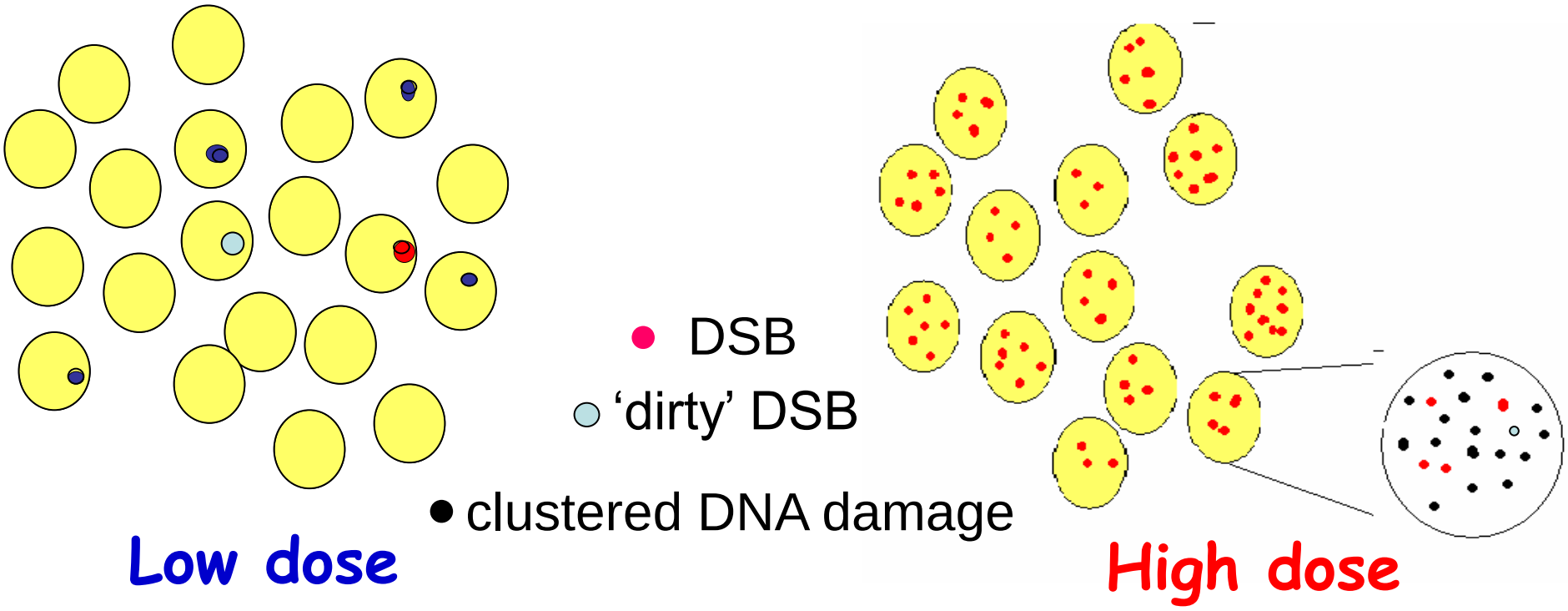
- **‘Dirty’ DSB pose problems to NHEJ pathway for DSB repair - role of DNAPKcs?**

- **BER protein levels dictate the hierarchy of initial processing of a clustered damage site- mutations or cytotoxic lesions formed**

Average number of lesions per cell at different doses for low LET radiation

Type of lesion	25 mGy	2 Gy
DSB	~0.5-1	~20-40
Complex DSB		
Assume		
5-10% for low LET	0.05-0.1 (1-2 cells in 20 cells)	2-4
25% for high LET	0.25	10
Non-DSB clustered damage	>8	>320

Considerations of ‘dirty’ DSB and clustered DNA damage distribution in cells on radiation dose

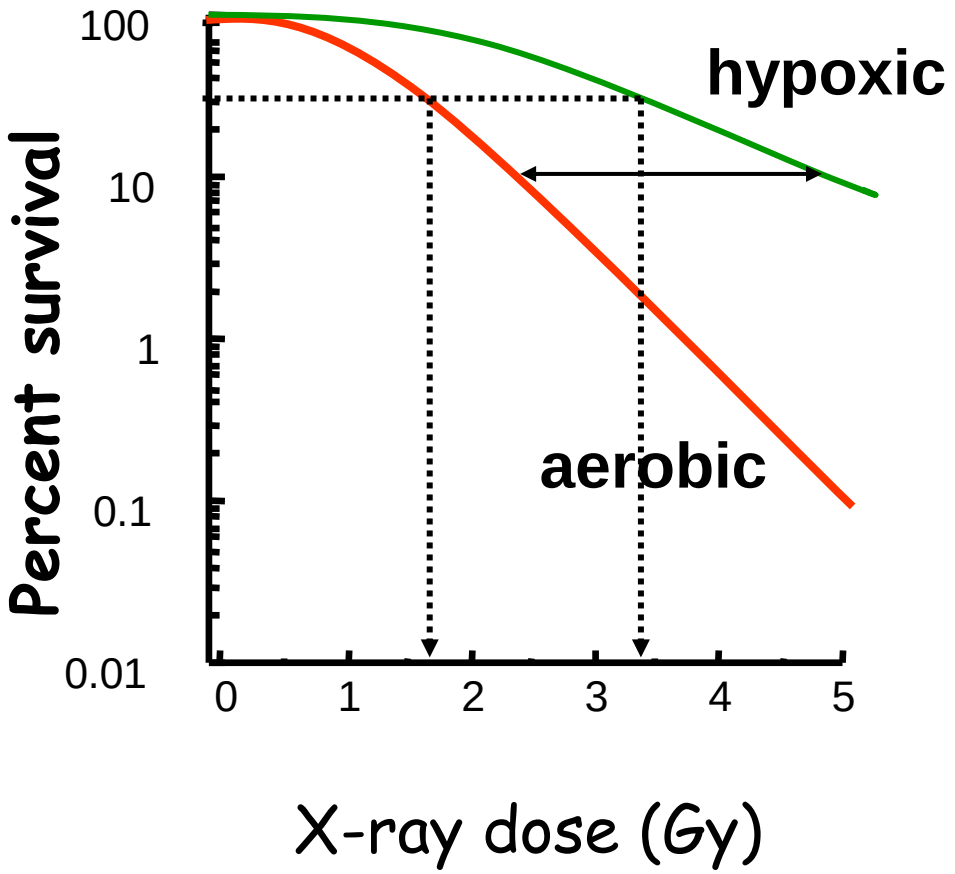


- Only a few cells will contain a DSB, others a clustered damage site at low dose. Most will not have radiation induced DNA damage. (see slide 47) At low dose, clustered damage may be important for mutations.

- At higher doses, most cells will contain all types of DNA damage when DSB effects may gain in importance in health effects

Oxygen effect (OER) – Enhances cell inactivation

- Tissues and cells become less radiosensitive under hypoxia
- Problem with treatment of some tumours



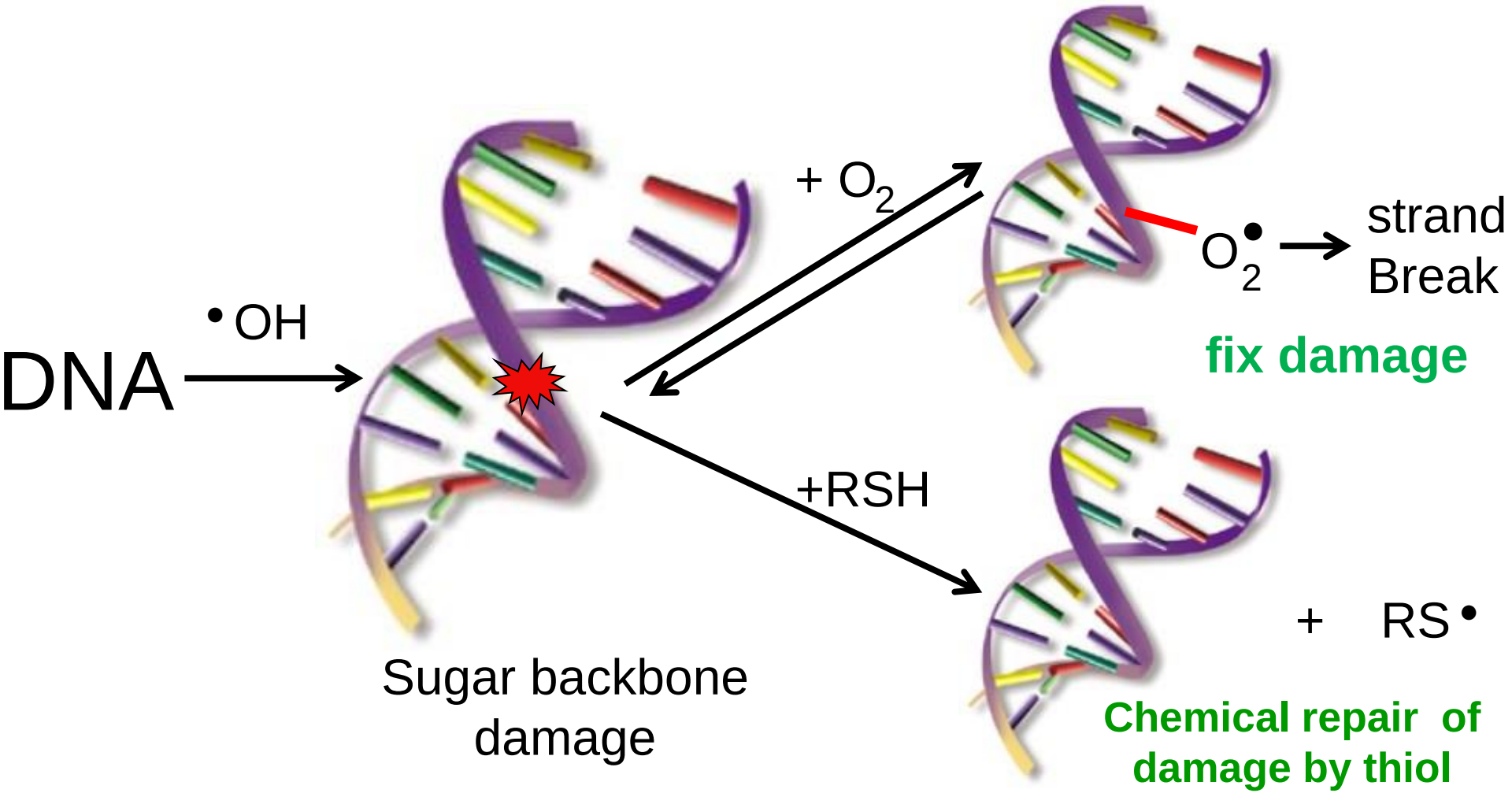
OER is the ratio of the doses to give the same level of survival under hypoxic and aerobic conditions

The ‘oxygen effect’ is a major factor in the outcome of radiotherapy at low LET

The mechanism involves chemical reactions of oxygen with DNA radicals during irradiation or within a few milliseconds following irradiation.

Held *et al*., Int. J. Radiat. Biol., 45, 627-636 (1982)

Oxygen effect for low LET radiation – fix damage

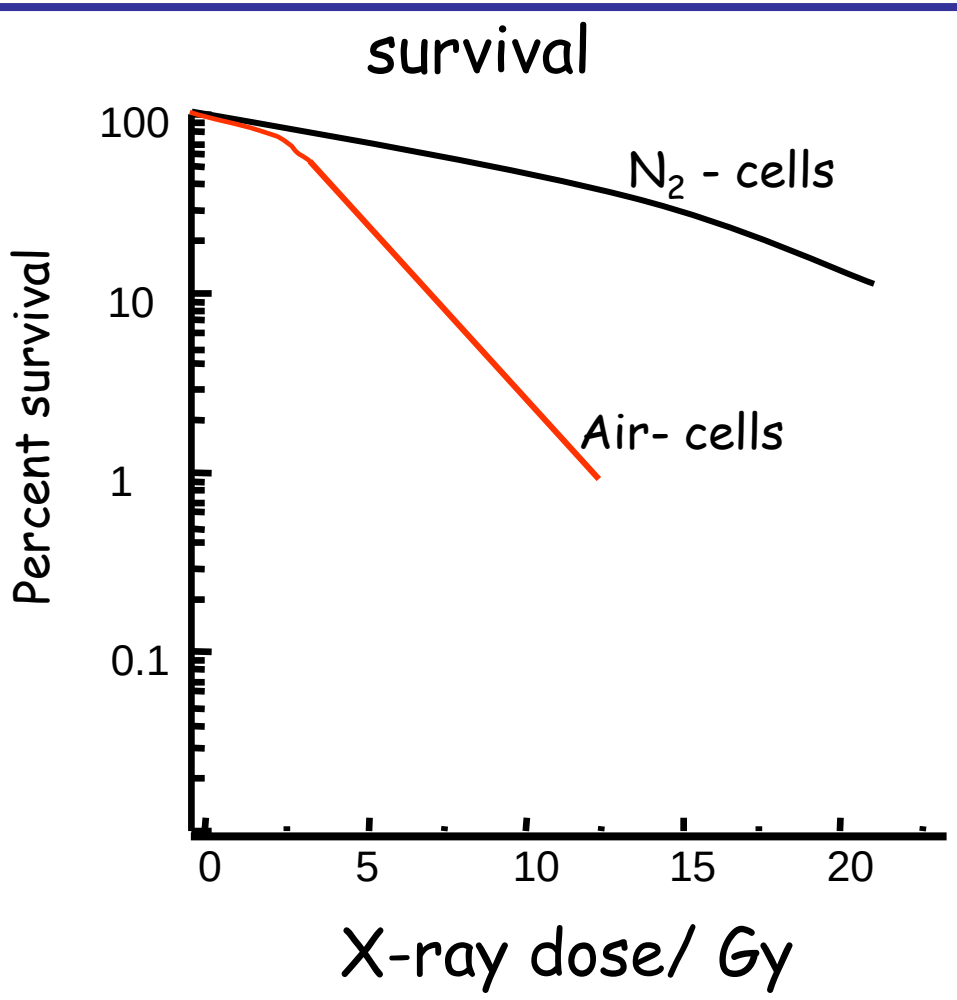


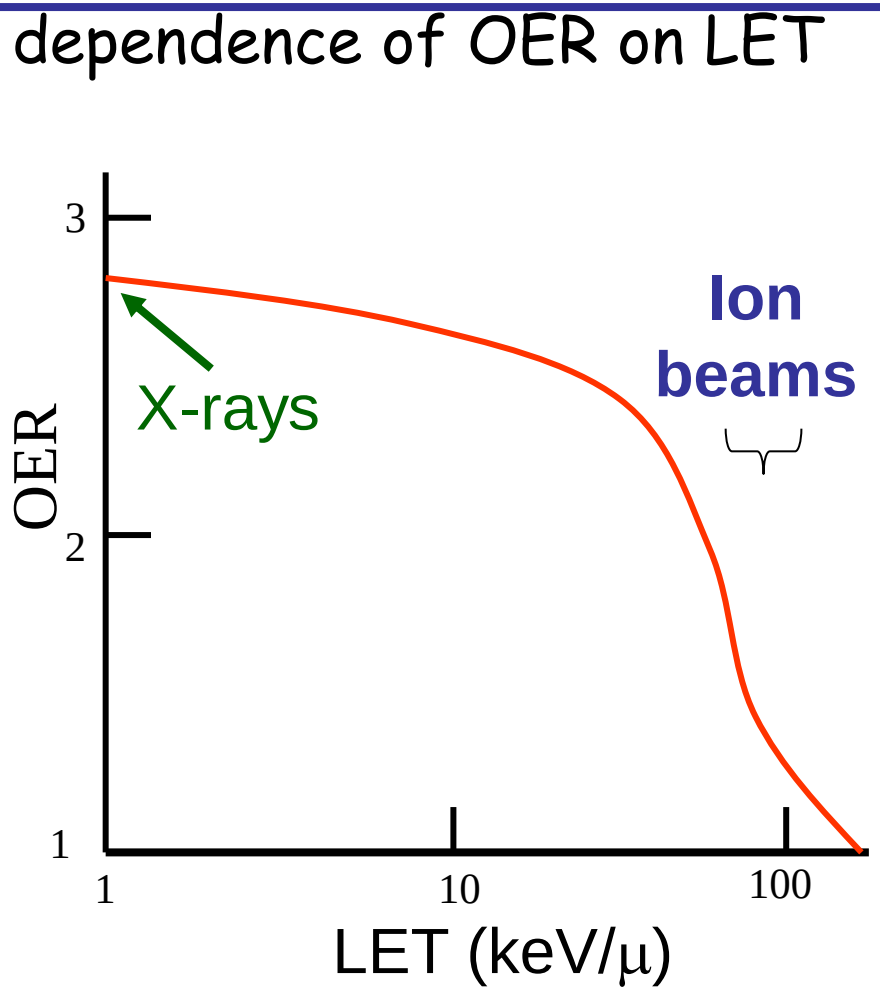
Oxygen needs to be present within 5 ms after irradiation to see any effect. Oxygen reacts with DNA target radicals in competition with other chemicals but importantly with thiols (RSH) and other electron/H-atom donors.

Held et al., IJRB, 45 627-636 (1984)

The oxygen effect in radiobiology

Oxygen enhancement ratio (OER)

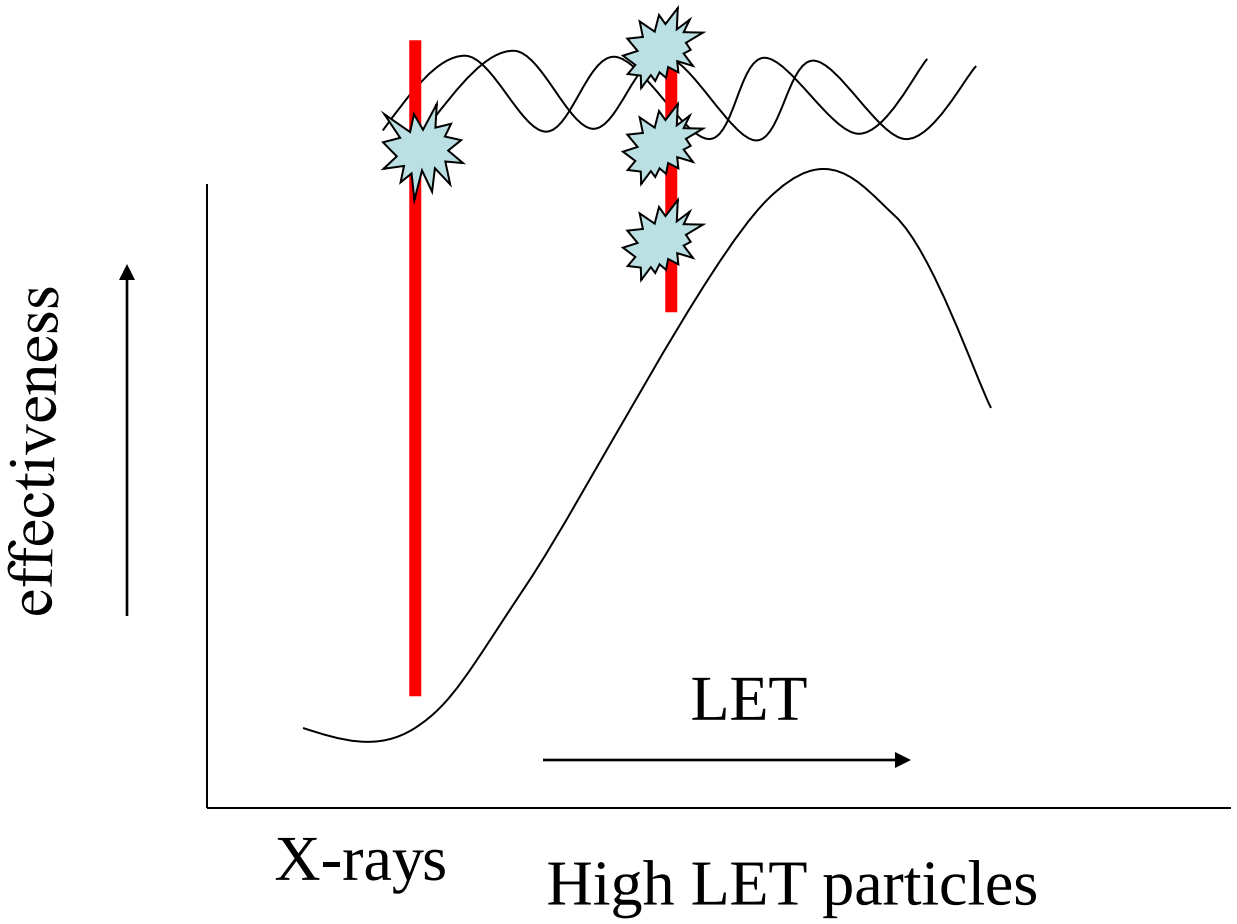




OER for low LET ~ 2.7 for survival and ~4-5 for DNA DSB induction

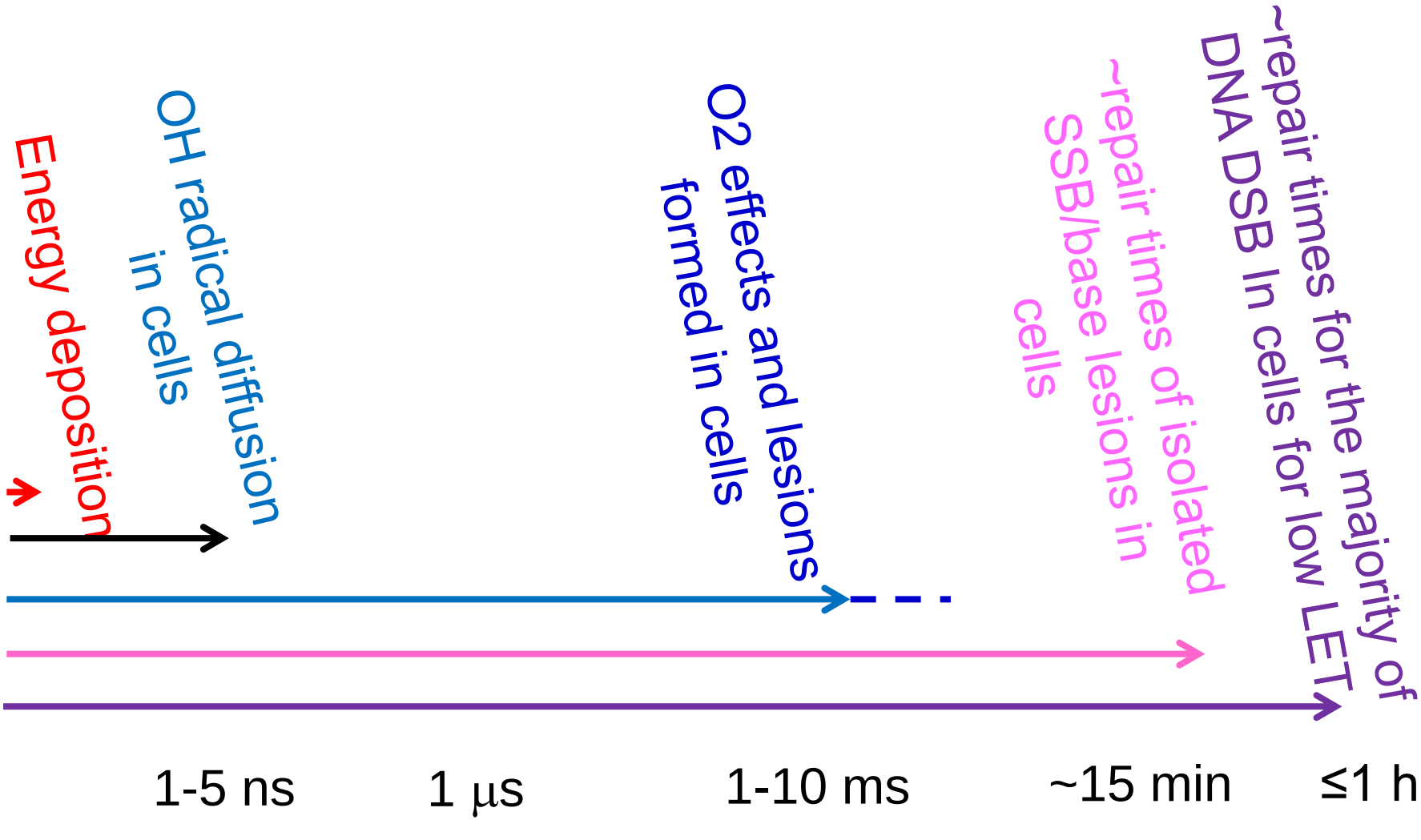
OER approaches 1 for high LET

Effectiveness of radiation quality at the genome level



The biological effectiveness of the radiation increases as the ionisation density (LET) increases.

Summary Overview of Timescales of Radiation Effects



Radiation tracks establish

- The site of energy deposition and the initial DNA radicals produced and the water radicals

- The spatial distribution of the radical species

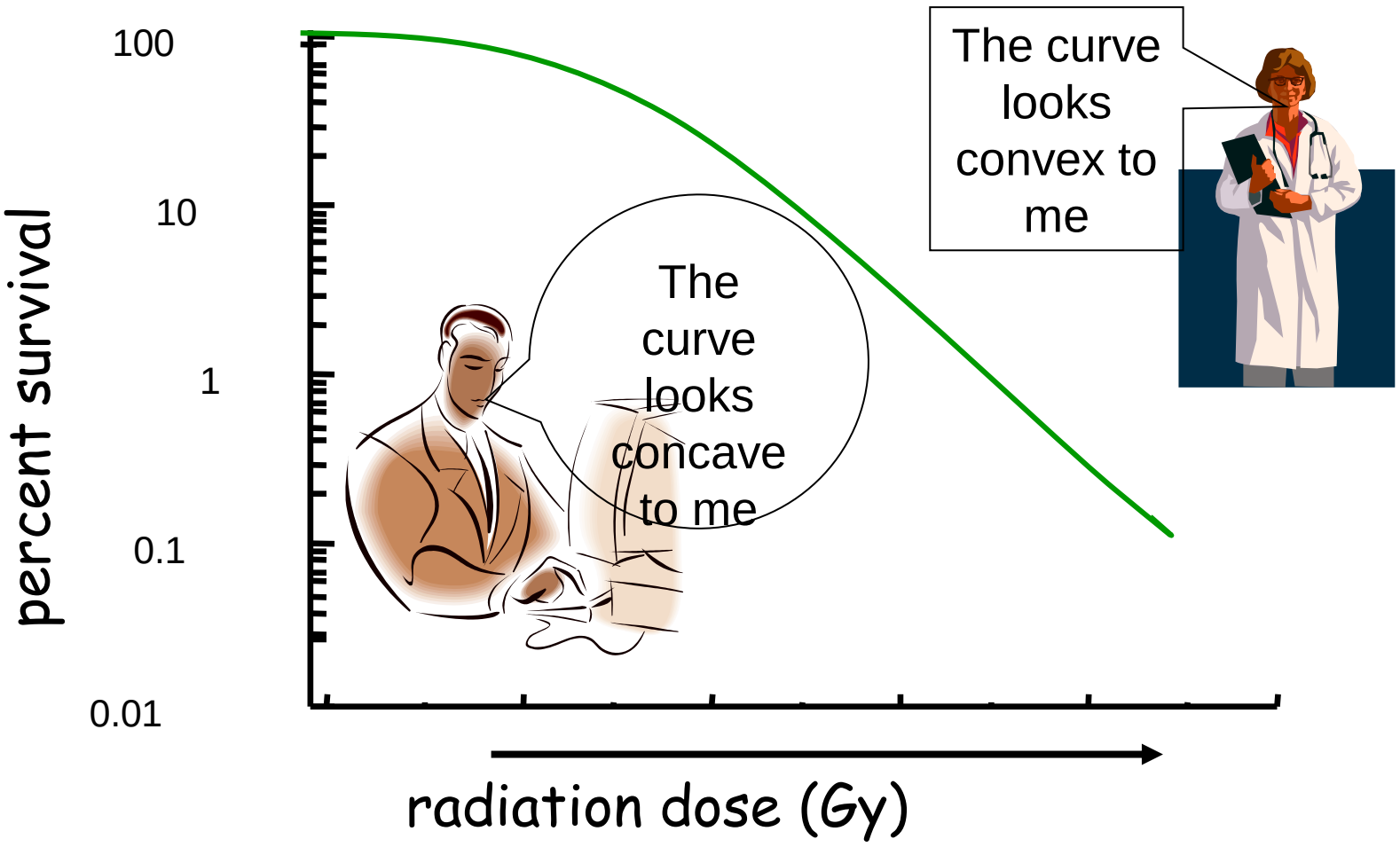
Chemical stage governs

- The ultimate site of persistent DNA damage

- The contribution of diffusible radical attack at DNA

- The type of DNA damage produced

- The influence of oxygen



Chemical mechanisms in radiobiology

von Sonntag, C. *Free-Radical-Induced DNA Damage and Its Repair. A Chemical Perspective*; Springer: Berlin, 2006.

Further Reading- Reviews

von Sonntag, C. Free-Radical-Induced DNA Damage and Its Repair. A Chemical Perspective; Springer: Berlin, 2006.

Reviews

1) Georgakilas,A.G., O'Neill,P. and Stewart,R.D.,
Induction and repair of clustered DNA lesions: what do we know so far?
Radiat. Res., **180**, 100–109 (2013).

2) Lomax ME, Folkes, L.K. and O'Neill,P.,
Biological Consequences of Radiation-induced DNA Damage: Relevance to Radiotherapy, *Clin Oncol.,* 25, 578-585 (2013)

3) Sage, E, Shikazono, N.,
Radiation-induced clustered DNA lesions: Repair and mutagenesis
Free Radicals Biology Medicine 107, 125-135 (2017).

4) Nickoloff, JA, Sharma,N. and Taylor L.,
Clustered DNA Double-Strand Breaks: Biological Effects and Relevance to Cancer Radiotherapy. *Genes* 11, 99 (2020).